ACADEMIE UNIVERSITAIRE WALLONIE-EUROPE

UNIVERSITE DE LIEGE

FACULTE DE MEDECINE VETERINAIRE

DEPARTEMENT MALADIES INFECTIEUSES ET PARASITAIRES

UNITE DE RECHERCHE EN EPIDEMIOLOGIE ET ANALYSE DE RISQUES APPLIQUEES AUX SCIENCES VETERINAIRES

Development of epidemiological methodologies to improve the clinical detection of emerging diseases in veterinary medicine

Développement de méthodes épidémiologiques pour améliorer la détection clinique des maladies émergentes en médecine vétérinaire

Sarah PORTER

Thèse présentée en vue de l'obtention du grade de Docteur en Sciences Vétérinaires

ANNEE ACADEMIQUE 2010-2011

To Maryse Scherotzke

Acknowledgements

I would like to express my deep and sincere gratitude to my promoter, Professor Claude Saegerman, for his support, motivation, efforts to seek funding, belief in my capacities and, I must admit, at times, for his patience. I am also deeply grateful to my co-promoter, Professor Niko Speybroek, for his help with the classification and regression analyses.

I wish to express my warm and sincere thanks to all the people who have contributed to the data used in this work: Prof H. Amory and all the staff from the Equine Teaching Hospital of Liège University for the data used in my retrospective study on equine endocarditis, as well as Lisette Trzpiot for her clerical assistance; Prof A. Leblond, Dr S. Lecollinet, Dr S. Zientara, Dr C. Cantile, Dr O. Kutai, and Dr P. Tritz for their contribution to the data used in my study on West Nile fever; Prof J. Mainil, Prof Yoichiro Horii, Prof Naoaki Misawa, Dr G. Czaplicki, and Prof Guatteo, for improving my reviews on Q fever; Prof B. Losson, Dr D. Vanhoff, Dr J.-P. Ganière, Dr D. Verwilgen, Dr G. Czaplicki and the practitioners who provided the sera for my laboratory analyses. Without all your precious contributions, my work would not have been possible.

I am particularly grateful to Dr Guy Czaplicki for accepting to collaborate for the laboratory work. I wish to thank him for his availability, his contribution to the bibliography and for sharing his laboratory and veterinary experience and knowledge. Furthermore, my warm thanks are due to all the staff from “l’ Agence régionale de santé et d’identification animales” (ARSIA), Loncin, for welcoming me. In particular, I thank Pascale Gotalle, Françoise Dohet and Béatrice Mutombo for their valuable advice and friendly help. Working among such great people was a wonderful experience.

I also wish to thank the members of the jury for reading this manuscript and in particular Dr Piet Deprez and Dr Eric Thys for being here today. I am extremely grateful to CEVA for sponsoring the editing of this work.

I owe my most sincere gratitude to Maryse Scherotzke and Dr Bureau for being there throughout the difficult times of the past few years and for being here today. This paper would not have been possible without them.

Finally, I extent my warmest thanks to my parents for their support, understanding and help during the preparation of this work. Their constant belief in my capacities has enabled me to rise from a childhood school failure and achieve my dream of becoming a veterinary surgeon.

And last but not least, I wish to thank my dog Tuppence for his constant presence by my side. His contribution must not be underestimated as he fulfilled the important role of protecting the office and data from undesirables. His psychological support and demand for exercise contributed to the equilibrium between mental and physical strain during the drafting of this work.

Table of contents

List of acronyms

ACERSA: Association pour la certification de la santé animale en élevage
AFSCA: Agence fédérale pour la sécurité de la chaine alimentaire
AFSSA : Agence française de sécurité sanitaire des aliments
ARSIA: agence régionale d'identification et de santé animales
BBB: blood-brain barrier
bp: base pair
BTM: bulk tank milk
CART: classification and regression trees
CBC: complete blood count
Cbmip: Coxiella burnetii macrophage infectivity potentiator
CDC: Centre of disease control and prevention
CFEP: Canadian Field Epidemiology Program
CFT: complement fixation test
CI: confidence interval
CNS: central nervous system
COPD: chronic obstructive pulmonary disease
CSF: cerebro-spinal fluid
E: envelope
EEE: eastern equine encephalomyelitis
E-Flanders: Eastern Flanders
EHV: equine herpesvirus
EID: emerging infectious diseases
ELISA: enzyme-linked immunosorbent assay
ELISA ProtG: ELISA using recombinant protein G conjugate
ELISA ProtA/G: ELISA using recombinant protein A/G conjugate
DISI: equine disease information system
FCV: feline calicivirus
FDA: Food and drug administration
FeLV: feline leukemia virus
FHV: feline herpesvirus
FIV: feline immunodeficiency virus
GAPDH: glyceraldehydes-3-phosphate dehydrogenase
HIV: human immunodeficiency virus
IFA: immunofluorescence assay
IFN: interferon
Ig: immunoglobulin
IHC: immunohistochemical
INRA: Institut national de recherché agronomique
IRS-PCR : infrequent restriction site-PCR
IUFD: intrauterine fetal death
IVIG: intravenous immunoglobulin G

JE : Japanese encephalitis
LCV: large-cell variant
LPS: lipopolyssacharide
MA: monoclonal antibody assay
MAF: modified acid-fast
MLVA: multiple locus variable tandem repeat analysis
MRI: Magnetic resonance imaging
n=N=No: number
NC= nc: negative control
No = N= n: number
OD: optic density
ODm: mean optic density
OIE: Office international des épizooties
OR: odds ratio
p: probability
P: positive
PC=pc: positive control
PCR: polymerase chain reaction
PFGE: pulsed-field gel electrophoresis
PMN: polymorphonuclear leukocyte
PrM: premembrane
PRNT: plaque-reduction neutralization test
rACP: recombinant acid phosphatase
RBC: red blood cell
ROC: receiver operating characteristic curve
ROI: reactive oxygen intermediates
r_s: Spearman's rank correlation
RT PCR: Real Time PCR
RTq PCR: Real Time Quantitative PCR
S: sample
SCV: small-cell variant
SD: standard deviation
Se: Sensitivity
Sp: Specificity
S/P: sample value related to positive control value= (mean sample optic density minus mean negative control optic density) / (mean positive control optic density minus mean negative control optic density)
RESPE: réseau d'épidémiosurveillance en pathologie équine
RFLP: restriction fragment length polymorphism
RNA: Ribonucleic acid
rRNA: ribosomal RNA
RTq-PCR: Real time Quantitative PCR
RT-nPCR: Real Time Nested PCR
USA: United States of America

UV: ultraviolet
VNTR: variable tandem repeat
WBC: white blood cells
W-Flanders: Western Flanders
WHO: World Health Organization
WN: West Nile
WNF: West Nile Fever
WNV: West Nile Fever virus
YF: Yellow fever
2D: two dimensional

INTRODUCTION

Preface

Emerging, and potentially emerging, diseases have become a subject of major interest for scientists worldwide.

Climate change, international transport of humans and goods, socio-economic changes, man-made or natural modifications of environment, development and commercialization of new diagnostic methods associated to an increased awareness and pro-active approach, have lead to the discovery of new pathogens and new clinical illnesses in Europe.

Infections transmitted by vectors are of predominant interest as distribution of known, or potential, vectors and hosts are in expansion. The dynamics of such diseases are in constant evolution allowing adaptation of pathogens to new epidemiological niches and conditions. This phenomenon can lead to the emergence of disease in an area previously non-infected. The pathogenic causal agent finds itself in a naïve population of animals or humans leading to potentially severe clinical manifestations and disastrous medical and economical consequences. Furthermore, many illnesses at risk of emerging in animals are zoonoses and several of them have currently become a severe threat to public health. Indeed, it is essential to promote early detection of emerging diseases in the veterinary field as well as in the medical field. The “One world, one health” concept is of major importance in these circumstances.

Clinical epidemiology applied to animal emerging diseases consists in the study of infectious and parasitic diseases. Its aim is to determine risk factors for emergence, development and maintenance of new pathogens in a new environment. As mentioned above, early detection of emerging animal diseases is essential. However, emerging diseases are usually very poorly known or understood in the countries concerned, rendering diagnosis very difficult. In this context, communication between countries and centralization of information is essential. In

this study, several epidemiological methods have been developed to facilitate diagnosis by veterinarians. However clinical epidemiology has its limitations and laboratory analyses or complementary examinations with more sophisticated equipment are sometimes unavoidable.

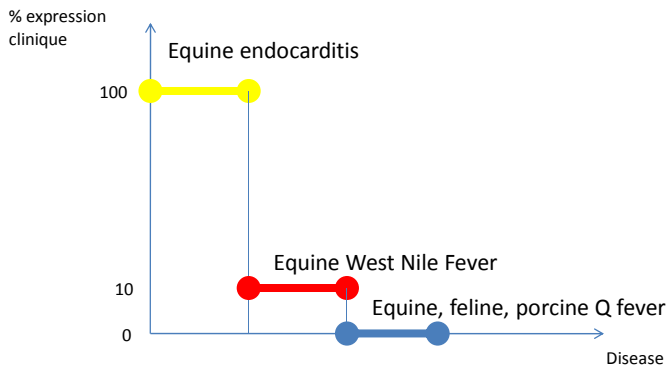
Widely used and very fashionable in the recent years, the definition of an emerging disease can lead to controversy. In this work, we will use the definition of the World Health Organization (WHO) that defines an emerging disease as "a pathogen that is newly recognized or newly evolved, or that has occurred previously but shows an increase in incidence or expansion in geographical, host, or vector range" (www.who.int/zoonoses/emerging_zoonoses/en).

At the beginning of emergence of a disease, clinical cases and their diagnoses are rare events. Prevalence of infection is very low. A certain lap of time is necessary for the transmission of the pathogen and, consequently, for infection of a larger proportion of the population. During this process the prevalence of disease increases gradually. The causal agent and its natural cycle must adapt to the new conditions present. With the gradual increase in number of immune individuals through the natural spread of disease or through large scale preventive vaccination programs, the prevalence of new infections and of clinical illness diminishes with time. Once completely abolished, the population or species or area is once more considered free from the disease. Further emergence of the same disease can however take place in new naïve populations.

Emerging zoonoses can either be symptomatic in 100% of cases, asymptomatic in a majority of cases, or seemingly completely asymptomatic in their reservoir host. Seroprevalence indicates circulation of the pathogen in the population and potential zoonotic risk.

This study was based on three different diseases:

- one considered 100% symptomatic (as long as the animal doesn't die from an independent cause beforehand): equine vegetative endocarditis
- one with only 10% of cases being symptomatic: West Nile fever in horses
- one with, to our knowledge, no clinical expression reported in western Europe (0% symptomatic): Q fever in western European horses, felines, pigs and wild boars



Vegetative endocarditis is a very rare condition in equids. Its prevalence is very low worldwide. As mentioned previously, an emerging disease initially has a very low prevalence. Equids with endocarditis generally develop associated clinical signs during their life span, except in cases where the animal dies as a consequent of an independent pathology (e.g., colic) with the endocarditis lesion being a fortuitous postmortem finding. In this study, vegetative endocarditis in equids was used as a model for the development of an epidemiological methodology for early clinical diagnosis of rare diseases, including future emerging diseases with a high rate of clinical expression.

West Nile Fever (WNF) is an example of emerging zoonosis in Europe. The aim of this section was to perform a direct application of the methodology developed for a disease with clinical expression (equine endocarditis) to a disease with a high percentage of asymptomatic infections. In this way, WNF emphasizes the limitations of clinical diagnoses and introduces the importance of laboratory testing in cases of emerging diseases implicating asymptomatic infections.

Previously an undiagnosed zoonosis, Q fever is currently an emerging public health problem. To our knowledge, no data concerning its prevalence and its potential clinical signs in western European equids, felines, pigs and wild boars is available. Seropositivity in equids, felines, pigs and wild boars would signify that the Q fever causes asymptomatic infections in these species. In infected ruminant herds, asymptomatic infections and chronic carriers are common and cause a problem for clinical diagnosis and control of the disease. The lack of clinical expression render laboratory testing essential to determine prevalence of infection.

1. Introduction to the diseases studied

1.1. Equine vegetative endocarditis

1.1.1. Definition and epidemiology

Bacterial endocarditis, also called vegetative or infective endocarditis, is a rare heart condition in horses (Figure 1). Because of the rareness of this disease, large scale epidemiological studies are lacking in this species. The few reported cases suggest that males could to be more frequently affected than females (Buergelt et al. 1985; Sage and Worth, 1999) and that young horses (under four years of age) could be predisposed to the development of endocarditis (Buergelt et al., 1985; Maxson and Reef, 1997). A later study by Maxson and Reef (1997) on a number of 10 horses concluded that no breed or sex predilection was obvious. Although this condition remains rare, its various forms of clinical expression make it an important element in the differential diagnosis of horses presented with vague clinical signs (such as weight loss, poor performance, fever of unknown origin, shifting lameness, etc.), or presented with signs suggestive of heart disease (Dedrick et al., 1988; Travers and Van den Berg, 1995; Patterson, 1996; Bonagura and Reef, 1998; Smith, 2002).

1.1.2. Clinical signs

Two different forms of vegetative endocarditis can be distinguished: an acute form and a subacute or chronic form. Signs characterizing the acute form are high fever, depression, reluctance to move due to thoracic discomfort or lameness, and the rapid development of signs of cardiac insufficiency (edema, increased jugular filling, etc.). In the subacute or chronic form, which is much more common, horses may show intermittent fever spikes, weight loss, shifting lameness, poor performance, lethargy/depression, and/or a significant

heart murmur lasting for weeks or months. Signs of cardiac failure usually occur only as a terminal event (Travers and Van den Berg, 1995). Over a period of 10 years from 1982 to 1992 at the New York State College of Veterinary Medicine, 19 horses suffering from vegetative endocarditis were observed (Ball and Weldon, 1992). All these horses exhibited lesions of endocarditis at necropsy, but only five of them (thus only 26%) presented with clinical signs related to the disease. The fourteen other horses (74%) presented with a variety of primary problems.

Although endocarditis is frequently associated with the presence of a heart murmur, this is not always the case. In humans, 10% of patients affected by the disease don't exhibit a heart murmur upon clinical examination (Weldon, 1992). The type of heart murmur obviously depends on the location and size of the lesion, which can lead to valvular insufficiency or valvular obstruction (Hillyer et al., 1990). Changes in the quality of a heart murmur can also be compatible with bacterial endocarditis (Dedrick et al., 1988; Hillyer et al., 1990).

The most commonly affected valve in equids appears to be the aortic valve, followed by the mitral valve. The tricuspid valve is the third most frequently affected valve. The pulmonary valve is rarely concerned in this species (Ball and Weldon, 1992). Mural atrial vegetative lesions have also been reported in horses (Collatos et al., 1990).

Vegetative endocarditis can be associated with cardiac arrhythmias on clinical examination and electrocardiogram. The reported arrhythmias include atrial fibrillation, atrial or ventricular premature beats and ventricular tachycardia (Buergelt et al., 1985; Roby and Reef, 1986; Dedrick et al., 1988; Collatos et al. 1990). Ventricular premature contractions and atrial fibrillations are the most common arrhythmias encountered in horses with bacterial endocarditis (Karzenski et al., 1997).

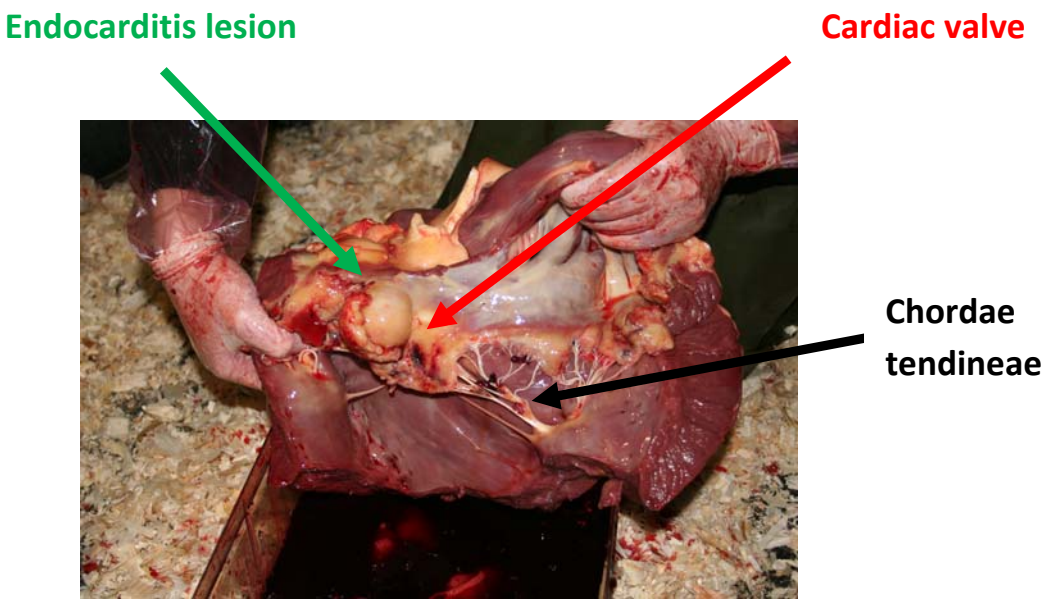


Figure 1. Vegetative endocarditis lesion on the mitral valve in a 3 year old cart horse (Liège University, 8/12/2006)

1.1.3. Pathogenesis

Each form of bacterial endocarditis (i.e. acute and subacute or chronic) is characterized by a specific pathway of valve infection (Figure 2).

The acute form of endocarditis consists of the infection by a primary pathogen attaching directly to the endothelium of the heart valves (Travers and Van den Berg, 1995). The only predisposing factor necessary is systemic infection with an organism capable of binding directly to the endothelium (Ball and Weldon, 1992). The most common organisms associated with this type of endocarditis are *Staphylococcus aureus* and some *Streptococcus spp*..

In the subacute or chronic form, an opportunistic pathogen colonizes the valve after the occurrence of certain predisposing events (Travers and Van den Berg, 1995). Indeed, under normal circumstances, vascular endothelium is resistant to infection by opportunistic bacteria. Therefore, the first step to valve infection is damage to the valve itself (Freedman, 1987). This

often occurs due to what is called the venturi or jet effect, during which turbulent blood flow traumatizes the endothelium (Travers and Van den Berg, 1995). This induces a lesion composed principally of platelets and fibrin forming a non-bacterial thrombotic vegetation. The next step is the discharge of microorganisms into the general circulation (bacteremia) from a peripheral site (Freedman, 1987). This bacteremia is usually of a transient nature. The inflammation focus source of infection can be a foot abscess, septic laminitis, phlebitis, etc. (Ball and Weldon, 1992). However, the primary source of infection is often not found (Travers and Van den Berg, 1995). The microorganisms become adherent to the non-bacterial thrombotic vegetation covering the damaged endothelium with subsequent deposition of additional fibrin and platelets, thereby covering the infecting agent (Freedman, 1987). In this way, the microorganisms are protected from circulating cellular immune system and can multiply within the platelet-fibrin vegetation. It also appears that the presence of a high agglutinating antibody titre toward the infecting organism is necessary to induce the endocarditis lesion. It has been shown experimentally that without a high antibody titre the development of the disease is impossible (Travers and Van den Berg, 1995). Clumping of free bacteria by antibodies in the blood could form a larger inoculum that could more readily be deposited on the platelet-fibrin thrombus. Moreover, antibody-antigen complexes formed are probably also responsible for the skin manifestations of infective endocarditis (e.g., petechiae, Osler and Janeway lesions), as well as associated arthritis and glomerulonephritis. Immune-complexes may be detected as cryoprecipitates and stimulate the formation of antiglobulin (rheumatoid factor) (Freedman, 1987).Once the infection is established, the bacteria can slip back into the circulation though little pores in the covering of fibrin and platelets, causing a new burst of bacteremia. The dislodgement of fragments of infected vegetation into the general circulation can produce peripheral emboli. These embolic showers cause either infarctions or metastatic infections of distant organs, causing secondary organ dysfunction

(Ball and Weldon, 1992; Travers and Van den Berg, 1995). Lesions on the right side of the heart most frequently cause embolic pneumonia (Travers and Van den Berg, 1995). Left sided heart lesions cause mainly renal and spleen involvement (Ball and Weldon, 1992; Travers and Van den Berg, 1995). Another after-effect of infective endocarditis is the development of congestive heart failure due to valvular insufficiency secondary to valvular damage. Non-septic arthritis can be associated with endocarditis (Freedman, 1987; Travers and Van den Berg, 1995). When arthritis is observed in association with endocarditis, three possibilities must be considered (Ball and Weldon, 1992): (1) the focus of the initial infection was a septic joint causing septicemia, then secondary infection of other joints and the development of infective endocarditis; (2) the infective endocarditis caused a bacteremia that has been at the origin of the development of septic arthritis; (3) the arthritis observed is the consequence of an immune-complex disposition in the synovial basement membrane. Glomerulonephritis, as mentioned above, could also be due to the disposition of immune-complexes on the basement membrane as has been shown in humans suffering from vegetative endocarditis. Myocardial infarcts also sometimes occur as a consequence of endocarditis (Ball and Weldon, 1992). They are more likely to occur in horses with left sided endocarditis and may contribute to the development of congestive heart failure (Maxson and Reef, 1997).

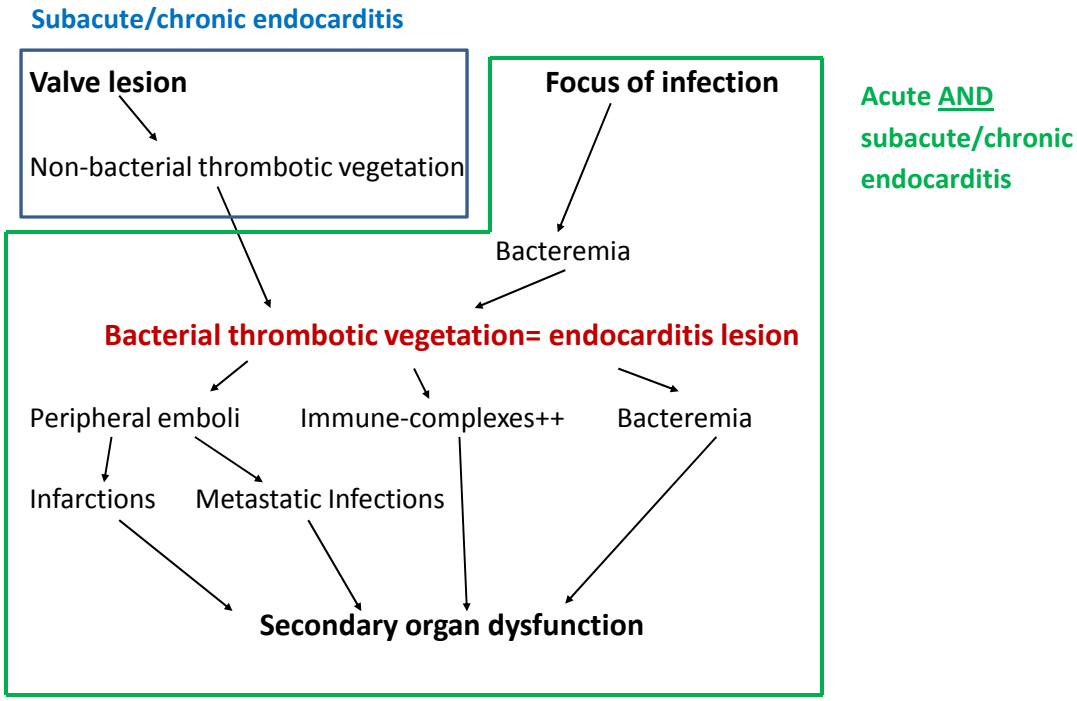


Figure 2. Pathogenesis of vegetative endocarditis

Legend:

Pathway surrounded in blue: pathogenesis of subacute/chronic endocarditis only; predisposing factor for endocarditis

Pathway surrounded in green: pathogenesis for acute AND subacute/chronic endocarditis

The organisms found in subacute or chronic equine endocarditis lesions vary considerably. The most commonly reported, even if it represents only a few cases, are *Streptococcus zooepidermicus* and *Actinobacillus equuli* (Hatfield et al., 1987; Travers and Van den Berg, 1995; Ramzan, 2000). Many other organisms have been reported, such as *Pseudomonas aeruginosa* (Travers and Van den Berg, 1995), *Erysipelothrix rhusiopathiae* (McCormick, 1985), *Escherichia coli*, *Candida parapsilosis* (Buergelt et al., 1985), *Pasteurella spp.* (Church et al., 1998), *Serratia marcescens* (Ewart et al., 1992), *Shigella equirulis* (Ball and Weldon, 1992) , *Mycobacterium tuberculosis* (Person, 1987), *Pseudomonas cepacia* (Travers and Van den Berg, 1995) and other *Streptococci* (Buergelt et al., 1985; Dowres, 1985; Robey

and Reef, 1986; Wagenaar and Kroneman, 1986; Freedman, 1987; Dedrick et al., 1988; Travers and Van den Berg, 1995; Kaplan and Moore, 1996). *Strongylus vulgaris* fourth stage larvae have also been incriminated as a cause of aortic semilunar valvulitis in the horse (Hatfield et al., 1987; Ball and Weldon, 1992; Travers and Van den Berg, 1995).

1.1.4. Diagnostic methods

Vegetative endocarditis cannot be diagnosed with certainty based on case history and clinical examination alone. To reach a definitive diagnosis, complementary examinations are necessary. Among the available diagnostic methods, echocardiography is the most useful.

Echocardiography and electrocardiography

Echocardiography and electrocardiography can be extremely beneficial in the diagnosis (Figure 3), prognosis and serial assessment of endocarditis by identifying anatomic lesions and characterizing arrhythmias (Karzenski et al., 1997).

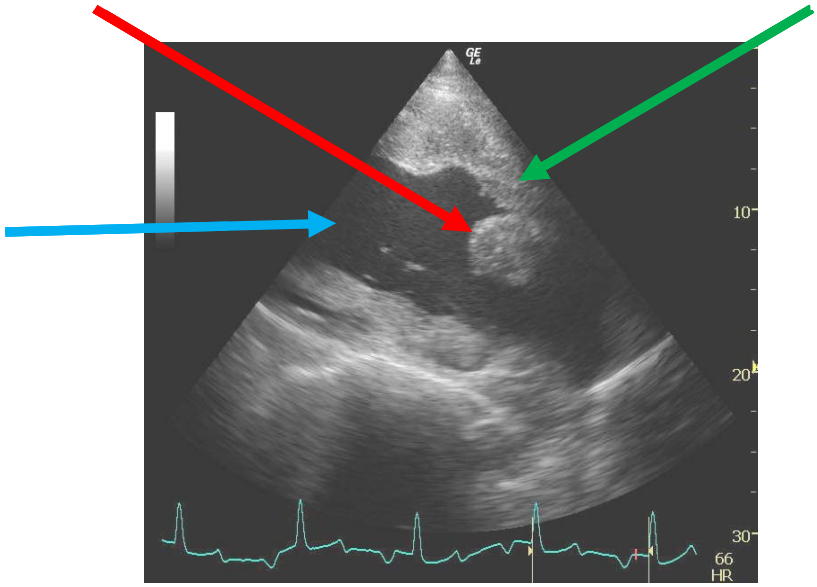


Figure 3. Echocardiography of an endocarditis lesion on the mitral valve in a 3 year old cart horse (Liège University, 8/12/2006)

Echocardiography remains the most specific, but not highly sensitive, modality for the diagnosis of endocarditis (Karzenski et al., 1997). Echocardiographic diagnosis of valvular endocarditis was first reported in man by Dillon et al. in 1973. Six years later, this method of diagnosis was used in a horse suffering from vegetative endocarditis by Pipes et al. (1979).

Two dimensional (2D) mode and M-mode echocardiography should be used systematically. The affected valve usually appears thickened or with an increased echogenicity. It may also appear shaggy or ragged or as parallel linear or irregular echoes. Increased echogenicity can be observed when calcification of the valve occurs. In domestic animals, valve calcification is usually secondary to bacterial endocarditis (Bonagura and Pipers, 1983). Vegetations should be differentiated from ruptured chordae tendineae, flail valvular leaflets or nodular thickening (Verdegaal and Sloet van Oldruitenborgh-Oosterbaan, 2006). If an endocarditis lesion is detected, Doppler echocardiography should be performed to examine the severity of valvular regurgitation. Active lesions can usually be differentiated from healing or old lesions by their echographic appearance. Decrease in size, smoothing, and increased echogenicity of the vegetative lesion is consistent with control of infection. On the other hand, enlargement of the valvular lesion indicates failure of treatment (Maxson and Reef, 1997). Moreover, serial echocardiography allows follow-up of the development or progress of heart failure. An indication of prognosis is based on the degree of enlargement of the cardiac chambers, severity of regurgitation and extent of the myocardial dysfunction. Observation of initial signs of congestive heart failure (increased size of cardiac cavities, shortening fraction decreased, etc.) worsens the vital prognosis for the patient (Maxson and Reef, 1997).

Electrocardiography can be employed to characterize arrhythmias resulting from myocardial damage (Karzenski et al., 1997).

Blood culture

Blood sample cultures and antibiograms are useful to identify the infecting pathogen and determine the choice of antibiotic if treatment is attempted (Ewart et al., 1992; Travers and Van den Berg, 1995; Karzenski et al., 1997; Maxson and Reef, 1997; Verdegaal and Sloet van Oldruitenborgh-Oosterbaan, 2006). It is thought that blood samples taken during a fever episode increases the likelihood of a positive blood culture. In practice, the phlebotomy site should be prepared aseptically. The samples are aseptically collected in a commercial broth media for blood culture. At least 3 samples should be collected from a different venipuncture over a two hour period. Moreover, the blood sampling should be done before any administration of antibiotic treatment or, if not possible, at least 24 hours after the last antibiotic treatment (Ball and Weldon, 1992).

Blood analyses and biochemistry results

Most commonly reported laboratory findings associated with endocarditis are hyperproteinemia, hyperfibrinogenemia, anemia, leukocytosis with mature neutrophilia, hypoalbuminemia and hyperglobulinemia associated to hypergammaglobulinemia (Hatfield et al., 1987; Dedrick et al., 1988; Kasari and Roussel, 1989; Hillyer et al., 1990; Ball and Weldon, 1992; Travers and Van Den Berg, 1995; Maxson and Reef, 1997). Those changes are indicative of a severe chronic inflammatory process (Van Lierde, 1988).

Other diagnostic aids

Other complementary examinations such as synovial fluid sampling, X-rays, urinalysis, broncho-alveolar lavages, etc. may be useful in cases showing metastatic manifestations of the disease.

1.1.5. Treatment

If a treatment is attempted, it should be instituted as early as possible in the course of the disease. However, due to the non-specific signs associated with endocarditis, early detection of the disease is often difficult.

Vegetative endocarditis being an infection of the valvular endothelium by a specific microorganism, treatment logically consists of a high dose of bactericidal antibiotic based on the results of the antibiogram for a minimum period of 4 to 6 weeks (Hillyer et al., 1980; Dedrick et al., 1988; Collatos et al., 1990). Ideally, the antibiotics should be administered intravenously. Good tissue penetration is necessary because the valvular vegetations consist of walled-off layers of avascular fibrous tissue with ennested organisms (Ball and Weldon, 1992; Travers and Van den Berg, 1995). Blood culture described above should be performed for directing an appropriate antibiotic therapy. While awaiting the results of the bacterial culture, a broad spectrum antibiotherapy should be initiated (Travers and Van den Berg, 1995). Indeed, early initiation of aggressive therapy seems to greatly affect the final outcome. The result of blood culture can be negative and thus, in such cases, broad spectrum antibiotics are the only option. On the contrary, when blood culture is positive, the antibiotherapy should be altered upon receipt of the antibiogram results and if initial clinical response is not favorable. The initial antibiotic therapy of choice is soluble penicillin G associated with gentamycin because of its broad spectrum and good penetration (Travers and Van den Berg, 1995). It is important to consider that poor penetration of antimicrobials into vegetations, high numbers of bacteria at the site of infection (inoculum effect) and slow growth of deep-seated bacterial colonies all hinder the effectiveness of antimicrobial treatment and complicate the interpretation of in vitro susceptibilities (Collatos et al., 1990). Therefore, when choosing a drug, one must consider not only the blood culture and antibiogram results, but also the

spectrum of activity, penetrability into the affected tissue, ability to maintain high tissue concentrations, bactericidal versus bacteriostatic effects, and potential for drug-induced toxicity during prolonged use (Karzenski et al., 1997). Antibiotic dosages should provide serum concentrations at least 4 times greater than the concentration needed to kill the organism in vitro (Dedrick et al., 1988).

Treatment of congestive heart failure secondary to vegetative endocarditis is occasionally necessary though it is rarely rewarding because in those cases, the extent of valvular damage is usually already large (Travers and Van den Berg, 1995; Karzenski et al., 1997). Valvular insufficiency may remain even if appropriate antibiotic therapy is instituted; in some cases, it may even increase as the valve lesion becomes fibrotic, resulting in unmanageable congestive heart failure (Ball and Weldon, 1992). Intermittent ventricular premature contractions and atrial fibrillation often resolve spontaneously once the underlying disease process is treated. If this is not the case, anti-arrhythmic drugs can be necessary (Karzenski et al., 1997).

Non-steroidal anti-inflammatory drugs can also be used for the treatment of endocarditis. Their anti-pyretic and anti-inflammatory effects improve the horse's demeanor and thus improve appetite and attitude (Collatos, 1992; Karzenski et al., 1997). Risks of nephrotoxicity and gastrointestinal ulceration should be considered with prolonged use. Concerning this later point, ketofen has been proven to be less toxic than phenylbutazon and flunixin meglumin (Karzenski et al., 1997). Flunixin meglumin is particularly useful to counteract the prostaglandins released in response to endotoxemia associated with Gram negative bacteria. The use of aspirin may also diminish further platelet aggregation on the vegetative lesion (Patteson, 1996).

Resolution of clinical signs, echocardiographic findings and reduction in leukocytosis and hyperfibrinogenemia should be used to evaluate the response and determine the duration of

therapy (Maxson and Reef, 1997). The monitoring of plasma fibrinogen levels is a good guide to follow the inflammatory process. It is advisable to maintain antibiotherapy for approximately two additional weeks after fibrinogen levels became normal and clinical signs resolved. It is wise to repeat this assay after cessation of the antibiotic treatment to be able to act at an early stage in case of reappearance of the complaint (Patteson, 1996).

1.1.6. Prognosis

In all cases of infective endocarditis, the prognosis is poor. As mentioned above, valvular insufficiency may remain despite reduction of the size and sterilization of the lesion. In addition, infarctions, metastatic infections and financial limitations may also complicate treatment and affect prognosis. Mortality rates have been calculated for dogs (80%) and cattle (70%) but not for horses, due to the rarity of this pathology (Ball and Weldon, 1992). The mortality rate is however considered to be very similar to the values calculated for dogs and cattle.

Marked cardiac chamber enlargement, severe deformation of the valve leaflets, severe valvular regurgitation and signs of congestive heart failure are grave prognosis indicators (Maxson and Reef, 1997). Pulmonary valve involvement may also carry a poor prognosis as a result of pulmonary stenosis and risks of development of a secondary *cor pulmonale* (Nilsfors et al., 1991).

Sudden death may occur even after an apparent clinical cure and is likely to be the result of a cardiac arrhythmia or myocardial dysfunction (Collatos et al., 1990; Maxson and Reef, 1997).

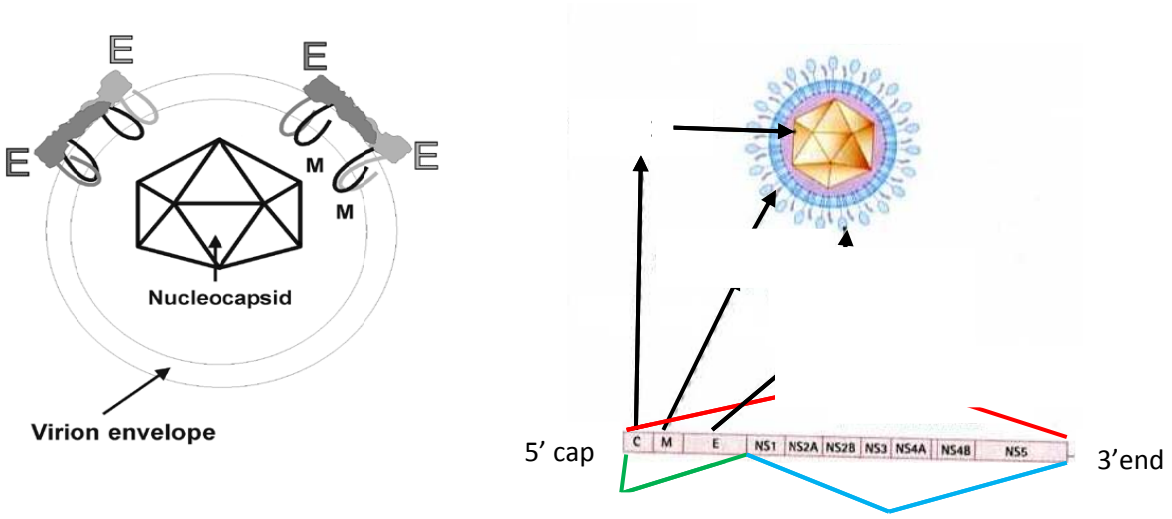
1.2. West Nile Fever

1.2.1. Definition and epidemiology

West Nile fever virus (WNV) was initially isolated from the blood of a mildly febrile woman in the West Nile district of Uganda in 1937 (Smithburn et al., 1940). Previously, WNV was rarely associated with disease in either humans or horses (Castillo-Olivares and Wood, 2004; Dauphin and Zientara, 2007). The only exception was the outbreak in Camargue (southern France) in 1962 and 1963 where at least 80 horses with ataxia and weakness were reported with a mortality rate of 25 to 30%. Since then, sporadic and major outbreaks mainly in humans but also in horses have occurred in Africa, Middle East and Europe (Murgue et al., 2001; Castillo-Olivares and Wood, 2004). Only recently has West Nile fever (WNF) become a major public health and veterinarian concern (Dauphin et al., 2004; Zeller and Schuffenecker, 2004). Indeed, in the last decades (mainly since the mid 1990's), WNV has re-emerged as an important pathogen for humans and horses (Autorino et al., 2002; Weese et al., 2003; Castillo-Olivares and Wood, 2004; Zeller and Schuffenecker, 2004; Dauphin and Zientara, 2007; Leblond et al., 2007a; Blitvich, 2008). An increased frequency of occurrence with, in parallel, an increased proportion of neurological cases (encephalitis, meningitis, meningoencephalitis) was been reported. During the USA outbreak in 2000, the number of severe human cases, increase in the severity of the neurological disease observed in infected horses, and high bird mortality (three elements present at the time) gave a whole new importance to this disease previously considered of minor consequences (Petersen and Roehrig, 2001; Sardelis et al., 2001; Castillo-Olivares and Wood, 2004; Dauphin and Zientara, 2007; Blitvich, 2008).

WNF is a viral zoonotic infection caused by a mosquito-borne *flavivirus* of the *Flaviviridae* family (Petersen and Roehrig, 2001; Autorino et al., 2002), within the Japanese encephalitis

(JE) antigenic complex. The JE complex includes 4 related viruses that cause infections of the central nervous system (CNS): JE virus in Asia, St Louis encephalitis virus in North and South America, Kunijn (considered a subtype of WNV) and Murray Valley encephalitis viruses in Australia (Marfin and Gubler, 2001). The mature virion consists of an icosahedral nucleocapsid enveloped in a lipid bilayer with a diameter of approximately 50nm (Figure 4). Envelope (E) proteins present in the lipid bilayer mediate cellular attachment and membrane fusion and are the major viral immunogen during infection (Chambers et al., 1998; Hobson-Peters et al., 2008). WNV's genome is a single positive–stranded linear message-sense RNA of approximately 11 Kb that encodes for a single open reading frame, which is flanked by a 5' (7-methylguanosine) cap and no polyadenylation at the 3'end. The resulting single polyprotein is post-translationally cleaved by host and viral proteases to produce 3 structural (capsid protein, premembrane (PrM), and E proteins) and 7 nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Chambers et al., 1998; Rossi et al., 2010). Five major genetic lineages of WNV have been distinguished: lineage 1 is found in North America, North Africa, Europe and Australia and includes most of the epidemic strains isolated since 1996 (Marfin and Gulber, 2001); lineage 2 in sub-Saharan Africa and Madagascar and includes many enzootic strains (Marfin and Gulber, 2001; Burt et al., 2002; Lanciotti et al., 1999); lineages 3 and 4 in central and eastern Europe (Lvov et al., 2004; Bakonyi et al., 2005) and lineage 5 in India (Bondre et al., 2007).



006, 87.

Figure 4. West Nile Virus

Legend:
E: envelope proteins
M: membrane proteins
C : nucleocapsid
NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5: RNA segments coding for nonstructural proteins

WNV can infect a remarkably large number of vertebrate and arthropod species, rendering the virus capable of successful spread over large geographical areas (Zeller and Schuffenecker, 2004; Del Piero et al., 2006; Blitvich, 2008). The identity of primary vectors and vertebrate host species is dependent on the geographical area and on the level of virus circulating (Castillo-Olivares and Wood, 2004). In nature, WNV is maintained in an enzootic transmission cycle that primarily involves ornithophilic mosquitoes (mainly *Culex* species but not exclusively) (Hubalek and Halouzka, 1999; Petersen and Roehrig, 2001; Sardelis et al., 2001; Castillo-Olivares and Wood, 2004; Dauphin et al., 2004; Epp et al., 2007; Jourdain et al., 2008) and a main amplifying host, birds (Cantile et al., 2000; Ostlund et al., 2000; Murgue et al., 2001; Autorino et al., 2002; Weese et al., 2003; Castillo-Olivares and Wood, 2004; Zeller and Schuffenecker, 2004; Leblond et al., 2007b; Blitvich, 2008). Birds are competent and amplifying hosts because they develop a viremia typically for up to 5 days and allow the

virus to replicate (intrinsic phase). A viremia of at least 10^5 pfu/ml is generally considered necessary for an infected vertebrate to serve as a reservoir (amplification) host (Turell, 2000; Sardelis, 2001). For transmission to proceed, vectors must feed on competent hosts during the viremic period. The virus is ingested during the bloodmeal and enters the vector's digestive tract. Next, the virus must replicate and enter the salivary glands. This "within the vector" cycle phase is called the extrinsic phase and is known to be temperature and humidity dependant (Epstein, 2001; Petersen and Roehrig, 2001; Castillo-Olivares and Wood, 2004; Blitvich, 2008). The cycle is completed once the competent vector infects a host through a new bloodmeal (Figure 5). At least 198 bird species are known to be implicated in the viral cycle, each with various amplifying role and susceptibility (Dauphin and Zientara, 2007). The house sparrow is considered to be a major reservoir in Europe and the USA because of its abundance in these regions. Migratory birds may introduce WNV into new areas (Cantile et al., 2000; Ostlund et al., 2000; Autorino et al., 2002; Mumcuoglu et al., 2005). The virus has been isolated in 60 species of mosquitoes but less than 10 are considered to be principal vectors (Ostlund et al., 2000; Dauphin and Zientara, 2007). Virus isolations have occasionally been reported from hematophagus arthropods (ticks) (Hubalek and Halouzka, 1999; Cantile et al., 2000; Ostlund et al., 2000; Dauphin et al., 2004; Mumcuoglu et al., 2005; Zeller and Schuffenecker, 2005; Blitvich, 2008), mites and hippoboscid flies (Hubalek and Halouzka, 1999; Farajollahi et al., 2005; Mumcuoglu et al., 2005; Blitvich, 2008). Some mosquito species are exclusively ornithophilic, thus are exclusively implicated in the enzootic, also called natural, cycle. Other species, known as bridging vectors, have more general feeding habits and can transmit WNV to humans, horses and other non-avian vertebrates after feeding upon viremic birds (Sardelis et al., 2001). Shifts in mosquito host selection have been documented. One study indicated that *Culex pipiens* mosquitoes in the northeastern USA shift their feeding behavior from highly competent American robins to mammals and humans in

the late summer to early fall, coinciding with the emigration of this avian species (Kilpatrick et al., 2006). A better knowledge of mosquitoes' feeding preferences would allow a better understanding of WNV ecology and hereafter the development of more efficient control strategies (Dauphin and Zientara, 2007). During warm periods, the extrinsic phase can take as little as two weeks (Castillo-Olivares and Wood, 2004). Mosquito reproduction and longevity are also dependent on environmental factors such as temperature, humidity and rainfall. As a result of this climatic dependence, a highly seasonal variation in WNV transmission and in disease outbreak is observed (Castillo-Olivares and Wood, 2004; Ward, 2005). Indeed, in most temperate regions such as Europe, Canada, Northern States of the USA, most clinical WNF cases occur in late summer or autumn when insect numbers and temperatures are high (Hubalek and Halouzka, 1999; Murgue et al., 2001; Castillo-Olivares and Wood, 2004; Zeller and Schuffenecker, 2004). However, in Europe, WNV outbreaks remain erratic and spatially and temporary limited phenomena, occurring quite unpredictably, even if all conditions appear to be present in a definite place (Dauphin et al., 2004; Zeller and Schuffenecker, 2004). For WNV's enzootic transmission cycle to be maintained in a location, a sufficient number of infected mosquitoes having completed the extrinsic phase must feed on a sufficient number of competent amplification hosts (Castillo-Olivares and Wood, 2004). Mammalian species including both horses and humans are thought to rarely develop titers sufficient to infect mosquito species and viremia is of short duration (Cantile et al., 2000). Thus, mammalian species are very unlikely to be able to sustain infectivity cycles. These species are called incidental (Joubert et al., 1971; Cantile et al., 2000; Cantile et al., 2001) or "dead end" host (Ostlund et al., 2000; Cantile et al., 2001; Murgue et al., 2001; Autorino et al., 2002; Weese et al., 2003; Dauphin et al., 2004; Zeller and Schuffenecker, 2004; Dauphin and Zientara, 2007; Epp et al., 2007; Blitvich, 2008; Castillo-Olivares and Wood, 2008). On the contrary to horses and humans, reptiles might be potential amplification hosts as they are

known to develop a viremia of long duration and can overwinter (Dauphin et al., 2004; Zeller and Schuffenecker, 2004). Direct bird to bird transmission has been documented experimentally (Weese et al., 2003) but remains to be proven in natural conditions. Moreover, WNV transmission through infected blood products and organs and from mother to child through breast feeding has been reported (Shireley et al., 2002; Campbell et al., 2002).

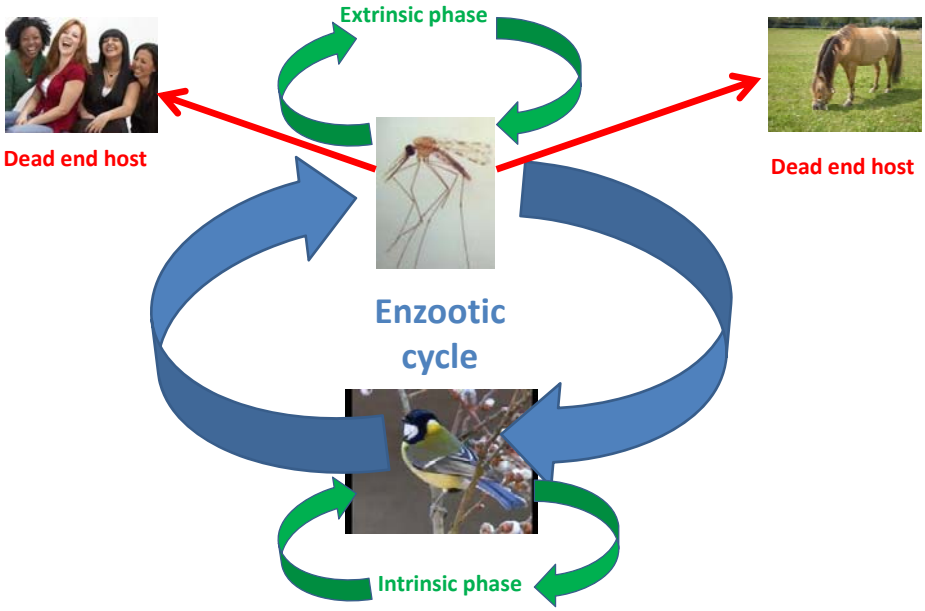


Figure 5. West Nile Virus cycle

Legend:

In blue: enzootic or natural cycle between mosquito and bird

In red: infection of dead end or incidental host

WNF is maintained in an area by its enzootic cycle between reservoir birds and competent mosquitoes. The mosquito gets infected during a bloodmeal on a viremic bird. The virus replicates in the mosquito during the extrinsic phase (humidity and temperature dependent). After viral replication, WNV enters the mosquito's salivary glands. The mosquito infects a bird during a bloodmeal through its saliva. Birds are competent host. A second replication phase takes place. This phase is the intrinsic phase (in the reservoir host). Birds develop a high viremia allowing infection of new mosquitoes during subsequent bloodmeals.

Mosquitoes can also feed on dead end hosts, also called incidental hosts. Dead end hosts do not permit sufficient amplification of WNV. Viremia remains insufficient for infection of mosquitoes during subsequent bloodmeals. The cycle is thus aborted. Humans and horses are dead end host.

It has been demonstrated that a higher proportion of dry bushes, open water, and woodlands in the vicinity of animals were risk factors for WNF cases. These biotopes are indeed favorable for the presence of birds and could represent areas of a higher contact rate among mosquitoes, reservoirs, and accidental hosts (Leblond et al., 2007b). A high WNV circulation level may be due to an enzootic status of the virus in that particular area or due to a reintroduction of the virus by other means (animal circulation, migratory birds). In Europe, bird mortality is rare (Murgue et al., 2001; Dauphin et al., 2004; Zeller and Schuffenecker, 2004; Dauphin and Zientara, 2007; Leblond et al., 2007a).

Individual factors of receptivity to the virus seem of major importance for the evolution and clinical expression of WNF (Joubert et al., 1971; Beasley et al., 2002). Indeed, Glass et al. (2005), Brault et al. (2007), and Lim et al. (2009) demonstrated that variations in certain loci influenced susceptibility to and clinical presentation of WN infection. The study by Glass et al. (2005) demonstrated that the locus CCR5, coding for CCR5 chemokine receptor, was an essential resistance factor against neuroinvasive infections. Indeed, the CCR5 deficient mice used in the study had increased viral titers in the CNS system and a higher mortality rate (Glass et al., 2005). Moreover, a genetic risk factor for initial WN infection found by Lim et al. in 2009 was the presence of a single point mutation in the gene OAS1, a member of an IFN-regulated gene family involved in degradation of viral RNA. Homozygotes for the OAS1 "A" allele at SNP rs10774671 were significantly more numerous among WN-positive individuals independently of the infection's clinical presentation. Furthermore, primary lymphoid tissue originating from human homozygotes was cultured in the ex-vivo model by Lim et al. (2009) and increased levels of WNV replication were observed. Later, Lim et al. (2010) studied the effect of a mutation named CCR5Δ32 in humans. Their study found that CCR5Δ32 mutation did not influence susceptibility to infection but did have a significant effect on early clinical presentation. Indeed, the symptoms retained by Lim et al. (2010) were

more frequently reported in homozygous CCR5Δ32 individuals than in heterozygous and wild-type CCR5 individuals. Moreover, the total number of symptoms reported per individual was superior in the WN-infected homozygous CCR5Δ32 group (Lim et al., 2010). The presence of antibodies against other *flaviviruses* is also thought to play a role in determining clinical presentation in some certain areas (e.g., Africa) (Beasley et al., 2002). Furthermore, clinical expression of a viral disease can vary depending on the pathogenicity of the isolate circulating within the population at that specific time and place. Indeed, a recent study by Vazquez et al. (2010) reported the circulation of two different WNV strains in Spain after genomic analyses: one avirulent strain and one pathogenic strain. Several scientific studies support the hypothesis that clinical differences are due to differences in viral strains, each viral strain potentially having a different pathogenicity (Cecilia and Gould, 1991; Havely et al., 1994; Chambers et al., 1998). Previous studies have suggested that N-linked glycosylation was implicated in attenuation of viral neuroinvasiveness and neurovirulence (Cecilia and Gould, 1991; Halevy et al., 1994). Chambers et al. (1998) studied the nucleotide sequence coding for the E protein of WNV strains with different virulence proprieties. Six different strains were sequenced by polymerase chain reaction (PCR) and analyzed. Differences in N-linked glycolsylations of the E protein were observed, as well as genomic sequence modifications at nucleotide and amino acid levels. The viral attenuation process could thus be more complicated than previously thought (Chambers et al., 1998). In a mouse model, Beasley et al. (2002) isolated groups of viruses in lineage 1 and lineage 2 with variable neuroinvasive potentials. According to the authors, their neuroinvasive potential was found not to be influenced by the source of the virus (mosquito, mammal, bird) or by its passage history. Venter et al. (2009) demonstrated that highly neuroinvasive lineage 1 and lineage 2 genotypes could be isolated from infected horses.

In a study during the 1999 WNV epizootic in New York on 25 symptomatic equine WNF cases, no breed, gender or age predilection for the development of the clinical disease was observed. Moreover, no consistent abnormalities on hematologic examination or in blood chemistry profile were reported in these horses (Ostlund et al., 2000).

1.2.2. Clinical signs

As mentioned previously, the major onset of clinical cases occurs during August and September (Marfin and Gulber, 2001).

In humans, after an incubation period of 3 to 15 days, WNF either remains asymptomatic or causes a mild febrile illness (Murgue et al., 2001; Autorino et al., 2002; Zeller and Schuffenecker, 2004; Epp et al., 2007). Commonly, infected patients suffer from 3 to 6 days of fever, headaches, backaches, myalgia, anorexia, and generalized lymphadenopathy. In 50% of cases a roseolar or maculopapular rash is observed (Marfin and Gulber, 2001). However, in a minority of cases, meningitis, meningoencephalitis, myelitis, optic neuritis or polyradiculitis occur (Murgue et al., 2001; Autorino et al., 2002; Kelley et al., 2003; Weese et al., 2003; Epp et al., 2007). Typically, neurologically affected patients suffer from fever and weakness during the prodromic phase followed by an acute onset of severe flaccid paralysis (Kelley et al., 2003). Despite maintenance of extremity reflexes and touch sensation, mental status can be modified due to involvement of the cerebral cortex (Asnis et al., 2001; Kelley et al., 2003). Furthermore, gastrointestinal complaints, pharyngitis, and conjunctivitis have been reported in several cases (Asnis et al, 2001). Factors disrupting the blood-brain barrier (BBB) (e.g., hypertension) and a less efficient immunity (elderly) are thought to enhance progression of CNS infections (Hayes, 1989). Indeed, older people have a significantly higher risk of severe neurological illness and death (Asnis et al., 2001; Marfin and Gulber, 2001). Severe neurological cases can also occur in children but are less common than in the elderly (Asnis et

al., 2001). Global fatality rates vary depending on the epidemic but remain low: 4.3% during the 1996 epidemic in Romania; 12% during the 1999 epidemic in the northeastern USA; and 6.7% during the 2000 epidemic in Israel (Marfin and Gubler, 20001).

In horses, WNF is usually asymptomatic (Cantile et al., 2000; Cantile et al., 2001; Castillo-Olivares and Wood, 2004; Zeller and Schuffenecker, 2004; Dauphin and Zientara, 2007; Epp et al., 2007; Blitvich, 2008; Sebastian et al., 2008). If clinical signs occur, they are non specific (Weese et al., 2003; Dauphin and Zientara, 2007). Except for fever, clinical signs are almost exclusively of a neurological nature and reflect the CNS pathology (Ostlund et al., 2000; Murgue et al., 2001; Castillo-Olivares and Wood, 2004; Zeller and Schuffenecker, 2004; Leblond et al., 2007a; Blitvich, 2008). Horses seem to be particularly sensitive to WNV with approximately 10% of infected animals presenting neurological disorders, as compared to 1% of humans (Petersen and Roehrig, 2001). West Nile (WN) infections may involve both central and peripheral nervous systems. A transitory febrile phase may occasionally be observed before the neurological clinical signs but this is not always the case (Murgue et al., 2001; Castillo-Olivares and Wood, 2004; Zeller and Schuffenecker, 2004). The clinical signs most commonly observed are ataxia, paresis, limb paralysis which can affect one, or two (usually the hindlimbs), or all four limbs, the latter cases usually progressing into recumbency (Cantile et al., 2000; Ostlund et al., 2000; Murgue et al., 2001; Castillo-Olivares and Wood, 2004; Zeller and Schuffenecker, 2004; Dauphin and Zientara, 2007; Leblond et al., 2007a). Other clinical signs reported are skin fasciculations, muscle tremors (Murgue, 2001), and muscle rigidity (Cantile et al., 2000; Ostlund et al., 2000; Castillo-Olivares and Wood, 2004; Dauphin and Zientara, 2007). A proportion of horses never recover from infection and die naturally or are euthanized (Cantile et al., 2000; Ostlund et al., 2000; Murgue et al., 2001; Weese et al., 2003; Castillo-Olivares and Wood, 2004; Leblond et al., 2007a). In clinically affected horses, the fatality rate has been estimated of 43 to 45% (Cantile et al., 2001).

1.2.3. Pathogenesis

Infected mosquitoes transmit the virus to humans and horses in saliva during subsequent bites. After local viral replication in tissues and lymph nodes, WNV is transported via lymphatics to blood. Viremia peaks between 4 and 8 days post-inoculation but viral concentration usually remains low (10^3pfu/ml) (Peiris and Amerasinghe, 1994; Hubalek and Halouzka, 1999). The mechanisms of viral infection of the CNS still remain uncertain. Currently, breakdown of the BBB is not thought to be necessary for CNS invasion and it is preferentially suggested that endothelial or axonal transport is the mean of infection (Rossi et al., 2010). Once in the neural cells, a second phase of viral replication occurs, causing severe inflammation and a cytotoxic immune response towards infected neural cells. Studies on mice suggested that host immune response could facilitate viral CNS infection by increasing BBB permeability and capillary leakage (Diamond and Klein, 2004; Wang et al., 2004; Arjona et al., 2007; Gracia-Tapia et al., 2007). Moreover, Samuel and Diamond (2005) found that in experimentally infected mice, high viremia easily lead to infection of the brain in the presence of a disrupted BBB. In their study, high viremia was correlated with severity of infection (Samuel and Diamond, 2005). Further research is required to highlight the means of CNS infection in humans and horses. In addition, the role of host immune response and of viral concentration in the host's blood has not yet been determined in these species.

1.2.4. Diagnostic methods

Clinically WNF cannot be distinguished from other CNS infections. Moreover, the majority of human encephalitis cases (75%) are not diagnosed accurately (Asnis et al., 2001). Differential diagnosis of human WNF includes encephalitides viruses (mainly *enteroviruses* and other *arboviruses*) and bacterial meningitis (Asnis et al., 2001; Rossi et al., 2010). Differential diagnosis of equine WNF in France and Belgium includes *equine herpesvirus-1*,

Borna disease, rabies, equine protozoal myeloencephalitis, trauma, moldy corn poisoning, hepatoencephalopathy, and cervical instability, also called Wobbler syndrome (Leblond et al., 2007a). Elsewhere, depending on the geographical and epidemiological context, African horse sickness (for sudden death cases only), equine encephalosis, eastern equine encephalomyelitis (EEE), Venezuelan equine encephalomyelitis and western equine encephalomyelitis can also be included (Venter et al., 2009). Common laboratory findings in WNF cases are non specific: leukocytosis or leukopenia in blood, and lymphocytic pleocytosis in the cerebrospinal fluid (CSF) with counts of 10 to 100 cells/mm^3, with elevated protein concentration (up to 900mg %) (Marfin and Gubler, 2001). Medical imaging can help localize the neurological inflammation. Magnetic resonance imaging (MRI) is the diagnostic method of choice for human encephalitis by visualization of acute meningeal enhancement (Marfin and Gulber, 2001).

Specific diagnosis of WNF is made by enzyme-linked immunosorbent assay (ELISA), immunohistochemical (IHC) staining, viral isolation, PCR or postmortem examination.

ELISA using the envelope protein E as the antigen of choice is the most commonly used serological assay (Hobson-Peters et al., 2008). Its sensitivity can approach 100% in appropriately timed CSF and serum samples (Tradei et al., 2000). A high level of WNV-specific immunoglobulin (Ig) M antibodies in a single acute phase serum sample associated to neurological clinical signs strongly suggests WNF. However, several problems are associated with IgM ELISA. Indeed, WNV-specific IgM antibodies are generally not detectable before the end of viremia (8-10 days post-infection) (Hayes, 1989; Ostlund et al., 2000) and can remain detectable for several months (2 to 3 months) in sera from infected individuals (Tardei et al., 2000). Furthermore, cross-reactions can occur with other *flaviviruses* including yellow fever virus (YF), dengue virus, and members of the JE antigenic complex preventing definitive diagnosis. Currently commercial testing for WNV-specific antibodies is limited for

this reason (CDC, 1997). Definitive diagnosis can only be obtained by means of plaque reduction neutralization test (PRNT) (Peris and Amerasinghe, 1994). In initial cases of an outbreak, PRNT comparing the antibody titers to WNV, St Louis encephalitis virus, and, when indicated, YF, dengue and JE should be used for serologic confirmation of infection (CDC, 1997). PRNT with an acute sample and a convalescent sample (collected at least 10 days later) are compared to document a 4-fold rise in antibody titer. Indeed, according to the CDC (1997), a 4-fold change in neutralizing antibody titer should still be sought to provide a specific diagnosis of WNF infection. A single negative assay of a sample obtained early during the clinical course of illness does not exclude infection (CDC, 1997). While PRNT is still considered the gold standard for specific diagnosis, ELISA is routinely used as it is less laborious, cheaper and more suited to high-throughput screening. Currently, attempts have been made to improve the specificity of E protein-based antigens by focusing on using peptides or individual domains of the E protein (Beasley et al., 2004; Herrmann et al., 2007; Roberson et al., 2007; Hobson-Peters et al., 2008). However, distinguishing WNV strains and sensitivity of such ELISA remain a problem (Hobson-Peters et al., 2008). Anti-WNV IgG ELISA is of little use for acute diagnosis as seropositivity only indicates past exposure. Indeed, IgG are only detectable 2 or more weeks post-infection and can persist for up to 2 years (Ostlund et al., 2000). Because IgM antibody does not cross the BBB, intrathecal IgM indicates intrathecal production (Asnis et al., 2001).

Another diagnostic method is IHC staining. IHC is capable of detecting the presence of a *flavivirus* on a formalin-fixed biopsy or necropsy material (Peris and Amerasinghe, 1994). However, its lack of specificity and the necessity of confirmation by other diagnostic means limit its use.

WNV isolation must be performed in a biosafety level 3 laboratory (Marfin and Gubler, 2001). Virus can be isolated from human serum, blood, and CSF early in the febrile stage and

from brain obtained during biopsy or autopsy (Peris and Amerasinghe, 1994). After sampling, virus growth is obtained by intracranial inoculation into suckling mice or on continuous cell lines of mosquito or mammalian origin (Peris and Amerasinghe, 1994). Hayes (1989) reported that in healthy humans the virus can be isolated from blood from two days before the onset of illness through the fourth day of illness. However, the rate of successful isolation drops rapidly after the first day of illness. In immunocompromised patients, viral isolation can be prolonged up to 22 to 28 days after infection. Not only is viral isolation difficult, but it also requires 3 weeks limiting its usefulness as a diagnostic method at the beginning of an epidemic (Johnson et al., 2003).

PCR assays have been developed to diagnose WNF by detecting viral RNA. A single-stage real time (RT)-PCR can detect viral RNA in birds and mosquitoes but its sensitivity is low for infected equine brain samples (Johnson et al., 2001). Johnson et al. (2001) developed a RT-nested PCR (RT-nPCR) for this purpose. A portion of the genomic region coding for the E protein was amplified during the assays. The results obtained by RT-nPCR were compared to results obtained by RT-PCR, viral isolation using cell cultures, and serology (IgM capture (MAC)-ELISA and PRNT). RT-nPCR was found to be 100,000-fold more sensitive than single-stage RT-PCR. Compared to viral isolation, RT-nPCR was also more sensitive. Moreover, RT-nPCR was completed in less than 24 hours. Specificity of RT-nPCR was also good compared to the other diagnostic assays. In conclusion, RT-nPCR is a reliable and rapid diagnostic assay for equine and avian samples. Furthermore, RT-nPCR can be standardized and performed on many samples at the same time. A nested multiplex RT-PCR that is capable of detecting and differentiating WNV and EEE virus has also been developed (Johnson et al., 2003).

Finally, postmortem examination can be useful in cases where WNF is suspected. Chronic inflammatory infiltrates of variable density can be visualized in the CNS. Lymphocytes are

the predominant cells present. Gliosis, scattered microglial nodules, focal perivascular cuffing, and infiltrating macrophages can be observed in the most affected area (Asnis et al., 2001; Kelley et al., 2003). Small hemorrhages and extensive neuronal degeneration have also been reported (Shieh et al., 2000; Sampson et al., 2000). The brainstem, anterior horn of the spinal cord and cerebrum appear to be preferentially involved (Kelley et al., 2003).

1.2.5. Treatment

In human and veterinary medicine, no specific treatment against WNF exists. To this day, supportive care remains essential. Respiratory support, management of cerebral edema, and prevention of secondary bacterial infections are the key to patient survival.

Makhoul et al. (2009) suggested administration of intravenous immunoglobulin G (IVIG) with a high concentration in anti-WNV antibodies, to prevent possible neuronal destruction and future disabilities. The IVIG used in their study was obtained from pooled healthy human sera and was administered at a dose of 0.4g/kg for 5 days. The authors found that early IVIG treatment associated with supportive care improved outcome.

1.2.6. Prognosis

If adequate supportive care is provided, recovery in humans is usually complete and rapid, despite being slower in adults than in children (Hubalek and Halouzka, 1999; Marfin and Gulber, 2001). Neutralizing antibodies directed against specific epitopes on the E protein appear to provide long-lived protection against reinfection (Marfin and Gubler, 2001). Non-neurological complications of infection such as myocarditis, pancreatitis, and fulminant hepatitis can occur but remain rare (Marfin and Gulber, 2001). However, more recent studies on WNF and its short- and long-term effects have found that long-term physical, functional, and cognitive problems after acute WNF were not as uncommon as previously thought

(Sejvar, 2007). Previously, permanent sequelae and long-term effects of WNV infection were not recorded, and were thus considered absent or of negligible importance (Hubalek and Halouzka, 1999). Extreme fatigue is the most frequently reported long-term complaint (Gottfried et al., 2005; Ou et al., 2005; Carson et al., 2006). Indeed, in the study by Watson et al. (2004), 96% of the 98 patients with otherwise uncomplicated WNF suffered from persistent fatigue for a median of 36 days. Weakness, concentration and memory difficulties several months after infection are also frequently reported (Ou et al., 2005; Carson et al., 2006; Haaland et al., 2006). Furthermore, an association between WNF and Parkinson disease is suspected. The study by Carson et al. (2006) on a total of 49 patients (11 with neuroinvasive and 38 with non-neuroinvasive WNF), post-illness, found that results at motor speed and manual dexterity tests were the most significantly abnormal. Parkinsonism associated with WNV infection is usually transient and self limited, but in this study, Parkinsonism was found to persist in some patients for more than 1.5 years following acute illness. Moreover, persistent tremor was observed or reported in 20% of the 49 patients. Myalgia, arthralgia and headaches can also occur despite apparent complete recovery from acute infection (Patnaik et al., 2006). A post-WNF syndrome similar to the post-poliovirus infection syndrome, with development of delayed onset or recurrence of limb weakness is considered possible or even likely (Sejvar, 2007). Persistence of WNV in the CNS has been suggested during experimental infections in monkeys but remains to be clarified and is hypothetical in humans (Perelman and Stern, 1974; Pogodina et al., 1983; Nash et al., 2001). Younger age is more predictive of eventual functional recovery than is severity of initial illness (Klee et al., 2004).

The most frequent cause of death in patients infected by WNV is cerebral edema after neuronal death and degeneration (Marfin and Gulber, 2001). Concerning WN encephalitis, in-hospital case-fatality rate is estimated to be approximately of 20%. Acute mortality appears to

be due to acute respiratory failure or sudden cardiac complications (Pepperell et al., 2003; Sejvar et al., 2005; Bode et al., 2006). Moreover, following hospitalization patients frequently require placement in rehabilitation centers (Bode et al., 2006). Physical, occupational or speech therapy may also be necessary (Klee et al., 2004). In addition, Green et al. (2005) reported that patients affected by WN encephalitis have a higher long-term mortality rate than the general population, with a 1-year post-infection mortality comparable to those of patients with chronic non-infectious diseases.

In equine cases, the severity of initial clinical signs has been reported not to necessarily hinder the ability to recover if appropriate supportive care is provided (Ostlund et al., 2000). However, studies on long-term effects of WNV infection are lacking at present. Late diagnosis in equids compared to humans, due to non specific clinical signs and cost of diagnostic methods, associated with the difficulty and cost of supportive treatment could explain the significantly higher fatality rate in this species (approximately 40% versus approximately 10%) (Cantile et al., 2001).

1.2.7. Preventive measures

Vaccination

Several strategies have been pursued for the WNV vaccine development. Inactivated, also called killed vaccines have been obtained by inactivation of WNV with formalin. In the past decade, plasmid DNA vaccines and live vectored vaccines have been developed. DNA vaccination consists in WNV structural antigens (PrM and E) being expressed from DNA plasmids (Davis et al., 2001). Recombinant vaccines use a heterologous virus backbone to produce WNV antigens. The vectors used are canarypox (e.g., Recombitek^{TM}), YF virus (e.g., Chimerivax^{TM}) and Dengue 4 (e.g., WNV-DEB4) (Rossi et al., 2010). The recombinant WNV canarypox vector ALVAC-WNV vaccine has been developed recently (Minke et al., 2004;

Siger et al., 2004). Canarypox virus vectors undergo an abortive replication cycle in mammalian cells during which the inserted gene product (transgene) is expressed which results in the stimulation of both humoral and cell-mediated immune responses in a manner similar to natural infection (Berencsi et al., 2001; El Garch et al., 2008). The study by El Garch et al. (2008) showed that immunity against WNV persisted for 9 months following a primary course of two injections and 5 months following the first booster vaccination with recombinant ALVAC-WNV vaccine. Furthermore, canarypox virus vectors allow for a strong immunogenicity of the recombinant antigen and appear to be free of inhibitory effects of vector immunity even after multiple injections (Franchini et al. 2004; El Garch et al., 2008). Another recent vaccine is a live-attenuated chimeric WN/YF vaccine, ChimerVax WN02, that comprises WNV NY 99 PrM and modified E sequences inserted into the backbone of the YF 17D vaccine genome (Smith et al., 2011). The PrM and E genes of the YF-17D vaccine strain were exchanged for those of the WNV NY 99 strain 385-399. Three attenuating mutations were introduced into the WNV E codon: E170 (leucine to phenylalanine), E336 (alanine to valine), and E440 (lysine to arginine). Smith et al (2011) found E430 specific CD8+ T cells through the first year following vaccination with chimeric WN/YF virus. It is thus a very promising vaccine. Finally vaccination by inoculation of purified viral proteins has been attempted (Quiao et al., 2004).

Currently there is no Food and Drug Administration (FDA)-licensed vaccine to prevent WN infection in humans but effective, licensed vaccines are available for horses (Rossi et al., 2010). Indeed, inactivated vaccines and Recombitek^{TM} has been licensed for use in horses.

Mosquito control

In a study by Epp et al. (2007) mosquito control methods were found to be significant protection factors against WNV infection. Indeed, in this study, horses in herds without any

mosquito control methods were 8.2 times more at risk of WN infection. When mosquito control methods (fans in barn, sealing at night, or both; smudges, insecticides) were compared the use of fans in barns housing the animals was the strongest protective factor.

1.3. Q fever

1.3.1. Definition and epidemiology

Q fever was first described in 1937 by E.H. Derrick in Queensland, Australia (Derrick, 1937). It was previously classified as a "Category "B" critical biological agent" by the Centre for Disease Control and Prevention (CDC) and is considered a potential weapon for bioterrorism (Alibek, 1999). Q fever is a public health concern throughout the world (Rousset et al., 2009a). However, it is rarely a notifiable disease and its surveillance is frequently severely neglected (Maurin and Raoult, 1999). Q fever is a zoonotic bacterial disease caused by *Coxiella burnetii*, an obligate intracellular Gram negative bacterium of the *Legionellales* order (Komiya et al., 2003; Berri et al., 2007; Rousset et al., 2007a; Hartzell et al., 2008; Frangoulidis et al., 2009). Domestic ruminants are considered the main reservoir for the pathogen but Q fever is known to infect a large variety of hosts, mammals (humans, caprids, bovids, ovids, small rodents, dogs, cats), as well as birds, fish, reptiles and arthropods (Marmion and Stoker, 1950; Davoli and Signorini, 1951; Slavin, 1952; Marmion et al., 1954; Stocker and Marmion, 1955; Blanc and Bruneau, 1956; Evans, 1956; Syrucek and Raska, 1956; Fiset, 1957; Hirai et To, 1998). It is a highly infectious disease (Benenson and Tigertt, 1956; Ormsbee et al., 1978). Infected mammals shed bacteria in milk, feces, urine, vaginal mucous and very importantly in birth products (Figure 6).

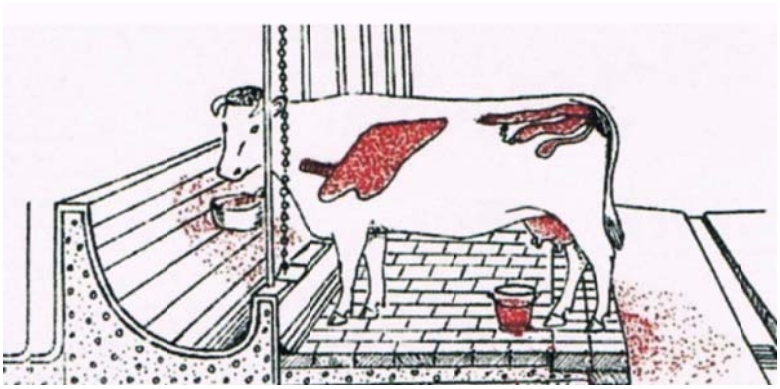


Figure 6. Infection and excretion routes of *Coxiella burnetii* by an infected host.

Legend:

In red: *C. burnetii* and reservoir organs

Inhalation of resistant bacteria present in the environment is the main route of animal and human infection. Infection by oral route remains subject to controversy (Figure 7).

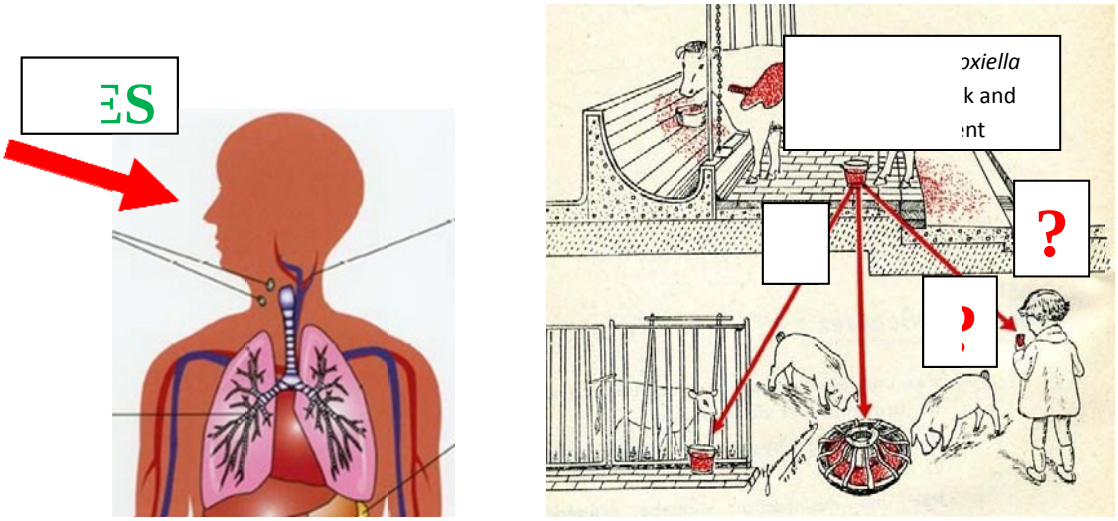


Figure 7. Routes of human and animal infection

Legend:

In red: *C. burnetii* and reservoir organs

Red arrows: transmission routes of *C. burnetii*

On the left: transmission by aerosol.

On the right: transmission of humans and other animals by oral route (consumption of infected milk).

1.3.2. Clinical signs

The main characteristic of Q fever is its clinical polymorphism (Kuroiwa et al., 2007; Hartzell et al., 2008; Million et al., 2009; Pape et al., 2009). After an incubation period of 1 to 3 weeks (Maurin and Raoult, 1999; Watanabe and Takahashi, 2008), Q fever can cause either an acute or a chronic disease. Q fever infections remain poorly understood (Rousset et al., 2007a; Pape et al., 2009) and their prevalence and importance have been underestimated for many years (Rousset et al., 2007a). Estimating the level of infection in animals remains problematic (Rousset et al., 2007a).

Complementary information on epidemiology, clinical signs, as well as the development of Q fever pathogenesis, diagnostic methods, treatment, and prognosis are available in the two reviews inserted hereby.

PORTER S.R., CZAPLICKI G., MAINIL J., HORII Y., MISAWA N., SAEGERMAN C. Q fever in Japan: an update review?

Vet. Microbiol., 2011, **149**, 298-306

Veterinary Microbiology 149 (2011) 298–306

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Review

Q fever in Japan: An update review

Sarah Rebecca Porter [a], Guy Czaplicki [b], Jacques Mainil [c], Yoichiro Horii [d], Naoaki Misawa [e], Claude Saegerman [a,*]

[a] Research Unit in Epidemiology and Risk Analysis applied to Veterinary Sciences (UREAR), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liege, Boulevard de Colonster 20, B42, 4000 Liege, Belgium
[b] Association Régionale de Santé et d'Identification Animales, Avenue Alfred Deponthière 40, B-4431 Loncin, Belgium
[c] Laboratory of Bacteriology, Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liege, Boulevard de Colonster 20, B43a, 4000 Liege, Belgium
[d] University of Miyazaki, Faculty of Agriculture, Department of Veterinary Science, Laboratory of Veterinary Parasitic Diseases, 1-1 Gakuenkibanadainishi, Miyazaki 889-2192, Japan
[e] University of Miyazaki, Faculty of Agriculture, Department of Veterinary Science, Laboratory of Veterinary Public Health, 1-1 Gakuenkibanadainishi, Miyazaki 889-2192, Japan

ARTICLE INFO

Article history:
Received 11 January 2010
Received in revised form 2 November 2010
Accepted 9 November 2010

Keywords:
Q fever
Coxiella burnetii
Zoonosis
Epidemiology
Clinical aspects
Animals
Birds
Humans
Cats

ABSTRACT

As neglected zoonosis for many years, Q fever is now ubiquitous in Japan. Similarly to elsewhere in the world, domestic animals are considered to be important reservoirs of the causal agent, *Coxiella burnetii*, a resistant intracellular bacterium. Infected animals shed bacteria in milk, feces, urine, vaginal mucous and birth products. Inhalation of bacteria present in the environment is the main route of animal and human infection. Shedding of *C. burnetii* in milk by domestic ruminants has a very limited impact as raw milk is seldom ingested by the Japanese population. The clinical expression of Q fever in Japan is similar to its clinical expression elsewhere. However clinical cases in children are more frequently reported in this country. Moreover, *C. burnetii* is specified as one of the causative organisms of atypical pneumonia in the Japanese Respiratory Society Guideline for the management of community-acquired pneumonia. In Japan, *C. burnetii* isolates are associated with acute illness and are mainly of moderate to low virulence. Cats are considered a significant source of *C. burnetii* responsible for human outbreaks in association with the presence of infected parturient cats. Since its recognition as a reportable disease in 1999, 7–46 clinical cases of Q fever have been reported by year. The epidemiology of Q fever in Japan remains to be elucidated and the exact modes of transmission are still unproven. Important further research is necessary to improve knowledge of the disease itself, the endogenous hosts and reservoirs, and the epidemiological cycle of coxiellosis in Japan.

Contents

* Corresponding author. Tel.: +32 4 366 45 79; fax: +32 4 366 42 61.
E-mail address: claude.saegerman@ulg.ac.be (C. Saegerman).

0378-1135/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2010.11.017

1. Introduction

Q fever was first described in 1937 (Derrick, 1937). Q fever is a ubiquitous neglected zoonosis caused by a resistant intracellular bacterium, *Coxiella burnetii* (Derrick, 1937; Mitscherlich and Marth, 1984; Babudieri, 1959; Maurin and Raoult, 1999; Rousset et al., 2009). Ignored for many years, Q fever is now thought to be ubiquitous in Japan since the reservoirs are present throughout the country (Hirai and To, 1998). Similarly to elsewhere in the world, domestic animals are considered to be important reservoirs of *C. burnetii* (Hirai and To, 1998). However Q fever is known to infect not only a large variety of hosts, mammals (humans, caprids, bovids, ovids, small rodents, dogs, and cats) but also birds, fish, reptiles and arthropods (Hirai and To, 1998; Maurin and Raoult, 1999; Bildfell et al., 2000; Berri et al., 2007; Rousset et al., 2007; Okimoto et al., 2007; Hartzell et al., 2008). It is a highly infectious disease (Ormsbee et al., 1978). Infected mammals shed bacteria in milk, feces, urine, vaginal mucous and very importantly in birth products. Inhalation of resistant bacteria present in the environment is the main route of animal and human infection. The main characteristic of Q fever is its clinical polymorphism (Kuroiwa et al., 2007; Hartzell et al., 2008; Million et al., 2009; Pape et al., 2009). After an incubation period of 1–3 weeks (Maurin and Raoult, 1999; Watanabe and Takahashi, 2008), Q fever can cause either an acute or a chronic disease.

This review describes the epidemiological situation of Q fever in Japan. The authors' choice of country was based firstly on the peculiar role of cats and additionally of wild birds in the epidemiology of Q fever in Japan and secondly the limited epidemiological investigation and awareness of Q fever in Japan incited further research on the subject.

2. Clinical expression

2.1. In humans

Since April 1999, the management of infection control and prevention in Japan has changed drastically and Q fever was designated as a national reportable disease. Q fever occurs almost all over the country. Under the revised surveillance system, clinical cases of Q fever have been reported 7–46 cases since 1999 for a total of 127 million inhabitants (Mahara, 2006). The clinical expression of Q fever in Japan is similar to its clinical expression elsewhere (Ejercito et al., 1993; Htwe et al., 1993). In the acute form, infections can be totally asymptomatic or can lead to self-limiting 'influenza-like' illness, pneumonia or hepatitis (To et al., 1998b; Sampere et al., 2003; Setiyono et al., 2005; Berri et al., 2007; Kuroiwa et al., 2007; Delsing and Kullberg, 2008; Hartzell et al., 2008; Schimmer et al., 2008; Frangoulidis et al., 2009; Million et al., 2009; Pape et al., 2009; Ughetto et al., 2009). Pneumonia is typically mild but progression to acute distress syndrome can occur (Hartzell et al., 2008; Watanabe and Takahashi, 2008). Indeed a study found Q fever to be involved in 2.5% of patients with an acute infection/exacerbation of a chronic lower respiratory tract disease state (Okimoto et al., 2007). In Japan, pneumonia is a common clinical presentation of acute Q fever. The prevalence of *C. burnetii* infection as causative agent for atypical pneumonia differs between different countries. Indeed, To et al. (1996) found that 39.7% of Japanese patients with atypical pneumonia were infected by *C. burnetii*. On the other hand, in the rural areas of Nova Scotia, a province of Canada, only 20% of patients admitted to hospital with atypical pneumonia were infected by Q fever (Marrie, 1990). In France, Tissot Dupont et al. (1992) reported a prevalence of atypical pneumonia caused by *C. burnetii* of 45.8%. The variability in prevalence between countries is most probably due to differences in incidence of Q fever in domestic ruminants (goats, sheep, and cattle). On the other hand, variability in occurrence of clinical illness could be explained by differences between local strains and their respective virulence, and/or by physiological differences in the host (To et al., 1996). Alimentary habits could also play a role. In France, for example, farmers and stock breeders are known to drink unpasteurized milk. Moreover, 61.9% of French patients infected by Q fever presented clinical signs of hepatitis (Tissot Dupont et al., 1992; Maurin and Raoult, 1999). However, currently, bacterial genotype and not route of infection is thought to determine clinical presentation. Furthermore transmission of infection by oral route remains a matter of debate (Marmoin and Stoker, 1958; Benson et al., 1963; Krumbiegel and Wisniewski, 1970; AFSSA, 2004; Dorko et al., 2008; Natale et al., 2009).

In Japan, *C. burnetii* isolates are mainly associated with acute illness and are of moderate to low virulence (Oda and Yoshiie, 1989; Hirai and To, 1998). *C. burnetii* is specified as one of the causative organisms of atypical pneumonia in the Japanese Respiratory Society Guideline for the management of community-acquired pneumonia (Okimoto et al., 2004; Watanabe and Takahashi, 2008).

In Japan, on the contrary to other countries, clinical expression of the disease has frequently been observed in children (Hirai and To, 1998) (Table 1). The study by To et al. (1996) suggested that Q fever was an important cause of atypical pneumonia in Japanese children. Cases of

Table 1
Seroprevalence and isolation of *Coxiella* in animals and humans from Japan, through 1990–2008.

Parameter	Kingdom	Species	Number of samples	% of positive	Reference
Seroprevalence	Animal	Healthy cattle	329	29.2	Yoshiie et al. (1991)
			562	46.6	Htwe et al. (1992)
			1501	25.4	Htwe et al. (1992)
			619	16.9	Nguyen et al. (1997)
		Reproductive disorder cattle	102	84.3	Htwe et al. (1992)
			166	78.9	To et al. (1995)
			207	60.4	To et al. (1996)
		Sheep	256	28.1	Htwe et al. (1992)
		Goat	85	23.5	Htwe et al. (1992)
		Dog	635	15	Htwe et al. (1992)
			589	10.2	Nguyen et al. (1997)
			81	9.9	Nagaoka et al. (1996)
			301	16.6	Hirai (1999)
		Cat	274	0	Htwe et al. (1992)
			100	16	Morita et al. (1994)
			150	15.3	Nguyen et al. (1997)
			101	6.7	Nagaoka et al. (1996)
			304	18.8	Hirai (1999)
		Pig	396	0	Htwe et al. (1992)
		Chicken	1589	2	To et al. (1996)
		Quail	174	2.9	To et al. (1996)
		Duck	158	2.2	To et al. (1996)
		Bear	36	77.8	Ejercito et al. (1993)
		Deer	133	61.7	Ejercito et al. (1993)
		Hare	8	62.5	Ejercito et al. (1993)
		Monkey	54	27.7	Ejercito et al. (1993)
		Nutria	32	12.5	Ejercito et al. (1993)
		Wild rodent	129	24.1	Hirai and To (1998)
		Crow	431	36	To et al. (1996)
		Rock Dove	201	6	To et al. (1996)
	Human	Veterinarians	9	22.2	Yoshiie et al. (1991)
		Healthy humans (adults)	60	3.3	Htwe et al. (1992)
			275	22.2	Htwe et al. (1992)
		Meat-processing workers	107	11.2	Htwe et al. (1992)
		Adults with respiratory disorders (in general)	184	15.2	Htwe et al. (1992)
		Adults with atypical pneumonia	284	1.4	Okimoto et al. (2004)
			120	4.17	Watanabe and Takahashi (2008)
		Children with flu-like symptoms	55	32.7	Nagaoka et al. (1996)
		Children with atypical pneumonia	56	35.7	To et al. (1996)
			58	46.55	Maurin and Raoult (1999)
		Hospitalized patients (adults)	3000	5.2	Nguyen et al. (1997)
		Veterinary students	275	35.64	Htwe et al. (1993)
		Adults with acute exacerbation of chronic respiratory disease	80	2.5	Okimoto et al. (2007)
		Adults with acute exacerbation of COPD[a]	240	0.4	Lieberman et al. (2001)
Isolation	Animal	Cattle with reproductive disorder (raw milk)	207	24.6	To et al. (1995)
		Healthy cattle (raw milk)	47	36.3	Nagaoka et al. (1996)
		Healthy cattle (fetus)	4	50.0	To et al. (1995)
		Tick (*Ixodes* spp.)	15	26.7	To et al. (1995)
		Dogs (sera)	5	100	To et al. (1996)
		Cat (sera, uterus swabs)	5	100	To et al. (1996)
	Human	Acute Q fever (adults)	1	100	Oda and Yoshiie (1989)
		Atypical pneumonia (children)	58	36.2	To et al. (1996)
		Hospitalized patients (adults)	17	76.5	Hirai (1997)
		Chronic Q fever endocarditis (adults)[b]	56	7.1	Yuasa et al. (1996)

From Hirai and To (1998), Hirai (1999), Nagaoka et al. (1998) and various sources.

[a] COPD: chronic obstructive pulmonary disease.
[b] Light microscopic observation.

hepatitis have also been reported and can potentially be fatal (Kuroiwa et al., 2007). The difference in prevalence of infection in Japanese children compared to children from other countries could be due to: (1) a more frequent clinical expression (as mentioned here above) due to a different virulence of the bacterial strain or to a greater sensitivity of the host, increasing the probability of diagnosis; (2) to a greater awareness of physicians of the possibility of Q fever infection in atypical and/or non-specific clinical cases.

In pregnant women, clinical expression of Q fever, initially asymptomatic, results in abortions, intrauterine

growth retardation, fetal and neonatal death, oligoamnios or premature delivery (Peter et al., 1987; Numazaki et al., 2000; Delsing and Kullberg, 2008; Hartzell et al., 2008; Schimmer et al., 2008; Vaidya et al., 2008b; Frangoulidis et al., 2009). Sporadically other clinical signs have been reported (such as osteomyelitis, septic arthritis, pericarditis, myocarditis, arteritis, hemolytic anemia, granulomatous hepatitis, lymphadenopathy, Guillain-Barré, optic neuritis, paralysis of the oculomotor nerve, meningitis, encephalitis, polyradiculonevritis, peripheral neuropathy, cranial nerve deficiency, and exanthema) (Hirai and To, 1998; Frangoulidis et al., 2009; Million et al., 2009; Pape et al., 2009).

In Japan, such as other countries, chronic infection leads commonly to endocarditis (Yuasa et al., 1996). Chronic hepatitis, osteomyelitis, septic arthritis, interstitial lung disease (Berri et al., 2007), and infection of aneurysm and vascular grafts (Delsing and Kullberg, 2008; Ughetto et al., 2009) have also been reported in chronic cases of Q fever (Frangoulidis et al., 2009; Pape et al., 2009). Individuals with underlying valvulopathy or other cardiovascular abnormalities are predisposed to the development of endocarditis (Maurin and Raoult, 1999; Kuroiwa et al., 2007; Delsing and Kullberg, 2008; Hartzell et al., 2008; Million et al., 2009; Pape et al., 2009; Ughetto et al., 2009). Furthermore, chronic fatigue syndrome has been diagnosed in previously infected individuals (Berri et al., 2007; Million et al., 2009).

2.2. In farm animals

C. burnetii is widespread among cattle population in Japan (4.4 millions of heads). Bovine coxiellosis is rarely an overt disease, except for reproductive disorders (such as abortion, infertility, metritis and mastitis) in females likewise to other parts of the world (To et al., 1995, 1998a; Vaidya et al., 2008a). Although high rates of abortions are rarely observed in cattle (Palmer et al., 1983), shedding of large quantities of germs remains a reality in the absence of any clinical sign. A retrospective study by Bildfell et al. (2000) demonstrated that *C.burnetii* only sporadically leads to abortion in cattle, but was significantly associated with placentitis. Some studies have reported an increase in seroprevalence of Q fever in Japanese cattle in recent years (Hirai and To, 1998). Cattle play an important role in maintaining infection and in dispersing the organism in the environment (Beaudeau et al., 2006; Guatteo et al., 2006; Rodolakis et al., 2007). They are one of the major reservoirs of *C. burnetii* in Japan (To et al., 1998a). The controversy associated to transmission of Q fever through milk ingestion is of minor importance for the Japanese population. Indeed shedding of *C. burnetii* in milk has a very limited impact as raw milk is seldom ingested by the native population (Okimoto et al., 2004). Raw milk is commonly pasteurized at 63 °C for 30 min or more therefore no problem is expected (Watanabe and Takahashi, 2008).

The rarity of sheep (10,000 heads) and goats (32,000 heads) populations renders these animals non significant for the spread of the disease (Hirai and To, 1998). Q fever has not been reported in pigs as yet but available data remains scarce (Hirai and To, 1998).

2.3. In pet animals

Dogs and cats have been found to be positive for *C. burnetii* by serology and bacteriology throughout the Japanese territory (Hirai and To, 1998; Komiya et al., 2003). Nagaoka et al. (1998) isolated *C. burnetii* in swabs of feline vaginal mucosa and suggested that the organism could be associated with reproductive disorders or abortions in the feline species. Further epidemiological study about the relationship between feline disorders and *C. burnetii* infection are suggested by the authors (Nagaoka et al., 1998). Small human outbreaks of coxiellosis associated with the presence of infected parturient cats have been reported in several studies (Marrie et al., 1988a,b, 1989; Pinsky et al., 1991). Cats are thus considered as a potential source for human infections in this country. However premature conclusions must not be made and supplementary evidence of feline to human transmission of Q fever is necessary with special attention to potential confounding factors. Outbreaks associated to infected dogs have not yet been reported to our knowledge. The dogs' role as reservoir of the pathogen remains poorly explored.

3. Epidemiological data

The epidemiology of Q fever in Japan remains to be elucidated and the exact modes of transmission are still unproven (Hirai and To, 1998). The review by Hirai and To (1998) attempted to explain the epidemiology of Q fever in Japan. Fig. 1 illustrates their hypotheses. Environment and ticks would be responsible for infection of domestic animals; infected domestic animals hereafter leading to human infections. Transmission directly from infected wild animals to humans would also be possible. Ticks could play a role in transmission of disease from the environment to domestic animals. Tick transmission from domestic and wild animals to humans (Hirai and To, 1998) is considered minor.

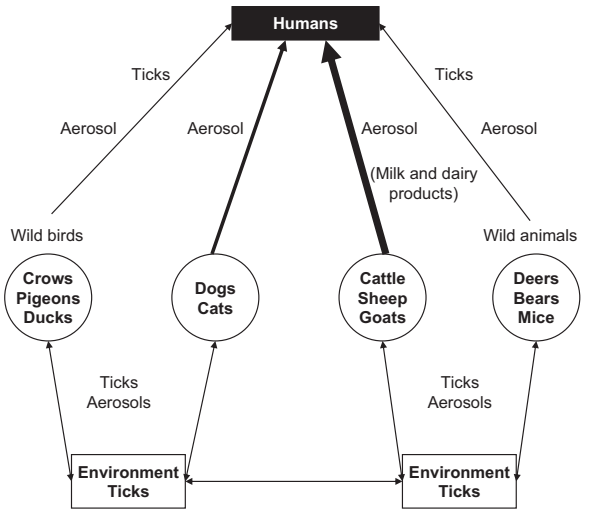


Fig. 1. Epidemiology of Q fever in Japan.From Hirai and To (1998).

3.1. Prevalence of Q fever in animals

Hirai and To (1998) reported the seroprevalence of Q fever in domestic and wild animals present in Japan (Table 1). Several authors contributed to these estimations. In domestic animals, cattle with reproductive disorders had the highest percentage of seroprevalence of coxiellosis. A significant level of seropositivity was detected in wild animals but the results must be interpreted with care as the sample size is often limited. Crows (seroprevalence of 36%) could be involved in transmission of *C. burnetii* from infected areas to non infected areas.

In a study performed by To et al. (1998b) many wild birds were found to be seropositive against *C. burnetii* by monoclonal antibody assay (MA). The polymerase chain reaction (PCR) also used in this study confirmed the serological results by detecting the bacterial DNA. Furthermore areas where infected livestock was present were associated with a higher seroprevalence of Q fever in birds. The authors suggested that wild birds could be used as indicators of foci of infection (To et al., 1996). Domestic birds were also found to be seropositive for Q fever and capable of infecting humans (Hirai and To, 1998) rendering additional investigation necessary, especially to determine the eventual role as natural reservoir of *C. burnetii* (dejection and soil). In 2006, chicken products were highly suspected as responsible for Q fever infections in humans (Muramatsu et al., 2006). *C. burnetii* was detected in market eggs and mayonnaise (Tatsumi et al., 2006). Initially the probability that the bacteria were alive was considered high (Tatsumi et al., 2006). However, the results of further investigations remain non precise and incomplete. PCR-based detection of *C. burnetii* DNA in dead bacterial fragments was reported but there are no reports demonstrating contamination with viable bacteria (Watanabe and Takahashi, 2008). No data is available concerning the extent of transmission of *C. burnetii* from wild animals and birds to humans and domestic animals (Hirai and To, 1998). Its epidemiological importance, however, is considered minor (Hirai and To, 1998). A previous study reported that four out of 11 Japanese wild species had a prevalence of infection higher to 50%, two had a prevalence of infection lesser to 50%, and five species were free from infection (Ejercito et al., 1993). Three hypotheses could explain absence of infection in the five species: the species were either isolated in a area free from Q fever, or they had an innate resistance to infection, or were false negative animals due to a lack of sensitivity of the laboratory diagnostic method (Ejercito et al., 1993). Further epidemiological studies are necessary to explain this apparent or real resistance to infection.

To et al. (1998a) studied the seropositivity rate in herds of dairy cattle with reproductive disorders in Japan. The three main reproductive problems studied were infertility, metritis and mastitis. The rates of positivity were assessed by indirect immunofluorescence assay (IFA) (with a distinction for phase 1 antibodies and phase 2 antibodies), by PCR (in sera and milk samples) and by isolation (in milk samples). Phase 2 antibodies and phase 1 antibodies are associated with acute and with chronic infections, respectively. The study showed that 60.4% of the cows considered were seropositive by IFA towards phase 2 bacteria. In addition, 3.9% and 24.6% were seropositive by PCR on sera and milk respectively. All PCR-positive samples were confirmed by isolation. Whatever be the laboratory method, a positive result was always obtained. This study by To et al. (1998a) demonstrated that in herds with reproductive disorders the prevalence of Q fever was far from nil.

In studies on feline seroprevalence, stray cats were found to have a higher incidence of infection than domestic cats (Nagaoka et al., 1998; Komiya et al., 2003) (Table 1). In this species it is suspected that Q fever could be responsible for breeding disorders (Nagaoka et al., 1998).

3.2. Prevalence of Q fever in the Japanese population

Table 1 reports the seroprevalence in humans in Japan (Hirai and To, 1998). It is interesting to notice that many healthy humans are seropositive to Q fever. In this study, the seroprevalence of infection was relatively high in children with respiratory disorders, flu-like symptoms, atypical pneumonia and in adults with

Table 2
Incidence of Q fever as a cause of community-acquired pneumonia in Japan, Europe, Asia, Africa and America from Watanabe and Takahashi (2003), Watanabe and Takahashi (2008) and varied sources.

Area	Report year	No. of patients	No. of Q fever cases	Incidence (%)	Reference
Africa	1997	65	6	9.2	Koulla-Shiro et al. (1997)
America (USA)	1996	149	4	2.7	Marrie et al. (1996)
	2001	170	4	2.4	Bochud et al. (2001)
Asia	1997	346	20	5.8	Lieberman et al. (1996)
Europe	1991	225	18	8.0	Albornoz et al. (1991)
	1996	124	3	2.4	Torres et al. (1996)
	1997	106	19	17.9	Zalacain et al. (1997)
	1998	173	4	2.3	Sopena et al. (1998)
	1999	395	11	2.8	Ruiz et al. (1999)
Japan	2000	232	2	0.9	Saito et al. (2006)
	2004	284	4	1.4	Okimoto et al. (2004)
	2004	400	10	2.5	Takahashi et al. (2004)

chronic respiratory disease (Hirai and To, 1998). Sample size is a problem for interpretation in certain categories of human beings. Table 2 reports the estimated number of cases of community-acquired pneumonia in different countries from 1989 to 2001. In Japan, the incidence rate of Q fever has significantly increased between 2000 and 2004. However, Q fever had been underdiagnosed for many years in Japan and increased awareness and recognition of the illness might be responsible for the increased observation in this study (Watanabe and Takahashi, 2008).

3.3. Isolation of *C. burnetii* from animals and humans

Hirai and To (1998) reported the isolation rates of *C. burnetii* in different animals present in Japan (Table 1). Fetuses of healthy cattle had a high content in bacteria. Ticks of the *Ixodes* order were significantly infected by *C. burnetii*. Indeed 75% of the sampled *Ixodes* ticks in the Toyama prefecture were infected by the bacteria.

As mentioned previously, cats are considered a significant source of *C. burnetii* in Japan (Marrie et al., 1988a; Marrie et al., 1988b; Marrie et al., 1989; Pinsky et al., 1991). In the study by Nagaoka et al. (1998) bacteria were isolated from vaginal swabs of asymptomatic cats as well as of cats with respiratory disorders, with fever or with fever and abortion. The bacteria were also isolated in cats with atypical clinical manifestations (compared to human clinical manifestations) such as peritonitis and mammary tumors.

Table 1 reports the isolation rates of *C. burnetii* in humans with various clinical signs (Hirai and To, 1998). The rate of isolation in children is particularly high compared to those observed in other parts of the world (To et al., 1996; Maurin and Raoult, 1999). Positive serologies, occasionally associated to bacterium isolation, were a relative frequent finding in adults with fever of unknown origin (Knockaert et al., 2003; Arnow and Flaherty, 1997; Hirschman, 1997; Lozano et al., 1996). In consequence, Q fever serology should be included in the standard work-up of fever of unknown origin in Japan. To confirm these results a second study with larger samples of humans would be necessary.

4. Diagnosis of Q fever and vaccination

Q fever is rarely mentioned in Japanese medical text books and many physicians are unaware of its existence (Watanabe and Takahashi, 2008). Similarly, Japanese veterinarians are insufficiently informed about the risks associated to manipulations of infected animals or infected biological matter (Abe et al., 2001). Thus the recognition of Q fever infections remains limited throughout the country (Watanabe and Takahashi, 2008). Reported clinical cases are rare with the first clinical case reported dating from 1989 (Watanabe and Takahashi, 2008). Increasing the physicians' awareness of the possibility of Q fever infections is essential as rapid diagnosis is known to improve prognosis (To et al., 1996). Table 3 reports the different aspects of the illness to facilitate diagnosis by a clinician (Watanabe and Takahashi, 2008). To reach definite diagnosis IFA, complement fixation test (CFT), enzyme linked immunoassay (ELISA) and PCR are available (e.g., Field et al., 2000; Ughetto et al., 2009). Imported IFA and ELISA kits present problems when used on Japanese individuals. Indeed, it has been observed that the increase in IgM antibodies in many Japanese patients infected in Japan is slow; whereas the increase in IgM antibodies is very rapid in Japanese patients infected abroad. This suggests that *Coxiella*

Table 3
Diagnostic points of acute Q fever from Watanabe and Takahashi (2008).

Area	Criteria	Key points
Clinical viewpoint	Opportunities for contact with animals	It should be noted that even slight contact may lead to an infection The risk of mass exposure is high around an animal after delivery An epidemic outbreak is possible at home or in an office
	Subjective and objective symptoms	Systemic symptoms such as high fever, arthralgia, and malaise are significant Influenza-like symptoms in the "off-season"
	Responsiveness to antimicrobial drugs	ß-Lactam antibiotics are basically ineffective (spontaneous remission during treatment is possible) Tetracyclines, macrolides and quinolones are effective
Etiological diagnosis	Measurement of antibody titers to phase II *Coxiella*	It is often impossible to evaluate antibody titers based only on acute phase serum samples It may take a few months for antibody titers to increase It is important to monitor antibody titers even after recovery from the disease
	PCR-based detection of the *Coxiella* gene	It is often necessary to use a nested PCR technique Detection is also possible in various samples from outside the respiratory system For suspected cases, acute phase samples should be kept in a freezer At present PCR should be considered as an adjunct diagnostic technique
	Overall evaluation	The clinical picture changes in antibody titers and PCR results should be integrated into the evaluation It is necessary to differentiate the pathogen from *Mycoplasma*, *Chlamydia*, and *Legionella*

strains vary between different countries (Watanabe and Takahashi, 2008). Moreover, the Japanese population might have a different physiological response to infection compared to Caucasians. Currently, results obtained with imported IFA and ELISA kits remain difficult to interpret. Furthermore, ELISA kits require a retest with the standard IFA before evaluating a patient (Watanabe and Takahashi, 2008). In conclusion, new rapid diagnostic tests specifically using the Japanese strain of *C. burnetii* are indispensable. In addition, a larger number of Japanese institutions and laboratories should be equipped with the diagnostic tests (Watanabe and Takahashi, 2008). Vaccination is uncommon in Japan because of the limited recognition of the disease (Watanabe and Takahashi, 2008).

5. Conclusion and perspectives

Q fever is a newly discovered disease in Japan. Previously it was considered completely absent. Knowledge of the illness is thus limited. Available epidemiological data consists frequently of small samples of animals or humans rendering the interpretation poorly accurate. The lack of knowledge of the epidemiological and geographical situation in certain areas of the country also causes problems. The estimation of the prevalence or incidence of Q fever is difficult due to the recent awareness of the illness, to the absence of previous data and to seroprevalences estimated on sampled individuals that are not necessarily representative of the endogenous population. Recently differential diagnoses are including Q fever and cases are being diagnosed and reported. Important further research is however necessary to improve knowledge of the disease itself, of the endogenous hosts and reservoirs (e.g., the role of domestic birds should be more investigated), and of the epidemiological cycle of coxiellosis in this country. Diagnostic tests must be improved to increase their sensitivity and avoid the necessity of retesting. They must be adapted to the Japanese strain of bacteria and to the Japanese conditions. The multidisciplinary approach needed would involve a large variety of scientists. To this day, Q fever remains a challenge for the veterinary and medical profession.

References

Abe, T., Yamaki, T., Hayakawa, T., Fukuda, H., Ito, Y., Kume, H., Komiya, T., Ishihara, K., Hirai, K., 2001. A seroepidemiological study of the risks of Q fever infection in Japanese veterinarians. Eur. J. Epidemiol. 17 (11), 1029–1032.

AFSSA, 2004. Fièvre Q: Rapport sur L'évaluation des Risqué Pour la Santé Publique et des Outils de Gestion des Risqué en Élevage de Ruminants. , pp. 1–88.

Albornoz, M.M.C., Heredia, J.H.P., Alvarez, J.S., Lopez, G.T., Palacios, R.H., Puente, A.R., 1991. Epidemiology of community-acquired pneumonia in the health area I from Navarra. Med. Clin. (Barc.) 97, 50–52.

Arnow, P.M., Flaherty, J.P., 1997. Fever of unknown origin. Lancet 350, 575–580.

Babudieri, B., 1959. Q fever: a zoonosis. Adv. Vet. Sci. 5, 82–182.

Beaudeau, F., Guatteo, R., Seegers, H., 2006. Voies d'excrétion de *Coxiella burnetii* par la vache laitière: implication pour le dépistage et la maîtrise de l'infection en élevage. Epidémiol. Et santé anim. 49, 1–4.

Benson, W.W., Brock, D.W., Mather, J., 1963. Serologic analysis of a penitentiary group using raw milk from a Q fever infected herd. Public Health Rep. 78, 707–710.

Berri, M., Rousset, E., Champion, J.L., Russo, P., Rodolakis, A., 2007. Goats may experience reproductive failures and shed *Coxiella burnetii* at two successive parturitions after a Q fever infection. Res. Vet. Sci. 83, 47–52.

Bildfell, R.J., Thomson, G.W., Haines, D.M., McEwen, B.J., Smart, N., 2000. *Coxiella burnetii* infection is associated with placentitis in cases of bovine abortion. J. Vet. Diagn. Invest. 12, 419–425.

Bochud, P.Y., Moser, F., Erard, P., Verdon, F., Studer, J.P., Villard, G., 2001. Community-acquired pneumonia. A prospective outpatient study. Medicine (Baltimore) 80, 75–87.

Delsing, C.E., Kullberg, B.J., 2008. Q fever in the Netherlands: a concise overview and implications of the largest ongoing outbreak. Neth. J. Med. 66, 365–367.

Derrick, E.H., 1937. "Q" fever, a new fever entity: clinical features, diagnosis and laboratory investigation. Med. J. Aust. 2, 281–299.

Dorko, E., Kalinova, Z., Weissova, T., Pilipcinec, E., 2008. Seroprevalence of antibodies to *Coxiella burnetii* among employees of the Veterinary University in Kosice, Eastern Slovakia. Ann. Agric. Environ. Med. 15, 119–124.

Ejercito, C.L., Cai, L., Htwe, K.K., Taki, M., Inoshima, Y., Kondo, T., Kano, C., Abe, S., Shirota, K., Sugimoto, T., Yamaguchi, T., Fukushi, H., Minamoto, N., Kinjo, T., Isogai, E., Hirai, K., 1993. Serological evidence of *Coxiella burnetii* infection in wild animals in Japan. J. Wildl. Dis. 29, 481–484.

Field, P.R., Mitchell, J.L., Santiago, A., Dickeson, D.J., Chan, S.W., Ho, D.W., Murphy, A.M., Cuzzubbo, A.J., Devine, P.L., 2000. Comparison of a commercial enzyme-linked imunosorbent assay with immunofluorescence and complement fixation tests for detection of *Coxiella burnetii* (Q fever) immunoglobulin. Med. J. Clin. Microbiol. 38, 1645–1647.

Frangoulidis, D., Rodolakis, A., Heiser, V., Landt, O., Splettstoesser, W., Meyer, H., 2009. DNA microarray-chip based diagnosis of Q-fever (*Coxiella burnetii*). Clin. Microbiol. Infect. 15 (2), 165–166.

Guatteo, R., Beaudeau, F., Berri, M., Rodolakis, A., Joly, A., Seegers, H., 2006. Shedding routes of *Coxiella burnetii* in dairy cows: implications for detection and control. Vet. Res. 37 (6), 827–833.

Hartzell, J.D., Wood-Morris, R.N., Martinez, L.J., Trotta, R.F., 2008. Q fever: epidemiology, diagnosis, and treatment. Mayo Clin. Proc. 83, 574–579.

Hirai, K., 1997. Q fever. J. Clin. Sci. 33, 1156–1161.

Hirai, K., To, H., 1998. Advances in the understanding of *Coxiella burnetii* infection in Japan. J. Vet. Med. Sci. 60, 781–790.

Hirai, K., 1999. Recent study of Q Fever. J. Vet. Med. Assoc. 52, 77–83 (in Japanese).

Hirschman, J.V., 1997. Fever of unknown origin in adults. Clin. Infect. Dis. 24, 291–300.

Htwe, K.K., Amano, K., Sugiyama, Y., Yogami, K., Minamoto, N., Hashimoto, A., Yamaguchi, T., Fukushi, H., Hirai, K., 1992. Seroepidemiology of *Coxiella burnetii* in domestic and companion animals. Vet. Rec. 131, 490.

Htwe, K.K., Yoshida, T., Hayashi, S., Miyake, T., Amano, K., Morita, C., Yamaguchi, T., Fukushi, H., Hirai, K., 1993. Prevalence of antibodies to *Coxiella burnetii* in Japan. J. Clin. Microbiol. 31, 722–723.

Knockaert, D.C., Vanderschueren, S., Blockmans, D., 2003. Fever of unknown origin in adults: 40 years on. J. Intern. Med. 253, 263–275.

Komiya, T., Sadamasu, K., Kang, M.I., Tsuboshima, S., Fukushi, H., Hirai, K., 2003. Seroprevalence of *Coxiella burnetii* infections among cats in different living environments. J. Vet. Med. Sci. 65, 1047–1048.

Koulla-Shiro, Kuaban, C., Belec, L., 1997. Microbial etiology of acute community-acquired pneumonia in adult hospitalized patients in Yaounde-Cameroon. Clin. Microbiol. Infect. 3, 180–186.

Krumbiegel, E.R., Wisniewski, H.J., 1970. Q fever in the Milwaukee area II. Consumption of infected raw milk by human volunteers. Arch. Environ. Health 21, 63–65.

Kuroiwa, Y., Oyanagi, R., Fuse, S., Mori, T., Ueno, H., Tsutsumi, H., 2007. Persistent hepatitis and repeated wheezing in an infant. Q fever. Pediatr. Infect. Dis. J. 26, 763 768–769.

Lieberman, D., Schleaffer, F., Boldur, I., Lieberman, D., Horowitz, S., Friedman, M.G., Leiononen, M., Horovitz, O., Manor, E., Porath, A., 1996. Multiple pathogens in adult patients admitted with community-acquired pneumonia: a one-year prospective study of 346 consecutive patients. Thorax 51, 179–184.

Lieberman, D., Lieberman, D., Ben-Yaakov, M., Lazarovich, Z., Hoffman, S., Ohana, B., Friedman, M.G., Dvoskin, B., Leinonen, M., Boldur, I., 2001. Infectious etiologies in acute exacerbation of COPD. Diagn. Microbiol. Infect. Dis. 40, 95–102.

Lozano, F., Torre-Cisneros, T., Bascuñana, A., Polo, J., Viciana, P., García-Ordóñez, M.A., Hernández-Quero, J., Márquez, M., Vergara, A., Díez, F., Pujol, E., Torres-Tortosa, M., Pasquau, J., Hernández-Burruezo, J.J., Suárez, I., 1996. Prospective evaluation of fever of unknown origin

in patients infected with the human immunodeficiency virus. Eur. J. Clin. Microbiol. Infect. Dis. 15, 705–711 (Grupo Andaluz para el Estudio de las Enfermedades Infecciosas.).
Mahara, F., 2006. Rickettsioses in Japan and the Far East. Ann. N.Y. Acad. 1078, 60–73.
Marmoin, B.P., Stoker, M.G., 1958. The epidemiology of Q fever in Great Britain; an analysis of the findings and some conclusions. Br. Med. J. 2, 809–816.
Marrie, T.J., Durant, H., Williams, J.C., Mintz, E., Waag, D.M., 1988a. Exposure to parturient cats: a risk factor for acquisition of Q fever in Maritime Canada. J. Infect. Dis. 158, 101–108.
Marrie, T.J., MacDonald, A., Durant, H., Yates, L., McCormick, L., 1988b. An outbreak of Q fever probably due to contact with a parturient cat. Chest 93, 98–103.
Marrie, T.J., Langille, D., Papukna, V., Yates, L., 1989. Truckin' pneumonia: an outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with newborn kittens. Epidemiol. Infect. 102, 119–127.
Marrie, T.J., 1990. Q Fever. The Disease. CRC Press, Boca Raton, Florida, pp. 125–160.
Marrie, T.J., Peeling, R.W., Fine, M.J., Singer, D.E., Coley, C.M., Kapoor, W.N., 1996. Ambulatory patients with community-acquired pneumonia the frequency of atypical agents and clinical course. Am. J. Med. 101, 508–515.
Maurin, M., Raoult, D., 1999. Q fever. Clin. Microbiol. Rev. 12, 518–553.
Million, M., Lepidi, H., Raoult, D., 2009. Q fever: current diagnosis and treatment options. Med. Maladies Infect. 39, 82–94.
Mitscherlich, E., Marth, E.H., 1984. Microbial survival in the environment. In: Bacteria and Rickettsiae Important in Human and Animal Health, Springer-Verlag, Düsseldorf, Germany, p. 802.
Morita, C., Katsuyama, J., Yanase, T., Ueno, H., Muramatsu, Y., Hondatsu, T., Koyama, H., 1994. Seroepidemiological survey of *Coxiella burnetii* in domestic cats in Japan. Microbiol. Immunol. 38, 1001–1003.
Muramatsu, Y., Hukuta, K., Satoh, S., Muramatsu, M., Nishimura, M., Nagahata, H., Ueno, H., Morita, C., Tamura, Y., 2006. Seroepidemiologic survey of *Coxiella burnetii* and attempt to detect *Coxiella* DNA in aged non-laying chickens in a prefecture of Japan where poultry farming prospers. J. Vet. Med. Sci. 68, 1007–1008.
Nagaoka, H., Akiyama, M., Sugieda, M., Nisho, T., Akahane, S., Hattori, H., Ho, T., Fushi, H., Hirai, K., 1996. Isolation of *Coxiella burnetii* from children with influenza-like symptoms in Japan. Microbiol. Immunol. 40, 147–151.
Nagaoka, H., Sugieda, M., Akiyama, M., Nishina, T., Akahane, S., Fujiwara, K., 1998. Isolation of *Coxiella burnetii* from the vagina of feline clients at veterinary clinics. J. Vet. Med. Sci. 60, 251–252.
Natale, A., Busani, L., Comin, A., De Rui, S., Buffon, L., Nardelli, S., Marangon, S., Ceglie, L., 2009. First report of bovine Q-fever in North-Eastern Italy: preliminary results. Clin. Microbiol. Infect. 15, 144–145.
Nguyen, S.A.V., To, H., Minamoto, N., Ogawa, M., Yamaguchi, T., Fukushi, H., Hirai, K., 1997. Evaluation of high-density agglutination test for *Coxiella burnetii* antibodies in animals. Clin. Diagn. Lab. Immunol. 4, 679–680.
Numazaki, K., Ueno, H., Yokoo, K., Muramatsu, Y., Chiba, S., Morita, C., 2000. Detection of serum antibodies to Bartonella henselae and *Coxiella burnetii* from Japanese children and pregnant women. Microbes Infect. 2, 1431–1434.
Oda, H., Yoshiie, K., 1989. Isolation of *Coxiella burnetii* strain that has low virulence for mice from a patient with acute Q fever. Microbiol. Immunol. 33, 969–973.
Okimoto, N., Asaoka, N., Osaki, K., Kurihara, T., Yamato, K., Sunagawa, T., Fujita, K., Ohba, H., Nakamura, J., Nakada, K., 2004. Clinical features of Q fever pneumonia. Respirology 9, 278–282.
Okimoto, N., Kibayashi, T., Mimura, K., Yamato, K., Kurihara, T., Honda, Y., Osaki, K., Asaoka, N., Ohba, H., 2007. *Coxiella burnetii* and acute exacerbations/infections in patients with chronic lung disease. Respirology 12, 619–621.
Ormsbee, R., Peacock, M., Gerloff, R., Tallent, G., Wike, D., 1978. Limits of Rickettsial infectivity. Infect. Immun. 19, 239–245.
Palmer, N.C., Kierstead, M., Key, D.W., Williams, J.C., Peacock, M.G., Vellend, H., 1983. Placentitis and abortion in goats and sheep in Ontario caused by *Coxiella burnetii*. Can. Vet. J. 24, 60–61.
Pape, M., Mandraveli, K., Arvanitidou-Vagiona, M., Nikolaidis, P., Alexiou-Daniel, S., 2009. Q fever in northern Greece: epidemiological and clinical data from 58 acute and chronic cases. Clin. Microbiol. Infect. 15 (2), 150–151.
Peter, O., Dupuis, G., Peacock, M.G., Burgdorferj, W., 1987. Comparison of enzyme-linked immunosorbent assay and complement fixation and indirect fluorescent-antibody tests for detection of *Coxiella burnetii* antibody. J. Clin. Microbiol. 25, 1063–1067.
Pinsky, R.L., Fishbein, D.B., Greene, C.R., Gensheimer, K.F., 1991. An outbreak of cat-associated Q fever in the United States. J. Infect. Dis. 164, 202–204.
Rodolakis, A., Berri, M., Héchard, C., Caudron, C., Souriau, A., Bodier, C.C., Blanchard, B., Camuset, P., Devillechaise, P., Natorp, J.C., Vadet, J.P., Arricau-Bouvery, N., 2007. Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine, and ovine herds. J. Dairy Sci. 90, 5352–5360.
Rousset, E., Duquesne, V., Russo, P., Thiéry, R., 2007. La fièvre Q: problématiques et risques sanitaires. Bull. Acad. Vet. Fr. 160, 107–114.
Rousset, E., Berri, M., Durand, B., Dufour, P., Prigent, M., Delcroix, T., Touratier, A., Rodolakis, A., 2009. *Coxiella burnetii* shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. Appl. Environ. Microbiol. 75, 428–433.
Ruiz, M., Ewig, S., Marcos, M.A., Martinez, J.A., Arancibia, F., Mensa, J., Torres, A., 1999. Etiology of community-acquired pneumonia: impact of age, comorbidity, and severity. Am. J. Respir. Crit. Care Med. 160, 397–405.
Saito, A., Kohno, S., Matsushima, T., Watanabe, A., Oizumi, K., Yamaguchi, K., 2006. Prospective multicenter study on the causative organisms of community-acquired pneumonia in adults in Japan. J. Infect. Chemother. 12, 63–69.
Sampere, M., Font, B., Font, J., Sanfeliu, I., Segura, F., 2003. Q fever in adults: review of 66 clinical cases. Eur. J. Clin. Microbiol. Infect. Dis. 22, 108–110.
Schimmer, B., Morroy, G., Dijkstra, F., Schneeberger, P.M., Weers-Pothoff, G., Timen, A., Wijkmans, C., van der Hoek, W., 2008. Large ongoing Q fever outbreak in the south of The Netherlands, 2008. Euro. Surveill. 13.
Setiyono, A., Ogawa, M., Cai, Y., Shiga, S., Kishimoto, T., Kurane, I., 2005. New criteria for immunofluorescence assay for Q fever diagnosis in Japan. J. Clin. Microbiol. 43, 5555–5559.
Sopena, N., Sabria-Leal, M., Pedro-Botet, M.L., Padilla, E., Dominguez, J., Morera, J., Tudela, P., 1998. Comparative study of the clinical presentation of Legionella pneumonia and other community-acquired pneumonias. Chest 113, 1195–1200.
Takahashi, H., Tokue, Y., Kikuchi, T., Kobayashi, T., Gomi, K., Goto, I., Shiraishi, H., Fukushi, H., Hirai, K., Nukiwa, T., Watanabe, A., 2004. Prevalence of community-acquired respiratory tract infections associated with Q fever in Japan. Diagn. Microbiol. Infect. Dis. 48, 247–252.
Tatsumi, N., Baumgartner, A., Qiao, Y., Yamamoto, I., Yamaguchi, K., 2006. Detection of *Coxiella burnetii* in market chicken eggs and mayonnaise. Ann. N.Y. Acad. Sci. 1078, 502–505.
Tissot Dupont, H., Raoult, D., Brouqui, P., Janbon, F., Peyramond, D., Weiller, P.J., Chicheportiche, C., Nezri, M., Poirier, R., 1992. Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. Am. J. Med. 93, 427–434.
To, H., Htwe, K.K., Yamasaki, N., Zhang, G.Q., Ogawa, M., Yamagichi, T., Fukushi, H., Hirai, K., 1995. Isolation of *Coxiella burnetii* from dairy cattle and ticks, and some characteristics of the isolates in Japan. Microbiol. Immunol. 39, 663–671.
To, H., Kako, N., Zhang, G.Q., Otsuka, H., Ogawa, M., Ochiai, O., Nguyen, S.V., Yamaguchi, T., Fukushi, H., Nagaoka, N., Akiyama, M., Amano, K., Hirai, K., 1996. Q fever pneumonia in children in Japan. J. Clin. Microbiol. 34, 647–651.
To, H., Htwe, K.K., Kako, N., Kim, H.J., Yamaguchi, T., Fukushi, H., Hirai, K., 1998a. Prevalence of *Coxiella burnetii* infection in dairy cattle with reproductive disorders. J. Vet. Med. Sci. 60, 859–861.
To, H., Sakai, R., Shirota, K., Kano, C., Abe, S., Sugimoto, T., Takehara, K., Morita, C., Takashima, I., Maruyama, T., Yamaguchi, T., Fukushi, H., Hirai, K., 1998b. Coxiellosis in domestic and wild birds from Japan. J. Wildl. Dis. 34, 310–316.
Torres, A., Dorca, J., Zalacain, R., Bello, S., EL-Elbiary, M., Molinos, L., Arévalo, M., Blanquer, J., Celis, R., Iriberri, M., Prats, E., Fernandez, R., Irigaray, R., Serra, J., 1996. Community-acquired pneumonia in chronic obstructive pulmonary disease: a Spanish multicenter study. Am. J. Respir. Crit. Care Med. 154, 1456–1461.
Ughetto, E., Gouriet, F., Raoult, D., Rolain, J.M., 2009. Three years experience of real-time PCR for the diagnosis of Q fever. Clin. Microbiol. Infect. 15 (2), 200–201.
Vaidya, V.M., Malik, S.V., Bhilegaonkar, K.N., Rathore, R.S., Kaur, S., Barbuddhe, S.B., 2008a. Prevalence of Q fever in domestic animals with reproductive disorders. Comp. Immunol. Microbiol. Infect. Dis. 33 (4), 307–321.
Vaidya, V.M., Malik, S.V.S., Simranpreet, K., Satish, K., Barbuddhe, S.B., 2008b. Comparison of PCR, immunofluorescence assay, and pathogen isolation for diagnosis of Q fever in humans with spontaneous abortions. J. Clin. Microbiol. 46, 2038–2044.

Watanabe, A., Takahashi, H., 2003. Epidemiology, diagnosis, and treatment of Q fever. Jpn. J. Chemother. 51 (8), 461–469 (in Japanese).
Watanabe, A., Takahashi, H., 2008. Diagnosis and treatment of Q fever: attempts to clarify current problems in Japan. J. Infect. Chemother. 14, 1–7.
Yoshiie, K., Oda, H., Nagano, N., Matayoshi, S., 1991. Serological evidence that the Q fever agent (*Coxiella burnetii*) has spread widely among dairy cattle. Microbiol. Immunol. 35, 577–581.
Yuasa, Y., Yoshiie, K., Takasaki, T., Yoshida, H., Oda, H., 1996. Retrospective survey of chronic Q fever in Japan by using PCR to detect *Coxiella burnetii* DNA in paraffin-embedded clinical samples. J. Clin. Microbiol. 34 (4), 824–827.
Zalacain, R., Talayero, N., Achotegui, V., Corral, J., Barrena, I., Sobradillo, V., 1997. Community-acquired pneumonia. Reliability of the criteria for deciding ambulatory treatment. Arch. Bronconeumol. 33, 74–79.

PORTER S.R., CZAPLICKI G., GUATTEO R., MAINIL J., SAEGERMAN C. Q fever: current status and perspectives.

In preparation.

Q FEVER: CURRENT STATUS AND PERSPECTIVES OF A NEGLECTED ZOONOSIS

Sarah Rebecca Porter[1], Guy Czaplicki[2], Jacques Mainil[3], Claude Saegerman[1*]

1: University of Liège, Faculty of Veterinary Medicine, Department of Infectious and Parasitic Diseases, Research Unit in Epidemiology and Risk Analysis applied to Veterinary Sciences, B42, Boulevard de Colonster 20, 4000 Liège, Belgium

2: Association Régionale de Santé et d'Identification Animales, 4431 Loncin, Belgique

3: University of Liège, Faculty of Veterinary Medicine, Department of Infectious and Parasitic Diseases, Laboratory of Bacteriology, Sart-Tilman B43a, B-4000 Liège, Belgium

* Corresponding author: claude.saegerman@ulg.ac.be

Short title: Q fever review.

Table of contents

ABSTRACT

Q fever is an ubiquitous neglected zoonosis caused by an extremely resistant intracellular bacterium, *Coxiella burnetii*. Knowledge of *C. burnetii* remains limited to this day. Its resistant (intracellular and environmental) and infectious proprieties have been poorly investigated. Understanding of the infected host's immune response remains vague and essentially hypothetical. Domestic ruminants are considered the main reservoir of bacteria but a large number of species can be infected. Infected animals shed the highly infectious organisms in milk, feces, urine, vaginal mucus and very importantly, in birth products. Inhalation is the main route of infection. Clinically Q fever is extremely polymorphic rendering its diagnosis difficult. Frequently asymptomatic in animals and humans, Q fever can cause acute or chronic infections. Consequences of infection can be dramatic and, at herd level, can lead to significant financial losses. Vaccination with inactive whole cell bacteria has been performed and proved effective in humans and animals. However inactive whole cell vaccines present several defects. Recombinant vaccines have been developed in experimental conditions and have great potential for the future. Q fever has become a severe public health problem and awareness of the disease must be promoted worldwide. Q fever is a challenging disease for scientists as significant further investigations are necessary. Great research opportunities are available to reach a better understanding and thus a better prevention and control of the infection.

Keywords: Q fever, zoonosis, *Coxiella burnetii*, reproductive disorders, atypical pneumonia

1. INTRODUCTION

Q fever was first described in 1937 by E. H. Derrick in Queensland, Australia (Derrick, 1944). It was previously classified as a "Category "B" critical biological agent" by the Centre for Diseases Control and Prevention and is considered a potential weapon for bioterrorism (Alibek, 1999). Q fever is a public health concern throughout the world (Rousset et al., 2009). However it is rarely a notifiable disease and its surveillance is frequently severely neglected.

Q fever is a zoonotic bacterial disease. Domestic ruminants are considered the main reservoir for the pathogen but Q fever is known to infect a large variety of hosts, mammals (humans, caprids, bovids, ovids, small rodents, dogs, cats) but also birds, fish, reptiles and arthropods (Marmion and Stoker, 1950; Davoli and Signorini, 1951; Slavin, 1952; Marmion et al., 1954; Blanc and Bruneau, 1956; Evans, 1956; Fiset, 1957; Syrucek and Raska, 1956; Stocker and Marmion, 1955; Hirai et To, 1998). It is a highly infectious disease (Benenson and Tigertt, 1956; Ormsbee et al., 1978). Q fever infections remain poorly understood (Rousset et al., 2007a; Pape et al., 2009) and their prevalence and importance have been underestimated for many years (Rousset et al., 2007a). Estimating the level of infection in animals remains problematic (Rousset et al., 2007a).

2. CAUSAL AGENT

The causal agent of Q fever is *Coxiella burnetii*, an obligate intracellular Gram negative bacterium of the *Legionellales* order (Mitscherlich and Marth, 1984; Ransom and Huebner, 1951; Babudieri, 1959). Its predilected target cells are the macrophages situated in body tissues (lymph nodes, spleen, lungs and liver, e.g.) and the monocytes circulating in the blood stream (Baca et al., 1983).

Two different antigenic forms of *C. burnetii* can be distinguished (Baca and Paretsky, 1983). The difference between phase I and phase II bacterial forms resides in the variation of the

surface lipopolysaccharide (LPS) (Amano and Williams, 1984). Only phase I bacteria have a complete LPS on their surface and are virulent bacteria (Moos and Hackstadt, 1987). Phase I bacteria can be isolated from a naturally infected individual or from animals infected in a laboratory (Krt, 2003; Setiyono et al., 2005). On the other hand, phase II bacteria have an incomplete LPS due to a spontaneous genetic deletion of 25,992 bp (Thompson et al., 2003) and are non virulent (Setiyono et al., 2005). Phase II bacteria occur during serial passage in an immunologically incompetent host, such as cell cultures or fertilized eggs (Krt, 2003; Thompson et al., 2003; Setiyono et al., 2005). The deleted chromosomal region comprises a high number of genes that are predicted to function in LPS or lipooligosaccharide biosynthesis, as well as in general carbohydrate and sulfur metabolism (Hoover et al., 2002). However, in Australia, the study by Thompson et al. (2003) on the genome of phase II human strains by polymerase chain reaction (PCR) reported the absence of truncated genes or of deletions. The Institute for Genomic Research has suggested that at least two other chromosomal regions are implicated in phase transition (Thompson et al., 2003). Antigenic variation of *C. burnetii* is important for serological diagnosis. Indeed, serologically, anti-phase II antibodies (IgG and IgM) are found at high levels in acute Q fever, whereas anti-phase I antibodies (IgG and IgA) are found at high levels only during the chronic infection (Setiyono et al., 2005).

Several genetic studies have been performed on *C. burnetii*. The genome of the American Nine Mile strain was sequenced completely in 2003 (Seshadri et al., 2003). The chromosome is circular and varies in size from 1.5 to 2.4 10^6 base pairs. Occasionally a 33- to 42-kb plasmid can be observed in the intracellular media but its function remains to be determined (Maurin and Raoult, 1999). Bacterial isolates can be identified by a probe to 16S ribosomal RNA (rRNA), which is highly conserved (Masuzawa et al., 1997a). Genetic heterogeneity of *C. burnetii* is limited with approximately 30 distinct variants (Million et al., 2009). According

to experimental studies, bacterial strains vary in their pathogenic effect (Stoenner and Lackman, 1960; Oda and Yoshiie, 1989; Kazr et al., 1993; To et al., 1995). Masuzawa et al. (1997b) studied the macrophage infectivity potentiator gene (Cbmip of 654-base DNA) and the sensor-like protein gene (qrsA of 1227-base DNA) sequences between eleven strains. Their results demonstrated that Cbmip and qrsA sequences were highly conserved (>99%) and didn't explain differences in pathogenicity (Masuzawa et al., 1997b). Furthermore, three different plasmids have been identified in *Coxiella* variants (Frazier et al., 1990): QpH1, QpRS and QpDG (Samuel et al., 1983; Mallavia, 1991). Another plasmid (QpDV) has been isolated in a strain from a human case of endocarditis (Valkova and Kazar, 1995). Plasmids differ by size and genomic sequence. However, several identical genomic sequences are present in all plasmids. In bacteria without plasmids, these sequences are found on the chromosome (Frazier et al., 1990). Generally, plasmids are of little interest for identification of microorganisms because they are not critical for survival and can infect a large variety of organisms (Frazier et al., 1990). However, *C. burnetii* plasmids have proven to be useful because different strains contain different plasmids. In fact, QpH1, QpRS and QpDV were present in different genotypes and were associated with difference in pathogenicity in the study by Frazier et al. (1990). Moreover, Savinelli et al. (1990) reported that in human patients, the QpH1 and the QpRS plasmids (or plasmidless strains containing QpRS-related plasmid sequences) were associated with acute and chronic infection, respectively. However, later studies by genomic restriction fragment length polymorphism analysis, plasmid typing or lipopolyssacharride analysis, on a larger number of strains did not confirm their results. Indeed, recent data shows that genetic variation has an apparent closer connection with the geographical source of the isolate than with clinical presentation (Maurin and Raoult, 1999; Glazunova et al., 2005). Moreover, host factors seem to be more important than genomic variation for development of acute or chronic infection (Yu and Raoult, 1994; La Scola et al.,

1998). According to the recent report by the OIE (2005), no specific genotype is associated to acute or chronic infection, to a particular clinical outcome, or to a specific host.

3. PATHOGENESIS

The first step of infection is entry of the bacterium into the host's cells by passive entry, followed by internalization in phagosomes. Phagolysosomes are formed after the fusion of phagosomes with cellular acidic lysosomes. The multiple intracellular phagolysosomes eventually fuse together leading to the formation of a large unique vacuole. *C. burnetii* has adapted to the phagolysosomes of eukaryotic cells and is capable of multiplying in the acidic vacuoles (Hackstadt et al., 1981). In fact, acidity is necessary for nutrients assimilation and for its metabolism, including synthesis of nucleic acids and amino acids (Thompson et al., 1990). Multiplication of *C. burnetii* can be stopped by raising the phagolysosomal pH using lysosomotropic agents such as chloroquine (Akporiaye et al., 1983; Raoult et al., 1990). The mechanisms of *C. burnetii* survival in phagolysosomes are still under study. Mo et al. (1995) and Akporiaye and Baca (1983) identified three proteins involved in intracellular survival: superoxide dismutase, catalase and a macrophage infectivity potentiator (Cbmip). Redd and Thompson (1995) found that secretion and export of Cbmip was triggered by an acid pH in vitro. Later, studies by Zamboni and Rabonovitch (2003) and by Brennan et al. (2004) demonstrated that growth of *C. burnetii* was reduced by reactive oxygen intermediates (ROI) and reactive nitrogen intermediates. Hill and Samuel (2011) analyzed C. *burnetii*'s genome and identified 2 acid phosphatase enzymes. They demonstrated experimentally that both a recombinant acid phosphatase (rACP) enzyme and *C. burnetii* extracts had a pH-dependent acid phosphatase activity. Moreover, rACP and bacterial extracts were capable of inhibiting ROI response by PMN despite their exogenous stimulation by a strong PMN stimulant. Inhibition of the assembly of the NADPH oxidase complex was found to be the mechanism involved (Hill and Samuel, 2011). The intracellular cycle of *C. burnetii* leads to the formation

of two development stages of the bacterium known as “small-cell variant”(SCV) and “large-cell variant” (LCV) (McCaul and Williams, 1981; McCaul et al., 1981; McCaul et al., 1991; Samuel et al., 2000). SCV is the extracellular form of the bacterium. Typically rod-shaped, SCV are compact measuring from 0.2 to 0.5 μm with an electron-dense core (McCaul and Williams, 1981). SCVs are metabolically inactive and are capable of resisting to extreme conditions such as heat, desiccation, high or low pH, disinfectants, chemical products, osmotic pressure and UV rays (Babudieri, 1950; Ransom and Huebner, 1951; McCaul et al., 1981; Mitscherlich and Marth, 1984; Samuel et al., 2000). Their extreme resistance in the environment (pseudo-spores) enables the bacteria to survive for long periods of time in the absence of a suitable host. This resistant form of *C. burnetii* is reversible (Rousset et al., 2007a). Indeed, once inhaled or ingested the SCV attaches itself to a cell membrane and is internalized. After phagolysosomal fusion, the acidity of the newly formed vacuole induces activation of SCV’s metabolism and its development into LCV. During the morphogenesis from SCV to LCV no increase in bacterial number is reported (Coleman et al., 2004). LCV is the metabolically active intracellular form of *C. burnetii*. They are more pleomorphic than SCV. Their cell wall is thinner and they have a more dispersed filamentous nucleoid region. They can exceed 1μm in length (McCaul and Williams, 1981). Intracellular growth is relatively slow with a doubling time of approximately 8 to 12 hours (Baca and Paretsky, 1983). LCVs can differentiate into spore-like bacteria by binary asymmetrical division. The endogenous spore-like forms can undergo further development and metabolic changes until finally reaching the SCV form. Finally cell lysis, or possibly exocytosis, releases the resistant bacteria into the extracellular media (Khavkin, 1977). Apoptosis of the infected cell can be induced by the intracellular bacterium itself leading to the destruction of the dead cell by other macrophages. This process permits *C. burnetii* to infect new cells and to continue its slow multiplication (Rousset et al., 2007a). The physical and biological factors responsible for the sporulation-like process remain unknown. Most natural infections by *C. burnetii* are probably

due to its resistant form (SCV or pseudo-spore) present in the environment. Decreasing the prevalence of Q fever infections using hygienic preventive measures requires a strict limitation of the environmental population of *Coxiella* pseudo-spores (Rousset et al., 2007a). During chronic infection the bacteria multiply at a slower rate (approximately 20h for one doubling), which is similar to the rate of the host cells mitosis. This phenomenon would allow persistence of the bacteria in the cell without causing cellular damage (Roman et al., 1986).

Studies on the immune reaction in naturally or experimentally infected individuals have suggested that cellular immunity and the synthesis of IFNγ are essential for control of *C. burnetii* infection (Izzo and Marmion, 1993; Helbig et al., 2003; Shannon et al., 2009). Helbig et al. (2003) demonstrated the predominant role of IFNγ, its level of production determining the outcome of infection. Indeed, IFNγ has been successfully tested to treat Q fever in patients not responding to antibiotic treatment (Morisawa et al., 2001; Maltezou and Raoult, 2002). A study by Shannon et al. (2009) reported that the development of protective antibody-mediated immunity *in vivo* was found to be independent of the cellular Fc receptors and of the complement (Shannon et al., 2009). The major part of vaccine-derived humoral response consists of IgG antibodies directed against proteins (Novak et al., 1992; Vigil et al., 2010). Several studies report that natural humoral response to *C. burnetii* is directed against both protein and glycolipid fractions (Hendrix et al., 1990; Zhang et al., 2003; Zhang et al., 2004; Zhang et al., 2005). Chen et al. (2011) identified 8 new CD4+ T cell epitopes. However, all the CD4+ T cell epitopes didn't lead to B cell stimulation and specific antibody production. Koster et al. (1985a) reported that in chronic infections, peripheral blood lymphocytes do not proliferate when exposed to *C. burnetii* antigens despite proliferating when exposed to other antigens or mitogens. This was not observed in acute infection (Koster et al., 1985b). In addition, Shannon et al. (2005) observed that *C. burnetii* phase I cells appeared almost invisible to dendritic cells.

Further research is more than necessary to fully understand the complex processes developed by *C. burnetii* to enter and infect a specific host's cell, to resist in the intracellular and extracellular environment, and its ability to cause illness. Moreover, a better understanding of the immune system's reaction to infection would give an insight into the processes developed by the bacterium and by the host that determine the final outcome of disease (asymptomatic, acute or chronic).

4. EPIDEMIOLOGICAL AND CLINICAL ASPECTS

4.1. Routes of infection

Inhalation is the most common route of infection in both animals and human (Welsh et al., 1957; Tissot-Dupont et al., 1999; Russell-Lodrigue et al., 2006). Inhalation of a small number of bacteria is sufficient for the development of an infection in a new host (Ormsbee et al., 1978). As mentioned above, domestic animals are considered the main reservoir for the pathogen (Lang, 1990; Guatteo et al., 2007; Vaidya et al., 2010). Infected animals contaminate the environment by shedding *C. burnetii* in milk, feces, urine, saliva (Hirai et To, 1998; Guatteo et al., 2006) and very importantly in vaginal secretions, placenta, amniotic fluids and other products of conception (To et al., 1996; Hirai et To, 1998; To et al., 1998a; Hatchette et al., 2002; Kim et al., 2005; Guatteo et al., 2006; Rodolakis et al., 2007; Berri et al., 2007; Rousset et al., 2009a). *C. burnetii* also spreads by wind causing infections at a distance from the initial source of bacteria (Marrie and Raoult, 1997; To et al., 1996; Okimoto et al., 2004).

In domestic ruminants, milk is the most frequent route of pathogen shedding (Rodolakis et al., 2007). Currently, controversy remains concerning the possibility of infection by oral route (AFSSA, 2004). Results of previous studies on the subject are considered inconclusive (Marmion et al., 1954; Marmion and Stoker, 1956; Benson et al., 1963; Krumbiegel and

Wisniewki, 1970; Dorko et al., 2008). OIE advises not to drink raw milk originating from infected farms (OIE, 2005). Further research is required to clarify the probability of infection by oral route. If infection by oral route is proven to be efficient, the sufficient number of pathogens capable of causing Q fever should be determined (Rousset et al., 2006).

Human-to-human transmission does not usually occur (OIE, 2005; Watanabe and Takahashi, 2008), although it has been described following contact with parturient women (Deutch and Peterson, 1950; Raoult and Stein, 1994). Currently, risk of transmission through blood transfusion is considered negligible (Anonymous, 1977; Desling and Kullberg, 2008). Transplacental transmission, intradermal inoculation and postmortem examinations have been associated to sporadic cases of Q fever (Harman, 1949; Gerth et al., 1982; Stein and Raoult, 1998; Anonymous, 1950). In addition, cases of sexual transmission of Q fever have been reported (Kruszewska et al., 1996; Milazzo et al., 2001; Miceli et al., 2010).

4.2. Q fever in domestic animals and wildlife

Cattle

Q fever is widespread in livestock and its seroprevalence is thought to have increased in recent years (Maurin and Raoult, 1999). Often neglected in the differential diagnosis, Q fever can persist in a herd causing great financial losses on the long term (To et al., 1998a).

In ruminants, well-known manifestations of Q fever are abortion, stillbirth, premature delivery and delivery of weak offspring (Rousset et al., 2009a). However these dramatic clinical manifestations are generally only expressed in sheep and goats. In bovids, Q fever is frequently asymptomatic. Clinical infected cows develop infertility, metritis and mastitis (To et al., 1998a). A retrospective study by Bildfell et al. (2000) found that *C.burnetii* was significantly associated with placentitis. Placental necrosis and fetal bronchopneumonia were also significantly associated with the presence of *C. burnetii* in the trophoblastes (Bildfell et

al., 2000). Unlike humans and cows infected experimentally (Plommet et al., 1973), naturally infected ruminant rarely present respiratory or cardiac signs. Beaudeau et al. (2006) and Guatteo et al. (2006) performed respective studies on shedding of *C. burnetii* by infected cows. In the study by Guatteo et al. (2006) the apparent proportion of shedders among the cows sampled was 45.5%. Milk was the most frequent positive sample for the bacterium compared to feces and vaginal mucus samples. The percentage of positive samples of each type was 24.4, 20.7 and 19% respectively. 65.4% of sampled cows excreted by one shedding route only, whereas 6.4% shed bacteria in the vaginal mucus, feces and milk simultaneously. A combined shedding in vaginal mucus and in feces was observed in 14.6% of cases. Unfortunately no value is available for the rate of combination of shedding in milk and vaginal mucus in this study (Guatteo et al., 2006). The study by Beaudeau et al. (2006) reported that 85% of their infected cows excreted by one shedding route only. In their study, only 2% of the infected cows shed bacteria in the vaginal mucus, feces and milk simultaneously. When combined shedding occurred, the combination of shedding in vaginal mucus and milk was the most frequently observed. The results of these two French studies are very similar and the differences observed could be due to differences in the sampled population of cattle. Differences in the diagnostic laboratory methods could also be an influential factor. Furthermore, different areas can have variable prevalence of Q fever and, not only is shedding in milk intermittent and its outset not associated with parturition, it also differs from one herd to another despite species being identical (Rodolakis et al., 2007). Milk being such a major shedding route, bulk tank milk (BTM) samples are useful for investigation of the sanitary grade of bovine (Czaplicki et al., 2009) and caprine herds (Dubuc-Forfait et al., 2009). Indeed, polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) performed on BTM samples, in addition to the analysis of serum samples of at least 10% of the animals in the herd, give rapid, economical and valuable information on the herd's status (Rodokalis et al., 2007). In cows' milk, Marrie (1990) identified *C. burnetii* for up to 32

months postpartum. Vaginal and fecal bacterial discharges seem to have a major impact on environmental contamination as a result of practices at kidding and effluent management (Rodolakis et al., 2007; Rousset et al., 2009a). Indeed, the incidence of Q fever has been observed to increase significantly during the lambing period (winter and early spring) (Evans, 1956). A seasonal correlation with spread of goat manure was reported in the Netherlands during the outbreaks of 2007-2008 (Desling and Kullberg, 2008).

Epidemiological data has shown that cows are more frequently chronically infected with persistent shedding of bacteria as a result (Lang, 1990). The sites of chronic *C. burnetii* infection are the uterus and mammary glands of females (Babudieri, 1959; Baca and Paretsky, 1983).

Goats

Numerous studies have suggested that epizootics of Q fever in goats are related to outbreaks in humans (Desling and Kullberg, 2008; Schimmer et al., 2008; Klassen et al., 2009; Rousset et al., 2009a). Indeed in many countries, goats are the most common source of human infection due to their extensive raising and close contact with humans (Berri et al., 2007).

Q fever in goats can induce pneumonia, abortions, stillbirth and delivery of weak kids; the latter two clinical signs being the most frequently observed (Berri et al., 2007; Vaidya et al., 2008; Rousset et al., 2009a). Abortions occur principally nearing the end of the pregnancy (Rousset et al., 2009a). The frequency of occurrence of Q fever abortions in goats is more important than in sheep with up to 90% of females being affected (Berri et al., 2007). Moreover, a study by Berri et al. (2007) reported that successive abortions at two kidding seasons can be observed. Similarly to cattle, pregnant animals are more susceptible to infection than non pregnant animals (Berri et al., 2007). These animals also frequently develop chronic infections with persistence of the bacteria in the uterus and mammary glands

(Sanchez et al., 2006). Hormonal changes during pregnancy cause immunomodulation in the female body causing reactivation of the organism. This immunomodulation has been advanced as an explanation for the increased multiplication of the organism in the placenta (Polydourou, 1981). Shedding of *C. burnetii* by infected goats is discontinuous (Arricau-Bouvery et al., 2003; Arricau-Bouvery et al., 2005; Berri et al., 2005; Berri et al., 2007; Rousset et al., 2009a). In naturally infected goats, shedding seemed to be limited to the kidding season following infection (Hatchette et al., 2003). However, an experimental study performed by Berri et al. (2007) reported that infected animals can shed *Coxiella* during two successive kidding seasons in vaginal mucus and milk. Moreover, shedding in milk can be maintained for a long period (Berri et al., 2005; Berri et al., 2007). Milk has been considered the major route of bacteria excretion for this species by several authors (Rodokalis et al., 2007).

Sheep

Sheep have a predisposition for abortion similarly to goats (Hirai et To, 1998; Berri et al., 2007). However, Q fever in sheep seldom causes chronic infections (Hirai et To, 1998; Rousset et al., 2007a; Vaidya et al., 2008). Infected sheep, like goats, shed *C. burnetii* in vaginal secretions, urine and feces and to a lesser extent in milk (Rodokalis et al., 2007). In naturally infected sheep, the bacterium has been isolated in vaginal discharges long after abortion (Berri et al., 2001) and can be shed at subsequent pregnancies (Berri et al., 2002). In the study by Rodokalis et al. (2007), no ewe was found to shed bacteria constantly in milk. Some sheep have been found to shed *C. burnetii* during 11 to 18 days postpartum in feces (Marrie, 1990). Most ewe shed bacteria by at least two routes, mainly in feces and vaginal mucus (Rodokalis et al., 2007). Rodokalis et al. (2007) reported a flock where no ewe ever shed by a single route (no exclusive shedding in milk or feces or vaginal mucus).

In certain areas (e.g. Basque country), sheep are an important source of human infections. Garcia-Pérez et al. (2009) studied the presence of *C. burnetii* DNA in BTM and its association with seroprevalence. The authors reported a lower seroprevalence in lambs and yearlings compared to older ewes. Herds with a history of abortion had a higher seroprevalence than other herds but the difference observed was not significant. Flocks with a level of seropositivity superior to 30% were more frequently positive on BTM-PCR analysis. However flocks with a low number of seropositive animals but with a positive result at BTM-PCR analysis were also observed in this study (Gracia-Pérez et al., 2009). In France, two cases of human Q fever were associated with the use of ovine manure as garden fertilizer. The flock of sheep that had provided the manure did not present any clinical signs, despite the presence of seropositive animals and excretion of bacteria in feces (Berri et al., 2003). The Q fever outbreak that occurred in Bulgaria in 2004 was also associated to infected sheep and goats (Panaiotov et al., 2009).

Pigs

Natural susceptibility has been demonstrated by the presence of antibodies to *C. burnetii* in their serum (Marmion and Stoker, 1958). However the role played by pigs in the epidemiology of Q fever remains unknown (Hirai et To, 1998).

Cats and dogs

Cats and dogs can be infected by Q fever and have been associated with human infections in rural and urban areas (Mantovani and Benazzi, 1953; Kosatsky, 1984; Marrie et al., 1985; Buhariwalla et al., 1996; Marrie et al., 1988a; Marrie et al., 1988b; Marrie et al., 1988c; Marrie et al., 1989; Higgins and Marrie, 1990; Pinsky et al., 1991; Mattewman et al., 1997). In 1952, Gillepsie and Baker performed successful feline experimental infections by subcutaneous inoculation, feeding infected yolk sacs and by contact with infected cats.

Despite the presence of pathogen in blood, urine and a serological response, clinical signs were not observed in all infected cats. In felines, Q fever is, seemingly, frequently asymptomatic and remains undiagnosed. However, infected cats excrete bacteria in the environment and become a potential source of human infections. In Japan, cats are widely considered to be one of the most important reservoirs of *C. burnetii* (Komiya et al., 2003). The study by Komiya et al. (2003) found that seroprevalence was significantly higher in stray cats than in domestic cats. Thus the felines' environment seems to influence the probability of *Coxiella* infection (Komiya et al., 2003).

The potential importance of dogs for the transmission of Q fever to humans is rarely mentioned. Dogs are however as close, if not closer, to humans as cats. Like other species, dogs can potentially be infected by inhalation, tick bites, consumption of placentas or milk from infected cows. Buhariwalla et al. (1996) reported three human cases of Q fever associated to an infected parturient dog. The puppies all died within 24 hours of birth. Indeed, Q fever in parturient dogs has previously been associated with early death of puppies (Mantovani and Benazzi, 1953). Similarily to cats, a previous study on dogs in California reported a higher prevalence of infection in stray than in domestic dogs (Willeberg et al., 1980).

Currently, the effect of infection and its clinical presentation remain poorly investigated in felines and canines.

Horses

In previous studies, horses have been found to be seropositive toward Q fever (Willeberg et al., 1980). Indeed, the study by Willeberg et al. (1980) reported a seroprevalence of 26% (31/121) in horses in California. To our knowledge no human case of Q fever has been associated with equids. Moreover, Q fever is not investigated routinely in cases of infertility or obstetric complications in this species.

Wild animals

At present, wildlife is considered of minor importance for Q fever epidemiology. Many wild mammals and birds have been found to be hosts to the infectious organism (Enright et al., 1971; Riemann et al., 1978; Webster et al., 1995; Hirai and To, 1998; Ruiz-Fons et al., 2008; Astobiza et al., 2011). A few cases of transmission of *C. burnetii* from wild animals to humans have been reported but merit further experimental research (Syrucek and Raska, 1956; Hirai et To, 1998).

Ticks and other potential vectors

Among ectoparasites, ticks are considered to be the natural primary reservoir of *C. burnetii*. Over 40 tick species are naturally infected (Cox, 1938; Parker and Davis, 1938; Davis, 1939; Cox, 1940; Smith, 1940; Smith and Derrick, 1940; Smith, 1941; Mantovani and Benazzi, 1953; Pope et al., 1960; To et al., 1995; Ruiz-Fons et al., 2008). Experimental infections have been obtained in guinea pigs with *Ixodes holocyclus*, *Haemaphysalis bispinosa*, *Rhipicephalus sanguineus* and *Dermacentor andersoni*. In Europe, *Ixodes ricinus* is the most common tick. *Rhipicephalus sanguineus* ticks are frequent on dogs (Smith, 1940; Smith, 1941; AFSSA, 2004). Thus, both these species of ticks could be (or become) reservoirs of *C. burnetii* in Europe.

Ticks excrete bacteria in saliva and feces. After multiplying in the cells of the midgut and stomach of an infected tick, extremely infectious bacteria are deposited onto the animal's skin during fecal excretion. The feces are extremely rich in bacteria and may reach a concentration of 10^{12} organisms per gram (Babudieri, 1959; Lang, 1990). Furthermore, transovarial transmission is suspected as bacteria have been isolated in the ovaries of infected ticks (Babudieri, 1959). However, despite all these factors, ticks are not thought to contribute to the maintenance of *coxiellosis* in endemic areas (Hirai and To, 1998; Rousset et al., 2007a). Indeed, in the study by Astobiza et al. (2011) none of the ticks analyzed were positive when

tested by PCR despite the area being endemic for Q fever. Furthermore, in an endemic area in Germany, only 3% of *Dermacentor spp.* were found to be infected (Pluta et al., 2010). Nevertheless ticks are suspected of having a significant role in the transmission of *C. burnetii* among wild vertebrates, especially rodents, lagomorphs and wild birds (Babudieri, 1959; Lang, 1990; Marie et al., 1986). In addition, Hirai and To (1998) hypothesized that they could play a role for transmission of *Coxiella* from infected wild animals to domestic naïve animals. Human infections through tick bites are rarely reported. Sporadic isolations of *C. burnetii* in chiggers, lice and flies have been reported (Cox, 1938; Philip, 1948; Giroud and Jardin, 1954).

4.3. Q fever in humans

The main characteristic of Q fever is its clinical polymorphism (Derrick, 1937; Benenson and Tigertt, 1956; Babudieri, 1959). Q fever is therefore considerably underdiagnosed and underreported (Gidding et al., 2009). After an incubation period of 1 to 3 weeks (Maurin and Raoult, 1999; Watanabe and Takahashi, 2008), Q fever can cause either an acute or a chronic disease. Size of the inoculum, geographical area, route and time of infection, as well as host factors influence the duration of the incubation period (Benenson and Tiggert, 1956; Williams; 1991; Marrie et al., 1996) and may contribute to the clinical expression of acute or chronic infection (Babudieri, 1959; Marrie, 1990).

Acute Q fever

In the acute form, infections can be totally asymptomatic in 50 to 60% of cases or cause a self-limiting illness associated with fever, fatigue, headache and myalgia (influenza-like syndrome). When clinically expressed, acute fever is frequently accompanied by atypical pneumonia and/or hepatitis. Pneumonia is an important manifestation of Q fever in humans (Derrick, 1937; Benenson and Tigertt, 1956; Babudieri, 1959). It is uncommon in Australia

and some parts of Russia, whereas in North America and Europe it is the major manifestation of infection (To et al., 1996; Okimoto et al., 2004). Pneumonia is typically mild but progression to acute distress syndrome can occur (Hartzell et al., 2007; Okimoto et al., 2007; Watanabe and Takahashi, 2008). Endocarditis can exceptionally be associated to acute infection in 0.76 % of cases (Fenollar et al., 2001; Fenollar et al., 2006). In pregnant women, Q fever can lead to spontaneous abortion, intrauterine fetal death (IUFD), premature delivery or intrauterine growth retardation. Transplacental infection of the fetus in utero has also been reported (Raoult and Stein, 1994; Kaplan et al., 1995; Raoult et al., 2002). Moreover, Carcopino et al. (2007) found that Q fever was significantly associated with oligoamnios, which is a recognized cause of neonatal morbidity and mortality (Barss et al., 1984; Moore et al., 1989). One case of endocarditis has been reported in a pregnant women with a bioprosthetic aortic valve and lead to maternofetal death at 27 weeks' gestation (Carcopino et al., 2007). Infection during the first trimester of the pregnancy is particularly associated with a negative outcome (Carcopino et al., 2007; Raoult et al., 2002). After infection, breast feeding is of course contraindicated (Raoult et al., 2002).

Mortality rate of acute Q fever is estimated of 1 to 2% (Tissot-Dupont et al., 1992; Delsing and Kullberg, 2008; Watanabe and Takahashi, 2008). Myocarditis, occurring in less than 1% of cases, is the first cause of death (Fournier et al., 2001). As yet, myocarditis has not been reported in pregnant women (Carcopino et al., 2009).

Chronic Q fever

Chronic Q fever consists in the persistence of infection for more than 6 months. Chronic Q fever concerns 5% of infected individuals (Fournier et al., 1998). Most commonly, endocarditis is observed in 60-70% of cases of chronic infections (Fenollar et al., 2004), but chronic hepatitis, osteomyelitis, septic arthritis, interstitial lung disease, and infection of aneurysm or vascular grafts can also occur (Derrick, 1937; Benenson and Tigertt, 1956;

Babudieri, 1959). Q fever-associated endocarditis has been estimated to be responsible for 3 to 5 % of all cases of human endocarditis (Fenollar et al., 2001; Parker et al., 2006). Individuals with underlying valvulopathy or other cardiovascular abnormalities are predisposed to the development of endocarditis (Fenollar et al., 2001; Fenollar et al., 2006). Over recent years the occurrence of rare clinical manifestations such as osteomyelitis, optic neuritis, pericarditis, lymphadenopathy and Guillan-Barre has significantly increased. Meningitis, encephalitis, polyradiculonevritis, peripheral neuropathy, cranial nerve deficiency, optic neuritis and paralysis of the oculomotor nerve have been reported in 3.5% of infected patients. The main clinical signs in neurological cases are headaches, behavioral problems, cognitive deficiency or confusion. Convulsions and even epileptic fits and aphasia are possible (Schuil et al., 1985; Shaked et al., 1989; Bernit et al., 2002). In pregnant women, chronic Q fever can lead to spontaneous abortions in future pregnancies (Raoult and Stein, 1994; Stein and Raoult, 1998; Langley et al., 2003). The prognosis of chronic infections is less favorable than for acute infections (Watanabe and Takahashi, 2008). Antibiotic treatment is less effective and the disease is usually long with mortality rates that can reach more than 50% (Watanabe and Takahashi, 2008).

Q fever in children

In children Q fever is thought to be rare. This could be explained by frequent asymptomatic or nonspecific presentations of infection leading to undiagnosed cases of *Coxiella* infection. Cases of hepatitis and pneumonia have however been reported in children and can be fatal (To et al., 1996; Kuroiwa et al., 2007). In Northern Ireland, McCaughey et al. (2008) reported a seropositivity rate inferior to 10% in children. The seropositivity rate increased markedly during the late teenage years and especially in young adults (McCaughey et al., 2008). Increased exposure with age is a plausible hypothesis to explain this phenomenon.

Post-illness follow-up

The study by Limonard et al. (2010) on follow-up of patients after the Q fever outbreak in the Netherlands, reported fatigue in 52% of patients 6 months post-illness and in 26% one year post-illness. They reported very high level of anti-phase I and anti-phase II antibodies up to 3 months after onset of disease, then a gradual decrease in the following 9 months. Post-Q fever chronic fatigue syndrome is also reported (Hickie et al., 2006; Delsing and Kullberg, 2008; Marmion et al., 2009). This syndrome has been attributed to dysregulation of cytokine production, induced by persistent antigens including LPS and proteins, rather than persistent latent *Coxiella* (Marmion et al., 2009).

5. ANIMAL MODELS FOR HUMAN Q FEVER

Animal models for human infection are commonly laboratory mice and guinea pig models. Guinea pigs are regarded as a model of acute Q fever in humans (La Scola et al., 1997). On the contrary to guinea pigs, mice develop a chronic infection, although endocarditis doesn't occur in normal adult mice. They remain chronically infected for months and excrete bacteria in feces and urine. Their susceptibility to infection varies in function of the mouse strain (Scott et al., 1987). Gonder et al. (1979) and Waag et al. (1999) reported a successful infection by aerosol route in cynomolgus and rhesus macaques. Post-infection, the macaques developed fever and pneumonia after 4 to 7 days. Their clinical expression of Q fever is thus unsurprisingly the closest to human clinical expression.

6. PREDISPOSITIONS

Recent experimental data indicates that host factors rather than specific genetic bacterial determinants are the main factors influencing the clinical course of *C. burnetii* infection (Yu and Raoult, 1994; La Scola et al., 1998; Raoult et al., 2005; Parker et al., 2006).

In humans, Q fever is mainly considered an occupational hazard. A study by Whitney et al. (2009) on 508 American veterinarians reported a prevalence rate (22.2%) far superior to the prevalence rate in the general adult US population. Veterinarians from a mixed or large animal practice were significantly more likely to be seropositive than veterinarians from a small animal practice. Furthermore, living on a farm, in the past or present, increased the probability of being seropositive for *coxiellosis*. Absence of protective clothing or mask, occupational risks (accidental cuts, needle sticks), and routine contact with water were also demonstrated as significant risk factors for infection (Whitney et al., 2009). Mc Quinston and Childs (2002) reported a seroprevalence of 7.8% among American veterinarians, farmers, slaughterhouse workers, and tannery workers. Furthermore, a Northern Irish study reported a seropositivity rate significantly higher among farmers than in the general population ($P<0.001$) (McCaughey et al., 2008).

On the contrary to countries where cats are the main reservoir of *C. burnetii* where no sex predisposition is reported, men between 30 and 70 years of age are the most frequently affected by clinical Q fever in countries where cattle are considered the main reservoir (Davies et al., 1997; Montejo et al., 1985; Tellez et al., 1988; Sanzo et al., 1993; Spelman, 1982; Thomas et al., 1994; Thomas et al., 1995). The study by Raoult et al. (2005) reported that being a male and over the age of 15 increased the risk of symptomatic disease. In Australia and France, males are 5-fold and 2.5-fold more likely than females to develop disease, respectively (Parker et al., 2006). In Northern Ireland, McCaughey et al. (2008) also reported a significantly higher seroprevalence in males than in females ($P=0.023$). Endocarditis is more frequently observed in men over 40 years of age (Brouqui et al., 1993). Although the precise reason for this difference remains unknown, it has been suggested that sex hormones play a role (Raoult et al., 2005; Parker et al., 2006). Indeed, Leone et al. (2004)

reported a protective role of 17beta-oestradiol towards Q fever infection rendering females more resistant.

Immunocompromised hosts, pregnant women, people with heart valve lesions, vascular abnormalities, liver cirrhosis and cancer are more susceptible to developing chronic Q fever (Raoult et al., 2000; Fenollar et al., 2006). In the study by Carcopino et al. (2007), 52.8% of the pregnant women with acute Q fever developed chronic serologic profiles. However, suprisingly, in a comparative study on clinical expression and outcome of Q fever after the outbreak in Chamonix Valley in France, pregnant women were found to be significantly less frequently symptomatic than other women and other patients (9.1% women were symptomatic versus 90.6% of men, 75% women and 33.3% children) (Tissot-Dupont et al., 2007).

7. TREATMENT

In non-pregnant women and other patients with acute Q fever, treatment consists in a daily dose of 200 mg doxycycline for 14 to 21 days. Hydroxychloroquine can be associated to doxycycline. Hydroxychloroquine increases the pH of the phagolysosomes and its association with doxycycline has a bacteriocidal effect (Maurin et al., 1992; Raoult et al., 1999). Rifampin, erythromycin, clarithromycin and roxithromycin can also be used as an alternative treatment (Gikas et al., 2001; Tissot-Dupont and Raoult, 2007). Fluoroquinolones are recommended in cases of meningoencephalitis as their penetration into the central nervous system is better compared to doxycycline. Mentioned previously, treatment with IFNγ has proven effective in certain cases (Morisawa et al., 2001; Maltezou and Raoult, 2002).

Treatment of chronically infected patients consists in a daily dose of 200mg of doxycycline associated with 600mg of hydroxychloroquine for duration of 18 to 36 months. The dosage of hydroxychloroquine is adjusted three months after initiation of treatment to obtain a plasmatic

level of 1±0.2mg/l (Maurin et al., 1992). During the treatment, an ophthalmologic follow-up is recommended every 6 months to detect accumulation of hydroxychloroquine in the retina. Rolain et al. (2003) advised a plasmatic concentration of 5mg/l of doxycycline for effective treatment. Minimal Inhibition Concentration (MIC) for doxycyline against *C. burnetii* varies from 1 to 4 µg/ml (Raoult et al., 1999). Bacterial resistance to doxycycline has been observed by Rolain et al. (2005a,b) in a patient with endocarditis. Thus, serological testing on a regular basis to evaluate response to treatment is advised. It is considered that an anti-phase I IgG titer inferior to 200 is predictive of a clinical cure (Karakpusis et al., 2006). A decrease of 2 dilutions in antibody titers in one year at the minimum signifies successful evolution (Raoult et al., 1999).

In infected pregnant women, administration of long-term cotrimoxazole (320 mg trimethoprim and 1600 mg sulfamethoxazole for at least 5 weeks), a bacteriostatic antibiotic, is advised (Carcopino et al., 2007). After delivery, a daily treatment with 200 mg of doxycycline associated with 600 mg of hydroxychloroquine for 1 year minimum is advised for chronically infected women. The study by Carcopino et al. (2007) reported that long-term cotrimoxazole therapy significantly reduced the number of obstetric complications, the frequency of placentits, and the development of a chronic serological profile. In their study, no IUFD was observed in treated women (0% versus 27% of IUFD in non treated women). The only complications reported were intrauterin growth retardation and premature delivery. No spontaneous abortions and no oligoamnios occurred (Carcopino et al., 2007).

8. DIAGNOSTIC METHODS

8.1. Direct diagnosis

Direct diagnostic methods identify the presence of the bacterium or of one of its components.

Coloration and direct visualization

Direct visualization of *C. burnetii* on smears or frozen tissue is a method of Q fever diagnosis. Smears are taken from the placenta of aborted ruminants, from the fetus's stomach content or from other body tissues. *C. burnetii* doesn't stain reliably with Gram stain and Gimenez stain is preferentially used (Gimenez, 1964). A Stamp-Macchiavello coloration, commonly called Macc staining, or routine Giemsa stain can also be performed. The specificity and sensitivity of direct visualization by bacterioscopic examination is poor, due to possible confusion with other pathogens such as *Brucella spp*., *Chlamydophila spp.* or *Chlamydia spp*. (Fournier et al., 2003).

Immunohistochemistry (IHC)

IHC has been performed for the diagnosis of chronic cases of Q fever. It can be utilized for detection of *C. burnetii* in tissues fixed in paraffin or acetone smears (Raoult et al., 1994). Dilbeck and McElwain (1994) developed an avidin-biotin-peroxidase complex IHC staining method for diagnosis and routine screening of ovine and caprine placental tissues after abortion. This technique is rapid and does not necessitate live bacteria or fresh tissues for diagnosis. Furthermore, it renders retrospective studies on stored samples possible.

Bacterial culture

In vitro cell culture of the bacteria is the gold standard for diagnosis of bacterial infections. *C. burnetii* can be cultured efficiently in the yolk sac of chicken embryos but also on diverse specimens, such as human embryonic lung fibroblasts, blood, cerebrospinal fluid, bone marrow, milk, etc. (Baca and Paretsky, 1983). However, technically, culture of *C. burnetii* remains a difficult process and sensitivity of this diagnostic method is low. Recently, *C. burnetii* can be grown outside a host cell in a cell-free laboratory medium (Omsland et al., 2009). This finding is revolutionary and will permit further studies on *C. burnetii*. Another

practical limitation to bacterial isolation is that it requires a Biosafety Laboratory 3 because of its high infectivity. As a result, culture is rarely performed, especially in veterinary medicine (Fournier et al., 1998).

Polymerase Chain Reaction (PCR)

PCR offers substantial benefits for the identification of *C. burnetii* compared to other laboratory techniques, especially in the early clinical stages of the illness (Fournier and Raoult, 2003; Frangoulidis et al., 2009). It has been successful in detecting *C. burnetii* DNA in various samples, including cell cultures, biopsy samples, blood, arthropods and serum samples (Fenollar et al., 2004; Ughetto et al., 2009). Its sensitivity and specificity are high. However, the usefulness of conventional PCR is limited by its inability to quantify the bacteria present. The development of Real Time Quantitative PCR (RTq PCR) not only renders PCR a rapid diagnostic tool but also provides quantifiable information. RTq PCR can be automated and thus can be used in large scale studies. The qualities of PCR make it very useful for early diagnosis of infection during the period when antibodies are not yet present (Fournier and Raoult, 2003). Several primers are available for diagnosis (Stein and Raoult, 1992; Willems et al., 1994; Frangoulidis et al., 2009; Schneeberger et al., 2009). A primer originating from the frequently repeated DNA sequence IS1111 (7 to 120 copies per genome) is commonly used and has proven to allow very sensitive testing (Frangoulidis et al., 2009; Schneeberger et al., 2009). In a study by Schneeberger et al. (2009), *C. burnetii* DNA was detected in 98% of seronegative acute Q fever patients and in 90% of anti-phase 2 IgM seropositive patients. PCR became progressively negative as the serological response developed (Schneeberger et al., 2009). Hou et al. (2011) noticed a significant association between the absence of IgM antibodies and a positive PCR result. Negative serology was most frequent in Q fever cases where sampling had been performed within the two weeks of illness. In such cases, the authors recommend routine PCR testing as well as serology. When

PCR was included in the diagnostic procedure primarily based on serology, a marked increase of sensitivity was observed (78% versus 29% when serology was used as unique diagnostic method) (Hou et al., 2011). PCR and RTq PCR are considered methods of choice for DNA detection in diverse samples (Beaudeau et al., 2006). However, they are currently only available in a limited number of laboratories (Fournier and Raoult, 2003). Furthermore, PCR and RTq PCR are incapable of distinguishing bacterial DNA from live and/or dead bacteria (Kramer et al., 2009). The advantage is that sampled tissues can be frozen, put into formalin or fixed with paraffin (Fournier and Raoult, 2003). The disadvantage is that interpretation of positive results in fresh samples can be difficult (Tatsumi et al., 2006; Watanabe and Takahashi, 2008). PCR kits are gradually becoming available (Arricau-Bouvery and Rodokalis, 2005).

8.2. Indirect diagnosis

Indirect diagnostic methods identify specific humoral or cellular immunity in response to *C. burnetii* infection. The diagnostic methods available in human and veterinary medicine differ.

Complement Fixation Test (CFT)

In veterinary medicine, CFT was the method of reference for serological diagnosis according to OIE (Herr et al., 1985; Peter et al., 1985; AFSSA, 2004). CFT usually utilizes phase 2 antigens only (Krt, 2003; AFSSA, 2004). It is capable of detecting approximately 65% of infected subjects during the second week after initial clinical signs and 90% during the fourth week (OIE, 2005). CFT is more laborious, less specific and less sensitive than indirect immunofluorescence assay (IFA) (Fournier et al., 1996) or ELISA (described hereafter) (Fournier et al., 1998). A study by Rousset et al. (2007b) on goats originating from different herds reported that CFT results obtained on sera of aborting goats and of non-aborting goats were not significantly different and confirmed the lack of sensitivity of CFT compared to

ELISA. CFT, on the contrary to ELISA, is incapable of detecting all IgG subclasses. In ruminants, only IgG1 fix the complement and can thus be detected by CFT. Moreover, IgG2, IgM and anti-complement substances potentially present in sera are capable of interfering with fixation of IgG1 to the complement lowering the titer of IgG1 detected by CFT. Rousset et al. (2007b) advised not to use CFT for serological animal screening because of its low sensitivity.

Enzyme-Linked Immunosorbent Assay (ELISA)

Another method of diagnosis for human and animal cases is ELISA. This method is more sensitive, easier to perform and to standardize than CFT (Fournier et al., 1998). Moreover, a strong association was reported between strongly positive ELISA results and the occurrence of abortion in goats (Rousset et al., 2007b). As mentioned above, the higher sensitivity of ELISA compared to CFT could be due to the detection of the IgG_2 subclass of antibodies, which are incapable of binding to the guinea pig complement (Krt, 2003). Commercially available human ELISA kits are frequently coated by phase 1 and phase 2 antigens. The antigens present are of two possible origins: antigens of the American Nine Mile strain of *C. burnetii* isolated from an endogenous tick, or antigens of a strain originating from infected European domestic ruminants. ELISA kits coated by the latter antigens are more sensitive and are advised for serological diagnosis (ACERSA, 2006). In France, the ELISAs available for veterinary diagnostic purposes do not differentiate anti-phase 1 and anti-phase 2 antibodies but detects total antibodies (AFSSA, 2004). ELISA tests can be automated which facilitates large scale studies (Rousset et al., 2007b).

Immunofluorescence assay (IFA)

In human medicine, IFA is commonly considered the reference diagnostic test and is the most frequently used worldwide (AFSSA, 2004). It is accurate, highly sensitive and specific

(Fournier et al., 1996). The study by Rousset et al. (2007b) on goats demonstrated an overall good agreement between IFA and ELISA results. The study also reported that IFA results obtained on sera of aborting goats and of non-aborting goats were significantly different and were associated with occurrence of abortion (Rousset et al., 2007b). Moreover, IFA is capable of detecting the two antigenic variants of *C. burnetii* (phase I and phase II). As mentioned previously, in acute infections the level of anti-phase II antibodies is higher than the level of anti-phase I antibodies. In chronic infections the reverse is observed (Fournier et al., 1998). As anti-phase II antibodies are present in all stages of infection, screening for epidemiological purposes is based on the detection of anti-phase II antibodies (Rousset et al., 2007b). Cross-reactions with *Legionella micdadei* and *Bartonella* have been reported but do not cause any problem for the interpretation of quantitative results (Musso and Raoult, 1997; La Scola and Raoult, 1996). In human medicine, IFA distinguish IgG and IgM. Titers of anti-phase II IgG superior or equal to 200 and of anti-phase II IgM superior or equal to 50 correspond to an acute infection with a predictive value of 100%. An isolated high titer of IgM (≥ 50) can correspond to the beginning of an acute infection as long as the possibility of a false positive can be rejected. A chronic infection is characterized by a high titer in anti-phase I IgG (≥800) with a predictive value of 98% for a titer of 800 and a predictive value of 100% for a titer of 1600 (Dupont et al., 1994). The preferential use of IFA instead of CFT in veterinary medicine would be advantageous for diagnosis and control of Q fever at animal level (AFSSA, 2004).

Skin test

A skin test method was proposed to investigate the cellular response and to improve detection of infected animals at herd level (Guatteo et al., 2008b). The skin test consists in an intradermal injection of extremely diluted inactivated vaccine (Coxevac®, *CEVA-Santé animale*, Libourne, France). The diluted vaccine induces an antigenic reaction. If the animal

has previously been infected by Q fever a nodule of variable size will appear at the site of injection. This test could easily be applied by rural practitioners.

Negative aspects of indirect diagnostic methods

The dependence of CFT, IFA and ELISA on the presence of antibodies limits their diagnostic value. Indeed, specific antibodies are often absent during the first 2 to 3 weeks of illness, making early diagnosis by serology difficult, if not impossible (Kuroiwa et al., 2007). In Q fever, phase II antibodies can be detected within two weeks of infection in most cases and within three weeks 90% are seropositive. AFSSA (2004) reports that a definitive diagnosis of human cases by IFA can only be confirmed one month and a half after the initial clinical signs. Cellular immunity also necessitates time before becoming detectable but the use of skin testing at herd level prevents it being a problem as different stages of infection are present simultaneously in the herd. None of these tests are capable of confirming an etiological diagnosis at an individual level. Moreover, in acute infections, rheumatic factor, anti-mitochondrial antibodies, anti-nuclear antibodies, anti-smooth muscle antibodies, anti-cardiolipin and lupus-anticoagulant and other autoantibodies often present a marked increase in their plasmatic levels and can interfere with diagnostic assays (Vardi et al., 2011). A high prevalence of IgM against Epstein-Barr virus, cytomegalovirus, parvcovirus, *Bordetella pertussis* and *Mycoplasma pneumonia* has also been detected. Vardi et al. (2011) concluded that diagnosis should not rely on a unique diagnostic approach. The global clinical and epidemiological context must be taken into account as well as the limitations of diagnostic assays. Bacteriological analyses are necessary to confirm or infirm any suspicion of Q fever (Rousset et al., 2007b).

8.3. Diagnosis by histopathology

In acute cases of hepatitis, the presence of doughnut granulomas can be visualized in histopathology hepatic specimens but there are not pathognomonic of Q fever. However, in chronic infections, granulomas are less organized but bacteria can be detected in larges vacuoles (Fournier et al., 1998). As mentioned above, in bovids, placentitis was found to be significantly associated with *C. burnetii* infection (Bildfell et al., 2000). On microscopic examination, an increased number of mononuclear cells (macrophages, lymphocytes, plasma cells) can be identified in the chorionic stroma. Increased stromal collagen is also frequently observed. Furthermore, placental necrosis is significantly associated with the presence of *C. burnetii*. Chorionic epithelial cells and villus tips are the most frequently affected. Infected trophoblasts are distended and contain basophilic granular to foamy material. The cytoplasms of infected cells appear bright red with the Macc stain and their nuclei are commonly eccentric. A modified Koster's modified acid-fast (MAF) staining of fresh placenta smears is considered a good screening test but confirmation by immunohistochemical techniques remains necessary (Bildfell et al., 2000). In endocarditis lesions, *C. burnetii* are visible as a voluminous intra-cytoplasmic mass within infected mononuclear cells (Brouqui et al., 1994).

9. CONTROL METHODS AND VACCINATION

In the case of a Q fever outbreak, sanitary and prophylactic measures should be applied at herd and human level, in order to limit disease transmission. Human and animal infections must be diagnosed early and treated immediately to prevent the development of chronic infections and secondary complications. Q fever being a zoonosis, prophylaxis at herd level is fundamental.

Control methods

In the Netherlands, spread of manure from infected herds is forbidden for at least 90 days after suspicion of infection (Schimmer et al., 2008). The effectiveness of this measure must be evaluated and modified if necessary. This measure, or its modified equivalent, should be applied worldwide if its effectiveness is proven. Transport of animals in and from infected areas are strictly controlled and restricted to days without wind.

In France, when Q fever has been diagnosed in a herd on a cheese producing farm, milk of the aborted females must be discarded. Indeed, sale, transformation and treatment of this milk are strictly forbidden (AFSSA, 2007). Milk of the remainder of the flock can be used for transformation, unless it is highly suspected that dairy products originating from these animals are dangerous for human consumption. In the latter case, the milk must be pasteurized at 72°C during 15 minutes or by an equivalent thermal treatment (Cerf and Condron, 2006; AFSSA, 2007). If Q fever is diagnosed on a farm producing raw milk for direct human consumption, sale of milk is forbidden during one year after the initial diagnosis of Q fever in an animal (AFSSA, 2007).

In the United Kingdom, Health Protection Agency guidelines suggest the use of 2% formaldehyde, 1% Lysol, 5% hydrogen peroxide, 70% ethanol, or 5% chloroform for decontamination of surfaces, and spills of contaminated material should be dealt with immediately using hypochlorite, 5% peroxide or phenol-based solutions (Health Protection Agency, 2010 at www.hpa.org.uk/deliberate_accidental_releases/biological). However, the guidelines note than decontaminating a large surface area is impossible. Moreover, Scott and Williams (1990) found that formaldehyde vapour was ineffective in the absence of a high relative humidity. High risk material (contaminated bedding, placenta, aborted fetuses) should be buried with lime or incinerated. Treatment of manure with lime or calcium cyanide is also recommended before spreading and spreading must be performed on a calm day.

At human level, prevention of exposure to animals or wearing gloves and masks during manipulation of animals or their litter is advised (AFSSA, 2004; Whitney et al., 2009). Post exposure prophylaxis guidelines for the general population have been established in the USA (United States Army Medical Research Institute for Infectious Diseases, 2004; Moodie et al., 2008). Doxycycline at a dose of 100 mg a day or 500 mg of tetracycline twice daily started 8-12 days post exposure is advised. No recommendation is available for pregnant women although cotrimoxazole has been suggested (United States Army Medical Research Institute for Infectious Diseases 2004; Moodie et al., 2008).

Pasteurization of all milk products should be performed if ingestion is proven an effective route of infection and after determination of the minimal infecting dose of bacteria. Awaiting further scientific research, caution is recommended especially for individuals at high risk of chronic infection.

Currently, antibiotic treatment of animals does not stop shedding (Astobiza et al., 2010).

Vaccination

Rodokalis et al. (2007) suggested a follow up of bovine and caprine herds by BTM analysis. In herds presenting a PCR-positive BTM result, pools of 10 individual milk samples should be tested by PCR to identify the shedding animals. If shedders are not very numerous, they can be eliminated, and the other animals should be vaccinated. Rodokalis et al. (2007) also suggested vaccination of herds in the proximity of infected herds or flocks.

In animals, vaccines considered the most effective are composed of inactivated whole phase 1 bacteria (OIE, 2005). Indeed, in goats, the inactivated phase I vaccine (Coxevac®, CEVA-Santé animale, Libourne, France) protects efficiently against abortion and has been shown to prevent bacterial shedding in vaginal mucus, feces and particularly in milk. However, vaccination proved more effective in nulliparous animals than in parous animals.

Furthermore, vaccination didn't clear infection in previously infected goats (Arricau-Bouvery et al., 2005; Rousset et al., 2009b; Hogerwerf et al., 2011).

The study by Guatteo et al. (2008) on dairy cattle reported that the probability of becoming a shedder for vaccinated naïve non-pregnant bovids was 5-fold inferior to the probability for naïve bovids receiving a placebo. However, vaccination had no effect on the bacterial load shed. Vaccination of previously infected animals and of naïve animals during pregnancy proved ineffective. Guatteo et al. (2008) hypothesized that the immunodepression induced by pregnancy was responsible for the lack of effective immune response after vaccination. This explanation seems plausible but a second study is necessary to reach confirmation of this hypothesis. No adverse effect was observed at the site of injection in this study. One abortion was reported in a vaccinated infected cow but no further investigations were performed on this animal except for a PCR on vaginal mucus at the time of abortion that gave a negative result.

The major problem associated to vaccination with inactivated phase 1 vaccine is the impossibility of distinguishing vaccinated and naturally infected animals. At herd level, the effectiveness of a vaccination program could be evaluated by monitoring bacterial shedding in BTM. Currently, prophylaxis includes vaccination with the non-fully licensed inactivated phase I vaccine, Coxevac® (CEVA-Santé animale, Libourne, France), when a focus of Q fever is declared. This vaccine contains formalin-inactivated *C. burnetii* strain RSA 493/Nine Mile phase I (CEVA, 2010 at www.ceva.com/en/Products/Cattle/Vaccines). A recent study by New vaccines, such as recombinant vaccines, have been developed (Zhang and Samuel, 2003). Several of these vaccines have proven to be antigenic but non protective. Others still require an investigation of their effectiveness in field conditions (Waag, 2007). An inactivated phase II vaccine, Chlamyvax FQ® has also been tested on animals but has failed to be effective (Arricau-Bouvery et al., 2005). In France, Chlamyvax FQ® was used in 2004-2005.

Vaccination of humans against Q fever could be effective in certain areas. Several different vaccines have been developed since the discovery of *C. burnetii*. In the Soviet Union in the 1960s, a live attenuated strain M-44 obtained after repeated passage through guinea pigs and mice was used extensively for vaccination. Because of the long-term persistence of the attenuated bacteria in animals and vaccinated human, this vaccine was never used in the West (Marmion, 1967). The Americans developed a chloroform-methanol residue vaccine based on the phase I Henzerling strain of *C. burnetii* (Fries et al., 1993). Despite being effective in protecting animals against aerosol challenges, humans were found to develop severe reactions to vaccination (Fries et al., 1993). A trichloroacetic acid extract of phase I Nine Mile strain which comprised proteins and LPS was also used for vaccination. However, Kazar et al. (1982) reported severe reactions in response to vaccination in humans. Vaccinating with phase I Nine Mile extracts treated with chloroform-methanol prevented severe reactions but the loss of protective immunogenicity rendered these vaccines ineffective (Williams and Cantrell 1982; Brooks et al., 1986; Kazar et al., 1987; Schmeer et al., 1987). Currently, a formalin-killed whole cell vaccine prepared from phase I Henzerling strain of *C. burnetii* (Qvax®, *CSL Limited*, Parkville, Victoria, Australia) is licensed in Australia (OIE, 2005). This vaccine proved effective in several studies (Ackland et al., 1994) but vaccination is only possible for individuals that haven't previously been in contact with *C. burnetii* to prevent serious reactions (Bell et al., 1964; OIE, 2005; Rousset et al., 2007a). Screening is thus required before vaccination (Ascher et al., 1983; Marmion et al., 1990), making this preventive measure time-consuming and costly. Gefenaite et al. (2011) performed a meta-analysis of previous studies on the effectiveness of Qvax® vaccine. All the studies included in their analysis reported a protective effect of vaccination (average effectiveness after pooling raw data: 97%, CI: 94-99%). However several biases were present in the latter studies. Gefenaite et al. (2011) concluded that generalization of the results to the general population or to a specific risk group was not possible. The authors advised more blinded, randomized and

unbiased research on Qvax®'s effectiveness. Development of recombinant protein subunit vaccines has proven disappointing (Zhang et al., 1994; Zhang and Samuel, 2003; Li et al., 2005; Tyczka et al., 2005). Before initiating a vaccination program, epidemiological knowledge of the area is necessary. Indeed, in endemic regions vaccination is impossible for practical reasons (screening) and non effective as a preventive measure. However, vaccination could be beneficial for at risk individuals such as pregnant or immunocompromised individuals, farmers, veterinarians, abattoir workers, and research and reference laboratory personnel in contact with *C. burnetii* (OIE, 2005). Availability of vaccines causes a real problem in most countries and vaccination is currently not performed in Europe.

10. OUTBREAK IN THE NETHERLANDS

The Netherlands has been confronted with a Q fever outbreak since 2007. In 2009, infected goats were at the origin of 2,361 human cases of Q fever (including six deaths) diagnosed in the Netherlands (Enserink, 2010). Most cases were diagnosed in the Noorth Brabant Province. A recent increase in high intensity dairy goat farming has lead to development of very large densily populated farms: from 100,000 goats in 2000 to 230,000 goats on approximately 350 farms in 2009 (Roest et al., 2011b). Transport of animals, manure spreading and wind have been reported as influential factors for humans infections. In a study by Karagiannis et al. (2009) on the outbreak in 2007 in the Netherlands, living east of the area in which a positive goat farm, cattle or small ruminants were situated, smoking and contact with agriculture products were found to be associated with recent infection. Scientists suspected the emergence of a more virulent subtype of *C. burnetii* than the initial subtype of bacterium identified in 2007 in the Netherlands (Klassen et al., 2009; Enserink, 2010). Genomic studies reported that multiple genotypes were involved in the Q fever outbreak. However, an identical subtype was identified in a dairy goat herd suffering from multiple abortions and in several human patients (Klassen et al., 2009). This particular subtype could thus have a survival and

propagation advantage compared to other bacterial subtypes (Klassen et al. 2009; Enserink, 2010). Roest et al. (2011a) performed multiple locus variable tandem repeat analyses on a large number of Q fever positive samples originating from domestic ruminants and associated with the outbreak. Their study found that one genotype predominated on all dairy farms in the southern part of the Netherlands. Indeed, on 12 out of 14 dairy goat farms, this genotype was found in 91% of samples, varying from 33% to 100%. Nine other genotypes occurred once, each representing only 0.8% of all found genotypes on the farms. The predominant genotype was found on 11 farms in the southern Netherlands and on a farm in the eastern part of the country. This suggests a clonal spread of *C. burnetii* with this predominant genotype. However, another study by Huijmans et al. (2011) found five distinct genotypes (3 in humans and 4 in livestock) implicated in the outbreak and concluded that environmental factors such as animal and human density and climate, rather than one particularly virulent strain favored the Dutch outbreak and its spread. Control measures were put in place following the 2007 outbreak. Vaccination of goats with Coxevac® vaccine on voluntary grounds began in 2008. Vaccination was exclusively performed in areas of the human outbreak. In 2009, vaccination of all dairy sheep and goats on farms with more than 50 animals became mandatory and area of vaccination was enlarged (Noorth-Brabant province and parts of adjacent provinces). Moreover, BTM testing was performed. A farm was considered infected when 2 consecutive BTM samples were positive by PCR. However, Hermans et al. (2011) reported the presence of bacterial DNA originating from the Coxevax® vaccine in goat milk after inoculation. *C. burnetii* DNA was detectable until 9 days post vaccination in quantities estimated by PCR up to approximately 100 genome equivalents per ml. The quantity of DNA detected in their study was around detection level. After the booster, the duration of vaccine-derived DNA excretion was shorter, quantitatively lower and detectable in fewer animals than after the first inoculation. This finding induced the modification of the Dutch control strategy. After this study, a two week interval between vaccination and BTM testing was imposed (Hermans et

al., 2011). In addition to these control methods, movement and breeding were banned for dairy goats and sheep on infected farms (Hogerwerf et al., 2011). Culling of all pregnant goats and sheep on these farms was also in application until May 2010.

11. PERSPECTIVES FOR THE FUTURE

A better knowledge of Q fever would allow better control and prevention of the disease in the future.

A better understanding of the bacterium and of its pathogenesis (entry into the cell, "sporulation-like" phenomenon, metabolism, mechanism of resistance to acidic conditions, infectivity of the bacterium, etc.) is essential. The immune response of the infected host must also be investigated further. The risk factors for illness must be determined (infectious dose, potential danger linked to pets or not, etc.). Moreover, studies are required to define more precisely the incidence, clinical spectrum, treatment, morbidity, and mortality associated with Q fever in children. Further studies on risk factors could confirm or contradict results of previous studies, such as the studies by Whitney et al. (2009) and Mc Caughey et al. (2008). Oral transmission of Q fever remains a controversial subject to this day. Further research is essential for the establishment of guidelines in case of an epidemic. Importantly, a high standard of laboratory diagnostic methods should be available in all accredited laboratories and a systematic search for *C. burnetii* should be performed when clinical signs render the individual or animal suspected of Q fever. New guidelines for general practitioners and gynecologists should be established to increase the rapidity of diagnosis of clinical Q fever. Moreover, guidelines for prevention of infection of the medical staff and for prevention of transmission from one patient to another are necessary. The veterinary aspects of Q fever also necessitate further investigations. Indeed, the effect of antibiotic treatment on shedding of bacteria in ruminants has been insufficiently studied. Identification of an antibiotic capable of severely diminishing or completely stopping shedding would radically modify the

management of *Coxiella* infection at a herd level. It could also prove useful to prevent infection of pregnant women by inhibiting shedding of bacteria by domestic cats and dogs. The prevalence and potential clinical consequences of Q fever in pets and equids must also be investigated. Veterinarians must include Q fever in their differential diagnoses of clinical cases whenever it cannot systematically be rejected as a potential diagnosis. Inactive whole cell phase I vaccines have proved to be effective but present several defects. Further research in this field and the development of new recombinant vaccines would permit a better management of foci of infection at a herd level but also in human populations in the future. Animal vaccination and vaccination of individuals at high risk of exposure or/and of severe clinical disease would diminish significantly the zoonotic risk.

12. CONCLUSIONS

Q fever is an underestimated disease that remains poorly understood to this day. The lack of awareness of this disease leads to underdiagnosing and underreporting of Q fever cases. Q fever is ubiquitous and the potential hosts for the infectious bacteria are extremely numerous. Q fever infections can have a significant economical impact on animal reproduction, animal trade and on the production and commercialization of animal products. In small ruminant flocks, the consequences of Q fever can be disastrous. Its zoonotic potential renders Q fever even more important. Human infections can lead to death. Furthermore with the advances in human medicine, the number of immunosuppressed, premature, elderly and chronically ill individuals has greatly increased compared to several years ago. Thus the population predisposed to infection, and especially to chronic infection, has also greatly increased. Chronic infections can lead to severe consequences that necessitate intense medical treatment increasing the patients' suffering and public health costs. An early diagnosis and early initiation of treatment are essential to improve prognosis by preventing the development of a chronic infection or other potential complications associated to *coxiellosis*.

ACKNOWLEDGEMENT

Dr Sarah Porter was funded by a research grant of the University of Liege (Belgium).

REFERENCES

ACERSA 2006 : Diagnostic d'un élevage cliniquement atteint de fièvre Q. 1-11.

Acha, P.N., and B. Szyfres 2005: Fièvre Q, CIM-10 A78. In: Acha, P.N., and B. Szyfres (eds), Zoonoses et maladies transmissibles communes à l'homme et aux animaux. Volume III : Chlamydioises, rickettsioses et viroses. 3ème Edn, pp. 22-32, OIE, Paris, France.

Ackland, J.R., D.A Worswick, and B.P. Marmion 1994: Vaccine prophylaxis of Q fever – a follow-up study of the efficacy of Q-Vax (CSL) 1985-1990. Med. J. Aust. 160, 704-708.

AFSSA, 2004: Fièvre Q : Rapport sur l'évaluation des risques pour la santé publique et des outils de gestion des risques en élevage de ruminants. 1-88.

Akporiaye, E.T., and O.G. Baca 1983: Superoxide anion production and superoxide dismutase and catalase activities in Coxiella burnetii. J. Bacteriol. 154, 520-523.

Akporiaye, E.T., J.D. Rowatt, A.A. Aragon, and O.G. Baca 1983: Lysosomal response of a murine macrophage-like cell line persistently infected with Coxiella burnetii. Infect. Immun. 40, 1155-1162.

Alibek, K. 1999: The chilling true story of the largest covert biological weapons program in the world. Random House, New York, USA.

Amano, K.I., and J.C. Williams 1984: Chemical and immunological characterization of lipopolysaccharides from phase I and phase II Coxiella burnetii. J. Bacteriol. 160, 994-1002.

Anonymous 1950: Experimental Q fever in man. Br. Med. J. 1, 1000 (Editorial).

Anonymous 1977: Comment on Q fever transmitted by blood transfusion-United States. Can. Dis. Weekly Rep. 3, 210 (Editorial).

Arricau Bouvery, N., A. Souriau, P. Lechopier, and A. Rodolakis 2003: Experimental *Coxiella burnetii* infection in pregnant goats: excretion routes. Vet. Res. 34, 423-433.

Arricau-Bouvery, N., and A. Rodolakis 2005: Is Q fever an emerging or re-emerging zoonosis? Vet. Res. 36, 327-349.

Arricau-Bouvery, N., I. Souriau, C. Bodier, P. Dufour, E. Rousset, and A. Rodolakis 2005: Effect of vaccines with phase 1 and phase 2 *Coxiella burnetii* vaccines in pregnant goats. Vaccine 23, 4392-4402.

Arricau-Bouvery, N., Y. Hauck, A. Bejaoui, D. Frangoulidis, C.C. Bodier, A. Sourieau, H. Meyer, H. Neubauer, A. Rodolakis, and G. Vergnaud 2006: Molecular characterization of *Coxiella burnetii* isolates by infrequent restriction site-PCR and MLVA typing. BMC Microbiol. 6, 38.

Astobiza, I., J.F. Barandika, A. Hurtado, R.A. Juste, and A.L. Garcia-Perez 2010: Kinetics of Coxiella burentii excretion in a commercial dairy sheep flock after treatment with oxytetracycline. Vet. J. 184, 172-175.

Astobiza, I., M. Barral, F. Ruiz-Fons, J.F. Barandika, X. Gerrikagoita, A. Hurtado, and A.L. Garcia-Perez 2011: Molecular investigation of the occurence of Coxiella burnetii in wildlife and ticks in an endemic area. Vet. Microbiol. 147, 190-194.

Babudieri, B. 1950: Research on the behavior of *Coxiella burnetii* in relation to various physical and chemical agents. Rend. Ist. Sup. Sanit. 13, 739-743.

Babudieri, B. 1959: Q fever: a zoonosis. Adv. Vet. Sci. 5, 82-154.

Baca, O.G., and D. Paretsky 1983: Q fever and Coxiella burnetii: a model for host-parasite interactions. Microbial. Rev. 47, 127-149.

Barss, V.A., B.R. Benacerraf, and F.D.Jr. Frigoletto 1984: Second trimester oligohydramnios, a predictor of poor fetal outcome. Obstet. Gynecol. 64, 608-610.

Beaudeau, F., R. Guatteo, and H. Seegers 2006: Excretion of *Coxiella burnetii* by dairy cows: consequences for disease screening and control. Epidémiol. et santé anim. 49, 1-4.

Benenson, A.S., and W.D. Tigertt 1956: Studies on Q fever in man. Trans. Assoc. Am. Physicians 69, 98-104.

Benson, W.W., D. W. Brock, and J. Mather 1963: Serologic analysis of a penitentiary group using raw milk from a Q fever infected herd. Public Health Rep. 78, 707-710.

Bernit, E., J. Pouget, F. Janbon, H. Dutronc, P. Martinez, P. Brouqui, and D. Raoult 2002: Neurological involvement in acute Q fever : a report of 29 cases and review of the literature. Arch. Intern. Med. 162, 693-700.

Berri, M., A. Souriau, M. Crosby, D. Crochet, P. Lechopier, and A. Rodolakis 2001: Relationships between the shedding of *Coxiella burnetii*, clinical signs and serological responses of 34 sheep. Vet. Rec. 148, 502-505.

Berri, M., A. Souriau, M. Crosby, and A. Rodolakis 2002: Shedding of *Coxiella burnetii* in ewes in two pregnancies following an episode of *Coxiella* abortion in a sheep flock. Vet. Microbiol. 85, 55-60.

Berri, M., E. Rousset, J.L. Champion, N. Arricau-Bouvery, P. Russo, M. Pepin, and A. Rodolakis 2003 :Ovine manure used as a garden fertilizer as a suspected source of human Q fever. Vet. Rec. 153, 269-270.

Berri, M., E. Rousset, C. Hechard, J.L. Champion, P. Dufour, P. Russo, and A. Rodolakis 2005: Progression of Q fever and *Coxiella burnetii* shedding in milk after an outbreak of enzootic abortion in a goat herd. Vet. Rec. 156, 548-549.

Berri, M., E. Rousset, J.L. Champion, P. Russo, and A. Rodolakis 2007: Goats may experience reproductive failures and shed *Coxiella burnetii* at two successive parturitions after a Q fever infection. Res. Vet. Sci. 83, 47-52.

Bildfell, R.J., G.W. Thomson, D.M. Haines, B.J. McEwen, and N. Smart 2000: *Coxiella burnetii* infection is associated with placentitis in cases of bovine abortion. J. Vet. Diagn. Invest. 12, 419-425.

Blanc, G., and J. Bruneau 1956: Etude épidémio-écologique dans la forêt de Nefifik. 1-Présence chez le lapin de garenne et ses arthropodes piqueurs de virus pathogènes pour l'homme. Arch. Inst. Pasteur Maroc 5, 87-200.

Brennan, R., K. Russell, G. Zhang, and J. Samuel 2004: Both inducible nitric oxide synthases and NADPH oxidase contribute to the control of virulent phase I Coxiella burnetii infections. Infect. Immun. 72, 6666-6675.

Brooks, D.L., R.W. Ermel, C.E. Franti, R. Ruppanner, D.E. Behymer, J.C. Williams, and J.C. Stephenson 1986: Q fever vaccination of sheep: challenge of immunity in ewes. Am. J. Vet. Res. 47, 1235-1238.

Brouqui, P., H.T. Dupont, M. Drancourt, Y. Berland, J. Etienne, C. Leport, F. Goldstein, P. Massip, M. Micoud, A. Bertrand, D. Raoult 1993: Chronic Q fever. Ninety-two cases from France, including 27 cases without endocarditis. Arch. Intern. Med. 153, 642-648.

Brouqui, P., J.S. Dumler, and D. Raoult 1994: Immunohistologic demonstration of Coxiella burnetii in the valves of patients with Q fever endocarditis. Am. J. Med. 97, 451-458.

Buhariwalla, F., B. Cann, and T.J. Marrie 1996: A dog related outbreak of Q fever. Clin. Infect. Dis. 23, 753-755.

Bustin, S.A. 2000: Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. J. Mol. Endocrinol. 25, 169-193.

Carcopino, X., D. Raoult, F. Bretelle, L. Boubli, and A. Stein 2007: Managing Q fever during pregnancy: the benefits of long-term cotrimoxazole therapy. Clin. Infect. Dis. 45, 548-555.

Cerf, O., and R. Condron 2006: For debate: *Coxiella burnetii* and milk pasteurization: an early application of the precautionary principle? Epidemiol. Infect. 134, 946-951.

CEVA, 2010. Vaccines, http://www.ceva.com/en/Products/Cattle/Vaccines. Accessed on 24 February 2011.

Chen, C., C. Dow, P. Wang, J. Sidney, A. Read, A. Harmsen, J.E. Samuel, and B. Peters 2011: Identification of CD4+ T cell epitopes in C. burnetii antigens targeted by antibody responses. PloS ONE 6, 3, e17712.

Chmielewski, T., K. Sidi-Boumedine, V. Duquesne, E. Podsiadly, R. Thiery, and S. Tylewska-Wierzbanowska 2009: Molecular epidemiology of Q fever in Poland. Pol. J. Microbiol. 58, 9-13.

Cox, H.R. 1938: A filter-passing infectious agent isolated from ticks. III. Description of organisms and cultivation experiments. Public Health Rep. 53, 2270-2276.

Cox, H.R. 1940: *Rickettsia diaporica* and American Q fever. Am. J. Trop. Med. Hyg. 20, 463-465.

Czaplicki, G., J.-Y. Houtain, C. Mullender, C. Manteca, and C. Saegerman 2009: Le lait de tank, outil fiable pour le diagnostic de la fièvre Q dans le troupeau bovin laitier? Epidémiol. et santé anim. 56, 117-127.

Davis, G.E. 1939: *Rickettsia diaporica*: recovery of three strains from *Dermacentor andersoni* collected in southeastern Wyoming: their identity with Montana strain 1. Public Health Rep. 54, 2219-2225.

Davoli, R., and L.F. Signorini 1951: Antibodies against Rickettsia burneti in serum of animals at a slaughter house in Tuscany. VI. Ann. Sanita Pubblica 12, 67-73.

Delsing, C.E., and B.J. Kullberg 2008: Q fever in the Netherlands: a concise overview and implications of the largest ongoing outbreak. Neth. J. Med. 66, 365-367.

Denison, A.M., H.A. Thompson, and R.F. Massung 2007: IS1111 insertion sequences of *Coxiella burnetii*: characterization and use for repetitive element PCR-based differentiation of *Coxiella burnetii* isolates. BMC Microbiol. 7, 91.

Derrick, E.H. 1937: Q fever, a new fever entity: clinical features, diagnosis and laboratory investigations. Med. J. Aust. 2, 281.

Deutch, D.L., and E.T. Peterson 1950: Q fever transmission from one human being to others. J. Am. Med. Assoc. 143, 348-354.

Dilbeck, P.M., and T.F. McElwain 1994: Immunohistochemical detection of *Coxiella burnetii* in formalin-fixed placenta. J. Vet. Diagn. Invest. 6, 125-127.

Dorko, E., Z. Kalinova, and E. Pilipcinec 2008: Seroprevalence of *Coxiella burnetii* antibodies among students of the Faculty of medicine in Kosice (Slovakia). Folia Microbiol. 53, 563-568.

Dubuc-Forfait, C., E. Rousset, J.L. Champion, M. Marois, P. Dufour, E. Zerhaoui, R. Thièry, and P. Sabatier 2009: Démarche d'appréciation du risque d'excrétion de *Coxiella burnetii* dans les troupeaux caprins laitirs dans le sud-est de la France. Epidémiol. et santé anim 55, 117-136.

Dupont, H.T., X. Thirion, and D. Raoult 1994: Q fever serology: cutoff determination for microimmunofluorescence. Clin. Diagn. Lab. Immunol. 1, 189-196.

Enright, J.B., C.E. Franti, W.M. Longhurst, D.E. Behymer, M.E. Wright, and V.J. Dutson 1971: Coxiella burnetii in a wildlife-livestock environment: antibody response of ewes and lambs in an endemic Q fever area. Am. J. Epidemiol. 94, 62-71.

Enserink, M. 2010: Questions abound in Q-fever explosion in the Netherlands. Science 327, 266-267.

Evans, A.D. 1956: Q fever in South Wales. Monthly Bull. Minist. Hlth. Lab. Serv. 15, 215-219.

Fenollar, F., P.E. Fournier, M.P. Carrieri, G. Habib, T. Messana, and D. Raoult 2001: Risk factors and prevention of Q fever endocarditis. Clin. Infect. Dis. 33, 312-316.

Fenollar, F., P.E. Fournier, and D. Raoult 2004: Molecular detection of *Coxiella burnetii* in the sera of patients with Q fever endocarditis or vascular infection. J. Clin. Microbiol. 42, 4919-4924.

Fenollar, F., F. Thuny, B. Xeridat, H. Lepidi, and D. Raoult 2006: Endocarditis after acute Q fever in patients with previously undiagnosed valvulopathies. Clin. Infect. Dis. 42, 818-821.

Fiset, P. 1957: Phase variation of Rickettsia (Coxiella) burneti; study of the antibody response in guinea pigs and rabbits. Can. J. Microbiol. 3, 435-445.

Fournier, P.E., T.J. Marrie, and D. Raoult 1998: Diagnosis of Q fever. J. Clin. Microbiol. 36, 1823-1834.

Fournier, P.E., J. Etienne, J.R. Harle, G. Habib, and D. Raoult 2001: Myocarditis, a rare but severe manifestation of Q fever : report of 8 cases and review of literature. Clin. Infect. Dis. 32, 1440-14407.

Frangoulidis, D., A. Rodolakis, V. Heiser, O. Landt, W. Splettstoesser, and H. Meyer 2009: DNA microarray-chip based diagnosis of Q-fever (*Coxiella burnetii*). Clin Microbiol Infect. 15 Suppl 2, 165-166.

Frazier, M.E., L.P. Mallavia, J.E. Samuel, and O.G. Baca 1990: DNA probes for the identification of *Coxiella burnetii* strains. Ann. N.Y. Acad. Sci. 590, 445-458.

Fries, L.F., D.M. Waag, and J.C. Williams 1993: Safety and immunogenicity in humans volunteers of a chloroform-methanol residue vaccine for Q fever. Infect. Immun. 61, 1251-1258.

Garcia-Pérez, A.L., I. Astobiza, J.F. Barandika, R. Atxaerandio, A. Hurtado, and R.A. Juste 2009: Short communication: investigation of *Coxiella burnetii* occurrence in dairy sheep flocks by bulk-tank milk analysis and antibody level determination. J. Dairy Sci. 92, 1581-1584.

Gefenaite, G., J.M. Munster, R. van Houdt, and E. Hak 2011: Effectiveness of the Q fever vaccine: a meta-anlysis. Vaccine 29, 395-398.

Gerth, H.J., U. Leidig, and T. Riemenschneider 1982: Q-fieber Epidemie in einem Institut for Humanpathologie. Deut. Med. Wochenschr. 107, 1391-1395.

Gidding, H.F., C. Wallace, G.L. Lawrence, and P.B. McIntyre 2009: Australia's national Q fever vaccination program. Vaccine 27, 2037-2041.

Gikas, A., D.P. Kofteridis, A. Manios, J. Pediaditis, and Y. Tselentis 2001: Newer macrolides as empiric treatment of acute Q fever infection. Antimicrob. Agents Chemother. 45, 3644-3646.

Gillepsie, J.H., and J.A. Baker 1952: Experimental Q fever in cats. Am. J. Vet. Res. 13, 91-94.

Gimenez, D.F. 1964: Staining rickettsiae in yolk-sac cultures. Stain technol. 39, 135-140.

Giroud, P., and J. Jardin 1954: Infection latent et conservation de Rickettsia burnettii chez l'homme: le rôle du pou. Bull. Soc. Pathol. Exot. 45, 764-765.

Glazunova O., V. Roux, O. Freylikman, Z. Sekeyova, G. Fournous, J. Tyczka, N. Tokarevitch, E. Kovacava, T.J. Marrie, and D. Raoult 2005: *Coxiella burnetii* genotyping. Emerg. Infect. Dis. 11, 1211-1217.

Gonder, J.C., R.A. Kishimoto, M.D. Kastello, C.E. Pedersen, and E.W. Larson 1979: Cynomolgus monkey model for experimental Q fever infection. J. Infect. Dis. 139, 191-196.

Guatteo, R., F. Beaudeau, D. Ledoux, E. Drean, and H. Seegers 2007: Risk of false-negative results when delaying PCR from sampling for *Coxiella burnetii* detection in dairy cows. Revue Méd. Vét. 158 (12), 641-644.

Guatteo, R., H. Seegers, A. Joly, and F. Beaudeau 2008a: Prevention of *Coxiella burnetii* shedding in infected dairy herds using a phase I *C. burnetii* inactivated vaccine. Vaccine 26, 4320-4328.

Guatteo, R., A. Joly, A. Rodolakis, D. Cochonneau, P. Sarradin, H. Seegers, D. Remmy, and F. Beaudeau 2008b : Prévention de l'excrétion de *Coxiella burnetii* à l'aide d'un vaccin dit phase I (Coxevac® en troupeaux bovines laitiers infectés). Renc. Rech. Ruminants 15, 59-62.

Hackstadt, T., and J. C. Williams 1981: Biochemical stratagem for obligate parasitism of eukaryotic cells by Coxiella burnetii. Proc. Natl. Acad. Sci. USA 78, 3240-3244.

Harman, J.B. 1949: Q fever in Great Britain; clinical account of eight cases. Lancet 2, 1028-1030.

Hartzell, J.D., S.W. Peng, R.N. Wood-Morris, D.M. Sarmiento, J.F. Collen, P.M. Robben, and K.A. Moran 2007: Atypical Q fever in United States armed soldiers. Emerg. Infect. Dis. 13, 1247-1249.

Hartzell, J.D., R.N. Wood-Morris, L.J. Martinez, and R.F. Trotta 2008: Q fever: epidemiology, diagnosis, and treatment. Mayo Clin. Proc. 83, 574-579.

Hatchette, T., N. Campbell, H. Whitney, R. Hudson, and T.J. Marrie 2002: Seroprevalence of *Coxiella burnetii* in selected populations of domestic ruminants in Newfoundland. Can. Vet. J. 43, 363-364.

Hatchette, T., N. Campbell, R. Hudson, D. Raoult, and T.J. Marrie 2003: Natural history of Q fever in goats. Vector Borne Zoonotic Dis. 3, 11-15.

Health Protection Agency (HPA). Centre for Infections, 2010. Guidelines for action in the event of a deliberate release: Q fever. Version 1.7, http://www.hpa.org.uk/deliberate_accidental_releases/biological. Accessed on 24 February 2011.

Helbig, K.J., S.L. Heatley, R.J. Harris, C.G. Mullighan, P.G. Bardy, and B.P. Marmion 2003: Variation in immune response genes and chronic Q fever. Concepts: preliminary test with post-Q fever fatigue syndrome. Genes Immun. 4, 82-85.

Hendrix, L.R., J.E. Samuel, and L.P. Mallavia 1991: Differentiation of *Coxiella burnetii* isolates by analysis of restriction-endonuclease-digested DNA separated by SDS-PAGE. J. Gen. Microbiol. 137, 269-276.

Hermans, M.H.A., C.R.J.J. Huijsmans, J.J.A. Schellekens, P.H.M. Savelkoul, and P.C. Wever 2011: Coxiella burentii DNA in goat milk after vaccination with Coxevac®. Vaccine 29, 2653-2656.

Herr, S., H.F. Huchzermeyer, L.A. Te Brugge, C.C. Williamson, J.A. Roos, and G.J. Schiele 1985: The use of a single complement fixation test technique in bovine brucellosis, Johne's disease, dourine, equine piroplasmosis and Q fever serology. Onderstepoort J. Vet. Res. 52, 279-282.

Hickie, I., T. Davenport, D. Wakefield, U. Vollmer-Conna, B. Cameron, S.D. Vernon, W.C. Reeves, and A. Lloyd 2006: Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. BMJ 333, 575.

Higgins, D., and T.J. Marrie 1990: Seroepidemiology of Q fever among cats in New Brunswick and Prince Edward Island. Ann. N.Y. Acad. Sci. 590, 271-274.

Hill, J., and J.E. Samuel 2011: Coxiella burnetii acid phosphatise inhibits the release of reactive oxygen intermediates in polymorphonuclear leukocytes. Inf. Immunol. 79, 414-420.

Hirai, K., and H. To 1998: Advances in the understanding of *Coxiella burnetii* infection in Japan. J. Vet. Med. Sci. 60, 781-790.

Hogerwerf, L., R. van den Brom, H.I.J Roest, A. Bouma, P. Vellema, M. Pieterse, D. Dercksen, and M. Nielen 2011: Reduction of Coxiella burentii prevalence by vaccination of goats and sheep, the Netherlands. Emerg. Infect. 17, 379-386.

Hoover, T.A., D.W. Culp, M.H. Vodkin, and E.I. Shaw 2002: Chromosomal DNA deletions explain phenotypic characteristics of two antigenic variants, phase 2 and RSA 514 (Crazy), of *Coxiella burnetii* Nine Mile strain. Infect. Immun. 70, 6726-6733.

Hou, M.Y., M.N. Hung, P.S. Lin, Y.C. Wang, C.C. Lin, P.Y. Shu, W.Y. Shih, H.S. Wu, and L.J. Lin 2011: Use of a single-tube nested real-time PCR assay to facilitate the early diagnosis of acute Q fever. Jpn J. Infect. Dis. 64, 161-162.

Htwe, K.K., T. Yoshida, S. Hayashi, T. Miyake, K. Amano, C. Morita, T. Yamaguchi, H. Fukushi, and K. Hirai 1993: Prevalence of antibodies to *Coxiella burnetii* in Japan. J. Clin. Microbiol. 31, 722-723.

Huijsmans, C.J.J., J.J.A. Schellekens, P.C. Wever, R. Toman, P.H.M. Savelkoul, I. Janse, and M.H.A. Hermans 2011: Single-Nucleotide-Polymorphism genotyping of Coxiella burnetii during a Q fever outbreak in the Netherlands. Appl. Environ. Microbiol. 77, 2051-2057.

Inokuma, H., S. Yamamoto, and C. Morita 1998: Survey of tick-borne diseases in dogs infested with *Rhipicephalus sanguineus* at a kennel in Okayama Prefecture, Japan. J. Vet. Med. Sci. 60, 761-763.

Izzo, A.A., and B.P. Marmion 1993: Variation in interferon-gamma responses to *Coxiella burnetii* antigens with lymphocytes from vaccinated or naturally infected subjects. Clin. Exp. Immunol. 94, 507-515.

Kaplan, B., D. Rabinerson, S. Ben-Ari, A. Neri, and P. Merlob 1995: An isolated case of Q fever during pregnancy. Acta Obstet. Gynecol. Scand. 74, 848-849.

Karagiannis, I., B. Schimmer, A. van Lier, A. Timen, P. Schneeberger, B. van Rotterdam, A. De Bruin, C. Wijkmans, A. Rietveld, and Y. van Duynhoven 2009: Investigation of a Q fever outbreak in a rural area of The Netherlands. Epidemiol. Infect. 137, 1283-1294.

Karakousis, P.C., M. Trucksis, and J.S. Dumler 2006: Chronic Q fever in the United States. J. Clin. Microbiol. 44, 2283-2287.

Kazar, J., R. Brezina, A. Palanova, B. Tvrda, and S. Schramek 1982: Immunogenicity and reactogenicity of a Q fever chemovaccine in persons professionally exposed to Q fever in Czechoslovakia. Bull. World Health Organ. 60, 389-394.

Kazar, J., S. Schramek, V. Lisak, and R. Brezina 1987: Antigenicity of a chloroform-methanol-treated Coxiella burentii preparation. Acta Virol. 31, 158-167.

Kazar, J., M. Lesny, P. Propper, D. Valkov, and R. Brezina 1993: Comparaison of virulence for guinea pigs and mice of different *Coxiella burnetii* phase 1 strains. Acta Virol. 37, 437-448.

Khavkin, T.N. 1977: Pathologo-anatomic and experimental study of the morphology of Q fever. Arkh. Patol. 39, 75-84.

Kim, S.G., E.H. Kim, C.J. Lafferty, and E. Dubovi 2005: *Coxiella burnetii* in bulk tank milk samples, United States. Emerg. Infect. Dis. 11, 619-621.

Klaassen, C.H.W., M.H. Nabuurs-Fransen, J.J.H.C. Tilburg, M.A.W.M. Hamans, and A.M. Horrevorts 2009: Multigenotype Q fever outbreak, The Netherlands. Emerg. Infect. Dis. 15, 613-614.

Komiya, T., K. Sadamasu, M.I. Kang, S. Tsuboshima, H. Fukushi, and K. Hirai 2003: Seroprevalence of *Coxiella burnetii* infections among cats in different living environments. J. Vet. Med. Sci. 65, 1047-1048.

Kosatsky, T. 1984: Household outbreak of Q-fever pneumonia related to a parturient cat. Lancet 2, 1447-1449.

Koster, F.T., J.C. Williams, and J.S. Goodwin 1985a: Cellular immunity in Q fever: specific lymphocyte unresponsiveness in Q fever endocarditis. J. Infect. Dis. 152, 1283-1289.

Koster, F.T., J.C. Williams, and J.S. Goodwin 1985b: Cellular immunity in Q fever – modulation of responsiveness by a suppressor T cell monocyte circuit. J. Immunol. 135, 1067-1072.

Kramer, M., N. Obermajer, B.B. Matijasic, I. Rogelj, and V. Kmetec 2009: Quantification of live and dead probiotic bacteria in lyophilized products by real-time PCR and by flow cytometry. Appl. Microbiol. Biotechnol. 84, 1137-1147.

Krt, B. 2003: The influence of *Coxiella burnetti* phase I and phase II antigens on the serological diagnosis of Q fever in cattle. Slov. Vet. Research 40, 203-207.

Krumbiegel, E.R., and H.J. Wisniewski 1970: Q fever in the Milwaukee area. II. Consumption of infected raw milk by human volunteers. Arch. Environ. Health 21, 63-65.

Kruszewska, D., K. Lembowicz, and S. Tylewska-Wierzbanowska 1996: Possible sexual transmission of Q fever among humans. Clin. Infect. Dis. 22, 1087-1088.

Kuroiwa, Y., R. Oyanagi, S. Fuse, T. Mori, H. Ueno, and H. Tsutsumi 2007: Persistent hepatitis and repeated wheezing in an infant. Q fever. Pediatr. Infect. Dis. J. 26, 763, 768-769.

Lang, G.H. 1990: Coxiellosis in animals. In: Marrie, T. J. (ed.), Q fever, vol 1. The Disease, pp. 23-48. CRC Press, Boca Raton, Florida, USA.

Langley, J.M., T.J. Marrie, J.C. Leblanc, A. Almudevar, L. Resch, and D. Raoult 2003: *Coxiella burnetii* seropositivity in parturient women is associated with adverse pregnancy outcomes. Am. J. Obstet. Gynecol. 189, 228-232.

La Scola, B., and D. Raoult 1996: Serological cross-reactions between Bartonella quintana, Bartonella henselae, and Coxiella burnetii. J. Clin. Microbiol. 34, 2270-2274.

La Scola, B., H. Lepidi, and D. Raoult 1997: Pathologic changes during acute Q fever: influence of route of infection and inoculum size in infected guinea pigs. Infect. Immun. 65, 2443-2447.

La Scola, B., H. Lepidi, M. Maurin, and D. Raoult 1998: A guinea pig model for Q fever endocarditis. J. Infect. Dis. 178, 278-281.

Leone, M., A. Honstettre, H. Lepidi, C. Capo, F. Bayard, D. Raoult, and J.L. Mege 2004: Effect of sex on *Coxiella burnetii* infection: protective role of 17beta-estradiol. J. Infect. Dis. 189, 339-345.

Lepidi, H., F. Gouriet, and D. Raoult 2009: Immunohistochemical detection of *Coxiella burnetii* in chronic Q fever hepatitis. Clin. Microbiol. Inf. 15 Suppl 2, 169-170.

Li, Q., D. Niu, B. Wen, M. Chen, L. Qiu, and J. Zhang 2005: Protective imunity against Q fever induced with a recombinant P1 antigen fused with HspB of Coxiella burnetii. Ann. N.Y. Acad. Sci. 1063, 130-142.

Ludlam, H. T.G. Wreghitt, S. Thornton, B.J. Thomson, N.J.Bishop, S. Coomber, and J. Cunniffe1997: Q fever in pregnancy. J. Infect. 34, 75-78.

Mallavia, L.P. 1991: Genetic of rickettsiae. Eur. J. Epidemiol. 7, 213-221.

Maltezou, H.C., and D. Raoult 2002: Q fever in children. Lancet Infect. Dis. 2, 689-691.

Mantovani, A., and P. Benazzi 1953: The isolation of *Coxiella burnetii* from *Rhipicephalus sanguineus* on naturally infected dogs. J. Am. Vet. Med. Asso. 122, 117-120.

Marmion, B. P., and M.G.P. Stoker 1950: Q fever in Great Britain: epidemiology of an outbreak. Lancet 2, 611-616.

Marmion, B. P. 1954: Q fever. II. Natural history and epidemiology of Q fever in man. Trans. R. Soc. Trop. Med. Hyg. 48, 197-203.

Marmion, B. P., and M.G. Stoker 1958: The epidemiology of Q fever in Great Britain; an analysis of the findings and some conclusions. BMJ 2, 809-816.

Marmion, B.P. 1967: Development of Q-fever vaccines 1937 to 1967. Med. J. Aust. 2, 1074-1078.

Marmion, B.P., R.A. Ormsbee, M. Kyrkou, J. Wright, D.A. Worswick, A.A. Izzo, A. Esterman, B. Feery, and R.A. Shapiro 1990: Vaccine prophylaxis of abattoir-associated Q fever: 8 years' experience in Australian abattoirs. Epidemiol. Infect. 104, 275-287.

Marmion, B.P., O. Sukocheva, P.A. Storm, M. Lockhart, M. Turra, T. Kok, J. Ayres, H. Routledge, and S. Graves, 2009: Q fever: persistence of antigenic non-viable cell residues of Coxiella burnetii in the host – implications for post Q fever infection fatigue syndrome and other chronic sequelae. QJM 102, 673-684.

Marrie, T.J., J. Van Buren, J. Fraser, E.V. Haldane, R.S. Faulkener, J.C. Williams, and C. Kwan 1985: Seroepidemiology of Q fever among domestic animals in Nova Scotia. Am. J. Public Health 75, 763-766.

Marrie, T.J., W.F. Schlech, J.C. Williams, and L. Yates 1986: Q fever pneumonia associated with exposure to wild rabbits. Lancet 2, 427-429.

Marrie, T.J. 1988a: Q fever, 1979-1987 –Nova Scotia. Can. Dis. Weekly Rep. 14, 69-70.

Marrie, T.J., A. MacDonald, H. Durant, L. Yates, and L. McCormick 1988b: An outbreak of Q fever probably due to contact with a parturient cat. Chest 93, 98-103

Marrie, T.J., H. Durant, J.C. Williams, E. Mintz, and D.M. Waag 1988c: Exposure to parturient cats is a risk factor for acquisition of Q fever in Maritime Canada. J. Infect. Dis. 158, 101-108.

Marrie, T.J., 1990: Acute Q Fever. In: Marrie, T.J. (ed), Q Fever. The disease, pp. 125-160. CRC Press, Boca Raton, Florida, USA.

Marrie, T.J., A. Stein, D. Janigan, and D. Raoult 1996: Route of infection determines the clinical manifestations of acute Q fever. J. Infect. Dis. 173, 484-487.

Marrie, T.J., and D. Raoult 1997: Q fever-a review and issues for the next century. Int. J. Antimicrob. Agents 8, 145-161.

Masuzawa, T., K. Sawaki, H. Nagaoka, M. Akiyama, K. Hirai, and Y. Yanagihara 1997a: Determination of rickettsiae isolated in Japan as *Coxiella burnetii* by 16S rRNA sequencing. Int. J. Syst. Bacteriol. 47, 883-884.

Masuzawa, T., K. Sawaki, H. Nagaoka, M. Akiyama, K. Hirai, and Y. Yanagihara 1997b: Relationship between pathogenicity of *Coxiella burnetii* isolates and gene sequences of the macrophage infectivity potentiator (Cmip) and sensor-like protein (qrsA). FEMS Microbiol. Lett. 154, 201-205.

Mattewman, L., P. Kelly, D. Hayter, S. Downie, K. Wray, N. Bryson, A. Rycroft, and D. Raoult 1997: Exposure of cats in southern Africa to Coxiella burnetii, the agent of Q fever. Eur. J. Epidemiol. 13, 477-479.

Maurin, M., A.M. Benoliel, P. Bongrande, and D. Raoult 1992: Phagolysosomal alkalinization and the bactericidal effect of antibiotics: the Coxiella burnetii paradigm. J. Infect. Dis. 166, 1097-1102.

Maurin, M., and D. Raoult 1999: Q fever. Clin. Microbiol. Rev. 12, 518-553.

Mc Caughey, C., J. Mc Kenna, C. Mc Kenna, P.V. Coyle, H.J. O'Neill, and D.E. Wyatt 2008: Human seroprevalence to *Coxiella burnetii* (Q fever) in Northern Ireland. Zoonoses Public Health 55, 189-194.

McCaul, T.F., and J.C. Williams 1981: Developmental cycle of Coxiella burnetii: structure and morphogenesis of vegetative and sporogenic differentiations. J. Bacteriol. 147, 1063-1076.

McCaul, T.F.T. Hackstadt, and J.C. Williams, 1981: Ultrastructural and biological aspects of Coxiella burnetii under physical disruptions. In: W. Burdorfer and R. L. Anacker (eds.), Rickettsiae and rickettsial diseases. pp. 267-280, Academic Press, Inc, New York, N.Y., USA.

McCaul, T.F., J.C. Williams, and H.A. Thompson 1991: Electron microscopy of Coxiella burnetii in tissue culture. Induction of cell types as products of developmental cycle. Acta Virol. 35, 545-556.

McQuiston, J.H., and J.E. Childs 2002: Q fever in humans and animals in the United States. Vector Borne Zoonotic Dis. 2, 179-191.

Miceli, M.H., A.K. Veryser, A.D. Anderson, D. Hofinger, S.A. Lee, and C. Tancik 2010: A case of person-to-person transmission of Q fever from an active duty serviceman to his spouse. Vector Borne Zoonotic Dis. 10, 539-541.

Milazzo, A., R. Hall, P.A. Strom, R.J. Harris, W. Winslow, and B.P. Marmion 2001: Sexually transmitted Q fever. Clin. Infect. Dis. 33, 399-402.

Million, M., H. Lepidi, and D. Raoult 2009: Q fever: current diagnosis and treatment options. Med. Mal. Infect. 39, 82-94.

Mitscherlich, E., and E.H. Marth, 1984: Bacteria and Rickettsiae. Important in Human and Animal Health. In: Mitscherlich, and E. H. Marth, Microbial Survival in the Environment, pp. 148-156. Springer-Verlag, New York, USA.

Mo, Y.Y., N.P. Cianciotto, and L.P. Mallavia 1995: Molecular cloning of a Coxiella burnetii gene encoding a macrophage infectivity potentiator (Mip) analog. Microbiology 141, 2861-2871.

Montejo Baranda, M., J. Corral Carranceja, and C. Aguirre Errasti 1985: Q fever in the Basque Country: 1981-1984. Rev. Infect. Dis. 7, 700-701.

Moodie, C.E., H.A. Thompson, M.I. Meltzer, and D.L. Swerdlow 2008: Prophylaxis after exposure to *Coxiella burnetii*. Emerg. Infect. Dis. 14, 1558-1566.

Moore, T.R., J. Longo, G.R. Leopold, G. Casola, and B.B. Gosink 1989: The reliability and predictive value of an amniotic fluid scoring system in severe second-trimester oligohydramnios. Obstet. Gynecol. 73, 739-742.

Moos, A., and T. Hackstadt 1987: Comparative virulence of intra- and interstrain lipopolysaccharide variants of Coxiella burnetii in the guinea pig model. Infect. Immun. 55, 1144-1150.

Morisawa, Y., H. Wakiguchi, T. Takechi, T. Kurashige, and H. Nagaoka 2001: Intractable Q fever treated with recombinant gamma interferon. Pediatr. Infect. Dis. J. 20, 546-547.

Musso, D., and D. Raoult 1997: Serological cross-reaction between Coxiella burnetii and Legionella micdadei. Clin. Diagn. Lab. Immunol. 4, 208-212.

Nagaoka, H., M. Sugieda, M. Akiyama, T. Nishina, S. Akahane, and K. Fujiwara 1998: Isolation of *Coxiella burnetii* from the vagina of feline clients at veterinary clinics. J. Vet. Med. Sci. 60, 251-252.

NHS 2006: Complement Fixation Tests. VSOP18. Standards Unit, Evaluations and Standards Laboratory, Centre for infections 2, 1-24. Available at: http://www.hpa-standardmethods.org.uk (accessed on 17 July 09).

Novak, M., R. Brezina, and J. Kazar 1992: Immunoblot analysis of antibody response in mice infected with Coxiella burentii phase I. Acta Virol. 36, 39-44.

Oda, H., and K. Yoshiie 1989: Isolation of a *Coxiella burnetii* strain that has low virulence for mice from a patient with acute Q fever. Microbiol. Immunol. 33, 969-973.

Okimoto, N., N. Asaoka, K. Osaki, T. Kurihara, K. Yamato, T. Sunagawa, K. Fujita, H. Ohba, J. Nakamura, and K. Nakada 2004: Clinical features of Q fever pneumonia. Respirology 9, 278-282.

Okimoto, N., T. Kibayashi, K. Mimura, K. Yamato, T. Kurihara, Y. Honda, K. Osaki, N. Asaoka, and H. Ohba 2007: *Coxiella burnetii* and acute exacerbations/infections in patients with chronic lung disease. Respirology 12, 619-621.

Omsland, A., D.C. Cockrell, D. Howe, E.R. Fischer, K. Virtaneva, D.E. Sturdevant, S.F. Porcella, and R.A. Heinzen 2009: Host cell-free growth of the Q fever bacterium Coxiella burnetii. Proc. Natl. Acad. Sci. USA 106, 4430-4434.

Ormsbee, R., M. Peacock, R. Gerloff, G. Tallent, and D. Wike 1978: Limits of rickettsial infectivity. Infect. Immun. 19, 239-245.

Panaiotov, S., M. Ciccozzi, N. Brankova, V. Levterova, M. Mitova-Tiholova, M. Amicosante, G. Rezza, and T. Kantardjiev 2009: An outbreak of Q fever in Bulgaria. Ann. Ist. Super Sanita 45, 83-86.

Pape, M., K. Mandraveli, M. Arvanitidou-Vagiona, P. Nikolaidis, and S. Alexiou-Daniel 2009: Q fever in northern Greece: epidemiological and clinical data from 58 acute and chronic cases. Clin. Microbiol. Infect. 15 Suppl 2, 150-151.

Parker, R.R., and G.E. Davis 1938: A filter-passing infectious agent isolated from ticks. II. Transmission by Dermacentor andersoni. Public Health Rep. 53, 2267-2269.

Peter, O., G. Dupuis, W. Burgdorfer, and M. Peacock 1985: Evaluation of the complement fixation and indirect immunofluorescence tests in the early diagnosis of primary Q fever. Eur. J. Clin. Microbiol. 4, 394-396.

Peter, O., G. Dupuis, M.G. Peacock, and W. Burgdorfer 1987: Comparaison of enzyme-linked immunosorbent assay and complement fixation and indirect fluorescent-antibody tests for detection of *Coxiella burnetii* antibody. J. Clin. Microbiol. 26, 1978-1982.

Philip, C.B. 1948: Observations on experimental Q fever. J. Parasitol. 34, 457-464.

Pinsky, R.L., D.B. Fishbein, C.R. Greene, K.F. Gensheimer 1991: An outbreak of cat-associated Q fever in the United States. J. Infect. Dis. 164, 202-204.

Plommet, M., M. Capponi, J. Gestin, and G. Renoux 1973: Fièvre Q expérimentale des bovins. Ann. Rech. Vet. 4, 325-346.

Polydorou, K. 1981: Q fever in Cyprus: a short review. Br. Vet. J. 137, 470-477.

Pope, J.H., W. Scott, and R. Dweyer 1960: *Coxiella burnetii* in kangaroos and kangorro-ticks in Western Queenland. Aust. Ann. Med. 9, 214-216.

Ransom, S.E., and R.J. Huebner 1951: Studies on the resistance of *Coxiella burnetii* to physical and chemical agents. Am. J. Hyg. 53, 110-119.

Raoult, D., M. Drancourt, and G. Vestris 1990: Bactericidal effect of doxycycline associated with lysosomotropic agents on Coxiella burnetii in P388D1 cells. Antimicrob. Agents Chemother. 34, 1512-1514.

Raoult, D. 1993: Treatment of Q fever. Antimicrob. Agents Chemother. 37, 301-302

Raoult, D., and A. Stein 1994: Q fever during pregnancy, a risk for women, fetuses and obstetricians. N. Engl. J. Med. 330, 371

Raoult, D., J.C. Laurent, and M. Mutillod 1994: Monoclonal antibodies to Coxiella burnetii for antigenic detection in cell culture and in paraffin-embedded tissues. Am. J. Clin. Pathol. 101, 318-320.

Raoult, D., H. Tissot-Dupont, C. Foucault, J. Gouvernet, P.E. Fournier, E. Bernit, A. Stein, M. Nesri, J.R. Harle, and P.J. Weiller 2000: Q fever 1985-1998: Clinical and epidemiological features of 1,383 infections. Medicine (Baltimore) 79, 109-123.

Raoult, D., P. Houpikian, H. Tissot-Dupont, J.M. Riss, J. Arditi-Djiane, and P. Brouqui 1999: Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydrochloroquine. Arch. Intern. Med. 159, 167-173.

Raoult, D., F. Fennolar, A. Stein 2002: Q fever during pregnancy: diagnosis, treatment and follow-up. Arch Int Med 162, 701-704.

Raoult, D., T. Marrie, and J. Mege 2005: Natural history and pathophysiology of Q fever. Lancet Infect. Dis. 5, 219-226.

Riemann, H.P., R.A. Thompson, D.E. Behymer, and C. Wijayasinghe 1978: Toxoplasmosis and Q fever antibodies among wild carnivores in California. J. Wildl. Manag. 42, 198-202.

Rodolakis, A., M. Berri, C. Hechard, C. Caudron, A. Souriau, C.C. Bodier, B. Blanchard, P. Camuset, P. Devillechaise, J.C. Natorp, J.P. Vadet, and N. Arricau-Bouvery 2007: Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine, and ovine herds. J. Dairy Sci. 90, 5352-5360.

Roest, H.I.J., R.C. Ruuls, J.J.H.C. Tilburg, M.H. Nabuurs-Franssen, C.H.W. Klaassen, P. Vellema, R. van den Brom, D. Dercksen, W. Woude, M.A.H. Spierenburg, A.N. ven der Spek, R. Buijs, A.G. de Boer, P.Th.J. Willemsen, and F.G. van Zijderveld 2011a: Molecular epidemiology of C. burnetii from ruminants in Q fever outbreak, the Netherlands. Emerg. Infect. Dis. 17, 668-675.

Roest, H.I., J.J. Tilburg, W. van der Hoek,P. Vellema, F.G. van Zijderveld, C.H. Klaassen, and D. Raoult 2011b: The Q fever epidemic in the Netherlands: history, onset, response and reflection. Epidemiol. Infect. 139, 1-12.

Rolain, J.M., M.N. Mallet, and D. Raoult 2003: Correlation between serum levels of doxycycline and serology evolution in patients treated for Coxiella burnetii endocarditis. J. Infect. Dis. 9, 1322-1325.

Rolain, J.M., A. Boulos, M.N. Mallet, and D. Raoult 2005a: Correlation between ratio of serum doxycycline concentration to MIC and rapid decline of antibody levels during treatment of Q fever endocarditis. Antimicrob. Agents Chemother. 49, 2673-2676.

Rolain, J.M., F. Lambert, and D. Raoult 2005b: Activity of telithromycin against thirteen new isolates of C. burnetii including three resistant to doxycycline. Ann. N.Y. Acad. Sci. 1063, 252-256.

Roman, M.J., P.D. Coriz, and O.G. Baca 1986: A proposed model to explain persistent infection of host cells with Coxiella burnetii. J. Gen. Microbiol. Clin. 15, 168.

Rousset, E., V. Duquesne, P. Russo, and R. Thiéry 2007a: Fièvre Q: problématiques et risques sanitaires. Bull. Acad. Vet. Fr. 160, 107-114.

Rousset, E., B. Durand, M. Berri, P. Dufour, M. Prigent, P. Russo, T. Delcroix, A. Touratier, A. Rodolakis, and M. Aubert 2007b: Comparative diagnostic potential of three serological tests for abortive Q fever in goats herds. Vet. Microbiol. 124, 286-297.

Rousset, E., M. Berri, B. Durand, P. Dufour, M. Prigent, T. Delcroix, A. Touratier, and A. Rodolakis 2009a: *Coxiella burnetii* shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. Appl. Environ. Microbiol. 75, 428-433.

Rousset, E., B. Durant, J.L. Champion, M. Prigent, P. Dufour, C. Forfait, M. Marois, T. Gasnier, V. Duquesne, R. Thiéry, and M.F. Aubert 2009b : Efficiency of a phase 1 vaccine for the reduction of vaginal *Coxiella burnetii* shedding in a clinically affected goat herd. Clin. Microbiol. Infect. 15 Suppl 2, 188-189.

Russell-Lodrigue, K.E., G.Q. Zhang, D.N. McMurray, and J.E. Samuel 2006: Clinical and pathologic changes in a guinea pig aerosol challenge model of acute Q fever. Infect. Immun. 74, 6085-6091.

Sampere, M., B. Font, J. Font, I. Sanfeliu, and F. Segura 2003: Q fever in adults: review of 66 clinical cases. Eur. J. Clin. Microbiol. Infect. Dis. 22, 108-110.

Samuel, J.E., M.E. Frazier, M.L.E. Kahn, L.S. Thomashow, and L.P. Mallavia 1983: Isolation and characterization of a plasmid from phase 1 *Coxiella burnetii*. Infect. Immun. 41, 448-493.

Samuel, J.E. 2000: Developmental cycle of Coxiella burnetii. In: Brun, Y.V., L.J. Shimkets (eds), Procaryotic development. pp. 427-440, ASM Press, Washington DC, USA.

Sanchez, J., A. Souriau, A.J. Buendia, N. Arricau-Bouvery, C.M. Martinez, J. Salinas, A. Rodolakis, and J.A. Navarro 2006: Experimental *Coxiella burnetii* infection in pregnant goats: a histopathological and immunohistochemical study. J. Comp. Pathol. 135, 108-115.

Sanzo, J.M., M.A. Garcia-Calabuig, A. Audicana, and V. Dehesa 1993: Q fever: prevalence of antibodies to Coxeilla burnetii in the Basque Country. Int. J. Epidemiol. 22, 1183-1187.

Savinelli, E.A., and L.P. Mallavia 1990: Comparison of *Coxiella burnetii* plasmids to homologous chromosomal sequences present in a plasmidless endocarditis-causing isolate. Ann. N.Y. Acad. Sci. 590, 523-533.

Schimmer, B., G. Morroy, F. Dijkstra, P.M. Schneeberger, G. Weers-Pothoff, A. Timen, C. Wijkmans, and W. van der Hoek 2008: Large ongoing Q fever outbreak in the south of The Netherlands, 2008. Euro Surveill. 13 (7-9), 1-3.

Schmeer, N.P. Muller, J. Langel, H. Krauss, J.W. Frost, and J. Wieda 1987: Q fever vaccines for animals. Zentbl. Bakteriol. Microbiol. Hyg. A. 267, 79-88.

Schneeberger, P.M., M.H.A. Hermans, E.J. van Hannen, J.J.A. Schellekens, A.C.A.P. Leenders, and P.C. Wever 2010: Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever. Clin. Vaccine Immunol. 17, 286-290.

Schuil, J., J.H. Richardus, G.S. Baarsma, G.J. Schaap 1985: Q fever as a possible cause of bilateral optic neuritis. Br. J. Ophthalmol. 69, 580-583.

Scot, G.H., J.C. Williams, and E.H. Stephenson 1987: Animal models in Q fever: pathological responses of inbred mice to phase I Coxiella burnetii. J. Gen. Microbiol. 133, 691-700.

Scot, G.H. and J.C. Willliams 1990: Susceptibility of Coxiella burnetii to chemical disinfectants. Ann. N.Y. Acad. Sci. 590, 291-296.

Seshadri, R., I.T. Paulsen, J.A. Eisen, T.D. Read, K.E. Nelson, W.C. Nelson, N.L. Ward, H. Tettelin, T.M. Davidsen, M.J. Beanan, R.T. Deboy, S.C. Daugherty, L.M. Brinkac, R. Madupu, R.J. Dodson, H.M. Khouri, K.H. Lee, H.A. Carty, D. Scanlan, R.A. Heinzen, H.A. Thompson, J.E. Samuel, C.M. Fraser, and J.F. Heidelberg 2003: Complete genome sequence of Q-fever pathogen *Coxiella burnetii*. Proc. Natl. Acad. Sci. USA 100, 5455-5460.

Setiyono, A., M. Ogawa, Y. Cai, S. Shiga, T. Kishimoto, and I. Kurane 2005: New criteria for immunofluorescence assay for Q fever diagnosis in Japan. J. Clin. Microbiol. 43, 5555-5559.

Shaked, Y., and Y. Samra 1989: Q fever meningoencephalitis associated with bilateral abducens nerve paralysis, bilateral optic neuritis and abnormal cerebrospinal fluid findings. Infection 17, 394-395.

Shannon, J.G., D.C. Cockrell, K. Takahashi, G.L. Stahl, and R.A. Heinzen 2009: Antibody-mediated immunity to the obligate intracellular bacterial pathogen *Coxiella burnetii* is Fc receptor- and complement-independent. BMC Immunol. 10, 26.

Slavin, G. 1952: 'Q' fever: the domestic animal as a source of infection for man. Vet. Rec. 64, 743.

Smith, D.J.W. 1940: Studies on the epidemiology of Q fever. III. The transmission of Q fever by the tick *Haemaphysalis humerosa*. Aust. J. Exp. Biol. Med. Sci. 18, 103-106.

Smith, D.J.W., and E.H. Derrick 1940: Studies on the epidemiology of Q fever. I. Isolation of six strains of *Rickettsia burnetii* from the tick *Haemaphysalis humerosa*. Aust. J. Exp. Biol. Med. Sci. 18, 1-5.

Smith, D.J.W. 1941: Studies on the epidemiology of Q fever. VIII. The transmission of Q fever by the tick *Rhipicephalus sanguineus*. Aust. J. Exp. Biol. Med. Sci. 19, 119-122.

Spelman, D.W. 1982: Q fever: a study of 111 consecutive cases. Med. J. Aust. 1, 547-553.

Stein, A., and D. Raoult 1992: Detection of *Coxiella burnetii* by DNA amplification using polymerase chain reaction. J. Clin. Microbiol. 30, 2462-2466.

Stein, A., and D. Raoult 1998: Q fever during pregnancy: a public health problem in Southern France. Clin. Infect. Dis. 27, 592-596.

Stoker, M.G.P., and B.P. Marmion 1955: The spread of Q fever from animals to man; the natural history of a rickettsial disease. Bull. Wld. Hlth. Org. 13, 781.

Stoenner, H.G., and D.B. Lackman 1960: The biologic properties of *Coxiella burnetii* isolated from rodents collected in Utah. Am. J. Hyg. 71, 45-51.

Svraka, S., R. Toman, L. Skultety, K. Slaba, and W.L. Homan 2006: Establishment of a genotyping scheme for *Coxiella burnetii*. FEMS Microbiol. Lett. 254, 268-274.

Syrucek, L., and K. Raska 1956: Q fever in domestic and wild birds. Bull. WHO 15, 329-337.

Tatsumi, N., A. Baumgartner, Y. Qiao, I. Yamamoto, and K. Yamaguchi 2006: Detection of *Coxiella burnetii* in market chicken eggs and mayonnaise. Ann. N.Y. Acad. Sci. 1078, 502-505.

Tellez, A., C. Sainz, C. Echevarria, S. De Carlos, M. V. Fernandez, P. Leon, and R. Brezina 1988: Q fever in Spain : acute and chronic cases 1981-1985. Rev. Infect. Dis. 10, 198-202.

Thiele, D., H. Willems, G. Köpf, and H. Krauss 1993: Polymorphism in DNA restriction patterns of *Coxiella burnetii* isolates investigated by pulsed field gel electrophoresis and image analysis. Eur. J. Epidemiol. 9, 419-425.

Thomas, D.R., R.L. Salmon, S.M. Kench, D. Meadows, T.J. Coleman, P. Morgan-Capner, and K.L. Morgan 1994: Zoonotic illness: determining risks and measuring effects of the association between current animal exposure and a history of illness in a well characterized rural population. J. Epidemiol. Community Health 48, 151-155.

Thomas, D.R., L. Treweek, R.L. Salmon, S.M. Kench, T.J. Coleman, D. Meadows, P. Morgan-Capner, and E.O. Caul 1995: The risk of acquiring Q fever on farms: a seroepidemiological study. Occup. Environ. Med. 52, 644-647.

Thompson, H.A., C.R. Bolt, T. Hoover, and J.C. Williams 1990: Induction of heat-shock proteins in Coxiella burnetii. N Y Acad Sci 590, 127-135.

Thompson, H.A., T.A. Hoover, M.H. Vodkin, and E.I. Shaw 2003: Do chromosomal deletions in the lipopolysaccharide biosynthetic regions explain all cases of phase variation in *Coxiella burnetii* strains? Ann. N.Y. Acad. Sci. 990, 664-670.

Tissot-Dupont, H., D. Raoult, P. Brouqui, F. Janbon, D. Peyramond, P.J. Weiller, C. Chicheportiche, M. Nezri, and R. Poirier 1992: Epidemiological features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. Am. J. Med. 93, 427-434.

Tissot-Dupont, H., S. Torres, M. Nezri, and D. Raoult 1999: Hyperendemic focus of Q fever related to sheep and wind. Am. J. Epidemiol. 150, 67-74.

Tissot-Dupont, H., and D. Raoult 2007: Clinical aspects, diagnosis and treatment of Q fever. In: Raoult, D., and P. Parola (eds), Rickettsial diseases, pp. 291-301, Informa Healthcare USA, New York, USA.

Tissot-Dupont, H., V. Vaillant, S. Rey, and D. Raoult 2007: Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. Clin. Infect. Dis. 44, 232-237.

To, H., K.K. Htwe, N. Yamasaki, G.Q. Zhang, M. Ogawa, T. Yamagichi, H. Fukushi, and K. Hirai 1995: Isolation of *Coxiella burnetii* from dairy cattle and ticks, and some characteristics of the isolates in Japan. Microbiol. Immunol. 39, 663-671.

To, H., N. Kako, G.Q. Zhang, H. Otsuka, M. Ogawa, O. Ochiai, S.V. Nguyen, T. Yamaguchi, H. Fukushi, N. Nagaoka, M. Akiyama, K. Amano, and K. Hirai 1996: Q fever pneumonia in children in Japan. J. Clin. Microbiol. 34, 647-651.

To, H., K.K. Htwe, N. Kako, H.J. Kim, T. Yamaguchi, H. Fukushi, and K. Hirai 1998a: Prevalence of *Coxiella burnetii* infection in dairy cattle with reproductive disorders. J. Vet. Med. Sci. 60, 859-861.

To, H., R. Sakai, K. Shirota, C. Kano, S. Abe, T. Sugimoto, K. Takehara, C. Morita, I. Takashima, T. Maruyama, T. Yamaguchi, H. Fukushi, and K. Hirai 1998b: Coxiellosis in domestic and wild birds from Japan. J. Wildl. Dis. 34, 310-316.

Tyczka, J., S. Eberling, and G. Balfer 2005: Immunization experiments with recombinant Coxiella burnetii proteins in a murine infection model. Ann. N.Y. Acad. Sci. 1063, 143-148.

Ughetto, E., F. Gouriet, D. Raoult, and J.M. Rolain 2009: Three years experience of real-time PCR for the diagnosis of Q fever. Clin. Microbiol. Infect. 15 Suppl 2, 200-201.

United States Army Medical Research Institute for Infectious Diseases 2004: Medical management of biological casualities handbook. 5th ed. Frederick (MD), The Institute, USA.

Vaidya, V.M., S.V.S. Malik, K. Simranpreet, K. Satish, and S.B. Barbuddhe 2008: Comparison of PCR, immunofluorescence assay, and pathogen isolation for diagnosis of Q fever in humans with spontaneous abortions. J. Clin. Microbiol. 46, 2038-2044.

Vaidya, V.M., S.V.S. Malik, K.N. Bhilegaonkar, R.S. Rathore, S. Kaur, and S.B. Barbuddhe 2010: Prevalence of Q fever in domestic animals with reproductive disorders. Comp. Immunol. Microbiol. Infect. Dis. 33, 307-321.

Valkova, D., and J. Kazar 1995: A new plasmid (QpDV) common to *Coxiella burnetii* isolates associated with acute and chronic Q fever. FEMS Microbiol. Lett. 125, 275-280.

Vardi, M., N. Petersil, A. Keysary, S. Rzotkiewicz, A. Laor, and H. Bitterman 2011. Immunological arousal during acute Q fever infection. Eur. J. Clin. Microbiol. Infect. Dis. DO: 10.1007/s10096-011-1255-5.

Vigil, A., R. Ortega, R. Nakajiima-Sasaki, J. Pablo, D.M. Molina, C.C. Chao, H.W. Chen, W.M. Ching, P.L. Felgner 2010: Genome-wide profiling of humoral immune response to Coxiella burnetii infection by protein microarray. Proteomics 10, 2259-2269.

Vodkin, M.H., J.C. Williams, and E.H. Stephenson 1986: Genetic heterogeneity among isolates of *Coxiella burnetii*. J. Gen. Microbiol. 132, 455.

Waag, D.M., W.R. Byrne, J. Estep, P. Gibbs, M.L.M. Pitt, and C.M. Banfield 1999: Evaluation of cynomolgus (Macaca fascicularis) and rhesus (Macaca mulatta) monkeys as experimental models of acute Q fever after aerosol exposure to phase I Coxiella burnetii. Lab. Anim. Sci. 49, 634-638.

Waag, D.M. 2007: *Coxiella burnetii*: host and bacterial responses to infection. Vaccine 25, 7288-7295.

Watanabe, A., and H.Takahashi 2008: Diagnosis and treatment of Q fever: attempts to clarify current problems in Japan. J. Infect. Chemother. 14, 1-7.

Webster, J.P., G. Lloyd, and D.W. Macdonald 1995: Q fever (Coxiella burnetii) reservoir in wild brown rats (Rattus norvegicus) populations in the UK. Parasitology 110, 31-35.

Welsh, H.H., E.H. Lennette, and F.R. Abinanti 1957: Air-borne transmission of Q fever: the role of parturition in the generation of infective aerosols. Ann. N.Y. Acad. Sci. 70, 528-540.

Whitney, E.A., R.F. Massung, A.J. Candee, E.C. Ailes, L.M. Myers, N.E. Patterson, and R.L. Berkelman 2009: Seroepidemiologic and occupational risk survey for *Coxiella burnetii* antibodies among US veterinarians. Clin. Infect. Dis. 48, 550-557.

Willeberg, P., R. Ruppanner, D.E. Behymer, S. Haghighi, J.J. Kaneko, and C.E. Franti 1980: Environmental exposure to *Coxiella burnetii*: a sero-epidemiologic survey among domestic animals. Am. J. Epidemiol. 111, 437-443.

Willems, H., D. Thiele, R. Frölich-Ritter, and H. Krauss 1994: Detection of *Coxiella burnetii* in cow's milk using the polymerase chain reaction (PCR). Zentralbl. Veterinarmed. B. 41, 580-587.

Willems, H., D. Thiele, C. Burger, M. Ritter, W. Oswald, and H. Krauss 1996: Molecular biology of *Coxiella burnetii*. In: Kazar, J., and R. Toman (eds), Rickettsiae and rickettsial diseases, pp. 363-378. Slovak Academy of Sciences, Bratislava, Slovakia.

Williams, J.C., and J.L. Cantrell 1982: Biological and immunological propreties of Coxiella burnetii vaccines in C57BL/10ScN endotoxin-non responder mice. Infect. Immun. 35, 1091-1102.

Williams, J.C. 1991: Infectivity, virulence, and pathogenicity of Coxiella burnetii for various hosts. In: Williams, J.C., and H.A. Thompson (eds), Q fever: the biology of Coxiella burnetii. pp. 21-71, CRC Press, Boca Raton, Florida, USA.

Yu, X., and D. Raoult 1994: Serotyping *Coxiella burnetii* isolates from acute and chronic Q fever patients by using monoclonal antibodies. FEMS Microbiol. Lett. 117, 15-19.

Zamboni, D.S., and M. Rabinovitch 2003: Nitric oxide partially controls Coxiella burnetii phase II infection in mouse primary macrophages. Infect. Immun. 71, 1225-1233.

Zhang, Y.X., N. Zhi, S.R. Yu, Q.J. Li, G.Q. Yu, and X. Zhang 1994: Protective immunity induced by 67-K outer-membrane protein of phase I Coxiella burnetii in mice and guinea pigs. Acta Virol. 38, 327-332.

Zhang, G., and J.E. Samuel 2003: Identification and cloning potentially protective antigens of Coxiella burnetii using sera from mice experimentally infected with Nine Mile phase I. Ann. N.Y. Acad. Sci. 990, 510-520.

2. Introduction to epidemiological tools

2.1. Retrospective study

A retrospective study, also called case-control study, consists in an analytical comparative study of two groups of individuals: a case group and a control group. The case group consists in individuals that are affected by the disease of interest, whereas the control group consists in individuals free from the disease. Definitive diagnosis (presence or absence of the disease) must be proven by diagnostic methods to ensure statistical and epidemiological accuracy of the study. Through an analysis of past exposures of the two groups, risk or/and protection factors are determined.

Retrospective studies were performed in the three studies.

2.2. Scoring of clinical signs and modified blood parameters

The scoring system permits the transformation of qualitative categorical data into quantitative analyzable data. Scoring of clinical signs and modified parameters consists in giving a value of 1 to the presence and a value of 0 to the absence of a clinical sign or modified parameter. The score of an animal is equal to the sum of the individual values (“1” and “0”) for all the clinical signs and modified parameters considered in the study. In more elaborate situations, different scores can be attributed depending on the level of gravity of the clinical signs or modified parameter observed.

A two-grade (presence or absence) scoring system was used in the three studies.

2.3. Receiver Operating Characteristic curve

Test performance for different cut-off values for a variable can be depicted graphically by plotting a Receiver Operating Characteristic (ROC) curve, which compares the true-positive rate, or Sensitivity, on the vertical axis with the false-positive rate (1 – Specificity) on the horizontal axis (Figure 8). The diagonal line reflects test values that are uninformative, e.g., where the true-positive rate equals the false-positive rate. A good test is one which has a high

true-positive rate and a low false-positive rate. In other words, the dot on the curve that is the closest to the left superior corner of the figure represents the optimal cut-off value.

A ROC curve was performed in the study on equine vegetative endocarditis.

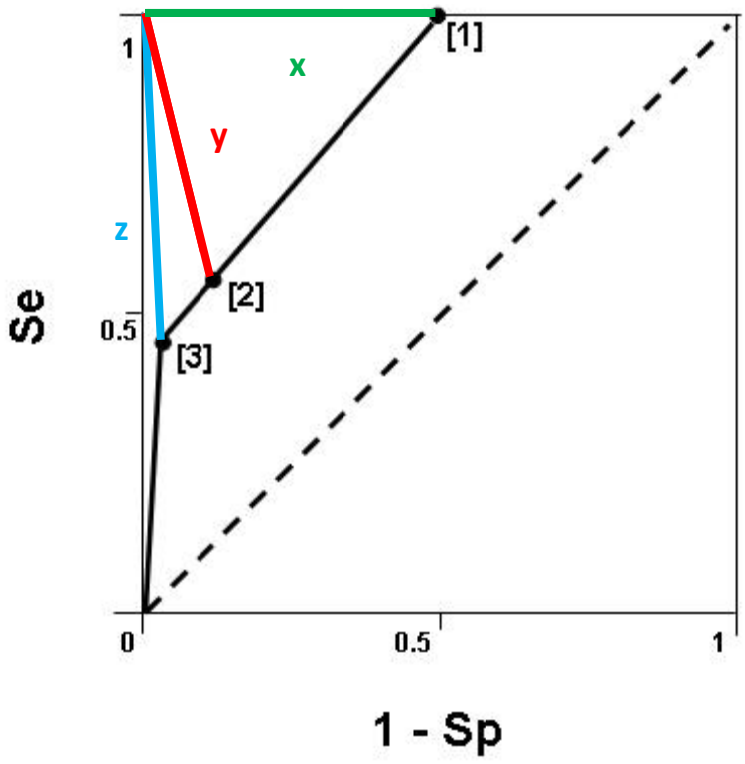


Figure 8. Receiver Operating Characteristic curve

abscissa: 1-Sp

ordinate: Se

Legend:

Sp = specificity

Se = sensitivity

[1]; [2] and [3]: different cut-off values

x: distance between left superior corner and cut-off value [1]

y: distance between left superior corner and cut-off value [2]

z: distance between left superior corner and cut-off value [3]

The true-positive rate is represented on the ordinate axis.

The false-positive rate is represented on the abscissa axis.

The different cut-off values are depicted graphically and represented by the points [1], [2] and [3].

The distances between the left superior corner and each cut-off point are compared.

The cut-off point [2] is the closest to the left superior corner in this graph (y<x<z).

Cut-off value [2] is the cut-off value of choice.

1.4. Classification and regression trees (CART)

Classification and regression trees (CART) analysis is described by Lewis (2000) as a powerful technique with significant potential and clinical utility. CART analysis is used to predict continuous or categorical variables from a set of continuous and/or categorical variables. The predicted variable is called the dependent variable whereas the other variables are called predictor or independent variables. A CART analysis is a non-linear and parametric model that is fitted by binary recursive partitioning of multidimensional covariate space determining in this way "if-then" logical conditions (StatSoft, 2011). At each binary division, or node, CART analysis chooses the predictor variable, or splitter, that generates the greatest improvement in homogeneity of the two subsequent subsets or child nodes. This potential of each independent variable to improve the homogeneity, also called purity, and in this way the predictive accuracy of the entire tree is called discriminating power (Lewis, 2000; StataSoft, 2011). Using CART 6.0 software (Salford Systems, San Diego, CA, USA), the analysis successively splits the dataset into increasingly homogeneous subsets until it is stratified to meet specified criteria (Saegerman et al., 2004; Thang et al., 2008) (Figure 9). When a primary splitting variable is missing for an individual observation, it is not discarded but, instead, a surrogate splitting variable is sought (vanEngelsdorp et *al.*, 2010). A surrogate splitter is a variable whose pattern within the dataset, relative to the outcome variable, is similar to the primary splitter. Thus, the program uses the best available information in the face of missing values. In datasets of reasonable quality, this allows all observations to be used (vanEngelsdorp et *al.*, 2010). This is a significant advantage of this methodology over

more traditional multivariate regression modeling, in which missing observations are often discarded. Furthermore, a significant advantage of CART analysis, compared to linear and regression models, is the simplicity of interpretation of the results (StatSoft, 2011). The best CART is the prediction model that has the least misclassified cases, also expressed as with minimum costs (proportion of misclassified cases). The "cost" notion gives a weight to each misclassification, some erroneous classifications having a more catastrophic impact than others (StataSoft, 2011).

The Gini index was used as the splitting method, and 10-fold cross-validation was used to test the predictive capacity of the obtained trees. CART performs cross-validation by growing maximal trees on subsets of data then calculating error rates based on unused portions of the dataset. To accomplish this, CART divides the dataset into 10 randomly selected and roughly equal parts, with each "part" containing a similar distribution of data from the populations of interest. CART then uses the first 9 parts of the data, constructs the largest possible tree, and uses the remaining 1/10 of the data to obtain initial estimates of the error rate of the selected subtree. The process is repeated using different combinations of the remaining 9 subsets of data and a different 1/10 data subset to test the resulting tree. This process is repeated until each 1/10 subset of data has been used. The results of the 10 mini-tests are then combined to calculate error rates for trees of each possible size; these error rates are applied to prune the tree grown using the entire dataset (vanEngelsdorp et *al.*, 2010). The consequence of this complex process is a set of fairly reliable estimates of the independent predictive accuracy of the tree, even when some of the data for independent variables are incomplete and/or specific events are either rare or overwhelmingly frequent (vanEngelsdorp et *al.*, 2010) (Figure 10).

CART analysis was used in the study on equine West Nile Fever.

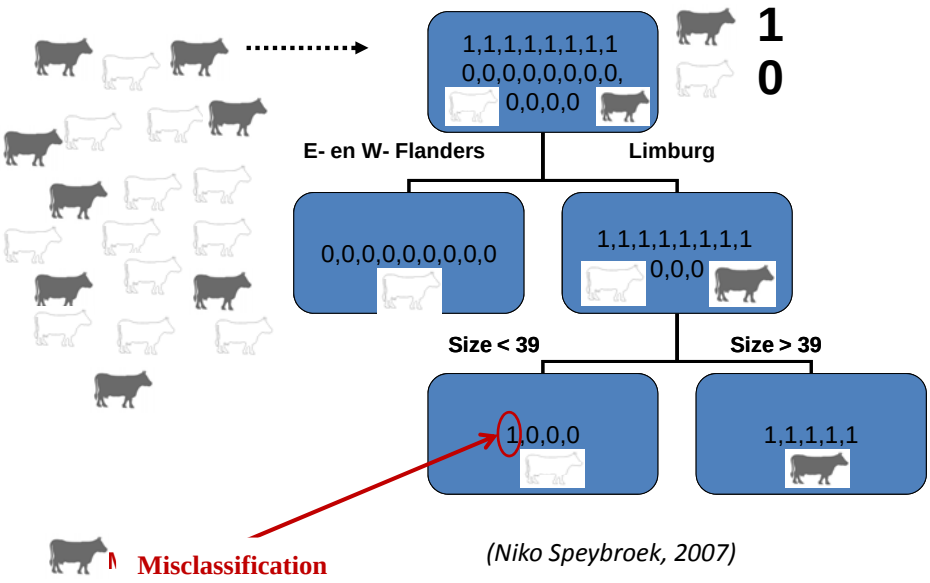


Figure 9. An example of classification and regression tree

Legend:

dark cow = cow with clinical sign or modified parameter (clinical score =1)

light cow = cow without clinical sign or modified parameter (clinical score=0)

E- and W- Flanders = eastern- and western-Flanders (northern Belgium)

Firstly, the cow herds are divided depending on their localization (eastern- or western-Flanders versus Limburg).

Localization is the primary predictor variable or splitter.

Two child nodes are obtained: one pure (100% homogeneity) on the left and one impure on the right.

The impure child node is analyzed by CART once more.

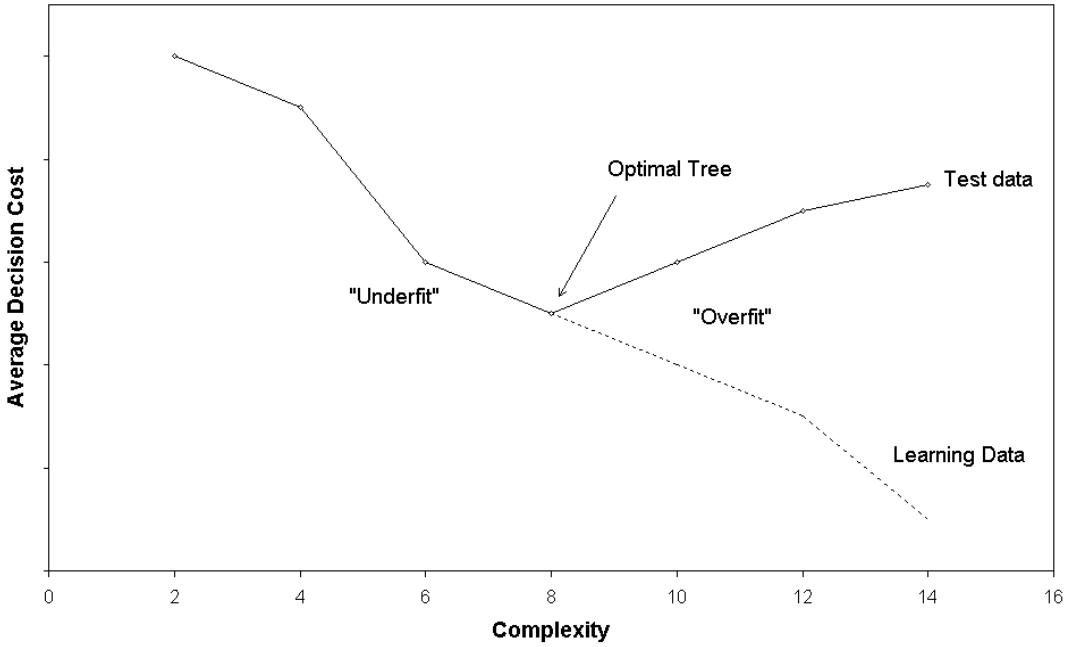
The herds of the latter impure node are divided depending on their size (number of bovids superior or inferior to 39).

Size is the second predictor variable or splitter.

Two final nodes are obtained.

The final node containing herds with more than 39 bovids per herd (on the right) is pure. All cows are positive for the clinical sign or modified parameter.

In the final node containing herds with less than 39 bovids per herd (on the left), one cow is misclassified.



(Niko Speybroek, 2007)

Figure 10. Graph for selection of the best classification and regression tree

Legend:

abscissa: complexity of the tree (number of nodes)

ordinate: average decision cost (number of misclassifications or impurity of nodes)

Each dot on the graph represents a classification and regression tree (CART). Each tree has a fixed number of nodes. The number of nodes of a CART increases with the number of binary recursive partitionings (or divisions). Thus, each binary recursive partitioning leads to a new tree. As the number of binary recursive partitionings increases, the trees grow in size and complexity.

As mentioned here above, in this study, the Gini index is used as the splitting method. This signifies that the dataset available for analysis is divided into 10 subsets of data by CART 6.0 software. An initial CART is obtained based on 9 of the 10 subsets of data. These 9 subsets of data are called learning data. The remaining tenth subset is used to test the accuracy of the tree obtained based on the 9 subsets of data and is called test data.

When based exclusively on learning data, as the number of nodes (complexity) increases, the impurity of the nodes (number of misclassifications) decreases. When the trees obtained by the initial CART analyses based on learning data are tested with the test data, as the number of nodes (complexity) increases, the impurity of the nodes (number of misclassifications) decreases until a certain point. Beyond this point (complexity of 8 nodes in this case), the impurity increases. This signifies that an overly complex tree whose elaboration is too faithful to the learning data is not adequate for application on other subsets of similar data. The optimal CART is situated at the intersection of the two curves (curve of CART based on learning data and curve of CART based on test data).

OBJECTIVES

The aim of this work was to develop epidemiological methods to facilitate the diagnosis of diseases poorly understood and/or poorly investigated by general practitioners. For many veterinarians, epidemiology is considered a theoretical science with no direct impact on individual clinical diagnosis. This study demonstrates the usefulness epidemiological methods can have in on the field situations. Emerging diseases as well as newly diagnosed diseases are a current scientific phenomenon. Our knowledge and clinical experience of such diseases are limited. Thus the development of methods emphasizing key points to reach a rapid diagnosis is of major interest.

In this study, several epidemiological methods have been developed to facilitate diagnosis by clinicians. However clinical epidemiology has its limitations and laboratory analyses or complementary examinations with more sophisticated equipment are sometimes unavoidable.

The aim of this work was:

- Firstly, to develop epidemiological methods based on a rare disease used as a model, equine vegetative endocarditis, to help veterinarians reach an early diagnosis when confronted with an emerging clinical disease at the beginning of its emergence
- Secondly, to apply the latter epidemiological methods to a current emerging disease, West Nile fever, and introduce the ongoing problem of asymptomatic infections, rendering laboratory analyses necessary to reach definitive diagnosis.
- Thirdly, to apply clinical epidemiological methods and laboratory skills introduced by the two earlier points, by investigating the prevalence of an emerging and seemingly asymptomatic disease, Q fever in horses, felines, pigs and wild boars, four species with an unknown and uninvestigated infection status in western Europe, and to assess the risk of human infection from these animals.

RESULTS

PORTER S.R., SAEGERMAN C., VAN GALEN G., SANDERSEN C., DELGUSTE C., GUYOT H., AMORY H. Vegetative Endocarditis in Equids (1994 –2006).

J. Vet. Intern. Med., 2008, **22**, 1411-1416.

Equine vegetative endocarditis

Preface

The first epidemiological study of this thesis was performed on equine vegetative endocarditis. Emerging diseases being initially rare in a population, their detection is difficult. The development of epidemiological methods facilitating detection of an “endemic” rare disease could lead to the adaptation of such methods to emerging diseases.

A retrospective study on a total of 153 equids admitted to the Veterinary Teaching Hospital of Liège University between 1994 and 2006 was performed. The purpose of this study was to determine the physical data, clinical signs, associations of clinical signs, and the abnormal blood parameters, which should lead the practitioner to consider a potential diagnosis of infective endocarditis. Previously epidemiological studies on infective endocarditis in equids have been performed but large scale studies remain rare. Many hypotheses on predisposition factors have been proposed but remain insufficiently proven.

J Vet Intern Med 2008;22:1411–1416

Vegetative Endocarditis in Equids (1994–2006)

S.R. Porter, C. Saegerman, G. van Galen, C. Sandersen, C. Delguste, H. Guyot, and H. Amory

Background: Endocarditis is a rare heart condition with variable clinical expressions in equids. Risk factors for this disease are incompletely understood.

Objective: Describe risk factors for endocarditis in equids.

Animals: One hundred and fifty-three equids admitted to Liège University, 9 diagnosed with endocarditis and 144 free from endocarditis but admitted to the hospital with a differential diagnosis including this disease.

Methods: Retrospective case-control study.

Results: Equids with endocarditis were significantly younger (mean age = 4.84 ± 5.74 years) than control equids (mean age = 10.8 ± 7.73 years) ($P = .01$). No sex or breed predisposition was observed. Animals with hyperthermia (odds ratio [OR] = 24.4; confidence interval [CI] = 1.40–428), synovial distension (OR = 13.4; CI = 3.00–59.8), lameness (OR = 6.52; CI = 1.63–26.1), hyperglobulinemia (OR = 26.4; CI = 3.03–229), hypoalbuminemia (OR = 11.4; CI = 1.34–96.8), hyperfibrinogenemia (OR = 9.81; CI = 1.16–82.7), or leukocytosis (OR = 7.12; CI = 1.40–36.4) presented a significantly higher risk of having endocarditis than control horses. The presence of two of the clinical signs mentioned above significantly increased the probability of a diagnosis of endocarditis ($P \leq .05$).

Conclusions and Clinical Importance: Age is associated with equine endocarditis. The diagnostic value of certain clinical signs and abnormalities in blood parameters in this disease are described.

Key words: Case-control study; Clinical study; Equine; Exploratory factors; Valvulitis.

Bacterial endocarditis, also called vegetative or infective endocarditis, is a rare cardiac disease in equids.[1–5] Two different forms of vegetative endocarditis can be distinguished: an acute form and a subacute or chronic form.[1,6,7] Signs characterizing the acute form are pyrexia, depression, reluctance to move due to thoracic discomfort or lameness, and the rapid development of signs of cardiac insufficiency including edema and increased jugular filling.[1] In the subacute or chronic form, which is much more common, equids might have intermittent pyrexia, weight loss, shifting lameness, poor performance, lethargy/depression, a heart murmur, or a combination of these signs lasting for weeks or months.[1] Signs of cardiac failure usually occur only as a terminal event.[1] Although endocarditis is frequently associated with the presence of a heart murmur, this is not always the case.[2,4–6,8] Vegetative endocarditis can also be associated with cardiac arrhythmias including atrial fibrillation and ventricular premature beats.[4,5,9] Atrial premature beats and ventricular tachycardia[10–13] also occur, but less frequently.

Vegetative endocarditis[2,8] can be suspected on the basis of history and physical examination but complementary examinations are required for definitive diagnosis. Echocardiography and electrocardiography can be useful in diagnosis, prognosis, and serial assessment of endocarditis by identifying anatomic lesions and characterizing arrhythmias.[3,4,7,9] Echocardiography remains the most specific, but not highly sensitive, modality in diagnosis of endocarditis.[1,6,9,14] Other complementary examinations can be useful in equids with manifestations of metastatic disease. Once established, the endocarditis lesion can release bacteria and emboli into the circulation, causing bacteremia, infarctions, and metastatic infections leading to secondary organ dysfunction.[1,2,6–8,15] The infection stimulates the immune system causing the formation of immune complexes and secondary immune-mediated complications.[1,2,4–7,15] Vegetative endocarditis being an infection of the valvular endothelium by a specific microorganism, treatment logically consists of a high dose of bactericidal antibiotic, ideally based on the results of the blood culture and antibiogram[1,4–7,9,16,17], for a minimum period of 4–6 weeks.[1,2,4–8,10,11,18] Resolution of clinical signs, improvement of echocardiographic findings, and reduction in leukocytosis and hyperfibrinogenemia should be used to evaluate the response and determine the duration of therapy.[4,17] The prognosis is poor[1,4–6,8] in all cases of infective endocarditis. Valvular insufficiency caused by vegetative endocarditis can remain despite reduction of the size and sterilization of the lesion.[2,6] In addition, infarctions, metastatic infections, and financial limitations can also complicate treatment and affect prognosis.[1,2,6] Because of the nonspecific signs associated with endocarditis, early detection of the disease is often difficult.[2] However, early initiation of aggressive therapy has been shown to greatly affect the final outcome.[4,5,7] An early diagnosis is thus essential to succeed in treating a patient.[4,5,7] A predisposition to infective endocarditis in

From the Equine Teaching Hospital, Department of Companion Animals and Equids Clinical Sciences, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium (Porter, van Galen, Sandersen, Delguste, Amory); Department of Infectious and Parasitic diseases, Faculty of Veterinary Medicine, Epidemiology and Risk Analysis applied to Veterinary Sciences, University of Liège, Liège, Belgium (Saegerman); and the Clinic for Ruminants, Clinical Department of Production Animals, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium (Guyot). This study was performed at the Equine Teaching Hospital, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium. The epidemiological and statistical analyses of data were performed at the Research Unit of Epidemiology and Risk Analysis applied to Veterinary Sciences, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.

Corresponding author: H. Amory, Equine Teaching Hospital, Department of Companion Animals and Equids Clinical Sciences, Faculty of Veterinary Medicine, University of Liège, Bat. B41, Sart-Tilman, 4000 Liège, Belgium; e-mail: helene.amory@ulg.ac.be.

Submitted October 27, 2007; Revised January 3, 2008; Accepted June 23, 2008.

10.1111/j.1939-1676.2008.0192.x

young[2,7,12,17], male[1,2,7,12] or both equids has been suggested by previous studies but contradictions between studies remain.[17] No breed predisposition was obvious in a study of 10 cases.[17]

The purpose of this study was to determine the physical data, the clinical signs, the associations of the clinical signs, and the abnormal blood parameters, which should lead the practitioner to consider a potential diagnosis of infective endocarditis.

Materials and Methods

Case Definition

Each animal was identified by its physical description, its name, its microchip number if present, and name and address of owner. Each animal admitted to the Veterinary Teaching Hospital of Liège University was always registered under a specific unique file with a record number identifying the animal throughout its stay at the hospital.

The diagnosis of vegetative endocarditis was made by echocardiography or by postmortem examination for the animals included in the case group.

For the control group, animals were selected from the files of equids admitted to the Veterinary Teaching Hospital during the period from 1994 to 2006. Vegetative endocarditis had to be included in their initial differential diagnosis, based on history and physical examination, but its diagnosis was thereafter excluded on the basis of echocardiography and/or postmortem examination. The clinical signs on which was based the choice of including vegetative endocarditis in the differential diagnosis were the presence of one or more of the following signs: hyperthermia, tachycardia, heart murmur, tachypnea/dyspnea, depression, weight loss, lameness, neurological symptoms, synovial distension, poor body condition, intensity variation of peripheral pulse, recurrent/persistent anorexia or inappetence, and arrhythmia. These clinical signs were the most frequently reported signs in the cases of endocarditis admitted to Liège University and in cases of endocarditis in the scientific literature.[1,6,9,10,11,16–33]

Animals

A total of 153 equids including horses, ponies, or donkeys were studied and consisted of 2 groups: a group of animals with endocarditis (case group, n = 9), and a group of control animals (control group, n = 144).

The case group included 9 cases (7 horses, 1 donkey, and 1 pony) suffering from vegetative endocarditis and admitted to the Veterinary Teaching Hospital of Liège University between 1994 and 2006.

The control group consisted of 144 cases (horses, ponies, or donkeys) that were admitted to the Veterinary Teaching Hospital of Liège University between 1994 and 2006.

Clinical Findings and Laboratory Analyses

For each animal included in this study, the description (breed, age, sex), the date of admission to the Veterinary Teaching Hospital, and all clinical signs reported in the history and physical examination at initial examination were abstracted from the medical record. The values of evaluated blood parameters were recorded. The blood parameters included CBC, serum protein profile, serum alkaline phosphatase activity, and serum haptoglobin and fibrinogen content. The reference values used for each blood parameter were the reference values used by Liège University's Biochemistry Laboratory. For the control group, the final diagnosis was recorded.

In addition, for clinical data, including physical abnormalities and abnormal clinical chemistry, on CBC, a value of 1 was attributed for each observed clinical sign, and the score of an individual animal is defined as the sum of all clinical signs present.

Statistical Analyses

The prevalence of infection among the equids included in the study, the sensitivity, the specificity, and the accuracy of each clinical sign were estimated with 95% confidence intervals (95% CI) assuming a binomial exact distribution. The mean ages and the mean values for each blood parameter in the 2 respective groups of animals, with unequal variance and considering nonnormal data, were compared with a Wilcoxon's rank-sum test.[34] The limit of statistical significance of the tests performed was defined as $P \leq .05$. The potential influence of age, sex, and breed was assessed. Statistical analysis of these data was performed using a χ^2-test and the tendency of each parameter to become a risk factor was evaluated by an odds ratio (OR) calculation with 95% CI (logarithmic approximation); a P value $\leq .05$ was considered significant.[35]

A receiver operating characteristic (ROC) curve is a graph of the relationship between the sensitivity (the true-positive rate) against 1 minus the specificity (the false-positive rate) for different cut-off values. A good test is 1 which has a high true-positive rate and a low false-positive rate and whose value, therefore, lies close to the top left-hand corner of the graph. The ROC curve is typically used to evaluate accuracy of different cut-off values for 1 variable and this variable can be the number of clinical signs (score of combination of clinical signs). In the present study, the determination of the best score of combination of clinical signs was made using the ROC curve. Epidemiological software for veterinary medicine was used.[36]

Results

Animals

Nine of the 153 equids in which vegetative endocarditis was included in the initial differential diagnosis had a final diagnosis of vegetative endocarditis (5.9% with 95% CI: 2.7–10.9%).

The mean age of the equids was 4.84 ± 5.74 and 10.8 ± 7.73 years in the case and in the control groups, respectively ($P = .01$).

Sex and breed were not risk factors to developing endocarditis in equids in this study. With geldings as the referent group, OR for stallions and females were 5.18 (95% CI: 0.885–30.3) and 1.32 (95% CI: 0.212–8.15), respectively.

Clinical Signs

The clinical signs presented by the 9 cases with endocarditis were hyperthermia (n = 9), tachycardia (n = 7), heart murmur (n = 7), tachypnea/dyspnea (n = 7), depression (n = 7), lameness (n = 5), recurrent/persistent anorexia or lack of appetite (n = 5), weight loss (n = 4), synovial distension (n = 4), neurological symptoms (n = 3), poor body condition (n = 3), intensity variation of peripheral pulse (n = 1), and arrhythmia (n = 1).

The 3 clinical signs associated with a significant higher risk of the presence of endocarditis were hyperthermia, synovial distension, and lameness (Table 1).

Blood Parameters

The abnormal blood parameters most frequently reported were hyperfibrinogenemia (n = 8), leukocytosis

Table 1. Odds ratio (OR), sensitivity, specificity, and accuracy calculated for the 13 clinical signs and the 9 blood parameters in equids with confirmed endocarditis and in equids with suspected endocarditis but free from the disease.

	Number of Observations							
	Cases		Controls					
Parameter[a]	Presence	Absence	Presence	Absence	OR (95% CI)[b]	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)
Clinical signs								
Hyperthermia	9	0	63	81	24.4 (1.40–428)*	100 (71.7–100)	56.3 (47.7–64.5)	58.8 (50.6–66.7)
Tachycardia	7	2	94	49	1.82 (0.365–9.12)	77.8 (40.0–97.2)	34.3 (26.5–42.7)	43.6(31.6–39.6)
Heart murmur	7	2	83	61	2.57 (0.516–12.8)	77.8 (40.0–97.2)	42.4 (34.2–50.9)	44.4 (36.4–52.7)
Tachypnea/dyspnea	7	2	88	55	2.19 (0.438–10.9)	77.8 (40.0–97.2)	38.5 (30.5–47.0)	40.8 (32.9–49.0)
Depression	7	2	64	79	4.32 (0.867–21.5)	77.8 (40.1–97.3)	55.2(46.7–63.6)	44.7(36.7–53.0)
Lameness	5	4	23	120	6.52 (1.63–26.1)*	55.6 (21.2–86.3)	83.9 (76.9–89.5)	82.2 (75.2–88.0)
Recurrent/ persistent anorexia or inappetence	5	4	48	96	2.5 (0.642–9.74)	56.6 (21.2–86.3)	66.7 (58.3–74.3)	66.0 (57.9–73.5)
Weight loss	4	5	81	62	0.612 (0.158–2.38)	44.4 (13.7–78.8)	43.4 (35.1–51.9)	43.4 (35.4–51.7)
Synovial distension	4	5	8	134	13.4 (3.00–59.8)*	44.4 (13.7–78.8)	94.4 (89.2–97.5)	90.7 (84.9–94.8)
Neurological symptoms	3	6	36	107	1.49 (0.353–6.25)	33.3 (7.49–70.1)	74.8 (66.9–81.7)	72.4 (64.5–79.3)
Poor body condition	3	6	53	90	0.849 (0.204–3.54)	33.3 (7.49–70.1)	62.9 (54.5–70.9)	61.2 (52.9–69.0)
Intensity variation of peripheral pulse	1	8	24	119	0.62 (0.0740–5.19)	11.1 (0.280–48.2)	83.2 (76.1–88.9)	79.0 (71.5–85.1)
Arrhythmia	1	8	36	107	0.372 (0.0449–3.07)	11.1 (0.280–48.2)	74.8 (66.9–81.7)	71.0 (63.2–78.1)
Blood parameters								
Fibrinogen	8	1	32	38	9.81 (1.16–82.7)*	88.9 (51.8–99.7)	54.3 (41.9–66.3)	58.2 (46.6–69.2)
Leukocytes	7	2	31	60	7.12 (1.40–36.4)*	77.8 (40.0–97.2)	65.9 (55.3–75.5)	67.0 (56.9–76.1)
Albumin	7	1	33	52	11.4 (1.34–96.8)*	87.5 (47.3–99.7)	61.2 (50.0–71.6)	63.4 (52.8–73.1)
Globulins	7	1	18	64	26.4 (3.03–229)*	87.5 (47.3–99.7)	78.0 (67.5–86.4)	78.9 (69.0–86.8)
Neutrophils	6	2	55	38	2.19 (0.419–11.5)	75.0 (34.9–96.8)	40.9 (30.8–51.5)	45.5 (35.6–55.8)
Hematocrit	5	4	35	60	2.18 (0.549–8.65)	55.6 (21.2–86.3)	63.2 (52.6–72.8)	62.5 (52.5–71.8)
Haptoglobin	5	1	23	12	2.86 (0.298–27.4)	83.3 (35.9–99.6)	34.3 (19.1–52.2)	41.5 (26.3–57.9)
Alkaline phosphatase	4	1	21	21	3.81 (0.391–37.1)	80.0 (28.4–99.5)	50.0 (34.2–65.8)	53.2 (38.1–67.9)
Total protein	2	6	23	69	2.19 (0.396–12.2)	25.0 (3.19–65.1)	71.0 (61.1–79.6)	71.0 (61.1–79.6)

[a]Thresholds for parameters: hyperthermia when temperature $>38.5\,^{\circ}C$, tachycardia when heart rate >44 beats/min, tachypnea when respiratory rate >16 respirations/min, anemia when hematocrit $<33\%$, hyperfibrinogenemia when concentration ([ ]) >3 g/L, leukocytosis when [ ] $>12\times10^9$/L, neutrophilia when [ ] $>6\times10^9$/L, hyperproteinemia when [ ] >75 g/L, hyperglobulinemia when [ ] >45 g/L, increased haptoglobin content when [ ] >500 mg/L, hypoalbuminemia when [ ] <25 g/L, increased alkaline phosphatase when >400 U/L.

[b]The 95% confidence interval (CI) (binomial exact).

*Values statistically significant at $P = .05$.

(n = 7), hypoalbuminemia (n = 7), hyperglobulinemia (n = 7), neutrophilia (n=6), anemia (n=5), increased serum haptoglobin content (n=5), increased serum alkaline phosphatase activity (n=4), and hyperproteinemia (n=2). The mean values for the 9 blood parameters in the case group and in the control group were significantly different for 5 parameters: fibrinogen, white blood cell count, albumin, total protein, and globulins (Table 2). The 4 abnormal blood parameters associated with a significant higher risk of the presence of vegetative endocarditis were hyperglobulinemia, hypoalbuminemia, hyperfibrinogenemia, and leukocytosis (Table 1).

Combination of the Significant Clinical Signs

A ROC curve shows that the association of 2 of the 3 of hyperthermia, synovial distension, and lameness gives the best test performance for a diagnosis of vegetative endocarditis (Fig 1). This result was not significantly different when a 2nd clinical pattern study was performed that considered lameness and synovial distension as being linked clinical signs. In the 1st pattern study, the specificity was higher than the sensitivity. Whereas in the 2nd pattern study, it was the contrary (Table 3).

Discussion

For the case-control study concerning the risk factors of vegetative endocarditis, age was identified as a significant risk factor. Animals with endocarditis were significantly younger than animals free from endocarditis. This supports previous suggestions on the subject.[12,17] The greater risk in young equids could be due to a more frequent occurrence of bacteremia in young animals than in adults, bacteremia being necessary for valve infection. Lower immune function, more frequent occurrence of severe worm infestation, or both could predispose young animals to infective endocarditis. No sex predisposition was found in the current study, which is consistent with some, but not all other studies.[12] In addition, because of the small sample size (n = 9) the ability to evaluate sex as an exploratory variable was

Table 2. Values for the 9 most frequently observed abnormal blood parameters in 9 equids suffering from endocarditis and in 144 equids with clinical signs suggestive of endocarditis but not suffering from endocarditis.

	Mean Value ± SD		
Blood Parameter	Case Group	Control Group	*P* Value
Hematocrit (%)	33.4 ± 10.6	35.0 ± 7.96	.36
Fibrinogen (g/L)	4.26 ± 1.07	3.09 ± 1.55	.01[a]
WBC (cells × 10^9/L)	17.9 ± 9.73	11.2 ± 6.81	.03[a]
Neutrophils (cells × 10^9/L)	13.7 ± 8.67	9.24 ± 7.06	.11
Albumin (g/L)	20.9 ± 3.45	25.3 ± 7.90	.01[a]
Total protein (g/L)	74.2 ± 14.6	61.8 ± 12.5	.01[a]
Globulins (g/L)	51.9 ± 19.4	37.7 ± 12.8	.005[a]
Alkaline phosphatase (U/L)	554 ± 138	530 ± 414	.33
Haptoglobin (mg/L)	2023 ± 1210	1014 ± 1005	.052

[a]Blood parameters with mean values significantly different in the case group and in the control group.

WBC, white blood cell count.

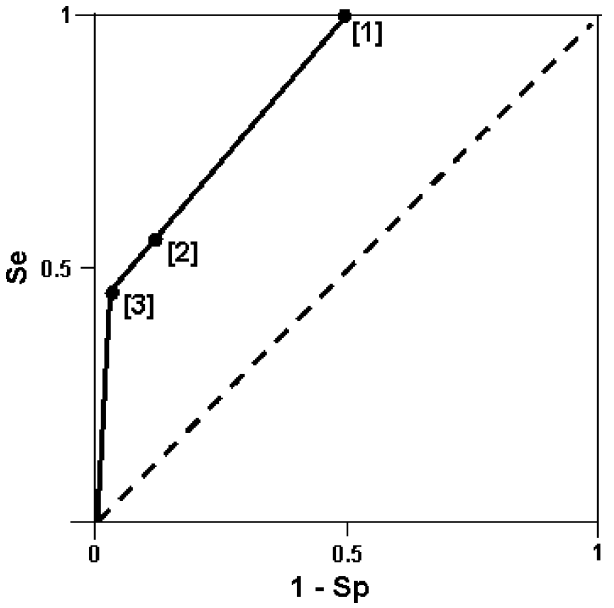


Fig 1. Receiver operating characteristic (ROC) curve of the score of the 3 most relevant clinical signs of vegetative endocarditis (hyperthermia, synovial distension, and lameness). Se, sensitivity; Sp, specificity; [1], [2], and [3]: endocarditis clinical signs score. Score [1] = hyperthermia or synovial distension or lameness. Score [2] = hyperthermia and synovial distension simultaneously present, or hyperthermia and lameness simultaneously present, or synovial distension and lameness simultaneously present. Score [3] = hyperthermia and synovial distension and lameness present. The test performance (clinical diagnostic) can be depicted graphically by plotting a ROC curve, which compares the true-positive rate, or Se, on the vertical axis with the false-positive rate (1−Sp) on the horizontal axis. The diagonal series of dashes reflect test values that are uninformative, eg, where the true-positive rate equals the false-positive rate. The dot on the continuous line that is the closest to the left superior corner of the figure represents the optimal number of clinical signs to reach a diagnosis of vegetative endocarditis in the horse.

limited. No breed predisposition was observed in the study. This could be attributable to the large variety of breeds present in the control and case groups and to an insufficiently large case group. Seasonal effect is impossible to identify in infective endocarditis because endocarditis can have initially vague clinical signs and usually following a chronic evolution in time, the date of diagnosis of the disease can differ greatly from the date of the initial infection.[2,4,5,7]

This study shows that the clinical signs significantly increasing the probability of diagnosis of vegetative endocarditis are hyperthermia, synovial distension, and lameness. It is interesting to notice that none of the cardiac clinical signs (tachycardia, heart murmur, arrhythmia, and intensity variation of peripheral pulse) was significant. These results suggest that endocarditis should not be considered merely a localized infection, but, rather, a systemic problem via associated bacteremia, or the circulation of antibody-antigen complexes, or both. The abnormal blood parameters whose presence significantly increases the probability of the diagnosis of vegetative endocarditis were hyperglobulinemia, hypoalbuminemia, hyperfibrinogenemia, and leukocytosis. Vegetative endocarditis following more frequently a subacute or chronic evolution, these blood abnormalities were likely as they occur in most cases of chronic inflammation.[8,37] It is however important to underline that in the present study, haptoglobin concentration and alkaline phosphatase activity were measured on a small number of animals. Thus, their importance could be increased or diminished in further studies with more clinical values.

The result of the statistical clinical pattern study of the present study shows that a way forward to an early diagnosis of vegetative endocarditis is the study of associations of clinical signs. Because infective endocarditis produces systemic effects on the organism, this is not surprising.[2,5–7] A statistical clinical pattern study of a larger number of clinical signs and animals could increase our knowledge on the clinical expression of vegetative endocarditis and could help to determine the multiple associations of clinical signs occurring most frequently in animals affected by the disease.

It must be noted that the only clinical signs and laboratory findings evaluated in this study were those reported frequently enough to ensure that the statistical analysis was valid. Moreover, it was assumed that all veterinary surgeons from Liège University proceeded in the same way while taking a history, conducting a physical examination, and a complementary examination. Their diagnoses were not reviewed.

Conclusion

This study is aimed in priority at the equine practitioner with a limited number of complementary examinations available. The determination of the risk factors, significant clinical signs, and abnormal blood parameters through the case-control study should increase the practitioners' awareness of equine endocarditis and its clinical expression, leading to an earlier diagnosis of endocarditis. An

Table 3. Characteristics[a] of 2 receiver operating characteristic (ROC) curves in function of the combination of clinical signs observed in 151 equids with clinical signs suggestive of endocarditis.

Combination of Clinical Signs[b]	Area under the ROC Curve (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)
Hyperthermia and synovial distension and lameness	84.7 (79.2–90.3)	55.6 (23.1–88.0)	88.0 (82.7–93.4)	86.1 (79.5–91.2)
Hyperthermia and synovial distension or lameness	84.0 (76.7–89.4)	100 (71.7–100)	50.0 (41.6–58.4)	84.0 (78.7–89.4)

[a]In brackets: the 95% confidence interval.

[b]Clinical signs associated with a significant higher risk of the presence of endocarditis (see Table 1).

early diagnosis being a major factor for the success of the medical treatment, it is therefore an essential aspect of this study. An increased success rate for the treatment of vegetative endocarditis would also diminish the financial losses associated with the numerous treatment failures.

Acknowledgments

The authors thank the staff of the Equine Teaching Hospital of Liège University for their contribution to the data of this study and for the management of hospitalized horses. The authors also thank Mrs Lisette Trzpiot from the Liège Biochemistry Laboratory, Belgium, for clerical assistance. Professor C. Saegerman and Professor H. Amory contributed to the work in equal proportions.

References

1. Travers CW, Van Den Berg JS. *Pseudomonas spp*. associated vegetative endocarditis in two horses. J S Afr Vet Assoc 1995;66: 172–176.

2. Jesty SA, Reef VB. Septicemia and cardiovascular infections in horses. Vet Clin Equine 2006;22:481–495.

3. Reef VB, Spencer P. Echocardiographic evaluation of equine aortic insufficiency. Am J Vet Res 1987;48:904–909.

4. Patteson MW. Equine cardiology. 2002.Available at: http://www.provet.co.uk./equinecardiology/5a66646.htm. Accessed November 5, 2006.

5. Patteson MW. Equine Cardiology. UK: Blackwell Science Ltd; 1996:150–154.

6. Ball MA, Weldon AD. Vegetative endocarditis in an Appaloosa gelding. Cornell Vet 1992;82:301–309.

7. Verdegaal EJMM, Sloet van Oldruitenborgh-Oosterbaan MM. Clinical commentary: Endocarditis in the horse. Equine Vet Educ 2006;18:196–198.

8. Brown CM. Symposium on cardiology: Acquired cardiovascular disease. Vet Clin North Am Equine Pract 1985;1:371–381.

9. Karzenski S, Crisman MV, Robertson J. Endocarditis in a stallion with a history of testicular torsion. Equine Pract 1997;19: 24–27.

10. Collatos C, Clark ES, Reef VB, Morris DD. Septicemia, atrial fibrillation, cardiomegaly, left atrial mass, and *Rhodococcus equi* septic osteoarthritis in a foal. J Am Vet Med Assoc 1990;197: 1039–1042.

11. Dedrick P, Reef VB, Sweeney RW, Morris DD. Treatment of bacterial endocarditis in a horse. J Am Vet Med Assoc 1988;193: 339–342.

12. Buergelt CD, Cooley AJ, Hines SA, Pipers FS. Endocarditis in six horses. Vet Pathol 1985;22:333–337.

13. Roby KA, Reef VB, Shaw DP, Sweeney CR. Rupture of an aortic sinus aneurysm in a 15-year-old broodmare. J Am Vet Med Assoc 1986;189:305–308.

14. Bonagura JD, Herring DS, Welker F. Symposium on cardiology: Echocardiography. Vet Clin North Am Equine Pract 1985;1: 312–333.

15. Freedman LR. The pathogenesis of infective endocarditis. J Antimicrob Chemother 1987;20(Suppl A):1–6.

16. Ewart S, Brown C, Derksen F, Kufuor-Mensa E. *Serratia marcescens* endocarditis in a horse. J Am Vet Med Assoc 1992;200: 961–963.

17. Maxson AD, Reef VB. Bacterial endocarditis in horses: Ten cases (1984–1995). Equine Vet J 1997;29:394–399.

18. Hillyer MH, Mair TS, Holmes JR. Treatment of bacterial endocarditis in a Shire mare. Equine Vet Educ 1990;2:5–7.

19. Fröhlich W, Wlaschitz S, Riedelberger K, Reifinger M. Fallbericht: Aortenklappenendocarditis beim Pferd. Dtsch Tierärztl Wochenschr 2004;111:370–373.

20. Deegen E, Lieske R, Schoon H. Klinische und kardiologische Untersuchungsbefunde bei 3 Deckhengsten mit Aortenklappeninsuffizienz. Tierärztl Prax 1980;8:211–222.

21. McCormick BS, Peet RL, Downes K. *Erysipelothrix rhusiopathiae* vegetative endocarditis in a horse. Aust Vet J 1985; 62:392.

22. Reef VB. Mitral valvular insufficiency associated with ruptured chordae tendineae in three foals. J Am Vet Med Assoc 1987; 191:329–331.

23. Kaplan NA, Moore BR. Case report: Streptococcus equi endocarditis, meningitis and panophtalmitis in a mature horse. Equine Vet Educ 1996;8:313–316.

24. Pace LW, Wirth NR, Foss RR, Fales WH. Endocarditis and pulmonary aspergillosis in a horse. J Vet Diagn Invest 1994;6: 506–508.

25. Bonagura JD, Pipers FS. Echocardiographic features of aortic valve endocarditis in a dog, a cow, and a horse. J Am Vet Med Assoc 1983;182:595–599.

26. Hatfield CE, Rebhun WC, Dietze AE. Endocarditis and optic neuritis in a quarter horse mare. Compendium Equine 1987;9:451–454.

27. Hines MT, Heidel JR, Barbee DD. Bacterial endocarditis with thrombus formation and abscessation in a horse. Vet Radiol Ultrasound 1993;34:47–51.

28. Ramzan PHL. Case report: Vegetative bacterial endocarditis associated with septic tenosynovitis of the digital sheath in a Thoroughbred racehorse. Equine Vet Educ 2000;12:120–123.

29. Sponseller BT, Ware WA. Case report: Successful treatment of staphylococcal endocarditis in a horse. Equine Vet Educ 2001;13: 298–302.

30. Froehlich W, Wlaschitz S, Riedelberger K, Reef VB. Case report: Tricuspid valve endocarditis in a horse with ventricular septal defect. Equine Vet Educ 2006;18:172–176.

31. Verdegaal EJMM, De Heer N, Meertens NM, et al. Case report: A right-sided bacterial endocarditis of dental origin in a horse. Equine Vet Educ 2006;18:191–195.

32. Nilsford L, Lombard CW, Weckner D, Kvart C. Case report: Diagnosis of pulmonary valve endocarditis in a horse. Equine Vet J 1991;23:479–482.

33. Church S, Harrigan KE, Irving AE, Peel MM. Endocarditis caused by *Pasteurella caballi* in a horse. Aust Vet J 1998;8:528–530.

34. Dagnelie P. Statistique théorique et appliquée. Tome 2. Inférence statistique à une et à deux dimensions. France: De Boeck & Larcier; 1998:1–659.

35. Grenier B. Utilisation des ODDS dans les tests associés ou séquentiels. In: Grenier B., ed. Décision médicale: analyse et stratégie de la décision dans la pratique médicale. France: Masson; 1990:102–106.

36. Thrusfield M, Ortega C, De Blas I, et al. WIN EPISCOPE 20: Improved epidemiological software for veterinary medicine. Vet Rec 2001;148:567–572.

37. Van Lierde H. Vademecum biologique du vétérinaire. Belgium: Prodim; 1988:1–612.

PORTER S.R., LEBLOND A., LECOLLINET S., TRITZ P., CANTILE C., KUTASI O., ZIENTARA S., PRADIER S., VAN GALEN G., SPEYBROEK N., SAEGERMAN C. West Nile Fever in Equids: the use of classification and regression trees for a clinical diagnosis and comparative study of clinical appearance in three European countries.

Transbound. Emerg. Dis., 2011, Jan 5. DOI: 10.1111/j.1865-1682.2010.01196.x.

West Nile Fever in equids

Preface

The second epidemiological study was performed on equine West Nile Fever (WNF). WNF is an emerging viral disease in Europe. The study consisted firstly in a retrospective study, secondly in a comparative study, and thirdly, in the creation of classification and regression trees based on French data originating from the Camargue area.

The purpose of the retrospective study was to determine physical data, clinical signs, and associations of clinical signs which should lead the practitioner to consider a potential diagnosis of WNF. Moreover, WNF is a vector-borne zoonosis highly influenced by climate and environmental changes. Thus, location and type of vegetation present in the area considered were taken into account to determine the potential risk of circulation of WNV. Comparison of the clinical presentation of WNF in France (cases from 2004 and 2008), Italy (2008) and Hungary (2008) was performed. Finally, classification and regression trees could increase veterinarian diagnostic effectiveness (Breiman, 1984; Grenier, 1990a; Clarck and Pregibon, 1992; Saegerman, 2005). The lack of clinical specificity and the high rates of asymptomatic infections of WNF limit the usefulness of exclusive clinical detection. Indeed, laboratory analyses are indispensable to reach definitive diagnosis and to determine the time of infection (recent or not). Increasing awareness and communication between countries on a European scale is essential (Brown, 2001; AFSSA, 2004).

Transboundary and Emerging Diseases

ORIGINAL ARTICLE

Clinical Diagnosis of West Nile Fever in Equids by Classification and Regression Tree (CART) Analysis and Comparative Study of Clinical Appearance in Three European Countries

R. S. Porter[1], A. Leblond[2], S. Lecollinet[3], P. Tritz[4], C. Cantile[5], O. Kutasi[6], S. Zientara[3], S. Pradier[7], G. van Galen[8], N. Speybroek[9] and C. Saegerman[1]

[1] Faculty of Veterinary Medicine, Department of Infectious and Parasitic diseases, Research Unit of Epidemiology and Risk Analysis applied to Veterinary Sciences, University of Liege, Liege, Belgium
[2] UR 346 Animal Epidemiology INRA Theix, National Veterinary Medicine School of Lyon, Lyon, France
[3] Agence nationale chargée de la Sécurité sanitaire de l'alimentation, de l'Environnement et du travail, UMR ANSES/INRA/ENVA, Mai sons-Alfort, France
[4] Réseau d'épidémioSurveillance en pathologie équine (RESPE), Mondeville, France and Veterinary Practice, Faluquemont, France
[5] Faculty of Veterinary Medicine, Department of Animal Pathology, University of Pisa, Pisa, Italy
[6] Faculty of Veterinary Medicine, University of Szent Istvan, Budapest, Hungary
[7] UR 346 Animal Epidemiology INRA Theix, National Veterinary Medicine School of Alfort, Maisons-Alfort, France
[8] Equine Teaching Hospital, Faculty of Veterinary Medicine, Department of Clinical Sciences, University of Liege, Liege, Belgium
[9] Faculty of Public Health, Institute of Health and Society, Université Catholique de Louvain, Clos Chapelle-aux-Champs, Bruxelles, Belgium

Keywords:
clinical epidemiology; data mining; arthropod-borne viral encephalomyelitis; West Nile Fever; equine

Correspondence:
Prof. C. Saegerman, Faculty of Veterinary Medicine, Research Unit of Epidemiology and Risk analysis applied to Veterinary Sciences, University of Liege, Boulevard de Colonster 20, B42, B-4000 Liege, Belgium. Tel.: +32 4 366 45 79; Fax: +32 4 366 42 61; E-mail: claude.saegerman@ulg.ac.be

Received for publication September 18, 2010

doi:10.1111/j.1865-1682.2010.01196.x

Summary

This retrospective study describes risk/protection factors for the development of clinical West Nile Fever (WNF) in equids, compares clinical presentation in three European countries, France, Italy and Hungary, and creates classification and regression trees (CART) to facilitate clinical diagnosis. The peak of WNF occurrence was observed in September whatever the country. A significant difference between Italy and France was observed in the delay between initial clinical signs and veterinary consultation. No clinical sign was significantly associated with WNF. Despite similar clinical presentations in the three countries, occurrence of hyperthermia was more frequently reported in France. Classification and regression tree demonstrated the major importance of geographical locality and month to reach a diagnosis and emphasized differences in predominant clinical signs depending on the period of detection of the suspected case (epizootic or not). However, definite diagnosis requires specific serological tests. Centralized reporting system and time-space risk mapping should be promoted in every country.

Introduction

West Nile fever (WNF) is a worldwide viral zoonotic infection caused by a mosquito-borne *Flavivirus* of the *Flaviviridae* family (Petersen and Roehrig, 2001; Autorino et al., 2002). The West Nile Fever virus (WNV) was initially isolated from the blood of a mildly febrile woman in the West Nile district of Uganda in 1937 (Smithburn et al., 1940). Recently has WNF become a major public health and veterinarian concern (Dauphin et al., 2004; Zeller and Schuffenecker, 2004). Indeed, in the last decades (mainly since the mid 1990s), WNV has emerged as an important pathogen for humans and horses (Autorino et al., 2002; Weese et al., 2003; Leblond et al., 2007a,b; Blitvich, 2008). In addition, the increased number of severe human cases, accompanied by an increase in the severity of the neurological disease observed in infected horses and the appearance of high bird mortality during

the outbreaks in the USA since 1999, has given a whole new importance to this disease previously considered of minor consequences (Castillo-Olivares and Wood, 2004; Dauphin et al., 2004; Blitvich, 2008).

In horses, WNF is usually asymptomatic (Blitvich, 2008; Cantile et al., 2000; Castillo-Olivares and Wood, 2004; Zeller and Schuffenecker, 2004; Dauphin and Zientara 2007; Epp et al., 2007b; Sebastain et al., 2008). If clinical signs occur, they are non-specific (Weese et al., 2003; Dauphin and Zientara, 2007). Except for fever, clinical signs are almost exclusively of a neurological nature and reflect the pathogenicity for the central and peripheral nervous system (Ostlund et al., 2000; Murgue et al., 2001; Castillo-Olivares and Wood, 2004; Zeller and Schuffenecker, 2004; Blitvich, 2008). Occasionally, a transitory febrile phase can be observed before the onset of neurological clinical signs, but this is not always the case (Murgue et al., 2001; Castillo-Olivares and Wood, 2004; Zeller and Schuffenecker, 2004).

The neurological signs most commonly observed are ataxia, paresis, limb paralysis of which can affect one or two limbs (usually the hind limbs) or all four, the latter usually progressing into recumbency (Cantile et al., 2000; Ostlund et al., 2000; Murgue et al., 2001; Castillo-Olivares and Wood, 2004; Zeller and Schuffenecker, 2004; Dauphin and Zientara, 2007; Leblond et al., 2007a). Other clinical signs reported are skin fasciculations, muscle tremors (Murgue et al., 2001), muscle rigidity (Cantile et al., 2000; Ostlund et al., 2000; Castillo-Olivares and Wood, 2004; Dauphin and Zientara, 2007). A proportion of horses never recover from infection and die naturally or are euthanized humanly (Cantile et al., 2000; Ostlund et al., 2000; Murgue et al., 2001; Weese et al., 2003; Castillo-Olivares and Wood, 2004; Leblond et al., 2007a). The severity of initial clinical signs does not necessarily hinder the ability to recover if appropriate supportive care is provided (Ostlund et al., 2000). Horses are particularly sensitive to WNV with approximately 10% of infected animals presenting neurological disorders, when compared to 1% of humans (Petersen and Roehrig, 2001; Leblond et al., 2007a), rendering its detection in equids highly pertinent in a public health perspective. The differential diagnosis in equids mainly includes arboviral encephalitis (e.g., Venezuelan, Eastern, and Western encephalomyelitis), equine protozoa myeloencephalitis (*Sarcosystis neurona* or *Neospora hughesi*), the neurological form of rhinopneumonia Equine Herpes Virus 1, Borna disease, and rabies.

The present retrospective comparative study investigates clinical presentation of WNF in three different European countries. To our knowledge, this is the first European comparative study. The purpose of the study was to determine the physical data, clinical signs, geographical and temporal information that could assist practitioners in considering a potential diagnosis of WNF. With this original approach, clinical impressions were rendered objective. The aim of the classification and regression tree (CART) analysis, never used until now for WNF, was to facilitate clinical diagnosis of the disease by veterinarians worldwide (e.g., Breiman et al., 1984; Saegerman et al., 2004).

Materials and Methods

Case definition

Data on the neurological syndromes were collected through the passive surveillance system of each country constituted by the veterinary practitioners and health authorities. In the area of study, every neurological syndrome observed in a horse was suspected of West Nile infection and was mandatorily to be declared to the health authorities by the attending veterinarian. Indeed, a suspicion of WNF was made after veterinary observation of neurological clinical signs with or without fever. Hereafter, the definitive diagnosis of WNF was reached exclusively by laboratory analyses. Horses presenting neurological clinical signs were classified as confirmed WNF cases when they tested positive to the ELISA IgM assay as performed by certified national laboratories. The cases were classified as probable WNF cases when the ELISA tests were positive for IgG and negative for IgM (Leblond et al., 2007b). Control animals used in the retrospective study were French equids suspected of WNF but later proven free from infection by laboratory analyses.

The antigens used for the detection of WNV antibodies were either recombinant prM/E antigens, used in indirect and competition ELISA or whole inactivated antigens, used in indirect or capture IgM ELISA. For WNV laboratory confirmation, a battery of tests was systematically used (confirmation never relied on a single test but on a combination of ELISA tests, for the detection of IgG and IgM antibodies, virus neutralization test, at least for the confirmation of the first recorded case and PCR tests, when central nervous system samples were available). As the proposed study is a retrospective one (with some samples that are no more available), the tests used differed over time, and for example, competition ELISA was used only recently for the confirmation of WNV outbreaks (Hungarian 2008 outbreak, for example). However, IgG ELISA tests (indirect with whole inactivated antigens, recombinant antigens or competition) gave comparable results, in terms of sensitivity, specificity and repeatability, on a panel of reference equine sera, sampled during French WNV outbreaks, while the IgM ELISA protocol did not change.

For French equids, to improve the passive surveillance, in 2004 an experimental integrated electronic system was developed based on the department's existing infrastructure for secure web-based and standardized electronic health information interchange with sentinel veterinary practitioners. This original system was called 'Equine Diseases Information System' and 2 years after was included in a computerized veterinary system allowing practitioners to report all neurological syndromes throughout the country (called 'Réseau d'épidémiosurveillance en pathologie equine (RESPE)'; http://www.respe.net/). Suspicions of WNF were retained after evaluation by a college of experts in equine neurology of the RESPE. Physical description, horse name, microchip number, name and address of owner, geographical location and environment in which the equid lived were retained.

Animals

Clinical presentation of WNF in European countries: To compare the clinical presentation of WNF in three European countries, three previously published datasets were used, which included raw clinical data of 14 Italian confirmed cases from 1998 (Cantile et al., 2000), 39 French confirmed or probable cases from 2004 (Leblond et al., 2007a) and 20 Hungarian confirmed cases from 2007 to 2008.[1]

French retrospective study: For the retrospective study, equine data from the French epizootic of 2004 were collected (39 cases and 61 controls). Data from suspected but infirmed cases from 2008 (i.e., 1 year after a phase test of the national network RESPE) were also included (39 other controls) to investigate the possible differences in the recruitment of suspected cases in a non-epizootic period.

Classification and regression tree analysis: Similarly to the retrospective study, the French data were analysed with CART. The first analyses were performed on all the French data available (2004 and 2008; $N = 139$). A CART analysis was further performed on data from the epizootic of 2004 exclusively ($N = 100$).

Clinical findings and laboratory analyses

The description (breed, age, sex), date of observation of initial clinical signs, and all clinical signs reported in the history and during physical examination were retained (Table 1). In addition, for clinical data, a value of 1 was attributed for each observed clinical sign, and the score of an individual animal is defined as the sum of all clinical signs present. The results were coming from certified

Table 1. Frequency of clinical signs in cases of West Nile fever in three different European countries ($N = 73$)

	Frequency of occurrence of clinical findings							
	France (2004)[a]		Italy (1998)[b]		Hungary (2008)[c]		Total	
Clinical characteristic	*N*[d]	%	*N*	%	*N*	%	*N*	%
Ataxia or incoordination	25	64	12	86*	10	50	47	64*
Muscular tremor, myoclonia and/or tetany	13	33	8	57	11	55	32	44
Paresis	12	31†	9	64†	6	30	27	37†
Hyperthermia	15	38‡	2	14	2	10	19	26‡
Behaviour disorders	10	26	1	7	7	35	18	25
Prostration	9	23	3	21	6	30	18	25
Weakness	2	5†‡	9	64†*	5	25	16	22‡†*
Paralysis	5	13†	7	50†*	2	10	14	19†*
Cranial nerves deficit	6	15	1	7	5	25	12	16
Recumbency	1	3‡	3	21	8	40	12	16‡
Hyperaesthesia	7	18	1	7	1	5	9	12
Anorexia	3	8	1	7	1	5	5	7
Hypermetria	0	0	0	0	2	10	2	3

[a]39 clinical cases of West Nile Fever (WNF).
[b]14 clinical cases of WNF.
[c]20 clinical cases of WNF.
[d]Number of cases showing the clinical sign.
*Significant difference between Italy and Hungary.
†Significant difference between France and Italy.
‡Significant difference between France and Hungary.

national laboratories and were recorded in a unique file for each equid.

Comparative statistical analyses

The statistical data analysis was conducted using chi-square tests, and the tendency of each parameter to become a risk/protector factor was evaluated by odds ratios (OR) with 95% confidence interval (CI). The frequency of occurrence of each clinical sign and the month of occurrence of WNF were compared in pairs with a Fisher's exact test. Comparisons of clinical scores between countries were performed using a two-sample Wilcoxon rank sum (Mann-Whitney) test assuming an unequal variance and non-normal data distribution. The clinical score of each animal was also compared with its level of WNV-IgM and WNV-IgG antibodies using Spearman's rank correlation, noted 'r_s' in the text. The statistical significance of the tests was set at a level of $P \leq 0.05$ (Petrie and Watson, 2006).

Classification and regression tree analysis

A CART analysis was conducted on the data set, where the presence or absence of West Nile infection determined by laboratory analyses was used as the dependent variable and the animal description (breed, age, sex), and clinical ($n = 13$; Table 1), as well as spatio-temporal (month of occurrence and geographical location), data were used as independent or predictor variables. A CART analysis is a non-linear and non-parametric model that is fitted by binary recursive partitioning of multidimensional covariate space (Breiman et al., 1984; Saegerman et al., 2004; Speybroeck et al., 2004). Using CART 6.0 software (Salford Systems, San Diego, CA, USA), the analysis successively splits the dataset into increasingly homogeneous subsets until it is stratified and meets the specified criteria. The Gini index was used as the splitting method, and 10-fold cross-validation was used to test the predictive capacity of the obtained trees. Classification and regression tree performs cross-validation by growing maximal trees on subsets of data and then calculating error rates based on unused portions of the data set. To accomplish this, CART divides the data set into 10 randomly selected and roughly equal parts, with each 'part' containing a similar distribution of data from the populations of interest (i.e., confirmed versus infirmed WNF suspects). Classification and regression tree then uses the first nine parts of the data, constructs the largest possible tree and uses the remaining 1/10 of the data to obtain initial estimates of the error rate of the selected subtree. The process is repeated using different combinations of the remaining nine subsets of data and a different 1/10 data subset to test the resulting tree. This process is repeated until each 1/10 subset of the data has been used as to test a tree that was grown using a 9/10 data sub set. The results of the 10 mini-tests are then combined to calculate error rates for trees of each possible size; these error rates are applied to prune the tree grown using the entire data set. The consequence of this complex process is a set of fairly reliable estimates of the independent predictive accuracy of the tree, even when some of the data for independent variables are incomplete and/or comparatively small. For each node in a CART generated tree, the 'primary splitter' is the variable that best splits the node, maximizing the purity of the resulting nodes. When the primary splitting variable is missing for an individual observation, that observation is not discarded but, instead, a surrogate splitting variable is sought. A surrogate splitter is a variable which pattern within the dataset, relative to the outcome variable and is similar to the primary splitter. Thus, the program uses the best *available* information in the face of missing values. In datasets of reasonable quality, this allows all observations to be used. This is a significant advantage of this methodology over more traditional multivariate regression modelling, in which observations which are missing *any* of the predictor variables are often discarded. Further details about CART are presented in previously published articles (e.g., Saegerman et al., 2004; Speybroeck et al., 2004).

In this study, four different analyses were performed. The first (I) and third (III) analyses were performed on all the French data available without distinguishing the year of occurrence of the suspected cases. The second (II) and fourth (IV) analyses only took the French data from 2004 into account. Analyses I and II only used the clinical signs for their tests, whereas analyses III and IV included all independent variables.

Results

Clinical presentation of WNF in European countries

French, Italian and Hungarian WNF case data were summarized and studied to determine the most frequently presented clinical signs for each subdataset (Table 1). The occurrence of paresis, weakness and paralysis was more frequently observed in Italian WNF cases than in French cases (Fisher's exact test; $P < 0.03$). The occurrence of hyperthermia was more frequently reported in France (Fisher's exact test; $P = 0.02$), whereas weakness and recumbency were more frequently reported in Hungary (Fisher's exact test; $P < 0.04$). Finally, ataxia, weakness and paralysis were significantly more frequently reported

in Italian cases than in Hungarian cases (Fisher's exact test; $P < 0.04$).

The score of clinical signs was significantly higher in Italy than in France (Wilcoxon rank sum test; $P = 0.03$) but non-significantly different between Italy and Hungary or between France and Hungary, respectively (Wilcoxon rank sum test; $P > 0.30$) (Fig. 1). Considering equine data from the French epizootic of 2004 for which quantitative serological data were available, the clinical score seems to be inversely proportional to the level of WNV-IgM antibodies ($r_s = -0.38$; $P = 0.04$) but not to the level of WNV-IgG ($r_s = 0.26$; $P = 0.18$).

In addition, the delay between observation of initial clinical signs and veterinary consultation was significantly longer for Italian cases in comparison with French cases (Wilcoxon rank sum test; $P = 0.006$) (Fig. 2). The month of occurrence of WNF was comparable in the three countries (Fisher's exact; $P = 0.83$). In each country, the peak of occurrence of WNF was observed during the month of September (Fig. 3). Hungary was the only country presenting a WNF case in November.

Of the 14 Italian horses, two died of a natural death and four were euthanized humanly. The eight other horses recovered. Among the Hungarian WNF cases, seven died, 10 recovered and three were lost for follow-up. Finally, seven horses died out of the French WNF cases, 31 survived and two were lost for follow-up. However, the mortality rate (including natural death and euthanized on human grounds) was not significantly different between France (82%), Italy (63%) and Hungary (53%) (Fisher's exact; $P = 0.09$).

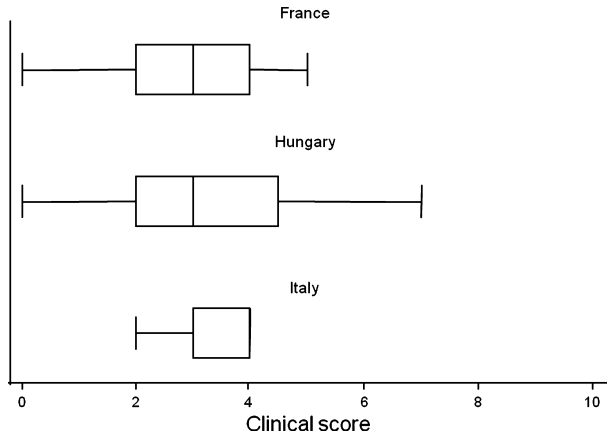


Fig. 1. Comparison of the clinical score for each country respectively. The graph shows the clinical score distribution; vertical line in the rectangle is the median score; right and left limits of the rectangle were the first and third quartiles; right and left limits of the Boxplot were the minimum and the maximum values; abscissa: clinical score (=sum of each presented clinical sign, each individual clinical sign presented having a value of 1); ordinate: each individual country.

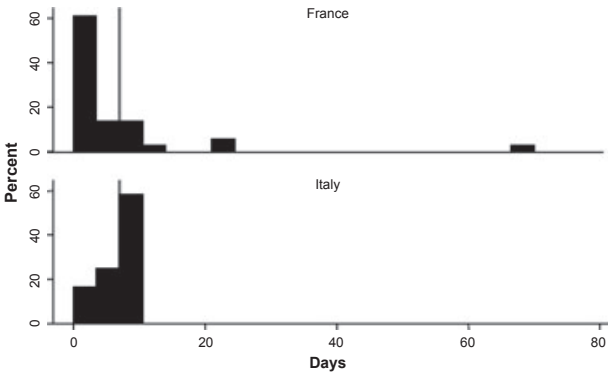


Fig. 2. Delay between observation of initial clinical signs and veterinary consultation in France and Italy. Abscissa: days; ordinate: percentage of confirmed West Nile Fever cases; vertical line: 7 days.

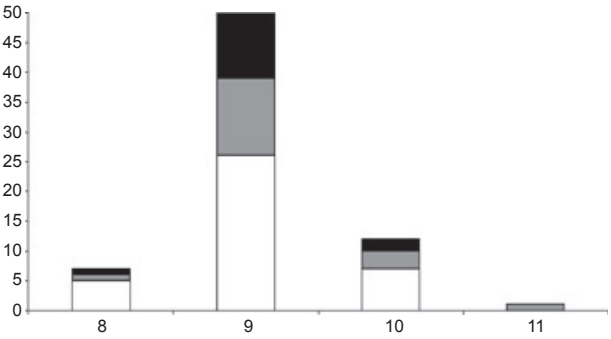


Fig. 3. Month of West Nile fever clinical appearance in France, Hungary and Italy. Abscissa: month; ordinate: number of West Nile Fever confirmed cases; data from France in white; data from Hungary in grey; data from Italy in black.

Retrospective French study

Considering equine data from the French epizootic of 2004, no clinical sign was found to be significantly associated with WNF. However, living in the location 'Saintes Maries de la Mer' with postal code 13 460 significantly increased the risk of WNF ($\chi^2 = 14.85$ with $P < 0.001$; OR = 10.67 with 95% CI: 2.76–41.17). In addition, during the non-epizootic period, the presence of cranial nerve deficit was found to significantly decrease the probability of the horses having WNF ($\chi^2 = 4.55$ with $P = 0.03$; OR = 0.34 with 95% CI: 0.12–0.94). No breed, age or gender effects were observed.

Classification and regression tree analyses

In CART analysis I (only clinical signs without distinguishing the year of occurrence of the suspected cases) (Data S1), paresis, weakness and recumbency were the

Table 2. Power of the different clinical signs splitters obtained after CART analysis (maximum score = 100)

CART I		CART II	
Predictor variable	Score	Predictor variable	Score
Paresis	100.00	Hyperesthesia	100.00
Weakness	81.37	Recumbency	93.60
Recumbency	75.45	Anorexia	81.41
Hyperthermia	63.59	Prostration	58.88
Paralysis	61.61	Cranial nerve deficit	57.87
Prostration	60.27	Hyperthermia	52.13
Cranial nerve deficit	46.13	Trembling, tetany, fasciculations	51.05
Trembling, tetany, fasciculations	42.15	Paralysis	49.65
Change in behaviour	40.81	Paresis	39.14
Anorexia	34.64	Change in behaviour	32.60
Hyperesthesia	34.27	Weakness	25.80
Ataxia	13.05	Ataxia	17.76

CART I, Classification and regression tree analysis that takes into account all the French suspected cases from 2004 and 2008 (N = 139; sensitivity = 82.1%; specificity = 78.0%); CART II, Classification and regression tree analysis that takes into account French suspected cases from 2004 epizootic exclusively (N = 100; sensitivity = 74.4%; specificity = 88.5%).

three most important predictors (or splitters). The sensitivity and the specificity of this tree were of 82.1% and 78%, respectively. However, in analysis II (only clinical signs in 2004 for the suspected cases) (Data S2), hyperesthesia, recumbency and anorexia were the most important. The sensitivity and the specificity of this tree were of 74.4% and 88.5%, respectively. Moreover, analysis III (all independent variables without distinguishing the year of occurrence of the suspected cases) reported an increase in specific anti-WNV IgM, postal code and month as major splitters. Analysis IV (all independent variables in 2004 for the suspected cases) reported positive anti-WNV IgM and anti-WNV-IgG serology assays and then postal code and month as major splitters. The importance of each splitter in their respective CART is reported in Table 2 for analyses I and II. The first splitter for CART III and CART IV confirms the case definition of WNF (circular process), but the second and the third splitters are of main interest in time-space risk mapping.

Discussion

Clinical detection of WNF cases in horses is interesting as a public health perspective because of their higher susceptibility to develop neurological clinical signs compared to humans. For this reason, every neurological syndrome observed in a horse was suspected of West Nile infection and was mandatorily to be declared to the health authorities by the attending veterinarian. However, clinical presentation of WNF is known to vary from one equid to another and during the course of illness. Indeed, some clinical signs are only observed at the initial stages and disappear as disease progresses, requiring great awareness of the owner/veterinarian during that period for their detection. Other clinical signs only appear during the final stages of the disease.

Hyperthermia potentially appears at different phases of the disease (3–6 days post-inoculation and second, in neurological animals, 5–22 days post-inoculation) (Cantile et al., 2001). However, hyperthermia was only frequently reported in French WNF cases. This may be because of a more attentive observation by horse owners in France than in Italy and Hungary, allowing detection of the initial hyperthermic phase, and to the shorter delay between initial clinical signs and veterinary consultation.

In a first step, the countries were compared in pairs. Paresis, weakness and paralysis were significantly more frequently observed in Italian WNF cases than in French cases. Between France and Hungary, hyperthermia was more frequently reported in France, whereas weakness and recumbency were more frequently reported in Hungary. These two latter results could be explained by lack of awareness of Italian and Hungarian horse owners at the time of this study: unaware of WNF circulation and of its clinical presentation, the owners/veterinarians could be less attentive to the more subtle clinical signs; severe clinical signs being more frequently reported. On the other hand, French owners living in endemic areas could be more attentive and seek prodromic clinical signs.

Finally, ataxia, weakness and paralysis were significantly more frequently reported in Italian than in Hungarian cases. This could be because of a closer observation and thus earlier detection in Hungary compared to Italy, as horses are in closer contact with humans in rural areas (working horses versus sport and leisure horses). Another hypothesis is that the differences observed in this compar-

ative study are because of differences in viral strains, each viral strain potentially having a different pathogenicity. Several scientific studies support the latter hypothesis (Cecilia and Gould, 1991; Halevy et al., 1994; Chambers et al., 1998). In a mouse model, Beasley et al. (2002) isolated groups of viruses in lineage 1 and lineage 2 with variable neuroinvasive potentials. According to the authors, their neuroinvasive potential was found not to be influenced by the source of the virus (mosquito, mammal, bird) or by its passage history. Individual factors of receptivity are also thought to be of major importance for the evolution and clinical expression of WNF (Joubert et al., 1971; Beasley et al., 2002). Indeed, several authors (Glass et al., 2005; Brault et al., 2007; Lim et al., 2009) demonstrated that variations in certain loci of the host's genome influenced susceptibility to and clinical presentation of WNV infection. Furthermore, the presence of antibodies against other flaviviruses is thought to play a role in determining clinical presentation in certain areas (e.g., Africa or Latin America) (Beasley et al., 2002). Our results are not in contradiction with literature, ataxia, recumbency, paresis and paralysis being considered as 'classic signs' of WNF (Epp et al., 2007a).

The delay between observation of initial clinical signs and veterinary consultation was significantly different between Italian and French cases. This difference is thought to be because of a lack of awareness of disease by Italian horse owners and to the absence of a centralized epidemiological surveillance system at the time of the outbreak in 1998. Indeed, an efficient surveillance system could permit registration of suspected and confirmed cases of WNF in a standardized manner. The peak of occurrence of WNF cases in the three countries was observed during September. This result is in accordance with previous studies on WNF and vector activity in temperate climates (Murgue et al., 2001; Zeller and Schuffenecker, 2004; Ludwig et al., 2010). Indeed, the study by Ludwig et al. (2010) performed on American crow populations demonstrated that time of year was a significant risk factor and that end of summer and autumn were associated with a higher mortality rate owing to WNF. Therefore, awareness must be increased before and during this period to allow early detection of WNV circulation. Hungary reported a case occurring in November. It would be interesting in the future, with the development of a centralized epidemiological surveillance system increasing the number of reported cases, to investigate if this isolated case is just a result of late clinical detection or if it is because of persistence of vector activity in the environment (climate change) or a false positive.

In the retrospective study, geographical location was found to be a significant risk factor. This result is in agreement with the previous studies (Epp et al., 2007a; Leblond et al., 2007b; Pradier et al., 2008; Soverow et al., 2009). In accordance with other retrospective studies (Cantile et al., 2001; Trock et al., 2001; Leblond et al., 2007a), no breed, age, gender or clinical signs (despite the fact that only clinical signs most frequently reported in WNF equine cases were included) were found to be significantly associated with WNF. Furthermore, proximity to host populations could be considered as a potential risk factor in future studies. In America, proximity of humans to colonies of white pelicans has been studied (Johnson et al., 2010). West Nile Fever virus-associated deaths in pelicans were found to increase by 5-fold the probability of neurological human WNF cases (Johnson et al., 2010). In Europe, proximity to large populations of magpies, crows, sparrows or any other avian species could be a risk factor (Jourdain et al., 2008; Calistri et al., 2010). Indeed, magpies were found to be seropositive in a study by Jourdain et al. (2008) and could thus serve as amplifying hosts.

The CART I and II analyses reported different clinical predictors depending on the inclusion (CART I) or not (CART II) of WNF suspected but not confirmed cases of 2008. Moreover, the sensitivity of the tree was more important for CART I in comparison with CART II, and the reverse was observed for the specificity. This observation could suggest the influence of different recruitment of suspected cases by owners/veterinarians during epizootic (CART II) or non-epizootic (CART I) periods. This observation stresses the necessity of regular awareness campaigns among owners and veterinarians. In accordance with the results of the retrospective study, the results of the CART analyses demonstrate that in a case of emergence of WNF, clinical suspicions are the first and essential step towards diagnosis, but confirmation by serology remains necessary. Indeed, CART III and IV emphasized the importance of specific diagnostic serological assays (case definition) to confirm a clinical suspicion and of time and place of occurrence of the suspected WNF case. This result is in agreement with the retrospective study, as well as with the study by Leblond et al. (2007a) and by Ward (2005).

Conclusion

Despite several previous studies on the subject, WNF remains a challenging disease and an important veterinary public health issue. Awareness of its potential emergence must be promoted. A centralized passive animal surveillance system reporting WNF and suspected WNF cases, in a standardized manner, must be organized in different countries to permit an early detection of its emergence. Communication between countries and between veteri-

nary and public authorities is essential for an efficient control of WNF in the currently unstable epidemiological situation. Spatio-temporal modelling could be of significant help for assessing risk of emergence and detecting high risk areas to allow active surveillance.

Acknowledgements

Dr Porter is a doctoral student of the Research Unit of Epidemiology and Risk Analysis applied to veterinary sciences (UREAR), Faculty of Veterinary Medicine, University of Liege. Her interests include field clinical epidemiology of emerging infectious diseases and rare events in cattle and horses. The first and last authors wish to thank in particular Prof. Leblond, Dr Lecollinet and Dr Zientara for their contribution to the French data of this study and for their persistent availability and motivation. They also wish to thank Dr Carlo Cantile and Dr Kutasi Orsolya for their essential contribution to data of this study and without whom the comparative clinical study would have been impossible. There are no potential conflicts of interest for any of the authors. This research was funded by the 'Fonds spéciaux pour la Recherche – Crédit de démarrage' (contrat D-08/02), University of Liège, Belgium.

Notes

[1]Kutasi O., Biksi I., Bakonyi T., Ferency E., Lecollinet S., Bahuon C., Zientara S., Szenci O., Equine encephalomyelitis outbreak caused by a genetic lineage 2 West Nile virus in Hungary. Annual Convention of the American Association of Equine Practitioners Proceedings, 2009, pp. 179.

References

Autorino, G.L., A. Battisti, V. Deubel, G. Ferrari, R. Forletta, A. Giovannini, R. Lelli, S. Murri, and M.T. Scicluna, 2002: West Nile Virus epidemic in horses, Tuscany Region, Italy. *Emerg. Infect. Dis.* 8, 1372–1378.

Beasley, D.W.C., M.T. Li Li Suderman, and A.D.T. Barrett, 2002: Mouse neuroinvasive phenotype of West Nile virus strains varies depending upon virus genotype. *Virology* 296, 17–23.

Blitvich, B.J., 2008: Transmission dynamics and changing epidemiology of West Nile virus. *Anim. Health Res. Rev.* 9, 71–86.

Brault, A.C., C.Y. Huang, S.A. Langevin, R.M. Kinney, R.A. Bowen, W.N. Ramey, N.A. Panella, E.C. Holmes, A.M. Powers, and B.R. Miller, 2007: A single positively selected West Nile mutation confers increased virogenesis in American crows. *Nat. Genet.* 39, 1162–1166.

Breiman, L., J.H. Friedman, R.A. Olshen, and C.J. Stone, 1984: *Classification and Regression Trees*. Wadsworth S, Pacific Grove, CA, USA.

Calistri, P., A. Giovannini, G. Savini, F. Monaco, L. Bonfanti, C. Ceolin, C. Terregino, M. Tamba, P. Cordioli, and R. Lelli, 2010: West Nile virus transmission in 2008 in north-eastern Italy. *Zoonoses Public Health* 57, 211–219.

Cantile, C., G. Di Guardo, C. Eleni, and M. Arispici, 2000: Clinical and neuropathological features of West Nile virus equine encephalomyelitis in Italy. *Equine Vet. J.* 32, 31–35.

Cantile, C., F. Del Piero, G. Di Guardo, and M. Arispici, 2001: Pathologic and immunohistochemical findings in naturally occurring West Nile virus infection in horses. *Vet. Pathol.* 38, 414–421.

Castillo-Olivares, J., and J. Wood, 2004: West Nile infection of horses. *Vet. Res.* 35, 467–483.

Cecilia, D., and E.A. Gould, 1991: Nucleotide changes responsible for loss of neuroinvasiveness in Japanese encephalitis virus neutralization-resistant mutants. *Virology* 181, 70–77.

Chambers, T.J., M. Halevy, A. Nestorowicz, C.M. Rice, and S. Lustig, 1998: West Nile virus envelope proteins: nucleotide sequence analysis of strains differing in mouse neuroinvasiveness. *J. Gen. Virol.* 79, 2375–2380.

Dauphin, G., and S. Zientara, 2007: West Nile virus: recent trends in diagnosis and vaccine development. *Vaccine* 25, 5563–5576.

Dauphin, G., S. Zientara, H. Zeller, and B. Murgue, 2004: West Nile: worldwide current situation in animals and humans. *Comp. Immunol. Microbiol. Infect. Dis.* 27, 343–355.

Epp, T., C. Waldner, F.A. Leighton, O. Berke, and H.G. Townsend, 2007a: Seroprevalence and risk factors for infection with West Nile virus in Saskatchewan, 2003. *Can. J. Vet. Res.* 71, 256–263.

Epp, T., C. Waldner, K. West, and H. Townsend, 2007b: Factors associated with West Nile virus disease fatalities in horses. *Can. Vet. J.* 48, 1137–1145.

Glass, W.G., L.K. Lim, R. Cholera, A.G. Pletnev, J.L. Gao, and P. Murphy, 2005: Chemokine receptor CCR5 promotes leukocyte trafficking to the brain and survival in West Nile infection. *J. Exp. Med.* 202, 1087–1098.

Halevy, M., Y. Akov, D. Ben-Nathan, D. Kobiler, B. Lachmi, and S. Lustig, 1994: Loss of active neuroinvasiveness in attenuated strains of West Nile virus: pathogenicity in immunocompetent and SCID mice. *Arch. Virol.* 137, 355–370.

Johnson, G., N. Nemeth, K. Hale, N. Lindsey, N. Panella, and N. Komar, 2010: Surveillance for West Nile virus in American white pelicans, Montana, USA, 2006–2007. *Emerg. Infect. Dis.* 16, 406–411.

Joubert, L., J. Oudar, C. Hannoun, and M. Chippaux III, 1971: Relations entre la virologie, la sérologie et l'évolution anatomo-clinique. Conséquences épidémiologiques et prophylactiques. In: Joubert, L., J. Oudar, C. Hannoun, and M. Chippaux (eds), *Reproduction expérimentale de la*

méningo-encéphalomyélite du cheval par l'arbovirus West Nile, France. Bull. Acad. Vet. Fr. 44, 159–167.

Jourdain, E., M. Gauthier-Clerc, P. Sabatier, O. Grége, T. Greenland, A. Leblond, M. Lafaye, and H.G. Zeller, 2008: Magpies as hosts for West Nile virus, Southern France. *Emerg. Infect. Dis.* 14, 158–160.

Leblond, A., P. Hendrikx, and P. Sabatier, 2007a: West Nile outbreak detection using syndromic monitoring in horses. *Vector Borne Zoonotic Dis.* 7, 403–410.

Leblond, A., A. Sandoz, G. Lefebvre, H. Zeller, and D.J. Bicout, 2007b: Remote sensing based identification of environmental risk factors associated with West Nile disease in horses in Camargue, France. *Prev. Vet. Med.* 79, 20–31.

Lim, J.K., A. Lisco, D.H. McDermott, L. Huynh, J.M. Ward, H. Johnson, J. Paper, G.A. Foster, D. Krysztof, D. Follmann, S.L. Stramer, L.B. Margolis, and P.M. Murphy, 2009: Genetic variation in OAS1 is a risk factor for initial infection with West Nile virus in man. *PLoS Pathog.* 5, e1000321.

Ludwig, A., M. Bigras-Poulin, P. Michel, and D. Bélanger, 2010: Risk factors associated with West Nile virus mortality in American crow populations in Southern Quebec. *J. Wildl. Dis.* 46, 195–208.

Murgue, B., S. Murri, S. Zientara, B. Durand, J.P. Durand, and H. Zeller, 2001: West Nile outbreak in horses in Southern France, 2000: the return after 35 years. *Emerg. Infect. Dis.* 7, 692–696.

Ostlund, E.N., J.E. Andresen, and M. Andresen, 2000: West Nile encephalitis. *Vet. Clin. North Am. Equine Pract.* 16, 427–441.

Petersen, L.R., and J.T. Roehrig, 2001: West Nile Virus: a reemerging global pathogen. *Emerg. Infect. Dis.* 7, 611–614.

Petrie, A., and P. Watson, 2006: *Statistics for Veterinary and Animal Science*, 2nd edn. Blackwell Science Ltd., Oxford.

Pradier, S., A. Leblond, and B. Durand, 2008: Land cover, landscape structure, and West Nile virus circulation in Southern France. *Vector Borne Zoonotic Dis.* 8, 253–263.

Saegerman, C., N. Speybroek, S. Roels, E. Vanopdenbosch, E. Thiry, and D. Berkvens, 2004: Decision support tools for clinical diagnosis of disease in cows with suspected bovine spongiform encephalopathy. *J. Clin. Microbiol.* 42, 172–178.

Sebastain, M.M., I. Stewart, N.M. Williams, K.B. Poonacha, S.F. Sellls, M.L. Vickers, and L.R. Harrison, 2008: Pathological, entomological, avian and meteorological investigation of a West Nile epidemic in a horse farm. *Transbound. Emerg. Dis.* 55, 134–139.

Smithburn, K.C., T.P. Hughes, A.W. Burke, and J.H. Paul, 1940: A neurotropic virus isolated from the blood of a native of Uganda. *Am. J. Trop. Med.* 20, 471–492.

Soverow, J.E., G.A. Wellenius, D.N. Fisman, and M.A. Mittleman, 2009: Infectious diseases in a warming world: how weather influenced West Nile virus in the United States (2001–2005). *Environ. Health Perspect.* 117, 1049–1052.

Speybroeck, N., D. Berkvens, A. Mfoukou-Ntsakala, M. Aerts, N. Hens, G. Van Huylenbroeck, and E. Thys, 2004: Classification trees versus multinomial models in the analysis of urban farming systems in Central Africa. *Agric. Syst.* 80, 133–149.

Trock, S.C., B.J. Meade, A.L. Glaser, E.N. Ostlund, R.S. Lanciotti, B.C. Cropp, V. Kulasekera, L.D. Kramer, and N. Komar, 2001: West Nile outbreak among horses in New York State, 1999 and 2000. *Emerg. Infect. Dis.* 7, 745–747.

Ward, M., 2005: Epidemic West Nile virus encephalomyelitis: a temperature-dependent, spatial model of disease dynamics. *Prev. Vet. Med.* 71, 253–264.

Weese, J.S., J.D. Baird, J. Delay, D.G. Kenney, H.R. Staempfli, L. Viel, J. Parent, L. Smith-Maxie, and R. Poma, 2003: West Nile virus encephalomyelitis in horses in Ontario: 28 cases. *Can. Vet. J.* 44, 469–473.

Zeller, H.G., and I. Schuffenecker, 2004: West Nile Virus: an overview of its spread in Europe and the Mediterranean Bassin in contrast to its spread in Americas. *Eur. J. Clin. Microbiol. Infect. Dis.* 23, 147–156.

Supporting Information

Additional supporting information may be found in the online version of this article:

Data S1. Classification and regression tree of the clinical signs for West Nile fever without distinguishing the year of occurrence of the suspected cases (N = 139; Sensitivity = 82.1% and Specificity = 78%).

Data S2. Classification and regression tree (CART) of the clinical signs for West Nile fever including suspected cases from 2004 only [In CART analysis I (only clinical signs without distinguishing the year of occurrence of the suspected cases) (N = 100; Sensitivity = 74.4% and Specificity = 88.5%)].

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

PORTER S.R., VERWILGEN D., GANIERE J.P., ZICOLA A., LOSSON B., CZAPLICKI G., SAEGERMAN C. Study on the seroprevalence of Q fever in cats, equids, pigs and wild boars in western Europe.

Vet J. Submitted.

Q fever

Preface

WNF has demonstrated the limits of clinical diagnosis when confronted with emerging diseases that can have an asymptomatic or non specific clinical presentation. Q fever is a typical example of an emerging, potentially asymptomatic, disease. The recent awareness and the lack of scientific knowledge on the subject make it an ideal choice for our study. After presenting the global epidemiological context of Q fever worldwide, the prevalence of infection in Belgian and Dutch horses, Belgian and French felines, Belgian pigs and wild boars was estimated. The epidemiological and zoonotic role of these species has not been investigated previously. Limitations of a purely clinical approach render laboratory analyses indispensable. The prevalence of Q fever was estimated by enzyme-linked immunosorbent assay (ELISA) and then confirmed by immunofluorescence assay (IFA). Our results are presented in the following article.

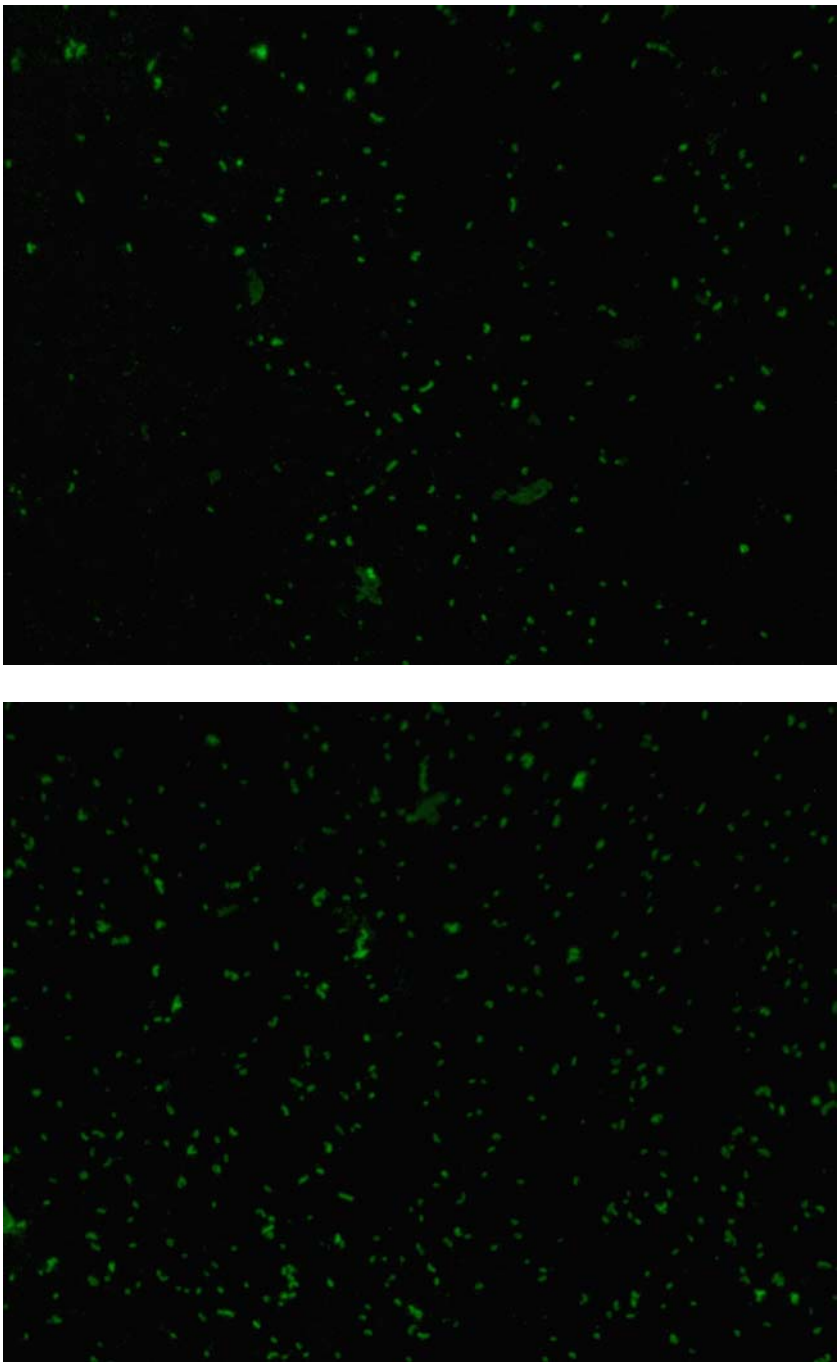


Figure 11. Isolation of *C. burnetii* by IFA in two different cats from a rescue center

Study on the seroprevalence of Q fever in cats, equids, pigs and wild boars in Western Europe

Sarah R. Porter[1], Guy Czaplicki[2], Christophe Manteca[3], Denis Verwilgen[4], Jean-Pierre Ganière[5], Sandra Jolly [6], Angélique Zicola[7], Bertrand Losson[8], Claude Saegerman[1*]

1. University of Liège, Faculty of Veterinary Medicine, Department of Infectious and Parasitic Diseases, Research Unit in Epidemiology and Risk Analysis applied to Veterinary Sciences (UREAR), Boulevard de Colonster 20, B42, 4000 Liège, Belgium

2. Association Régionale de Santé et d'Identification Animales (ARSIA), Avenue A. Deponthière 40, 4431 Loncin, Belgium

3. CEVA Animal Health, La Ballastière, BP 126-F33501, Libourne, France

4. University Large Animal Hospital, Swedish University of Agricultural Sciences, Uppsala, Sweden

5. Nantes-Atlantic National College of Veterinary Medicine, Food and Science and Engineering (ONIRIS), Unité mixte 1300 Bio-agression, Epidémiologie et Analyses de Risques en Santé Animale, Atlanpole-Chautrerie, BP 40706, 44307 Nantes cedex 03, France

6. University of Liège, Faculty of Veterinary Medicine, Department of Morphology and Pathology, Boulevard de Colonster 20, B43, 4000 Liège, Belgium

7. University of Liège, Faculty of Veterinary Medicine, Department of Infectious and Parasitic Diseases, Virology and Pathogenesis of Viral Diseases, B43, Boulevard de Colonster 20, 4000 Liège, Belgium

8. University of Liège, Faculty of Veterinary Medicine, Department of Infectious and Parasitic Diseases, Parasitology and Parasitic Diseases, B43, Boulevard de Colonster 20, 4000 Liège, Belgium

* Corresponding author. Tel.: +32 366 45 79; fax: +32 366 42 61

Email address: claude.saegerman@ulg.ac.be (Claude Saegerman)

Abstract

Several human Q fever cases associated with infected cats have been reported. Moreover, *Coxiella burnetii* is known to infect numerous hosts. This study investigates Q fever seroprevalence in felines, equids, pigs and wild boars, and then compares enzyme-linked immunosorbent assay (ELISA) protein G, ELISA protein A/G and immunofluorescent assay (IFA) phase II. Sera from domestic, stray, and rescue center cats (N=318), horses (N=344), pigs (N=264) and wild boars (N=272) were tested by ELISA Prot G and IFA. Moreover, feline sera (N=247) were tested by ELISA ProtA/G. The seroprevalence in the feline subgroups were compared by Chi2 test. Distributions of S/P ratio were compared between species by a two-sample Mann-Whitney test. A Bonferroni correction was applied for multiple comparisons. Statistical significance was defined as $P < 0.05$/k, with k being the number of comparisons made. All animals were negative by ELISA Prot G. Several cats were IFA positive at dilution 1:40, whereas, only one horse was positive. No pig or wild boar was IFA positive. A significant difference in IFA seroprevalence was observed between domestic and stray cats (P=0.007). In conclusion, felines, especially strays, represent a potential zoonotic risk. Reasons for the difference in seroprevalence between species and felines remains to be elucidated.

Keywords: Q fever; *Coxiella burnetii*; Diagnostic; Seroprevalence; Uninvestigated species

Introduction

Q fever is a ubiquitous neglected zoonosis caused by a resistant intracellular bacterium, *Coxiella burnetii* (Derrick, 1937; Mitscherlich and Marth, 1984; Babudieri, 1959; Ormsbee et al., 1978). Domestic ruminants are considered the main reservoir for the pathogen (Lang, 1990; Guatteo et al., 2007; Vaidya et al., 2010). However Q fever is known to infect a large variety of hosts (Marmion and Stoker, 1950; Davoli and Signorini, 1951; Slavin, 1952; Marmion, 1954; Stocker and Marmion, 1955; Blanc and Bruneau, 1956; Evans, 1956; Syrucek and Raska, 1956; Fiset, 1957; Hirai and To, 1998). Inhalation is the main route of animal and human infection (Welsh et al., 1957; Tissot-Dupont et al., 1999; Russell-Lodrigue et al., 2006). Epidemiological studies on Q fever in animals have found that cats can be infected by *C. burnetii* (Marrie et al., 1985; Higgins & Marrie, 1990; Pinsky et al., 1991; Mattewman et al., 1997). Moreover, in 1952, Gillepsie and Baker performed successful feline experimental infections by subcutaneous inoculation, feeding infected yolk sacs and by contact with infected cats. Despite the presence of pathogen in blood, urine and a serological response, clinical signs were not observed in all infected cats. In felines, Q fever is, seemingly, frequently asymptomatic and remains undiagnosed. However, infected cats excrete *C. burnetii* in the environment and are a potential source of human infections. Indeed, human Q fever cases associated to infected cats have been reported previously (Marrie et al., 1989; Langley et al., 1988; Marrie et al., 1988). In Europe, the role played by cats in Q fever transmission remains poorly investigated. Moreover, Q fever has rarely been investigated in equids, pigs and wild boars despite some contacts with infected domestic ruminants.

The aim of this study was to investigate Q fever-seroprevalence in western European cats, equids, pigs and wild boars, allowing, in this way, an estimation of their zoonotic importance. Finally, the authors suggest cut-off values for the enzyme-linked immunosorbent assay (ELISA) kits used in this study.

Materials and Methods

Animals

A total of 30 bovine, 344 equine, 318 feline, 264 swine and 272 wild boar sera were analyzed. The bovine sera were used as a reference to gain the laboratory skills.

Bovines: The bovine sera were obtained from southern Belgian and Luxemburgish cattle between February and October 2010, which had a history of abortion associated or not with a proven *Coxiella* infection.

Felines: The French feline sera (n=130) originated from domestic cats from the Rennes area, France. The cats were sampled between January and November 2004 at two different veterinary clinics; one at Vern sur Seiche, mainly with an urban and suburban feline population and, one at Chateaugiron with a predominant suburban and rural feline population. Belgium feline samples were provided by two different sources. 62 samples were obtained through an eradication of stray cats program started in March 2010, in Ciney. Serological tests were performed to evaluate the feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) status of each animal. Euthanasia was decided based on the latter results or on general condition. Other feline samples (n=126) were provided by animal rescue centers localized in southern Belgium.

Horses: 163 sera originated from horses admitted in private clinic in the Netherlands for arthroscopy from 2007 to 2009.

181 sera originated from Belgian Sport horses admitted to the Veterinary Teaching Hospital of Liège University, Belgium, from 2007 to 2009 for a pre-purchase examination, arthroscopy, or for a complete standard examination before registration in the Belgian Sport Horse Stud Book.

Pigs and wild boars: The wild boar sera were obtained during a previous study performed by Association Régionale de Santé et d'Identification Animales (ARSIA) on classical swine

fever in 2007 (n=272). The wild boars sampled originated from the free roaming southern Belgium population. The pigs were sampled in 2010 and were housed in various farms in southern (n=232) and northern Belgium (n=32).

Conservation of the serum samples

The blood obtained was centrifuged and the serum conditioned in aliquots, identified and frozen. All samples were stored at a temperature of –20 degrees C° before analyses and were thawed the least times possible to prevent any modification of sera quality.

Data retained for each sample

Age, breed, sex, environmental habitat, date of sampling, vaccination status and motif of consultation were retained for each French feline sera. For the Belgian felines from Ciney, age, breed, sex, weight, general condition, environmental habitat (stray or domestic), date of sampling, FIV and FeLV status, necessity of euthanasia or not and its motif were included in the sample data. The data concerning the Belgian blood samples from animal rescues included date of sampling and localization only.

Age, breed, sex, weight, motif of consultation, and date of sampling were retained for every equine sample included in the study.

A large panel of data was available for pig and wild boar sera including identification, herd number, geographical localization and results of previous analyses.

Laboratory analyses

All the laboratory analyses were performed at ARSIA, Loncin, Belgium.

Firstly, an ELISA using a recombinant protein G conjugate (ELISA Prot G) was performed, followed by an ELISA using a recombinant protein A/G conjugate (ELISA ProtA/G). The differences between recombinant proteins A, G, and A/G are summarized in **Table 1**. LSIVET Ruminant Milk/Serum Q fever ELISA kits, licensed by “Institut National de Recherche Agronomique” (INRA), provided by LSI (France), were used.

In ELISA test, the ratio optical density of the sample / optical density of the positive control (S/P) expressed in percent was calculated using the following formula:

$$\left(\frac{ODs - ODnc}{ODpc - ODnc} \right) \quad x \quad 100$$

with "*OD*" for optical density values measured at 450 nm, "*s*" for sample; "*nc*" for negative control and "*pc*" for the positive control supplied by the manufacturer.

Secondly, immunofluorescent assays (IFA) were performed on approximately 30 randomly selected samples from each group. The VMRD* IFA kit and VIRCELL** *Coxiella burnetii* slides were used. Each slide contains 10 wells coated with grown in MRC-5 cells, with *C. burnetii* phase II, Nine Mile strain (ATCC 616-VR), formaldehyde inactivated and acetone fixed. Dilutions from 1:40 to 1:160 were obtained successively by two-fold dilutions. Tests were carried out according to standard procedures and slides were read using a fluorescent light microscope at * 400 magnification. Titers of 1:40 or more were considered positive. This cut-off value was determined following a previous study by Matthewans et al. (1997) on Q fever seroprevalence in felines.

Sera selection for analyses

All samples were tested by ELISA Prot G. Feline samples only (n=247) and 9 bovine sera, as controls, were tested by ELISA ProtA/G. For IFA, due to lack of time and cost of analyses, samples to be tested were selected randomly. The number of samples tested by ELISA and IFA per species is summarized in **Table 2**.

Statistical analyses

The Chi 2 or Fisher's exact test was used to compare seroprevalences in the different feline subgroups. The relation between distributions of the S/P ratio in function of groups was evaluated by pair using two-sample Wilcoxon rank-sum (Mann-Whitney) test. A Bonferroni correction for multiple comparisons was applied. Statistical significance was defined as $P < 0.05/k$, with k is the number of comparisons made (Petrie and Watson, 2006).

As the true infectious status of the animals was unknown, a theoretical cut-off value was proposed based on the distribution of the S/P ratio values of IFA negative sera for each species. The latter distributions were considered normal and the cut-off value was determined as follows: mean + 3 * standard deviation (SD).

Results

The results obtained by ELISA Prot G and ELISA ProtA/G and by IFA are summarized in **Table 2**.

IFA: Only four French felines (17.4%) out of the 24 tested were found to be positive for *C. burnetii* at dilution 1:40. Two and none remained positive at dilution 1:80 and 1:160, respectively. The two feline subgroups from Belgium presented a higher seroprevalence at dilution 1:40: 41.2% (14/34) for the group from rescue centers and 51.4% (18/35) for the group of stray cats. The difference between the two latter subgroups was not significant (Chi2=0.73; 1ddl; *P*=0.39). After pooling both subgroups, 11 and 6 animals remained positive at dilution 1:80 and 1:160, respectively. In addition, a non significant difference occurs between domestic cats and cats originating from rescue centers (Chi2=3.95; 1 ddl; *P*=0.05). However, a significant difference was observed between domestic cats and stray cats (Chi2=7.36; 1 ddl; *P*=0.007) after Bonferroni adjustment.

Exclusively one horse was positive at dilution 1:40 only. No pig or wild boar was positive.

ELISA: The distribution of the S/P ratio for IFA negative sera, in function of ELISA tests and species is presented in **Figure 1.** For ELISA Prot G, except for pigs and wild boars, each species has a significantly different distribution (Wilcoxon rank-sum test; *P*<0.008). Distribution of feline S/P ratio was very different depending on the ELISA test used (Prot G *versus* ProtA/G) (Wilcoxon rank-sum test *P*<0.0001). The theoretical cut-off values per species are summarized in **Table 3**. For cats, the theoretical cut-off value for ELISA Prot G

was lower in comparison with other species. Moreover, the theoretical cut-off value was higher for ELISA ProtA/G than for ELISA Prot G.

Considering each value of S/P ratio above the theoretical cut-off value, the number of positive sera was determined for each group (species) (**Table 2**). Except for abortive bovines (as reference sub-population) and wild boars (only one positive serum with S/P ratio close to the cut-off value), other species were negative for ELISA Prot G. For felines, the use of ELISA ProtA/G permitted to identify 4 and 2 positive sera for rescue and stray cats, respectively. The proportion of positive sera between the two subgroups was not significantly different (Fischer's exact test; P=0.56).

Discussion

This study on Q fever suggests that horses, pigs and wild boars don't represent an important zoonotic hazard for human infections in Western Europe. Felines, however, especially strays, could be a source of human infections.

Currently, farming has become more specialized leading to bigger farms with a higher density of animals of one unique species. Horses and pigs are frequently housed separately from ruminants, in studs, yards or riding clubs and in specialized pig farms. Furthermore, the horses included in this study are predominantly sport horses with generally very little contact, if any, with domestic ruminants.

Wild boars, however, can have indirect contact with ruminants' environment and are frequently infested by ticks. In Northern Spain, *C. burnetii* was detected by PCR in 4.3% of wild boars (Astobiza et al., 2011). The absence of seropositive wild boars in this study could be related to the absence or low rate of infection in ticks. Indeed, in Germany, only 3% of *Dermacentor spp.* were found to be infected despite the study area being endemic for Q fever (Pluta et al., 2010). Further studies on a larger number of Belgian wild boars are necessary.

Among the felines sampled in this study, the subgroup of strays presented a significantly higher seropositivity rate. Free roaming cats can have direct or indirect (contaminated litter) contact with environment, milk, feces, urine, vaginal mucus and birth products of infected ruminants. Thus, greater proximity to the main source of *C. burnetii* could explain their higher seroprevalence compared to horses, pigs and wild boars. Moreover, free roaming cats are more likely to be infected by FIV and FeLV, predisposing those to secondary viral and bacterial infections (Hosie et al., 1989; Fuchs et al., 1994; Bandecchi et al., 2006; Gleich et al., 2009; Skykes, 2010). In this study, in the subgroup of 11 stray cats with IFA positive results, 8 were positive for FIV and/or FeLV (73%). In the subgroup of 23 stray cats with IFA negative results, 11 were positive for FIV and/or FeLV (48%) (data not shown). Strays are more likely to be under fed, unvaccinated, flea, tick and worm ridden, leading to an immunodepressed state. Furthermore, ticks are known reservoirs and vectors of *C. burnetii* and are suspected of having a role in environmental maintenance of Q fever. *Ixodes ricinus*, the main species of tick present in Europe, has low host specificity and readily feeds on small mammals such as cats, dogs and rodents (Parola and Raoult, 2001). Stray animals in the absence of preventive anti-parasitic treatments have an increased likelihood of tick infestation and consequently, of arthropod-borne infection. Furthermore, rodents are preferential hosts for *I. ricinus* larva and occasional hosts for nymphs and adults. A closer and more frequent contact with rodents and rodents' environment could increase the risk of tick bites. However, as mentioned previously, prevalence of Q fever in ticks is apparently low in Western Europe, rendering the true contribution of tick-borne infections to feline seroprevalence minimal. In the United Kingdom, Webster et al. (1995) found that wild brown rats were frequently infected by *C. burnetii* (seroprevalence: 7 to 53%). The authors hypothesized that rats could represent a major reservoir of the organism from which domestic animals may become contaminated. Strays are more likely to have hunted for survival. Thus infection by oral route

must not be discarded, especially as this route was proven effective by Gillepsie and Baker (1952) in their experimental infection. However, currently, controversy on this subject remains. The study by Komiya et al. (2003) reported a higher seroprevalence of Q fever in stray than in domestic cats in Japan. A previous study on dogs also reported a higher seroprevalence in stray than in domestic dogs (Willeberg et al., 1980). These two studies reinforce the hypothesis of increased vulnerability of stray animals due to immunodepression and/or exposure.

In our study one horse only proved to be positive at dilution 1:40. The seropositive horse originated from Mont Saint Aubert, in the province of Hainaut, in southern Belgium. This could be explained by contact or proximity with infected ruminants or by aerosols transported by wind. Testing the other horses on the yard and local farm animals by IFA, as well as meteorological studies to evaluate the main direction of the wind, would allow confirmation of direct or indirect exposure to *C. burnetii*. In previous studies, horses have been found to be seropositive toward Q fever (Willeberg et al., 1980). The difference in seroprevalence observed between studies could be due to a difference in level of exposure to ruminants or to another reservoir of *C. burnetii* (Enright et al., 1971; Riemann et al., 1978).

The results of studies based exclusively on IFA for Q fever diagnosis must be interpreted with care as cross-reactions are known to occur between *C. burnetii*, *Legionella micdadei* and *Bartonella spp.* (La Scola and Raoult, 1996; Musso and Raoult, 1997).

In the study by Chomel et al. (1995), cats from shelters and former strays had a significant higher *Bartonella*-seropositivity rate than the group of pet cats (92% versus 71.40%). Several studies have obtained similar results (Childs et al., 1994; Gurfield et al., 2001; Juvet et al., 2010). However, a study on Danish felines reported no significant difference in seroprevalence between rescue center and stray cats compared to pet cats (46.9% versus 44.2%). In their study, 45.6% of the cats sampled were seropositive (Chomel et al., 2002). In

the Netherlands, stray and pet cats were also found to have the same risk of being seropositive to *Bartonella henselae* (Bergmans et al., 1997). If cross-reactions between *C. burnetii* and *Bartonella* spp. occurred in our study, the *Bartonella*-seroprevalence observed in the domestic cat group would be abnormally low in comparison to previous studies (Chomel et al., 1995; Bergmans et al., 1997; Chomel et al., 2002). However, to confirm our results cross-absorption followed by Western blot analysis is advised. However, this additional analysis was not performed due to insufficient sera volumes.

The study by Collins et al. (1982) demonstrated that horses are frequently seropositive for *L. pneumophila* and related organisms. In the study by Barth et al. (1983), 83% of horses included in this study were seropositive towards *L. micdadei* at dilution 1:16. Among these seropositive horses, 52% had an antibody titer of 1/64 or more. This high seroprevalence of antibodies against *L. micdadei* indicates that if cross-reactions occurred during our IFA testing, many horses should have been seropositive at dilution 1:40. In our study, only one horse was seropositive at dilution 1/40. Considering the number of horses tested by IFA (n=83), the likelihood of the presence of only one horse seropositive to *L. micdadei* is small. Furthermore, Barth et al. (1983) reported a *L. micdadei*-seropositivity rate of 72% in pigs. In our study no pig was found to be seropositive. We can thus conclude that the likelihood that the equine positive IFA reaction represents the presence of antibodies towards *C. burnetii* is high.

This study confirms that IFA is currently the diagnostic method of choice for sero-diagnosis of Q fever. However, the ELISA cut-off value for bovids might not be adequate for other species. In this study, ELISA Prot A/G seems to be a better assay than ELISA Prot G for Q fever diagnosis in cats. The latter result could be expected as Prot A/G has a greater affinity for feline immunoglobulins than Prot G (http://www.piercenet.com). This test needs further

research on conjugate calibration to improve properties of detectability, specificity and sensitivity.

Conclusion and perspectives

This original study investigates neglected aspects of the Q fever such as uninvestigated potential hosts and laboratory diagnostic methods for these species. This study demonstrates that felines can be infected by Q fever and thus could represent a reservoir for zoonotic transmission. Limiting human contacts, especially of at risk individuals, with pregnant and parturient cats is advised. If these cats have to be manipulated, protective clothing, masks, and gloves should be worn. Birth products should be destroyed as soon as possible. Separating pregnant and parturient cats from other animals would limit propagation of bacteria. In breeding catteries, euthanasia of infected cats could be considered.

Further investigations are necessary to explain the differences in seroprevalence observed. IFA on indoor domestic cats could confirm or infirm the hypothesis on the importance of exposure. Detection of FIV, FeLV, and evaluation of general physical condition, would allow a study on the correlation between the presence of viral infections, immunodepression and infection by *C.burnetii*. The role of other pets in transmission of Q fever to humans should be investigated.

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Acknowledgements

This research was funded by the “Fonds spéciaux pour la Recherche – Crédit de démarrage” (contrat D-08/02), University of Liège, Belgium.

The authors are very grateful to ARSIA for the use of its laboratory for the sample testing and to LSI (France) for the free serological kits making this study possible.

The authors wish to thank all the staff of the private clinic in the Netherlands and of the Veterinary Equine Teaching Hospital of Liege University (Belgium), that have participated in the sampling and care of the horses used in this study.

Moreover, the authors wish to thank the staff of the two French veterinary clinics for the sampling and care of the felines included in the study as well as the members of ONIRIS (France) for the collection and organization of this data.

The authors also wish to thank all the staff implicated in the Ciney (Belgium) stray cat eradication program and in the sampling of cats in rescue centers. In particular, the authors are extremely grateful to Dr Marc Dive and Dr Daniel Vanhoff.

And last but not least, the authors wish to thank the owners of the animals for giving their permission for the blood sampling allowing this study to take place.

References

Babudieri, B., 1959. Q fever: a zoonosis. Advances in Veterinary Science 5, 82-154.

Bandecchi, P., Dell’Omodarme, M., Magi, M., Palamidessi, A., Prati, M.C., 2006. Feline leukaemia virus (FeLV) and feline immunodeficiency virus infections in cats in the Pisa district of Tuscany and attempts to control FeLV infection in a colony of domestic cats by vaccination. Veterinary Record 158, 555-557.

Barth, T.C., Renner, E.D., Gabrielson, D.A., 1982. A survey of domestic animals to detect serological responses against Legionella spp. by indirect fluorescent antibody. Canadian Journal of Comparative Medicine 47, 341-346.

Bergmans, A.M.C., De Jong, C.M.A., Van Amerongen, G., Schot, C.S., Schouls, L.M., 1997. Prevalence of Bartonella species in domestic cats in the Netherlands. Journal of Clinical Microbiology 35, 2256-2261.

Blanc, G., Bruneau, J., 1956. Isolement du virus de la fièvre Q de deux rongeurs sauvages provenant de la forêt de Nefifik (Maroc). Bulletin de la Société de Pathologie Exotique et de ses filiales 49, 431–434.

Childs, J.E., Rooney, J.A., Cooper, J.L., Olson, J.G., Regnery, R.L., 1994. Epidemiologic observations on infection with Rochalimaea species among cats living in Baltimore, Md. Journal of American Veterinary Medical Association 204, 1775-1778.

Chomel, B.B., Abbott, R.C., Kasten, R.W., Floyd-Hawkins, K.A., Kass, P.H., Glaser, C.A., Pedersen, N.C., Koehler, J.E., 1995. Bartonella henselae prevalence in domestic cats in California : risk factors and association between bacteremia and antibody titers. Journal of Clinical Microbiology 33, 2445-2450.

Chomel, B.B., Boulouis, H.-J., Petersen, H., Kasten, R.W., Yamamoto, K., Chang, C.-C., Gandoin, C., Bouillin, C., Hew, C.M., 2002. Prevalence of Bartonella infection in domestic cats in Denmark. Veterinary Research 33, 205-213.

Collins, M.T., Cho, S.-N., Reif, J.S., 1982. Prevalence of antibodies to Legionella pneumophila in animal populations. Journal of Clinical Microbiology 15, 130-136.

Davoli, R., Signorini, F.L., 1951. Antibodies against Rickettsia burneti in the serum of animals at a slaughter house in Tuscany. VI. Annali della Sanita Pubblica 12, 67-73.

Derrick, E. H., 1937. Q fever, a new fever entity: clinical features, diagnosis and laboratory investigations. The Medical Journal of Australia 2, 281.

Enright, J.B., Franti, C.E., Longhurst, W.M., Behymer, D.E., Wright, M.E., Dutson, V.J., 1971. Coxiella burnetii in a wildlife-livestock environment: antibody response of ewes and lambs in an endemic Q fever area. American Journal of Epidemiology 94, 62-71.

Evans, A. D., 1956. 'Q' fever in South Wales. Monthly Bulletin of the Ministry of Health 15, 215.

Fiset, P., 1957. Phase variation of *Rickettsia (Coxiella) burnetii*; study of antibody response in guinea pigs and rabbits. Canadian Journal of Microbiology 3, 435.

Fuchs, A., Binzel, L., Lonsdorfer, M., 1994. Epidemiology of FeLV and FIV infection in Federal Republic of Germany. Tierarztliche Praxis 22, 273-277.

Gillepsie, J.H., Baker, J.A., 1952. Experimental Q fever in cats. American Journal of Veterinary Research 13, 91-94.

Gleich, S.E., Krieger, S., Hartmann, K., 2009. Prevalence of feline immunodeficiency virus and feline leukaemia virus among client-owned cats and risk factors for infection in Germany. Journal of Feline Medicine and Surgery 11, 985-992.

Guatteo, R., Beaudeau, F., Ledoux, D., Drean, E., Seegers, H., 2007. Risk of false-negative results when delaying PCR from sampling for *Coxiella burnetii* detection in dairy cows. Revue de Médecine Vétérinaire 158, 641-644.

Higgins, D., Marrie, T.J., 1990. Seroepidemiology of Q fever among cats in New Brunswick and Prince Edward Island. Annals New York Academy of Sciences 590, 271-274.

Hirai, K., To, H., 1998. Advances in the understanding of *Coxiella burnetii* infection in Japan. Journal of Veterinary Medical Science 60, 781-790.

Hosie, M.J., Robertson, C., Jarrett, O., 1989. Prevalence of feline leukaemia virus and antibodies to feline immunodeficiency virus in cats in the United Kingdom. Veterinary Record 125, 293-297.

Juvet, F., Lappin, M.R., Brennan, S., Mooney, C.T., 2010. Prevalence of selected infectious agents in cats in Ireland. Journal of Feline Medicine and Surgery. 12, 476-482.

Komiya, T., Sadamasu, K., Kang, M.I., Tsuboshima, S., Fukushi, H., Hirai, K., 2003. Seroprevalence of *Coxiella burnetii* infections among cats in different living environments. Journal of Veterinary Medical Science 65, 1047-1048.

Lang, G.H., 1990. Coxiellosis (Q fever) in animals. In: Marrie, T.J (Ed), Q fever. The disease, Vol 1, CRC Press, Boca raton, Florida, USA, pp 23-48.

Langley, J.M., Marrie, T.J., Covert, A., Waag, D.M., Williams, J.C., 1988. Poker players' pneumonia. An urban outbreak of Q fever following exposure to a parturient cat. The New England Journal of Medicine 319, 354-356.

La Scola, B., Raoult, D., 1996. Serological cross-reactions between Bartonella Quintana, Bartonella henselae, and Coxiella burnetii. Journal of Clinical Microbiolgy 34, 2270-2274.

Marmion, B. P., Stoker, M.G.P., 1950. Q fever in Great Britain: epidemiology of an outbreak. Lancet 2, 611-616.

Marmion, B. P., 1954. Q fever II. Natural history and epidemiology of Q fever in man. Transactions of the Royal Society of Tropical Medicine and Hygiene 48, 197-207.

Marrie, T.J., Van Buren, J., Fraser, J., Haldane, E.V., Faulkener, R.S., Williams, J.C., Kwan, C., 1985. Seroepidemiology of Q fever among domestic animals in Nova Scotia. American Journal of Public Health, 75, 763-766.

Marrie, T.J., MacDonald, A., Durant, H., Yates, L., McCormick, L., 1988. An outbreak of Q fever probably due to contact with a parturient cat. Chest 93, 98-103.

Marrie, T.J., Langille, D., Papukna, V., Yates, L., 1989. Truckin' pneumonia – an outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with newborn kittens. Epidemiology and Infection 102, 119-127.

Mattewman, L., Kelly, P., Hayter, D., Downie, S., Wray, K., Bryson, N., Rycroft, A., Raoult, D., 1997. Exposure of cats in southern Africa to Coxiella burnetii, the agent of Q fever European Journal of Epidemiology 13, 477-479.

Mitscherlich, E., Marth, E.H., 1984. Bacteria and Rickettsiae. Important in human and animal health. In: Mitscherlich, E., Marth, E.H. (Eds), Microbial Survival in the Environment, Springer-Verlag, New York, USA, pp. 148-156.

Musso, D., Raoult, D., 1997. Serological cross-reactions between Coxiella burnetii and Legionella micdadei. Clinical and Diagnostic Laboratory Immunology 4, 208-212.

Ormsbee, R., Peacock, M., Gerloff, R., Tallent, G., Wike, D., 1978. Limits of rickettsial infectivity. Infection and Immunity 19, 239-245.

Parola, P., Raoult, D., 2001. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. Clinical Infectious Diseases 32, 897-928.

Petrie, A., Watson, P., 2006. Statistics for veterinary and animal science. Volume 14. 2nd edition. Edited by Science B. Edinburgh: Blackwell Science, pp. 299.

Pinsky, R.L., Fishbein, D.B., Greene, C.R., Gensheimer, K.F., 1991. An outbreak of cat-associated Q fever in the United States. The Journal of Infectious Diseases 164, 202-204.

Pluta, S., Hartelt, K., Oehme, R., Mackenstedt, U., Kimmig, P., 2010. Prevalence of *Coxiella burnetii* and *Rickettsia* spp. in ticks and rodents in southern Germany. Ticks and Tick-borne Diseases 1, 145-147.

Riemann, H.P., Thompson, R.A., Behymer, D.E., Wijayasinghe, C., 1978. Toxoplasmosis and Q fever antibodies among wild carnivores in California. Journal of Wildlife Management 42, 198-202.

Russell-Lodrigue, K.E., Zhang, G.Q., McMurray, D.N., Samuel, J.E., 2006. Clinical and pathologic changes in a guinea pig aerosol challenge model of acute Q fever. Infection and Immunity 74, 6085-6091.

Skykes, J.E., 2010. Immunodeficiencies caused by infectious diseases. Vet Clinical North American Small Animal Practice 40, 409-423.

Slavin, G., 1952. 'Q' fever: the domestic animal as a source of infection for man. Veterinary Record 64, 743.

Stocker, M.G., Marmion, B.P., 1955. The spread of Q fever from animals to man; the natural history of a rickettsial disease. Bulletin of the World Health Organisation 13, 781-806.

Syrucek, L., Raska, K., 1956. Q fever in domestic and wild birds. Bulletin of the World Health Organisation 15, 329-337.

Tissot-Dupont, H., Torres, S., Nezri, M., Raoult, D., 1999. Hyperendemic focus of Q fever related to sheep and wind. American Journal of Epidemiology 150, 67-74.

Vaidya, V. M., Malik, S.V.S., Bhilegaonkar, K.N., Rathore, R.S., Kaur, S., Barbuddhe, S.B., 2010. Prevalence of Q fever in domestic animals with reproductive disorders. Comparative Immunology, Microbiology and Infectious Diseases 33, 307-321.

Webster, J. P., Lloyd, G., Macdonald D.W., 1995. Q fever (*Coxiella burnetii*) reservoir in wild brown rats (Rattus norvegicus) populations in the UK. Parasitology 110, 31-35.

Welsh, H. H., Lennette, E.H., Abinanti, F.R., 1957. Air-borne transmission of Q fever: the role of parturition in the generation of infective aerosols. Annals of the New York Academy of Sciences 70, 528-540.

Willeberg, P., Ruppanner, R., Behymer, D.E., Haghighi, S., Kaneko, J.J., Franti, C.E., 1980. Environmental exposure to *Coxiella burnetii*: a sero-epidemiologic survey among domestic animals. American Journal of Epidemiology 111, 437-443.

Internet:

Thermo Fisher Scientific, 2011. Tech Tip #34. Binding characteristics of antibody-binding proteins: Protein A, Protein G, Protein A/G and Protein L, http://www.piercenet.com/Objects/View.cfm?type=Page&ID=75D6C066-AB3E-4B74-87F2-C38A85F0DBC4. Accessed 20 January 2011.

Tables and Figures

Table 1. General characteristics of immunoglobulin binding proteins produced by *E. coli*

Recombinant	Molecular weight	Number of binding sites for Ig (binding target = Fc)	Optimal binding pH
Protein A	44.6	4	8.2
Protein G	21.6	2	5
Protein A and G	50.46	6	5 to 8.2

Legend: * Ig: Immunoglobulin

Table 2. The number of samples tested and positive by test and per species

Samples			Nb. of animals tested			Nb. of positive animals*				
Species	Country	Period	ELISA Prot G	ELISA ProtA/G	IFA 1/40	ELISA Prot G	ELISA ProtA/G	IFA 1/40	IFA 1/80	IFA 1/160
Horses	The Netherlands	2007 to 2009	163	-	68	0	-	0	-	-
	Belgium	2007 to 2009	181	-	15	0	-	1	0	-
Cats (rescue centers)	Belgium	2008-2009	126	72	34	0	4	11	6	3
Cats (strays)	Belgium	2010	62	47	35	0	2	17	5	3
Cats (domestic)	France	2004	130	126	24	0	0	4	2	0
Pigs	Belgium	2010	264	-	15	0	-	0	-	-
Wild boars	Belgium	2007	272	-	24	1[#]	-	0	-	-
Cows (as reference)	Belgium	2010	30	9	30	17	4	19	18	18

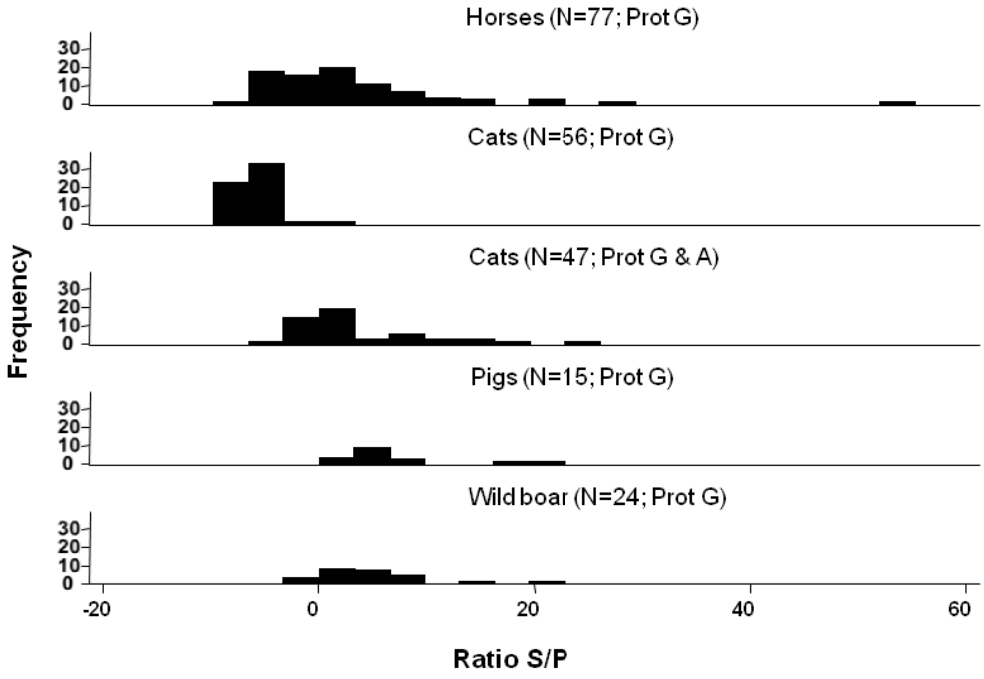
Legend: Nb.: Number; *: according to the determination of a theoretical cut-off value for ELISA Prot G and ELISA Prot A/G (see section of results); [#]: borderline value.

Table 3. Main statistical characteristics of the S/P ratio in the ELISA Prot G and ELISA Prot A/G, for IFA negative sera, in function of species

Species	S/P ELISA Prot G				S/P ELISA ProtA/G			
	Number	Mean	SD	Cut-off*	Number	Mean	SD	Cut-off*
Horses	77	2.89	8.93	29.67	-	-	-	-
Cats	56	-6.20	2.56	1.49	47	3.01	6.00	20.99
Pigs	15	6.36	5.81	23.79	-	-	-	-
Wild boars	24	4.83	5.08	20.08	-	-	-	-
Bovines	11	0.12	5.37	16.23	-	-	-	-

Legend: SD, Standard Deviation; * Theoretical cut-off as the mean of the S/P ratio plus 3 standard deviations.

Figure 1. Distribution of the S/P ratio obtained by ELISA Prot G and ELISA Prot A/G, for IFA negative sera, in function of species



Legend: N: number; Prot G: ELISA Prot G; Prot G &A: ELISA Prot A/G

DISCUSSION

New challenges, new opportunities

According to Rhyan and Spraker (2010), any significant change occurring in the ecosystem may imbalance the host/agent/environment equilibrium, allowing a newly introduced agent or subclinical infection to manifest as an emerging disease. Among emerging infectious diseases (EID), zoonoses are considered to represent between 60 to 75% (Taylor et al., 2001; Kuiken et al., 2005; Woolhouse and Gowtage-Sequeria, 2005; Jones et al., 2008). Domestic animals and, especially, wildlife's importance for emerging diseases must not be underestimated. In fact, involvement of wildlife in livestock and human diseases is considered to have increased in recent decades (Rhyan and Spraker, 2010). In the close future, surveillance of diseases of wildlife, domestic animals, and vectors must become a priority to prevent major outbreaks of zoonoses. For many zoonotic agents, the potential to cause infection in accidental hosts, such as humans, exist, but often this represents a dead-end host (Cutler et al., 2010).

The reasons for the major recent emergence of zoonotic infections are numerous. This increase is essentially due to anthropogenic factors.

Firstly, increase in human population has cause encroachment of humans and their domestic animals into wildlife habitat, which is becoming more and more fragmented (Rhyan and Spraker, 2010). Wildlife can serve as vectors for non-zoonotic diseases of livestock, leading to potentially devastating economical and dietary consequences, and also as reservoirs for zoonotic diseases (Rhyan and Spraker, 2010). The interface between wildlife, other free-living or captive wildlife, domestic animals and humans has increased. Moreover, fragmented landscapes are known to provide more habitats for edge species and a greater diversity of resources. For example, high tick densities are generally found between forests and open areas, leading to a higher rate of tick-borne infections (Daniel et al., 1998; Kantso et al., 2010). Increasing interest in and popularity of wildlife has led to more human contact with

wild animals. Wildlife associated and captive wildlife industries have developed with sometimes intensive management of selected species. Management actions, such as supplementary feeding, lead to an unnatural increase in animal density. Increase in animal density result in behavioral changes (concentration of animals around feeding areas) and thus, increase exposure to infectious animals and discharges (Rhyan and Spraker, 2010). Furthermore, deliberate or accidental introduction of non-native animals into areas already containing high population densities can cause emergence of disease (Cutler et al., 2010; Rhyan and Spraker, 2010). In addition, Cutler et al. (2010) in their article on emerging and neglected zoonoses, reported that hunting and eating wildlife are currently risk factors of increasing global concern. Indeed, tracking, capturing, handling, butchering in the field and transport of carcasses are associated with a high risk of zoonotic infection. Transport of live wildlife and of wildlife products can also lead to emerging diseases (Rhyan and Spraker, 2010). Intensification of agricultural practices with associated deforestation can have unexpected effects (Cutler et al., 2010; Rhyan and Spraker, 2010). In Malaysia in 1998-1999, an outbreak of Nipah virus occurred due to an intensification of domestic pig farming and plantation and cultivation of fruit trees. The fruit trees attracted subclinically infected shedding fruit bats. The pigs' proximity to the attractive trees led to their infection. The high density of pigs and trade allowed a rapid transmission within the pig population and next, to an outbreak of human infections (Daszak et al., 2001). Loss of biodiversity caused by increase in human populations has been associated with increase in pathogen transmission and disease incidence (Keesing et al., 2010). Indeed, several case studies have reported that species most likely to be lost from ecosystems as diversity declines are those most likely to reduce pathogen transmission (Kosoy et al., 1997; LoGuidice et al., 2008; Allan et al., 2009; Keesing et al., 2009; Suzan et al., 2009).

Secondly, many zoonotic infections are transmitted by vectors. If host availability is reduced, vectors seek alternative hosts, such as domestic animals or humans, increasing in this way, opportunities for zoonotic transmission (Cutler et al., 2010). In recent decades, vector distribution has expanded due to climate change, leading to introduction of new pathogens into naïve populations (Cutler et al., 2010). Furthermore, according to Lambin et al. (2010), human behavior is a crucial controlling factor of vector-human contacts, and of infection.

Thirdly, collapse of public health programs during political upheavals has been associated with reemergence of zoonotic pathogens (Cutler et al., 2010). Concentration of humans in refugee camps with lack of hygiene and medical care are known to be preferential sites for disease emergence (plague, typhoid, cholera, dengue hemorrhagic fever, etc.) (Elias et al., 1990). Moreover, economic crisis's sometimes modify people's livelihood strategies (e.g., fruit/mushroom picking, collecting wood in forests), exposing them to a new zoonotic risk. Education and awareness are influential risk/protection factors (Sumilo et al., 2008).

Fourthly, increased globalization and international transport of humans and animals have created great opportunities for rapid geographical spread of pathogens. Tourism, with an increased interest in exotic destinations and adventure holidays, has been associated with an increase in imported zoonotic infections. Human activities such as water sports, wilderness camping, contact with companion animals (cats, dogs, pet rats, psittacine birds) and exotic pets (reptiles, prairie dogs, etc.) have also been found to be risk factors for zoonotic infections (Cutler et al., 2010).

Fifthly, factors such as increased longevity and aging of the population, therapies for sick people, Human Immunodeficiency Virus (HIV), and organ transplants, have modified the susceptibility of the human host, facilitating infection by pathogens (Cutler et al., 2010).

Finally, at the pathogen level, variants with altered pathogenic, persistence and spreading potential can arise and cause major disease outbreaks (Cutler et al., 2010).

Surveillance as a major tool in preventing emerging infectious diseases

High density clustering of human population paves the way for potential outbreaks on an immense scale, rendering a better surveillance a fundamental priority for the future (Cutler et al., 2010). As reported previously by Koplan (2001) and Sosin (2003) in their respective report and manuscript, good surveillance is the first major tool in preventing EID that arise naturally or through terrorist activities. Surveillance is defined by the Last and International Epidemiological Association (2001) as a systematic ongoing collection, collation and analysis of data with the timely dissemination of information to responsible decision-makers. Brillant (2008) advanced the idea of "being ahead of the curve", in other words, identifying risky situations before the first cluster of hospital cases are identified. Regarding the high proportion of zoonoses among EID, Kruse et al. (2004) proposed surveillance systems based on animal health information for early warning purposes. The number of human-only surveillance systems in the literature increased in 1998, leveled off then increased again in 2003-2005; animal-only surveillance systems were relatively steady with a marked jump in 2006; and systems including both showed a steady rate with a jump in 2000 followed by a leveling off (Vrbova et al., 2010). The majority of surveillance systems (70%) are designed for known diseases (Vrbova et al., 2010). In Canada, the Canadian Field Epidemiology Program (CFEP) in the Public Health Agency of Canada evaluated surveillance systems between 1999 and 2007. The reports often used multiple evaluation criteria depending on the system attributes, data availability and the specific objectives of each evaluation. The most common evaluation criteria were timeliness, acceptability, utility or relevance, flexibility, sensitivity, specificity or positive predictive value, data quality, simplicity, and sustainability

(Vrbova et al., 2010). Recently the “one world one health” trend aims at centralizing animal and human data in one global and unique surveillance system. Unfortunately, surveillance of animal and zoonotic diseases in animals is often not legally mandated to the same extent as in humans, particularly in wildlife (Vrbova et al., 2010).

Surveillance of zoonoses is necessarily a multi-disciplinary endeavor. Domestic animals, wildlife, humans and environment are interconnected in a complex manner, rendering effective surveillance extremely challenging. For surveillance and management of wildlife diseases, for example, public affairs specialists must be involved and have thorough understanding of the disease situation and needed management actions. Communication and education of the public are essential in the complex context of emerging diseases in wildlife (Rhyan and Spraker, 2010). Passive surveillance of wildlife consists in observation of clinically ill or dead animals and on the opportunistic collection of specimens. The general public and hunters can be involved in this type of surveillance. The difficulties of passive surveillance of wildlife include lack of any baseline data on the disease and population in question. Indeed, basic knowledge such as susceptibility, carrier status, and transmission potential are unavailable for many diseases in wildlife. Furthermore, survival behavior often masks clinical signs, and predation and scavenging hinder observation of dead animals and diagnostic processes (Rhyan and Spraker, 2010). To this day, surveillance and management of diseases in wildlife remains a challenge rendering further wider and more intense interdisciplinary collaboration essential (Rhyan and Spraker, 2010).

Improving surveillance, a challenging task for scientists worldwide

Temporal and spatial, also called landscape, epidemiology are fundamental for development of effective surveillance. Temporal epidemiology consists in the study of variation of disease incidence in relation to time. Disease incidence can vary depending on season and year.

Knowledge of this temporal variation of disease incidence allows detection of a sudden increase above the natural variation of incidence, and thus, early detection of emergence. Moreover, temporal epidemiology allows detection of clusters of cases and thus, detection of high risk periods. Detection of high risk periods is useful to permit concentration of surveillance means at specific times. Spatial epidemiology is defined as the study of spatial variation in disease risk or incidence (Lambin et al., 2010). Landscape and meteorological factors control not just emergence, but also spatial concentration and spatial diffusion of infection risk. Factors influencing people-vector contacts include the relative population densities, as well as spatial and temporal distributions of both vectors and people, and their movements and behaviors. Landscape, connectivity of habitats for vectors and hosts, and vector-host ratio are the main influential factors. Proximity of vector and host habitats may not necessarily result in a high level of risk if these critical habitats are not connected spatially by landscape features favorable to the circulation of vectors and hosts (Lambin et al., 2010).

Improved surveillance and disease detection may be achieved through use of a syndromic approach rather than searching for specific diagnoses (Cutler et al., 2010). In 2005, Leblond et al. suggested that horses could be considered as good sentinels for the WNV circulation in the Camargue area, France. Later, in 2007, Leblond et al. (2007a) developed an equine syndromic surveillance system based on the 2004 WNV epizootic in Camargue. This system proved to provide a warning 4 weeks before the epidemic period. In the same study, by comparison, the laboratory-case confirmation in horses could only provide a warning less than one week before the epizootic. However, the stability of such surveillance depends on voluntary or mandatory reporting, generally without compensation. In the future, more emphasis should be focused on regular feedback as motivating practitioners to provide timely input is essential (Leblond et al., 2007a). Moreover, further evaluation of syndromic surveillance in horses for WNF should be undertaken. Effectiveness must be evaluated depending on the area

concerned. Indeed, in the USA in 1999 and 2000, equine WN cases did not precede detection of the virus in mosquitoes and wild birds, nor did it predict human case occurrences (Leblond et al., 2007a). In the study by Nielsen et al. (2008), however, immunologically naïve horses in California were found to be more sensitive for detection of WNV transmission than mosquito sampling alone, and were thus considered a sensitive indicator of WNV activity. Vaccination programs and naturally acquired immunity can hinder the effectiveness of equine passive surveillance (Nielsen et al., 2008).

Novel contribution to improve surveillance

Animal surveillance and using animal data for precocious clinical detection of emerging zoonoses before their emergence in human populations are the basis of this work. This original work emphasizes the need for collaboration between countries, as well as for a multidisciplinary approach with involvement of clinical and laboratory skills. The epidemiological methods developed in this study are methods that can be applied to a large panel of diseases, emerging or not. It is not limited by the species affected or by the necessity of having a good knowledge of the illness diagnosed. Clinical epidemiology does not require special equipment to be put into place and is relatively easy and fast to develop. Furthermore, the great originality of this work is its acknowledgement of the fact that emerging zoonotic diseases can potentially be asymptomatic in animal reservoirs. This study goes from a methodological approach of a rare clinical disease used as a model of an emerging disease (equine endocarditis), to a more complex situation where the emerging zoonotic disease is occasionally symptomatic (equine WNF) or considered asymptomatic (Q fever in felines, equids, pigs and wild boars). The aim of this work was to develop epidemiological methods to facilitate the diagnosis of diseases poorly understood and/or poorly investigated by general

practitioners in western Europe. It is an important step forward towards a better surveillance of zoonotic diseases; improved surveillance allowing earlier detection of emergence.

The study on equine vegetative endocarditis demonstrates that epidemiological methodologies can easily be applied to clinical diseases. Being a rare disease, equine endocarditis permits the development of a methodology that can be applied to any emerging disease. Determination of significant risk factors can facilitate diagnosis for the field practitioner, leading to an earlier treatment and a better prevention of spread of infection. Earlier diagnosis and improved management of infectious diseases would have local financial advantages; national and international commercial advantages, by preventing spread of infection by fast control of the identified focus of infection and by escaping any sanctions for international trade. The epidemiological methodologies used in this study can be applied at individual or herd level. Large scale studies on risk factors increase awareness but also improve surveillance of a specific proportion of the population, of a period of the year or of an area, identified as being at high risk of emergence. This study also emphasizes the major importance of association of different risk factors.

The second study on WNF is a more sophisticated application of the methodology developed for equine endocarditis. This study contributes to improvement of surveillance by emphasizing the importance of standardized centralization of data, and of focusing on geographical areas at high risk of emergence. Standardization is facilitated by the fact that clinical signs and risk factors seem constant in Europe. Communication with veterinarians and the public was found to be lacking in certain countries and must be promoted in the future for a more effective surveillance. Development of classification and regression trees emphasized the importance of major risk factors for WNF emergence and is a methodology that can be applied to a great variety of situations.

This study demonstrates the need for increased awareness among clinicians and veterinarians about the possibility of WNF cases of encephalitis and meningoencephalitis during periods of potential transmission (Zeller and Schuffenecker, 2004). Among many other unanswered questions, the mechanism of WNV (re)introduction in Europe and the cycle of maintenance in infected areas remain to be elucidated (AFSSA, 2004; Zeller and Schuffenecker, 2004). The major difficulty with WNV circulation and infections is the fact that most infections are asymptomatic and that the etiology of a large number of encephalitis and/or meningoencephalitis cases (both human and equine cases) remain unknown (AFSSA, 2004). It is essential to continue studying the evolution of the epidemiological situation of WNV to prevent its harmful effect on human and animal populations in the future (Toma et al., 2001; AFSSA, 2004). Moreover, a better knowledge of the epidemiology of WNV, that is complex and deeply influenced by environmental factors and hosts (Leblond et al., 2007b), as well as by climatic conditions (Sardelis et al., 2001; Khasnis and Nettleman, 2005; Haines et al., 2006) and vector biology (Cantile et al., 2000), in each country or area would justify the use or not of equine vaccination (AFSSA, 2004; Morse, 2004). Extensive studies to identify both the invertebrate vector and the vertebrate reservoir species of WNV seem necessary (Cantile et al., 2000).

The third original study is a major contribution to a better understanding of the epidemiology of Q fever in western Europe. Q fever can have disastrous consequences (e.g., in the Netherlands (Karagiannis et al., 2009; Enserink, 2010)) and is currently of major concern; improving its surveillance is fundamental. This study investigates neglected aspects of Q fever: potential domestic animal reservoirs, diagnostic methods and their application to different species. Importantly, this work increases our awareness of pet associated zoonotic risk. To our knowledge, Q fever seroprevalence has not previously been investigated in western European equids, felines, pigs and wild boars. This study found that several cats and

one horse were seropositive for Q fever. The presence of seropositive animals signifies that these species could potentially represent a zoonotic threat and a source of bacteria for environmental contamination. Risk and protection factors for seropositivity were determined within each species and subgroup. Stray cats were the most likely to be seropositive for Q fever. This signifies that cats could be a sentinel for circulation of Q fever in areas where ruminants are scarce. Cats are easier to catch than wild rodents (Webster et al. (1995) suggested wild brown rats with a seroprevalence of 7 to 53% as sentinel in the United Kingdom) and sampling could be performed at the same time as eradication or sterilization programs. According to this study, usefulness of equids as sentinel animals seems minor in western Europe. However, equine sentinels could prove effective in other areas (e.g., California (Willeberg et al., 1980)). In the future, a closer observation and follow-up of felines and equids could potentially allow detection of clinical signs associated to Q fever infection, which were previously unnoticed, or, erroneously, defined as being the consequence of another independent condition. Indeed, in the USA, in 1952, Gillespie and Baker infected farm cats experimentally by subcutaneous inoculation with infected yolk sac (n=3) or guinea pig spleen (n=1) suspensions, by feeding infected yolk sacs (n=3) or guinea pig spleens (n=1), or by exposure (n=4) to subcutaneously infected cats. In their study, 3 out of the 4 cats infected subcutaneously developed fever, lethargy and lack of appetite two days after inoculation and for a period of 3 days. The only subcutaneously infected cat that didn’t develop clinical signs was the cat infected with a guinea pig spleen suspension of bacteria. None of the cats infected orally or by exposure developed clinical signs. *C. burnetii* were isolated from blood or urine of all subcutaneously infected cats (n=4), all cats fed infected yolk sacs (n=3) and of one cat out of 4 exposed cats. All these cats became seropositive between 4 and 8 weeks post-infection. Thus, according to this study, subclinical infections are frequent in cats (only 3 out of 8 infected cats developed clinical signs). However, no long

term clinical follow-up is available in this experimental study. Moreover, no postmortem examinations or blood tests evaluating organ functions (kidneys, liver, etc.) are available. This study demonstrates that cats can be infected by *C. burnetii*, can develop clinical signs associated to infection, and can be a source of environmental contamination by excreting bacteria in urine (Gillespie and Baker, 1952). Most reports of cat associated human outbreaks do not describe clinical signs in the cat (Marrie et al., 1988a; Marrie et al., 1989; Pinsky et al., 1991). However, a case report from Marrie et al. (1988b) reported the presence of vaginal bleeding in an infected cat before parturition and the birth of stillborn kittens. In conclusion, a better understanding of Q fever infection in felines could contribute to clinical veterinary medicine. In our study, no pig or wild boar was found to be positive for *C. burnetii*. However, the number of animals tested by IFA, the gold standard for definite diagnosis, was small. Further IFA on a larger number of pigs and wild boars are necessary to confirm our results and hypotheses. ELISA ProtG, ELISA ProtA/G, and IFA were compared in this study. ELISA kits and cut-off values adequate for diagnosis in bovids are not adequate for Q fever diagnosis in felines or equids. This study emphasizes the need for improvement of ELISA testing and proposes new cut-off values after distribution analyses.

CONCLUSIONS AND PERSPECTIVES

Human disease surveillance must be associated with enhanced longitudinal veterinary surveillance in food-producing animals, pets, and wildlife (Cutler et al., 2010). Indeed, control of zoonotic infections in reservoir hosts is known to have a protective effect in human populations (Cutler et al., 2010). Spatial and temporal surveillance of vectors, and potential vectors, is essential, as vector distribution is expanding and in continual evolution. A more intense interdisciplinary approach and international collaboration must be promoted. Surveillance networks must be organized involving specialists in each domain (doctors, veterinarians, epidemiologists, entomologist, etc.). The role of each specialist must be defined precisely for the best effectiveness of the network. Prioritization of emerging, and potentially emerging, diseases must be performed depending on risk and consequences of emergence. Standardized classification and regression trees are a great help for prioritization and also for rapid decision making in case of emergence. Worldwide standardization of data collecting is fundamental and must be developed further.

Future studies on wildlife are necessary to identify potential hosts, reservoirs, vectors and sentinels. Follow-up of wildlife migration and movements is necessary as they can lead to infection of new areas by asymptomatic pathogen shedding, leading to outbreaks in naïve populations.

Adequate animal sentinels should be placed (domestic animals) or selected (wildlife) in at risk areas. As mentioned previously, sentinel species must be chosen depending on the epidemiological context to permit disease detection before human cases occur.

A syndromic approach would allow fast and cheap detection of disease circulation and prevent major outbreaks in human populations. Early detection of major risk factors would permit to focus controls on key points or areas. Thus, syndromic approach and risk factors must be emphasized more in the future. Classification and regression trees could be used to standardize the diagnostic approach for a large variety of diseases.

Medical and laboratory staff, people involved in public health and food-processing, farmers, breeders and traders must received regular training sessions concerning emerging diseases. Communication with the public must be promoted to increase awareness and emphasize the importance of preventive measures. At risk individuals (elderly, HIV, transplant patients, etc.) must be informed of the risk of exotic destinations, animal contact, petting farms, outdoor activities (e.g., mushroom picking), and food-borne zoonoses.

Concerning diagnosis of emerging diseases, further development of laboratory assays is necessary in veterinary medicine, as well as in human medicine. Cut-off values must be adapted depending on the species concerned and on the area (variability in strains). ROC analyses are a fast and easy method to orientate the choice of an accurate cut-off value (best compromise between sensitivity and specificity) but require testing of samples with “gold standard” test beforehand. Sensitivity of the ELISA kits for emerging zoonoses must be improved. Using a more sensitive conjugate (e.g., for *C. burnetii*, ProtA/G versus ProtG), prolonging incubation period with the conjugate/substrate, increasing temperature or developing more sensitive techniques could lead to a better detection of seropositive animals. Laboratories must be aware of the need for collaboration and be ready for rapid diagnosis of new pathogens. They should be equipped with diagnostic tests for diseases at high risk of emergence in their area. Communication and their capacity of helping one another in case of a crisis are important to permit early diagnosis. Development of laboratory assays allowing distinction of different emerging strains (e.g., *WNV*, *C. burnetii*) would be useful as pathogenicity can vary greatly between strains. Detection of a particularly virulent strain early after its emergence would prevent disastrous consequences for veterinary and public health.

In conclusion, this work is a direct application of epidemiology to clinical situations. Through its original and broad approach, it emphasizes aspects that must be improved for a better surveillance of emerging diseases in the future.

SUMMARY IN FRENCH

Introduction

Les maladies émergentes, zoonotiques dans 60 à 75% des cas (Taylor *et al.*, 2001), sont actuellement un sujet de grand intérêt pour les scientifiques du monde entier. Les changements climatiques et socio-économiques, le transport international, les modifications environnementales, le développement et la commercialisation de méthodes diagnostiques ont mené à la découverte de nouveaux pathogènes et de nouvelles maladies.

L'épidémiologie clinique des maladies émergentes est définie comme l'application du raisonnement épidémiologique à l'étude des maladies nouvellement apparues dans une population ou qui ont existé précédemment mais qui augmentent en incidence ou s'étendent géographiquement de manière importante (Morse, 1995), en vue d'en améliorer le diagnostic, la prévention ou le traitement (Toma *et al.*, 1991). La détection précoce des maladies émergentes est un point clé pour la prévention de conséquences désastreuses au niveau de la santé publique et animale. Les maladies émergentes sont souvent très peu connues dans les régions concernées, rendant le diagnostic clinique problématique. Dans ce contexte, la communication entre pays et la centralisation d'informations sont essentielles.

Ce travail est basé sur l'étude de trois maladies différentes. La première consiste en une étude rétrospective de l'endocardite végétante équine, maladie considérée comme étant symptomatique dans tous les cas (tant que le cheval ne meurt pas d'une autre pathologie avant le développement des signes cliniques). L'endocardite végétante équine est une maladie rare, infectieuse, non contagieuse, non émergente mais connue (Patteson, 1996). Elle est utilisée comme « modèle » d'une maladie émergente qui est rare au début de son introduction dans un territoire donné. Développer des méthodes épidémiologiques sur l'endocardite végétante équine à partir de données rétrospectives permet, par la suite, de transférer ces méthodes aux maladies réellement émergentes. La deuxième étude porte sur la Fièvre de la vallée du Nil Occidental (FNO) chez les chevaux, maladie émergente et symptomatique dans 10% des cas (Petersen et Roehrig, 2001). Les méthodes épidémiologiques développées dans la première étude ont été adaptées dans ce nouveau contexte et ont permis l'identification de facteurs de risque significatifs. En outre, des arbres de classification et de régression ont été mis au point afin

d'aider au diagnostic clinique de terrain (Lewis, 2000). Une étude comparative a été réalisée à partir de données françaises, italiennes et hongroises permettant la détection de différences fondamentales entre la présentation clinique et l'épidémio-vigilance dans les trois pays. La troisième étude investigue la prévalence de la fièvre Q chez les chevaux, chats, porcs et sangliers. La fièvre Q est une zoonose émergente en Europe de l'ouest. En l'absence de signes cliniques rapportés dans ces espèces, la séropositivité indiquerait une circulation du pathogène et un risque zoonotique. Face à une maladie d'allure asymptomatique, l'approche de l'épidémiologie clinique est limitée. Dès lors, des analyses de laboratoire ont été nécessaires pour évaluer la prévalence de la fièvre Q chez les 4 espèces. Ensuite, les facteurs de risque pour la séropositivité ont été déterminés. De même, une étude comparative des différentes analyses de laboratoire et la détermination d'une valeur seuil idéale pour un diagnostic par espèce a été réalisée.

Résultats

Etude sur l'endocardite végétante équine

Cette étude a permis de déterminer les signes cliniques et paramètres sanguins modifiés qui doivent pousser le vétérinaire à une investigation cardiologique des équidés présentés en consultation. L'identification des facteurs de risque a pour avantage de faciliter le diagnostic menant ainsi à un traitement plus précoce. Appliquer la méthodologie développée dans cette étude aux maladies infectieuses permettrait un diagnostic plus précoce et une meilleure gestion de l'infection grâce à un contrôle rapide du foyer infectieux identifié. De plus, les méthodes épidémiologiques utilisées dans cette étude peuvent être appliquées au niveau individuel ou de troupeau. Des études à large échelle sur les facteurs de risque amélioreraient la surveillance ciblée d'une proportion spécifique de la population, à une période déterminée de l'année et/ou d'une zone géographique donnée, identifiée comme étant à haut risque d'émergence. Les résultats de l'analyse faisant usage d'une courbe ROC (*Receiver Operating Characteristic*) (Toma *et al.*, 1991) mettent en évidence l'importance majeure de l'association

de différents facteurs de risque, en proposant un seuil de score clinique (somme des signes cliniques présents) pour permettre un diagnostic clinique plus précoce.

Etude sur la fièvre du Nil occidentale (FNO) chez les équidés

La deuxième étude porte sur la FNO et est un développement plus sophistiqué de la méthodologie mise au point pour l'endocardite équine. Cette étude contribue à l'amélioration de la surveillance de la FNO en mettant en avant l'importance de la communication et de la centralisation de données standardisées. L'étude sur les facteurs de risque associés à la FNO a démontré l'importance de focaliser l'effort de surveillance sur des périodes et régions géographiques à haut risque d'émergence. L'étude comparative des cas cliniques de FNO de France, Italie et Hongrie a permis la mise en évidence d'un manque de communication au sujet de la maladie envers les vétérinaires et les propriétaires de chevaux. Les effets néfastes d'un manque de sensibilisation concernant le risque d'émergence de FNO sont rendus explicites dans cette étude. En effet, dans le pays le moins informé, le délai de diagnostic clinique était significativement prolongé. La communication doit être améliorée à l'avenir afin d'effectuer une meilleure surveillance. Le développement d'arbres de classification et de régression dans cette étude a permis de mettre en évidence l'importance des facteurs de risque géographique et saisonnier dans l'émergence de la FNO. Cette méthodologie novatrice peut être appliquée à une grande variété de situations et mérite davantage d'intérêt (Lewis, 2000).

Etude sur la fièvre Q chez les chevaux, chats, porcs et sangliers

La troisième étude est une contribution originale permettant une meilleure compréhension de l'épidémiologie de la fièvre Q en Europe de l'ouest. La fièvre Q peut avoir des conséquences désastreuses (par ex. aux Pays-Bas (Karagiannis *et al.*, 2009 ; Enserink, 2010)) et est actuellement un sujet d'intérêt majeur. Améliorer sa surveillance est crucial. Cette étude explore des aspects négligés de la fièvre Q: réservoirs potentiels non explorés parmi les

animaux domestiques, méthodes de diagnostic actuelles et leur application à ces espèces. Ce travail augmente de manière importante la connaissance du risque zoonotique associé aux animaux domestiques. De plus, cette étude propose un nouvel animal « sentinelle » (Toma *et al.*, 1991), le chat, qui pourrait se montrer utile dans de futurs programmes de surveillance. Finalement, cette étude met en évidence le besoin d'amélioration des tests Enzyme Linked Immunosorbent Assay (ELISA) et propose de nouvelles valeurs seuils par espèce en recourant à l'usage de courbes ROC.

Conclusions et Perspectives

Ces trois études mettent en évidence des points clés pour améliorer la surveillance des maladies émergentes à l'avenir et propose une méthodologie pour augmenter son efficacité. Elles se basent sur l'utilisation de données animales pour une détection clinique plus précoce des zoonoses avant leur émergence dans la population humaine. Ce travail original met en évidence la nécessité d'une collaboration internationale, d'une approche interdisciplinaire et d'une standardisation des données. Les méthodes épidémiologiques développées dans les trois études sont des méthodes qui peuvent être appliquées à une grande variété de maladies, émergentes ou non. Leur application n'est pas limitée ni par l'espèce affectée ni par la nécessité d'avoir une connaissance approfondie de la maladie (ce qui ne sera pas toujours possible immédiatement face à une maladie nouvelle). De plus, l'épidémiologie clinique ne requière pas d'infrastructure spécialisée et est relativement facile et rapide à mettre en place. L'originalité de ce travail est la reconnaissance du fait que les zoonoses émergentes peuvent potentiellement être asymptomatiques chez les animaux réservoirs. Ce travail commence par une approche méthodologique d'une maladie rare (l'endocardite équine), utilisée comme « modèle » d'une maladie émergente (première étude), puis évolue vers des situations plus complexes où la zoonose est occasionnellement symptomatique (FNO chez les chevaux) ou considérée comme asymptomatique (fièvre Q chez les chevaux, chats, porcs, sangliers) (deuxième et troisième études).

L'objectif de ce travail était de développer des méthodes épidémiologiques pour faciliter le diagnostic précoce de maladies émergentes, peu comprises et/ou peu investiguées par les vétérinaires de terrain

en Europe de l'ouest. Ce travail démontre que la surveillance des maladies humaines doit être associée à une surveillance vétérinaire accrue des animaux de production, domestiques et de la faune sauvage. En effet, le contrôle des zoonoses dans les hôtes réservoirs a été reconnu comme ayant un effet protecteur pour les humains (Cutler *et al.*, 2010). Ce travail est une contribution significative à l'amélioration de la surveillance des maladies zoonotiques. Cette amélioration de la surveillance permettra une détection précoce de l'émergence.

A l'avenir, une approche interdisciplinaire plus intense et une collaboration internationale doivent être davantage développées. Des réseaux de surveillance doivent être organisés impliquant des spécialistes de domaines variés (médecins, vétérinaires, épidémiologistes, entomologistes, etc.). Le rôle de chaque spécialiste doit être défini précisément pour une efficacité optimale du réseau. La priorisation des maladies émergentes et potentiellement émergentes doit être réalisée après une étude approfondie des risques et conséquences d'émergence. Les arbres de classification et de régression sont une aide pour la priorisation mais aussi pour la prise de décision rapide en cas d'émergence. La standardisation internationale de la récolte de données est fondamentale et doit être stigmatisée et rendue accessible par tous. Des études approfondies sur la faune de sauvage sont nécessaires pour identifier les hôtes potentiels, les réservoirs, vecteurs et animaux « sentinelles » (Rhyan and Spraker, 2010). Un suivi des migrations et mouvements de la faune sauvage est nécessaire puisqu'ils peuvent mener à l'infection de nouveaux territoires par excrétion asymptomatique de pathogènes, provoquant ainsi une épidémie dans des populations naïves. La surveillance spatio-temporelle des vecteurs et vecteurs potentiels est essentielle étant donné que leur distribution est en expansion et en évolution (Lambin *et al.*, 2010). Des animaux « sentinelles » adéquats doivent être placés (animaux domestiques) ou sélectionnés (faune sauvage) dans les zones à risque (Toma *et al.*, 1991). Ils doivent être choisis en fonction du contexte épidémiologique local pour permettre la détection précoce de l'émergence avant l'émergence de cas humains (Leblond *et al.*, 2007; Nielsen *et al.*, 2008). Une approche syndromique permettrait une détection rapide et peu coûteuse de la circulation de maladies et préviendrait des épidémies majeures dans les populations humaines (Leblond *et al.*, 2007; Cutler *et al.*, 2010). La détection précoce des facteurs de risques permettrait une focalisation de la surveillance et des contrôles sur des

points ou endroits clés. L'importance de l'approche syndromique et des facteurs de risque ne doit donc pas être sous-estimée et doit être valorisée à l'avenir. Les arbres de classification et régression pourraient être utilisés pour standardiser l'approche diagnostique d'une grande variété de maladies. Les personnels médicaux et de laboratoire, le personnel impliqué dans la santé publique et la chaîne alimentaire, les agriculteurs, éleveurs et marchands d'animaux doivent recevoir des formations concernant les maladies émergentes. De même, la communication avec le public doit être développée pour augmenter la vigilance et sensibiliser à l'importance des mesures préventives. Les individus à risque (jeunes enfants, personnes âgées, femmes enceintes, immunodéprimés, malades chroniques) doivent être informés des risques associés aux voyages exotiques, aux contacts avec des animaux de rente et domestiques, aux fermes pédagogiques, aux activités extérieures et aux zoonoses alimentaires.

Concernant le diagnostic des maladies émergentes, le développement des tests de laboratoire est nécessaire en médecine vétérinaire aussi bien qu'en médecine humaine. Les valeurs seuils des analyses doivent être adaptées en fonction de l'espèce concernée. L'analyse de courbes ROC est une méthode facile et rapide pour orienter le choix de la valeur seuil la plus appropriée (meilleur compromis entre sensibilité et spécificité) mais nécessite l'existence préalable d'un test de référence (Toma *et al.*, 1991). Actuellement, la sensibilité des kits ELISA pour les zoonoses émergentes doit être améliorée. Utiliser un conjugué plus sensible (protéine A/G versus protéine G), prolonger la période d'incubation avec le conjugué/substrat, augmenter la température ou développer des nouvelles analyses plus sensibles permettraient une meilleure détection des animaux séropositifs. Les laboratoires doivent être conscients de la nécessité de collaboration et être prêts pour le diagnostic pour de nouveaux pathogènes. Ils doivent être équipés avec des tests diagnostiques pour les maladies à haut risque d'émergence dans leur secteur. La communication et leur capacité d'entre-aide en cas de crise sont importantes pour permettre un diagnostic précoce. Le développement d'analyses de laboratoire permettant la distinction entre différentes souches émergentes (par ex. pour FNO ou *C. burnetti*) pourrait être utile puisque la pathogénicité peut varier de manière importante en fonction des souches (Klaassen *et al.*, 2009 ; Vazquez *et al.*, 2010). La détection précoce d'une souche émergente

particulièrement virulente préviendrait des conséquences désastreuses pour la santé publique et vétérinaire.

Références

CUTLER S.J., FOOKS A.R., VAN DER POEL W.H.M. Public Health Threat of new reemerging, and neglected zoonoses in the industrialized world. *Emerg. Infect. Dis.*, 2010, **16**, 1-7.

ENSERINK M. Questions abound in Q-fever explosion in the Netherlands. *Science*, 2010, **327**, 266-267.

KARAGIANNIS I., SCHIMMER B., VAN LIER A., TIMEN A., SCHNEEBERGER P., VAN ROTTERDAM B., DE BRUIN A., WIJKMANS C., RIETVELD A., VAN DUYNHOVEN Y. Investigation of a Q fever outbreak in a rural area of The Netherlands. *Epidemiol. Infect.*, 2009, **137**, 1283-1294.

KLAASSEN C.H.W., NABUURS-FRANSEN M.H., TILBURG J.J.H.C., HAMANS M.A.W.M., HORREVORTS A.M. Multigenotype Q fever outbreak, The Netherlands. *Emerg. Infect. Dis.*, 2009, **15**, 613-614.

LAMBIN E.F., TRAN A., VANWANBEKE S.O., LINARD C., SOTI V. Pathogenic landscapes: interactions between land, people, disease vectors, and their animal hosts. *Int. J. Health Geogr.*, 2010, **9**, 54.

LEBLOND A., HENDRIKX P., SABATIER P. West Nile outbreak detection using syndromic monitoring in horses. *Vector Borne Zoonotic Dis.*, 2007, **7**, 403-410.

LEWIS R.J. An introduction to classification and regression tree (CART) analysis. In : The 2000 Annual Meeting of the Society for Academic Emergency Medicine, San Francisco, California, USA, 25 May 2000, 2000, 1-14.

MORSE S.S. Factors in the emergence of infectious diseases. *Emerg. Infect. Dis.*, 1995, **1**, 7−15.

NIELSEN C.F., REISEN W.K., ARMIJOS M.V., MACLACHLAN N.J., SCOTT T.W. High subclinical West Nile virus incidence among non vaccinated horses in northern California associated with low vector abundance and infection. *Am. J. Trop. Med. Hyg.*, 2008, **78**, 45-52.

PATTESON M.W., Endocarditis. In: PATTESON M.W., Equine Cardiology. Blackwell Science Ltd: United Kingdom, 1996, 150–154.

PETERSEN L.R., ROEHRIG J.T. West Nile Virus: A reemerging global pathogen. *Emerg. Infect. Dis.*, 2001, **7**, 611-614.

RHYAN J.C., SPRAKER T.R. Emergence of diseases from wildlife reservoirs. *Vet. Pathol.*, 2010, **47**, 34-39.

TAYLOR L.H., LATHMAN S.M., WOOLHOUSE M.E. Risk factors for human disease emergence. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 2001, **356**, 983-989.

TOMA B., BENET J.-J., DUFOUR B., ELOIT M., MOUTOU F., SANAA M. Glossaire d'épidémiologie animale. Point Vétérinaire (Eds) : Maison-Alfort, 1991, 365p.

VAZQUEZ A., SANCHEZ-SECO M.P., RUIZ S., MOLERO F., MAGALLANES A., TEJEDOR C.G., TENORIO A. Putative new lineage of West Nile virus, Spain. *Emerg. Infect. Dis.*, 2010, **16**, 549-552.

REFERENCES

A

ABE T., YAMAKI T., HAYAKAWA T., FUKUDA H., ITO Y., KUME H., KOMIYA T.,

ISHIHARA K., HIRAI K. A seroepidemiological study of the risks of Q fever infection in Japanese veterinarians. *Eur. J. Epidemiol.*, 2001,**17**, 1029–1032.

ACERSA. Diagnostic d'un élevage cliniquement atteint de fièvre Q. 2006, 1-11.

ACHA P. N., SZYFRES B. Fièvre Q, CIM-10 A78. In : Acha, P. N., Szyfres B., Zoonoses et maladies transmissibles communes à l'homme et aux animaux. Volume III : Chlamydioises, rickettsioses et viroses. 3ème Edn, OIE: Paris, France, 2005, 22-32.

ACKLAND J.R., WORSWICK D.A., MARMION B.P. Vaccine prophylaxis of Q fever – a follow-up study of the efficacy of Q-Vax (CSL) 1985-1990. *Med. J. Aust.*, 1994,**160**, 704-708.

AFSCA. Fièvre Q: Rapport sur L'évaluation des Risques Pour la Santé Publique et des Outils de Gestion des Risques en Elevage de Ruminants. 2004, 1–88.

AFSSA. Bilan de la surveillance West Nile en France en 2004. *Bulletin épidémiologique*, 2004, **17**, 3.

AFSSA. Évaluation du dispositif de surveillance de l'infection à virus West Nile en France. *Bulletin épidémiologique*, 2005, **17**,2.

AKPORIAYE E.T., BACA O.G. Superoxide anion production and superoxide dismutase and catalase activities in *Coxiella burnetii*. *J. Bacteriol.*, 1983, **154**, 520-523.

AKPORIAYE E.T., ROWATT J.D., ARAGON A.A, BACA O.G. Lysosomal response of a murine macrophage-like cell line persistently infected with *Coxiella burnetii*. *Infect. Immun.*, 1983, **40**, 1155-1162.

ALBORNOZ M.M.C., HEREDIA J.H.P., ALVAREZ J.S., LOPEZ G.T., PALACIOS R.H., PUENTE A.R. Epidemiology of community-acquired pneumonia in the health area I from Navarra. *Med. Clin. (Barc.)*, 1991, **97**, 50–52.

ALIBEK K. Biohazard: The chilling true story of the largest covert biological weapons program in the world, Told From the Inside by the Man Who Ran It. Random House Inc: New York, USA, 1999, 319 p.

ALLAN B.F., LANGERHANS R.B., RYBERG W.A., LANDESMAN W.J., GRIFFIN N.W., KATZ R.S., OBERLE B.J., SCHUTZENHOFER M.R., SMYTH K.N., DE ST MAURICE A., CLARK L., CROOKS K.R., HERNANDEZ D.E., MCLEAN R.G., OSTFELD R.S., CHASE J.M. Ecological correlates of risk and incidence of West Nile virus in the United States. *Oecologia*, 2009, **155**, 699-708.

AMANO K.I., WILLIAMS J.C. Chemical and immunological characterization of lipopolysaccharides from phase I and phase II *Coxiella burnetii*. *J. Bacteriol.*, 1984, **160**, 994-1002.

ANONYMOUS Experimental Q fever in man. *Br. Med. J.*, 1950, **1**, 1000 (Editorial).

ANONYMOUS Comment on Q fever transmitted by blood transfusion-United States. *Can. Dis. Weekly Rep.*, 1977, **3**, 210 (Editorial).

ARJONA A., FOELLMER H.G., TOWN T., LENG L., MCDONALD C., WANG T., WONG S.J., MONTGOMERY R.R., FIKRIG E., BUCALA R. Abrogation of macrophage migration inhibitory factor decreases West Nile virus lethality by limiting viral neuroinvasion. *J. Clin. Invest.*, 2007, **117**, 3059-3066.

ARNOW P.M., FLAHERTY J.P. Fever of unknown origin. *Lancet*, 1997, **350**, 575–580.

ARRICAU-BOUVERY N., SOURIAU A., LECHOPIER P., RODOLAKIS A. Experimental *Coxiella burnetii* infection in pregnant goats: excretion routes. *Vet. Res.*, 2003, **34**, 423-433.

ARRICAU-BOUVERY N., RODOLAKIS A. Is Q fever an emerging or re-emerging zoonosis? *Vet. Res.*, 2005a, **36**, 327-349.

ARRICAU-BOUVERY N., SOURIAU I., C. BODIER C., DUFOUR P., ROUSSET E., RODOLAKIS A. Effect of vaccines with phase 1 and phase 2 *Coxiella burnetii* vaccines in pregnant goats. *Vaccine*, 2005b, **23**, 4392-4402.

ARRICAU-BOUVERY N., HAUCK Y., BEJAOUI A., FRANGOULIDIS D., BODIER C.C., SOURIEAU A., MEYER H., NEUBAUER H., RODOLAKIS A., VERGNAUD G. Molecular characterization of *Coxiella burnetii* isolates by infrequent restrictionsite-PCR and MLVA typing. *BMC Microbiol.*, 2006, **6**, 38.

ASNIS D.S., CONETTA R., WALDMAN G., TEIXEIRA A.A. The West Nile virus encephalitis outbreak in the United States (1999-2000): from Flushing, New York, to beyond its borders. *Ann. N.Y. Acad. Sci.*, 2001, **951**, 161-171.

ASTOBIZA I., BARANDIKA J.F., HURTADO A., JUSTE R.A., GARCIA-PEREZ A.L. Kinetics of *Coxiella burentii* excretion in a commercial dairy sheep flock after treatment with oxytetracycline. *Vet. J.*, 2010, **184**, 172-175.

ASTOBIZA I., BARRAL M., RUIZ-FONS F., BARANDIKA J.F., GERRIKAGOITA X., HURTADO A., GARCIA-PEREZ A. L. Molecular investigation of the occurence of *Coxiella burnetii* in wildlife and ticks in an endemic area. *Vet. Microbiol.*, 2011, **147**, 190-194.

AUTORINO G.L., BATTISTI A., DEUBEL V., FERRARI G., FORLETTA R., GIOVANNINI A., LELLI R., MURRI S., SCICLUNA M.T. West Nile Virus epidemic in horses, Tuscany Region, Italy. *Emerg. Infect. Dis.*, 2002, **8**, 1372-1378.

B

BABUDIERI, B. Research on the behavior of *Coxiella burnetii* in relation to various physical and chemical agents. *Rent. Ist. Sup. Sanit.*, 1950, **13**, 739-743.

BABUDIERI B. Q fever: a zoonosis. *Adv. Vet. Sci.*, 1959, **5**, 82-182.

BACA O.G., PARESTSKY D. Q fever and *Coxiella burnetii*: a model for host-parasite interactions. *Microbial. Rev.*, 1983, **47**, 127-149.

BAKONYI T., HUBALEK Z., RUDOLF I., NOWOTNY N. Novel *flavivirus* or new lineage of West Nile virus, central Europe. *Emerg. Infect. Dis.*, 2005, **11**, 225-231.

BALL M.A., WELDON, A.D. Vegetative endocarditis in an Appaloosa gelding. *Cornell Vet*., 1992, **82**, 301–309.

BANDECCHI P., DELL'OMODARME M., MAGI M., PALAMIDESSI A., PRATI M.C. Feline leukaemia virus (FeLV) and feline immunodeficiency virus infections in cats in the Pisa district of Tuscany and attempts to control FeLV infection in a colony of domestic cats by vaccination. *Vet. Rec.*, 2006, **158**, 555-557.

BARSS V.A., BENACERRAF B.R., F.D.Jr. FRIGOLETTO F.D.Jr. Second trimester oligohydramnios, a predictor of poor fetal outcome. *Obstet. Gynecol.*, 1984, **64**, 608-610.

BEASLEY D.W.C., LI L., SUDERMAN M.T., BARRETT A.D.T. Mouse neuroinvasive phenotype of West Nile virus strains varies depending upon virus genotype. *Virology*, 2002, **296**, 17-23.

BEAUDEAU F., GUATTEO R., SEEGERS H. Voies d'excrétion de *Coxiella burnetii* par la vache laitière: implication pour le dépistage et la maîtrise de l'infection en élevage. *Epidémiol. et Santé anim.*, 2006, **49**, 1–4.

BENENSON A.S., TIGERTT W.D., Studies on Q fever in man. *Trans. Assoc. Am. Physicians*, 1956, **69**, 98-104.

BENSON W.W., BROCK D.W., MATHER J. Serologic analysis of a penitentiary group using raw milk from a Q fever infected herd. *Public Health Rep.*, 1963, **78**, 707–710.

BERGMANS A.M.C., DE JONG C.M.A., VAN AMERONGEN G., SCHOT C.S., SCHOULS L.M. Prevalence of *Bartonella* species in domestic cats in the Netherlands. *J.Clin. Microbiol.*, 1997, **35**, 2256-2261.

BERNIT E., POUGET J., JANBON F., DUTRONC H., MARTINEZ P., BROUQUI P., RAOULT D. Neurological involvement in acute Q fever : a report of 29 cases and review of the literature. *Arch. Intern. Med.*, 2002, **162**, 693-700.

BERRI M., SOURIAU A., CROSBY M., CROCHET D., LECHOPIER P., RODOLAKIS A. Relationships between the shedding of *Coxiella burnetii*, clinical signs and serological responses of 34 sheep. *Vet. Rec.*, 2001,**148**, 502-505.

BERRI M., SOURIAU A., CROSBY M., RODOLAKIS A. Shedding of *Coxiella burnetii* in ewes in two pregnancies following an episode of *Coxiella* abortion in a sheep flock. *Vet. Microbiol.*, 2002, **85**, 55-60.

BERRI M., ROUSSET E., CHAMPION J.L., ARRICAU-BOUVERY N., RUSSO P., PEPIN M., RODOLAKIS A. Ovine manure used as a garden fertilizer as a suspected source of human Q fever. *Vet. Rec.*, 2003, **153**, 269-270.

BERRI M., ROUSSET E., HECHARD C., CHAMPION J.L., DUFOUR P., RUSSO P., RODOLAKIS A. Progression of Q fever and *Coxiella burnetii* shedding in milk after an outbreak of enzootic abortion in a goat herd. *Vet. Rec.*, 2005, **156**, 548-549.

BERRI M., ROUSSET E., CHAMPION J.L., RUSSO P., RODOLAKIS A. Goats may experience reproductive failures and shed *Coxiella burnetii* at two successive parturitions after a Q fever infection. *Res. Vet. Sci.*, 2007, **83**, 47–52.

BILDFELL R.J., THOMSON G.W., HAINES D.M., MCEWEN B.J., SMART N. *Coxiella burnetii* infection is associated with placentitis in cases of bovine abortion. *J. Vet. Diagn. Invest.*, 2000, **12**, 419–425.

BLANC G., BRUNEAU J. Isolement du virus de la fièvre Q de deux rongeurs sauvages provenant de la forêt de Nefifik (Maroc). *Bull. Soc. Pathol. Exot. Filiales*, 1956, **49**, 431–434.

BLANC G., BRUNEAU J. Etude épidémio-écologique dans la forêt de Nefifik. 1-Présence chez le lapin de garenne et ses arthropodes piqueurs de virus pathogènes pour l'homme. *Arch. Inst. Pasteur Maroc*, 1956, **5**, 87-200.

BLITVICH B.J. Transmission dynamics and changing epidemiology of West Nile virus. *Anim. Health Res. Rev.*, 2008, **9**, 71-86.

BOCHUD P.Y., MOSER F., ERARD P., VERDON F., STUDER J.P., VILLARD G. Community-acquired pneumonia. A prospective outpatient study. *Medicine (Baltimore)*, 2001, **80**, 75–87.

BODE A.V., SEJVAR J.J., PAPE W.J., CAMPBELL G.L., MARFIN A.A. West Nile virus disease: a descriptive study of 228 patients hospitalized in a 4-county region of Colorado in 2003. *Clin. Infect. Dis.*, 2006, **42**, 1234-1240.

BONAGURA J.D., PIPERS F.S. Echocardiographic features of aortic valve endocarditis in a dog, a cow, and a horse. *J. Am. Vet. Med. Assoc.*, 1983, **182**, 595-599.

BONAGURA J.D., HERRING D.S., WELKER F. Symposium on cardiology: Echocardiography. *Vet. Clin. North Am. Equine Pract.*, 1985, **1**, 312–333.

BONDRE V.P., JADI R.S., MSIHRA A.C., YERGOLKAR P.N., ARANKALLE V.A. West Nile virus isolates from India: evidence for a distinct genetic lineage. *J. Gen. Virol.*, 2007, **88**, 875-884.

BRAULT A.C., HUANG C.Y., LANGEVIN S.A., KINNEY R.M., BOWEN R.A., RAMEY W.N., PANELLA N.A., HOLMES E.C., POWERS A.M., MILLER B.R. A single positively selected West Nile mutation confers increased virogenesis in American crows. *Nat. Gen.*, 2007, **39**, 1162-1166.

BREIMAN L., FRIEDMAN J.H., OLSHEN R.A., STONE C.J. Classification and regression Trees. Wadsworth Inc.: Montery, Pacific Grove, California, USA, 1984, 368.

BRENNAN R., RUSSELL K., ZHANG G., SAMUEL J. Both inducible nitric oxide synthases and NADPH oxidase contribute to the control of virulent phase I *Coxiella burnetii* infections. *Infect. Immun.*, 2004, **72**, 6666-6675.

BRILLIANT L. Emerging threats: can we predict and prevent them? In: International Conference on Emerging Infectious Diseases (ICEID). Atlanta, GA, 2008.

BROOKS D. L., ERMEL R.W., FRANTI C.E., RUPPANNER R., BEHYMER D.E., WILLIAMS J.C., and J. C. STEPHENSON J.C. Q fever vaccination of sheep: challenge of immunity in ewes. *Am. J. Vet. Res.*, 1986, **47**, 1235-1238.

BROUQUI P., DUPONT H.T., DRANCOURT M., BERLAND Y., ETIENNE J., LEPORT C., GOLDSTEIN F., MASSIP P., MICOUD M., BERTRAND A., RAOULT D. Chronic Q fever. Ninety-two cases from France, including 27 cases without endocarditis. *Arch. Intern. Med.*, 1993, **153**, 642-648.

BROUQUI P., DUMLER J.S., RAOULT D. Immunohistologic demonstration of *Coxiella burnetii* in the valves of patients with Q fever endocarditis. *Am. J. Med.*, 1994, **97**, 451-458.

BROWN C. Importance des maladies émergentes pour la santé publique et animale et pour les échanges commerciaux. In: OIE, 69ème Session Générale du Comité International de l'Organisation mondiale de la santé animale, Paris, France, 27 mai au 1er juin 2001, 2001, document 69 SG/9 OIE, 6 pages.

BROWN C.M. Symposium on cardiology: Acquired cardiovascular disease. *Vet. Clin. North Am. Equine Pract.*, 1985, **1**, 371–381.

BUERGELT C.D., COOLEY A.J., HINES S.A., PIPERS F.S. Endocarditis in six horses. *Vet. Pathol.*, 1985, **22**, 333–337.

BUHARIWALLA F., CANN B., MARRIE T.J. A dog related outbreak of Q fever. *Clin. Infect. Dis.*, 1996, **23**, 753-755.

BURT F.J., GROBBELAAR A.A., LEMAN P.A., ANTHONY F.S., GIBSON G.V., SWANEPOEL R. Phylogenic relationships of Southern African West Nile virus isolates. *Emerg. Infect. Dis.*, 2002, **8**, 820-826.

BUSTIN S. A. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J. Mol. Endocrinol.*, 2000, **25**, 169-193.

C

CALISTRI P., GIOVANNINI A., SAVINI G., MONACO F., BONFANTI L., CEOLIN C., TERREGINO C., TAMBA M., CORDIOLO P., LELLI R. West Nile virus transmission in 2008 in north-eastern Italy. *Zoonoses Public Health*, 2010, **57**, 211-219.

CANTILE C., DI GUARDO G., ELENI C., ARISPICI M. Clinical and neuropathological features of West Nile virus equine encephalomyelitis in Italy. *Equine Vet. J.*, 2000, **32**, 31-35.

CANTILE C., DEL PIERO F., DI GUARDO G., ARISPICI M. Pathologic and immunohistochemical findings in naturally occurring West Nile virus infection in horses. *Vet. Pathol.*, 2001, **38**, 414-421.

CARCOPINO X., RAOULT D., BRETELLE F., BOUBLI L., STEIN A. Managing Q fever during pregnancy: the benefits of long-term cotrimoxazole therapy. *Clin. Infect. Dis.*, 2007, **45**, 548-555.

CARSON P.J., KONWEKO P., WOLD K.S., MARIANA P., GOLI S., BERGLOFF P, CROSBY R.D. Long-term clinical and neuropsychological outcomes of West Nile virus infection. *Clin. Infect. Dis.*, 2006, **43**, 723-730.

CASTILLO-OLIVARES J., WOOD J. West Nile infection of horses. *Vet. Res.*, 2004, **35**, 467-483.

CECILIA D., GOULD E.A. Nucleotide changes responsible for loss of neuroinvasiveness in Japanese encephalitis virus neutralization-resistant mutants. *Virology*, 1991, **181**, 70-77.

CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC). Case definitions for infectious conditions under public health surveillance. *Morb. Mortal. Wkly. Rep.*, 1997, **49**, 25-28.

CERF O., CONDRON R. For debate: *Coxiella burnetii* and milk pasteurization: an early application of the precautionary principle? *Epidemiol. Infect.*, 2006, **134**, 946-951.

CEVA. Vaccines. [on line] (2010) URL Address: http://www.ceva.com/en/Products/Cattle/Vaccines. Accessed on 24 February 2011.

CHAMBERS T.J., HALEVY M., NESTOROWICZ A., RICE C.M., LUSTIG S. West Nile virus envelope proteins: nucleotide sequence analysis of strains differing in mouse neuroinvasiveness. *J. Gen. Virol.*, 1998, **79**, 2375-2380.

CHEN C., DOW C., WANG P., SIDNEY J., READ A., HARMSEN A., SAMUEL J.E., PETERS B. 2011: Identification of CD4+ T cell epitopes in *C. burnetii* antigens targeted by antibody responses. *PloS ONE*, 2011, **6**, 3, e17712.

CHILDS J.E., ROONEY J.A., COOPER J.L., OLSON J.G., REGNERY R.L., 1994. Epidemiologic observations on infection with *Rochalimaea* species among cats living in Baltimore, Md. *J. Am. Vet. Med. Assoc.*, 1994, **204**, 1775-1778.

CHMIELEWSKI T., SIDI-BOUMEDINE K., DUQUESNE V., PODSIADLY E., THIERY R., TYLEWSKA-WIERZBANOWSKA S. Molecular epidemiology of Q fever in Poland. *Pol. J. Microbiol.*, 2009, **58**, 9-13.

CHOMEL B.B., ABBOTT R.C., KASTEN R.W., FLOYD-HAWKINS K.A., KASS P.H., GLASER C.A., PEDERSEN N.C., KOEHLER J.E. *Bartonella henselae* prevalence in domestic cats in California : risk factors and association between bacteremia and antibody titers. *J. Clin. Microbiol.*, 1995, **33**, 2445-2450.

CHOMEL B.B., BOULOUIS H.-J., PETERSEN H., KASTEN R.W., YAMAMOTO K., CHANG C.-C., GANDOIN C., BOUILLIN C., HEW C.M. Prevalence of *Bartonella* infection in domestic cats in Denmark. *Vet. Res.*, 2002, **33**, 205-213.

CHURCH S., HARRIGAN K.E., IRVING A.E., PEEL M.M. Endocarditis caused by *Pasteurella caballi* in a horse. *Aust. Vet. J.*, 1998, **8**, 528–530

CLARCK L.A., PREGIBON D.A. Tree-based models. In: Hastie T.J. (ed), Statistical models in S. Wadsworth and Brooks: Pacific Grove, California, USA, 1992, 377-420.

COLLATOS C., CLARK E.S., REEF V.B., MORRIS D.D., Septicemia, atrial fibrillation, cardiomegaly, left atrial mass, and *Rhodococcus equi* septic osteoarthritis in a foal. *J. Am. Vet. Med. Assoc.*, 1990, **197**, 1039–1042.

COLLINS M.T., CHO S.-N., REIF J.S. Prevalence of antibodies to *Legionella pneumophila* in animal populations. *J. Clin. Microbiol.*, 1982, **15**, 130-136.

COX H.R. A filter-passing infectious agent isolated from ticks. III. Description of organisms and cultivation experiments. *Public Health Rep.*, 1938, **53**, 2270-2276.

COX H.R. *Rickettsia diaporica* and American Q fever. *Am. J. Trop. Med. Hyg.*, 1940, **20**, 463-465.

CUTLER S.J., FOOKS A.R., VAN DER POEL W.H.M. Public Health Threat of new reemerging, and neglected zoonoses in the industrialized world. *Emerg. Infect. Dis.*, 2010, **16**, 1-7.

CZAPLICKI G., HOUTAIN J.-Y., MULLENDER C., MANTECA C., SAEGERMAN C. Le lait de tank, outil fiable pour le diagnostic de la fièvre Q dans le troupeau bovin laitier? *Epidémiol. et santé anim.*, 2009, **56**, 117-127.

D

DAGNELIE P. Statistique théorique et appliquée. Tome 2. Inférence statistique à une et à deux dimensions. De Boeck & Larcier: France, 1998, 659p.

DANIEL M., KOLAR J., ZEMAN P., PAVELKA K., SADLO J. Predictive map of *Ixodes ricinus* high-incidence habitats and a tick-borne encephalitis risk assessment using satellite data. *Exp. App. Acarol.*, 1998, **22**, 417-433.

DAUPHIN G., ZIENTARA S., ZELLER H., MURGUE B. West Nile: worldwide current situation in animals and humans. *Comp. Immunol. Microbiol. Infect. Dis.*, 2004, **27**, 343-355.

DAUPHIN G., ZIENTARA S. West Nile virus: Recent trends in diagnosis and vaccine development. *Vaccine*, 2007, **25**, 5563-5576.

DAVIS G. E. *Rickettsia diaporica*: recovery of three strains from *Dermacentor andersoni* collected in southeastern Wyoming: their identity with Montana strain 1. *Public Health Rep.*, 1939, **54**, 2219-2225.

DAVIS B.S., CHANG G.J., CROPP B., ROEHRIG J.T., MARTIN D.A., MITCHELL C.J., BOWEN R., BUNNING M.L. West Nile virus recombinant DNA vaccine protects mouse and horse from virus challenge and expresses in vitro a noninfectious recombinant antigen that can be used in enzyme-linked immunosorbent assays. *J. Virol.*, 2001, **75**, 4040-4047.

DAVOLI R., SIGNORINI F.L. Antibodies against *Rickettsia burneti* in the serum of animals at a slaughter house in Tuscany. VI. *Ann. Sanita Pubblica.*, 1951, **12**, 67-73.

DASZAK P., CUNNINGHAM A.A., HYATT A.D. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Trop.*, 2001, **78**, 103-116.

DEEGEN E., LIESKE R., SCHOON H. Klinische und kardiologische Untersuchungsbefunde bei 3 Deckhengsten mit Aortenklappeninsuffizienz. *Tierärztl. Prax.*, 1980, **8**, 211–222.

DEDRICK P., REEF V.B., SWEENEY R.W., MORRIS D.D. Treatment of bacterial endocarditis in a horse. *J. Am. Vet. Med. Assoc.*, 1988, **193**, 339–342.

DEL PIERO F., STREMME D.W., HABECKER P.L., CANTILE C. West Nile *Flavivirus* Polioencephalomyelitis in a harbor seal (*Phoca vitulina*). *Vet. Pathol.*, 2006, **43**, 58-61.

DELSING C.E., KULLBERG B.J. Q fever in the Netherlands: a concise overview and implications of the largest ongoing outbreak. *Neth. J. Med.*, 2008, **66**, 365–367.

DENISON A. M., THOMPSON H.A., MASSUNG R.F. IS1111 insertion sequences of *Coxiella burnetii*: characterization and use for repetitive element PCR-based differentiation of *Coxiella burnetii* isolates. *BMC Microbiol.*, 2007, **7**, 91.

DERRICK E.H. Q fever, a new fever entity: clinical features, diagnosis and laboratory investigations. *Med. J. Aust.*, 1937, **2**, 281-299.

DEUTCH D.L., PETERSON E.T. Q fever transmission from one human being to others. *J. Am. Med. Assoc.*, 1950, **143**, 348-354.

DIAMOND M.S., KLEIN R.S. West Nile virus: crossing the blood-brain barrier. *Nat. Med.*, 2004, **10**, 1294-1295.

DILBECK P. M., MC ELWAIN T.F. Immunohistochemical detection of *Coxiella burnetii* in formalin-fixed placenta. *J. Vet. Diagn. Invest.*, 1994, **6**, 125-127.

DORKO E., KALINOVA Z., PILIPCINEC E. Seroprevalence of *Coxiella burnetii* antibodies among students of the Faculty of medicine in Kosice (Slovakia). *Folia Microbiol.*, 2008a, **53**, 563-568.

DORKO E., KALINOVA Z., WEISSOVA T., PILIPCINEC E. Seroprevalence of antibodies to *Coxiella burnetii* among employees of the Veterinary University in Kosice, Eastern Slovakia. *Ann. Agric. Environ. Med.*, 2008b, **15**, 119–124.

DUBUC-FORFAIT C., ROUSSET E., CHAMPION J.L., MAROIS M., DUFOUR P., ZERHAOUI E., THIERY R., SABATIER P. Démarche d'appréciation du risque d'excrétion de *Coxiella burnetii* dans les troupeaux caprins laitirs dans le sud-est de la France. *Epidémiol. et santé anim.*, 2009, **55**, 117-136.

DUPONT H.T., THIRION X., RAOULT D. Q fever serology: cut off determination for microimmunofluorescence. *Clin. Diagn. Lab. Immunol.*, 1994, **1**, 189-196.

E

EJERCITO C.L., CAI L., HTWE K.K., TAKI M., INOSHIMA Y., KONDO T., KANO C., ABE S., SHIROTA K., SUGIMOTO T., YAMAGUCHI T., FUKUSHI H., MINAMOTO N., KINJO T., ISOGAI E., HIRAI K.,. Serological evidence of *Coxiella burnetii* infection in wild animals in Japan. *J. Wildl. Dis.*, 1993, **29**, 481–484.

EL GARCH H., MINKE J.M., REHDER J., RICHARD S., EDLUND TOULEMONDE C., DINIC S., ANDREONI C., AUDONNET J.C., NORDGREN R., JUILLARD V. A West Nile virus (WNV) recombinant *canarypox* virus vaccine elicits WNV-specific neutralizing antibodies and cell-mediated immune responses in the horse. *Vet. Immunol. Immunopath.*, 2008, **123**, 230-239.

ENRIGHT J.B., FRANTI C.E., LONGHURST W.M., BEHYMER D.E., WRIGHT M.E., DUTSON V.J. *Coxiella burnetii* in a wildlife-livestock environment: antibody response of ewes and lambs in an endemic Q fever area. *Am. J. Epidemiol.*, 1971, **94**, 62-71.

ENSERINK M. Questions abound in Q-fever explosion in the Netherlands. *Science*, 2010, **327**, 266-267.

EPP T., WALDNER C., LEIGHTON F.A., BERKE O., TOWNSEND H.G. Seroprevalence and risk factors for infection with West Nile virus in Saskatchewan, 2003. *Can. J. Vet. Res.*, 2007a, **71**, 256-263

EPP T., WALDNER C., WEST K., TOWNSEND H. Factors associated with West Nile virus disease fatalities in horses. *Can. Vet. J.*, 2007b, **48**, 1137-1145.

EPSTEIN P.R. West Nile virus and the climate. *J. Urban Health*, 2001, **78**, 367-371.

EVANS A.D. 'Q' fever in South Wales. *Mon. Bull. Minist. Public Health Lab. Serv.*, 1956, **15**, 215-219.

EWART S., BROWN C., DERKSEN F., KUFUOR-MENSA E., *Serratia marcescens* endocarditis in a horse. *J. Am. Vet. Med. Assoc.*, 1992, **200**, 961–963.

F

FARAJOLLAHI A., CRANS W.J., BRYANT P., WOLF B., BURKHALTER K.L., GODSEY M.S., ASPEN S.E., NASCI R.S. Detection of West Nile viral RNA from an overwintering pool of *Culex pipens pipiens* (*Diptera: Culicidae*) in New Jersey, 2003. *J. Med. Entomol.*, 2005, **42**, 490-494.

FENOLLAR F., FOURNIER P.E., CARRIERI M.P., HABIB G., MESSANA T., RAOULT D. Risk factors and prevention of Q fever endocarditis. *Clin. Infect. Dis.*, 2001, **33**, 312-316.

FENOLLAR F., FOURNIER P.E., RAOULT D. Molecular detection of *Coxiella burnetii* in the sera of patients with Q fever endocarditis or vascular infection. *J. Clin. Microbiol.*, 2004, **42**, 4919-4924.

FENOLLAR F., THUNY F., XERIDAT B., LEPIDI H., RAOULT D. Endocarditis after acute Q fever in patients with previously undiagnosed valvulopathies. *Clin. Infect. Dis.*, 2006, **42**, 818-821.

FIELD P.R., MITCHELL J.L., SANTIAGO A., DICKESON D.J., CHAN S.W., HO D.W., MURPHY A.M., CUZZUBBO A.J., DEVINE P.L. Comparison of a commercial enzyme-linked imunosorbent assay with immunofluorescence and complement fixation tests for detection of *Coxiella burn*etii (Q fever) immunoglobulin. *Med. J. Clin. Microbiol.*, 2000, **38**, 1645–1647.

FISET P., Phase variation of *Rickettsia (Coxiella) burnetii*; study of antibody response in guinea pigs and rabbits. *Can. J. Microbiol.*, 1957, **3**, 435.

FOURNIER P. E., MARRIE T.J., RAOULT D. Diagnosis of Q fever. *J. Clin. Microbiol.*, 1998, **36**, 1823-1834.

FOURNIER P.E., ETIENNE J., HARLE J.R., HABIB G., RAOULT D. Myocarditis, a rare but severe manifestation of Q fever: report of 8 cases and review of literature. *Clin. Infect. Dis.*, 2001, **32**, 1440-14407.

FRANGOULIDIS D., RODOLAKIS A., HEISER V., LANDT O., SPLETTSTOESSER W., MEYER H. DNA microarray-chip based diagnosis of Q-fever (*Coxiella burnetii*). *Clin. Microbiol. Infect.*, 2009, **15**, 165–166.

FRAZIER M.E., MALLAVIA L.P., SAMUEL J.E., BACA O.G. DNA probes for the identification of *Coxiella burnetii* strains. *Ann. N.Y. Acad. Sci.*, 1990, **590**, 445-458.

FREEDMAN L.R., The pathogenesis of infective endocarditis. *J. Antimicrob. Chemother.*, 1987, **20**: Suppl A, 1–6.

FRIES L.F., WAAG D.M., WILLIAMS J.C. Safety and immunogenicity in humans volunteers of a chloroform-methanol residue vaccine for Q fever. *Infect. Immun.*, 1993, **61**, 1251-1258.

FROEHLICH W., WLASCHITZ S., RIEDELBERGER K., REEF V.B. Case report: Tricuspid valve endocarditis in a horse with ventricular septal defect. *Equine Vet. Educ.*, 2006, **18**, 172–176.

FROHLICH W., WLASCHITZ S., RIEDELBERGER K., REIFINGER M. Fallbericht: Aortenklappenendocarditis beim Pferd. *Dtsch. Tierärztl. Wochenschr.*, 2004, **111**, 370–373.

FUCHS A., BINZEL L., LONSDORFER M. Epidemiology of FeLV and FIV infection in Federal Republic of Germany. *Tierarztl. Prax.*, 1994, **22**, 273-277.

G

GARCIA-PEREZ A. L., ASTOBIZA I., BARANDIKA J.F., ATXAERANDIO R., HURTADO A., JUSTE R.A. Short communication: investigation of *Coxiella burnetii* occurrence in dairy sheep flocks by bulk-tank milk analysis and antibody level determination. *J. Dairy Sci.*, 2009, **92**, 1581-1584.

GEFENAITE G., MUNSTER J.M., VAN HOUDT R., HAK E. Effectiveness of the Q fever vaccine: a meta-analysis. *Vaccine*, 2011, **29**, 395-398.

GERTH H.J., LEIDIG U., RIEMENSCHNEIDER T. Q-fieber Epidemie in einem Institut for Humanpathologie. *Deut. Med. Wochenschr.*, 1982, **107**, 1391-1395.

GIDDING H.F., WALLACE C., LAWRENCE G.L., MCINTYRE P.B. Australia's national Q fever vaccination program. *Vaccine*, 2009, **27**, 2037–2041.

GIKAS A., KOFTERIDIS D.P., MANIOS A., PEDIADITIS J., TSELENTIS Y. Newer macrolides as empiric treatment of acute Q fever infection. *Antimicrob. Agents Chemother.*, 2001, **45**, 3644-3646.

GILLESPIE J.H., BAKER J.A., Experimental Q fever in cats. *Am. J. Vet. Res.*, 1952, **13**, 91-94.

GIMENEZ D.F. Staining *rickettsiae* in yolk-sac cultures. *Stain technol.*, 1964, **39**, 135-140.

GIROUD P., JARDIN J. Infection latent et conservation de *Rickettsia burnettii* chez l'homme: le rôle du pou. *Bull. Soc. Pathol. Exot.*, 1954, **45**, 764-765.

GLASS W.G., LIM L.K., CHOLERA R., PLETNEV A.G., GAO J.L., MURPHY P.M., Chemokine receptor CCR5 promotes leukocyte trafficking to the brain and survival in West Nile infection. *J. Exp. Med.*, 2005, **202**, 1087-1098.

GLAZUNOVA O., ROUX V., FREYLIKMAN O., SEKEYOVA Z., FOURNOUS G., TYCZKA J., TOKAREVITCH N., KOVACAVA E., MARRIE T.J., RAOULT D. *Coxiella burnetii* genotyping. *Emerg. Infect. Dis.*, 2005, **11**, 1211-1217.

GLEICH S.E., KRIEGER S., HARTMANN K. Prevalence of feline immunodeficiency virus and feline leukaemia virus among client-owned cats and risk factors for infection in Germany. *J. Feline Med. Surg.*, 2009, **11**, 985-992.

GONDER J.C., KISHIMOTO R.A., KASTELLO M.D., PEDERSEN C.E., LARSON E.W. Cynomolgus monkey model for experimental Q fever infection. *J. Infect. Dis.*, 1979, **139**, 191-196.

GOTTFRIED K., QUINN R., JONES T. Clinical description and follow-up investigation of human West Nile virus cases. *South Med. J.*, 2005, **98**, 603-606.

GREEN M.S. Long-term death rates, West Nile virus epidemic, Israel, 2000. *Emerg. Infect. Dis.*, 2005, **11**, 1754-1757.

GRENIER B. Chapitre 6 : Arbre de décision. In : Grenier B. Décision médicale: analyse et stratégie de la décision dans la pratique médicale. Masson: Paris, 1990a, 107-136.

GRENIER B. Utilisation des ODDS dans les tests associés ou séquentiels. In: Grenier B. Décision médicale: analyse et stratégie de la décision dans la pratique médicale. Masson: France, 1990b, 102-106.

GUATTEO R., BEAUDEAU F., BERRI M., RODOLAKIS A., JOLY A.,SEEGERS H. Shedding routes of *Coxiella burnetii* in dairy cows: implications for detection and control. *Vet.Res.*, 2006, **37**,827–833.

GUATTEO R., BEAUDEAU F., LEDOUX D., DREAN E., SEEGERS H., Risk of false-negative results when delaying PCR from sampling for *Coxiella burnetii* detection in dairy cows. *Rev. Med. Vet.*, 2007, **158**, 641–644.

GUATTEO R., SEEGERS H., JOLY A., BEAUDEAU F. Prevention of *Coxiella burnetii* shedding in infected dairy herds using a phase I *C. burnetii* inactivated vaccine. *Vaccine*, 2008a, **26**, 4320-4328.

GUATTEO R., JOLY A., RODOLAKIS A., COCHONNEAU D., SARRADIN P., SEEGERS H., REMMY D., BEAUDEAU F. Prévention de l'excrétion de *Coxiella burnetii* à l'aide d'un vaccin dit phase I (Coxevac® en troupeaux bovines laitiers infectés). *Renc. Rech. Ruminants*, 2008b, **15**, 59-62.

H

HAALAND K.Y., SADEK J., PERGAM S., ECHEVARRIA L.A., DAVIS L.E., GOADE D., HARNAR J., NOFCHISSEY R.A., SEWEL C.M., ETTESTAD P. Mental status after West Nile virus infection. *Emerg. Infect. Dis.*, 2006, **12**, 1260-1262.

HACKSTADT T., WILLIAMS J.C. Biochemical stratagem for obligate parasitism of eukaryotic cells by *Coxiella burnetii*. *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 3240-3244.

HAINES A., KOVATS R.S., CAMPBELL-LENDRUM D., CORVALAN C. Climate change and human health: impacts, vulnerability, and mitigation. *Lancet*, 2006, **367**, 2101-09.

HALEVY M., AKOV Y., BEN-NATHAN D., KOBILER D., LACHMI B., LUSTIG S. Loss of active neuroinvasiveness in attenuated strains of West Nile virus: pathogenicity in immunocompetent and SCID mice. *Arch. Virol.*, 1994, **137**, 355-370.

HARMAN J.B. Q fever in Great Britain; clinical account of eight cases. *Lancet*, 1949, **2**, 1028-1030.

HARTZELL J.D., PENG S.W., WOOD-MORRIS R.N., SARMIENTO D.M., COLLEN J.F., ROBBEN P.M., MORAN K.A. Atypical Q fever in US soldiers. *Emerg. Infect. Dis.*, 2007, **13**, 1247-1249.

HARTZELL J.D., WOOD-MORRIS R.N., MARTINEZ L.J., TROTTA R.F. Q fever: epidemiology, diagnosis, and treatment. *Mayo Clin. Proc.*, 2008, **83**, 574-579.

HATCHETTE T., CAMPBELL N., WHITNEY H., HUDSON R., MARRIE T.J. Seroprevalence of *Coxiella burnetii* in selected populations of domestic ruminants in Newfoundland. *Can. Vet. J.*, 2002, **43**, 363-364.

HATCHETTE T., CAMPBELL N., HUDSON R., RAOULT D., MARRIE T.J. Natural history of Q fever in goats. *Vector Borne Zoonotic Dis.*, 2003, **3**, 11-15.

HATFIELD C.E., REBHUN W.C., DIETZE A.E. Endocarditis and optic neuritis in a quarter horse mare. *Compendium Equine*, 1987, **9**, 451–454.

HAYES C.G. West Nile Fever. In: Monath T.P., The arboviruses: epidemiology and ecology. CRC Press: Boca Raton, Florida, 1989, 59-88.

HEALTH PROTECTION AGENCY (HPA). CENTRE FOR INFECTIONS. Guidelines for action in the event of a deliberate release: Q fever. Version 1.7 [on line] (14 June 2010) URL Address: http://www.hpa.org.uk/deliberate_accidental_releases/biological. Accessed on 24 February 2011.

HELBIG K. J., HEATLEY S.L., HARRIS R.J., MULLIGHAN C.G., BARDY P.G., MARMION B.P. Variation in immune response genes and chronic Q fever. Concepts: preliminary test with post-Q fever fatigue syndrome. *Genes Immun.*, 2003, **4**, 82-85.

HENDRIX L. R., SAMUEL J.E., MALLAVIA L.P. Differentiation of *Coxiella burnetii* isolates by analysis of restriction-endonuclease-digested DNA separated by SDS-PAGE. *J. Gen. Microbiol.*, 1991, **137**, 269-276.

HERMANS M.H.A., HUIJSMANS C.R.J.J., SCHELLEKENS J.J.A., SAVELKOUL P.H.M., WEVER P.C. *Coxiella burentii* DNA in goat milk after vaccination with Coxevac®. *Vaccine*, 2011, **29**, 2653-2656.

HERR S., HUCHZERMEYER H.F., TE BRUGGE L.A., WILLIAMSON C.C., ROOS J.A., SCHIELE G.J. The use of a single complement fixation test technique in bovine brucellosis, Johne's disease, dourine, equine piroplasmosis and Q fever serology. *Onderstepoort J. Vet. Res.*, 1985, **52**, 279-282.

HICKIE I., DAVENPORT T., WAKEFIELD D., VOLLMER-CONNA U., CAMERON B., VERNON S.D., REEVES W.C., LLOYD A. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. *BMJ*, 2006, **333**, 575.

HIGGINS D., MARRIE T.J. Seroepidemiology of Q fever among cats in New Brunswick and Prince Edward Island. *Ann. N.Y. Acad. Sci.*, 1990, **590**, 271-274.

HILL J., SAMUEL J.E. *Coxiella burnetii* acid phosphatase inhibits the release of reactive oxygen intermediates in polymorphonuclear leukocytes. *Inf. Immunol.*, 2011, **79**, 414-420.

HILLYER M.H., MAIR T.S., HOLMES J.R. Treatment of bacterial endocarditis in a Shire mare. *Equine Vet. Educ.*, 1990, **2**, 5–7.

HINES M.T., HEIDEL J.R., BARBEE D.D. Bacterial endocarditis with thrombus formation and abscessation in a horse. *Vet. Radiol. Ultrasound.*, 1993, **34**, 47–51.

HIRAI K. Q fever. *J. Clin. Sci.*, 1997, **33**, 1156–1161.

HIRAI K., TO H. Advances in the understanding of *Coxiella burnetii* infection in Japan. *J. Vet. Med. Sci.*, 1998, **60**, 781-790.

HIRAI K. Recent study of Q Fever. *J. Vet. Med. Assoc.*, 1999, **52**, 77–83.

HIRSCHMAN J.V. Fever of unknown origin in adults. *Clin. Infect. Dis.*, 1997, **24**, 291–300.

HOGERWERF L., VAN DEN BROM R., ROEST H.I.J., BOUMA A., VELLEMA P., PIETERSE M., DERCKSEN D., NIELEN M. Reduction of *Coxiella burentii* prevalence by vaccination of goats and sheep, the Netherlands. *Emerg. Infect.*, 2011, **17**, 379-386.

HOOVER T. A., CULP D.W., VODKIN M.H., SHAW E.I. Chromosomal DNA deletions explain phenotypic characteristics of two antigenic variants, phase 2 and RSA 514 (Crazy), of *Coxiella burnetii* Nine Mile strain. *Infect. Immun.*, 2002, **70**, 6726-6733.

HOSIE M.J., ROBERTSON C., JARRETT O. Prevalence of feline leukaemia virus and antibodies to feline immunodeficiency virus in cats in the United Kingdom. *Vet. Rec.*, 1989, **125**, 293-297.

HOU M.Y., HUNG M.N., LIN P.S., WANG Y.C., LIN C.C., SHU P.Y., SHIH W.Y., WU H.S., LIN L.J. Use of a single-tube nested real-time PCR assay to facilitate the early diagnosis of acute Q fever. *Jpn J. Infect. Dis.*, 2011, **64**, 161-162.

HTWE K.K., AMANO K., SUGIYAMA Y., YOGAMI K., MINAMOTO N., HASHIMOTO A., YAMAGUCHI T., FUKUSHI H., HIRAI K. Seroepidemiology of *Coxiella burnetii* in domestic and companion animals. *Vet. Rec.*, 1992, **131**, 490.

HTWE K.K., YOSHIDA T., HAYASHI S., MIYAKE T., AMANO K., MORITA C., YAMAGUCHI T., FUKUSHI H., HIRAI K. Prevalence of antibodies to *Coxiella burnetii* in Japan. *J. Clin. Microbiol.*, 1993, **31**, 722–723.

HUBALEK Z., HALOUZKA J. West Nile Fever - a Reemerging Mosquito-Borne Viral Disease in Europe. *Emerg. Infect. Dis.*, 1999, **5**, 643-650.

HUIJSMANS C.J.J., SCHELLEKENS J.J.A., WEVER P.C., TOMAN R., SAVELKOUL P.H.M., JANSE I., HERMANS M.H.A. Single-Nucleotide-Polymorphism genotyping of *Coxiella burnetii* during a Q fever outbreak in the Netherlands. *Appl. Environ. Microbiol.*, 2011, **77**, 2051-2057.

I

INOKUMA H., YAMAMOTO S., MORITA C. Survey of tick-borne diseases in dogs infested with *Rhipicephalus sanguineus* at a kennel in Okayama Prefecture, Japan. *J. Vet. Med. Sci.*, 1998, **60**, 761-763.

IZZO A.A., MARMION B.P. Variation in interferon-gamma responses to *Coxiella burnetii* antigens with lymphocytes from vaccinated or naturally infected subjects. *Clin. Exp. Immunol.*, 1993, **94**, 507-515.

J

JESTY S.A., REEF V.B. Septicemia and cardiovascular infections in horses. *Vet. Clin. Equine*, 2006, **22**, 481–495.

JONES K.E., PATEL N.G., LEVY M.A., STOREYGARD A., BALK D., GITTLE J.L., DASZAK P. Global trends in emerging infectious diseases. *Nature*, 2008, **451**, 990-993.

JOHNSON D.J., OSTLUND E.N., PEDERSEN D.D., SCHMITT B.J. Detection of North American West Nile virus in animal tissue by a reverse transcription-nested polymerase chain reaction assay. *Emerg. Infect. Dis.*, 2001, **7**, 739-741.

JOHNSON D.J., OSTLUND E.N., SCHMITT B.J. Nested multiplex RT-PCR for detection and differentiation of West Nile virus and eastern equine encephalomyelitis virus in brain tissues. *J. Vet. Diagn. Invest.* 2003, **15**, 488-493.

JOHNSON G., NEMETH N., HALE K., LINDSEY N., PANELLA N., KOMAR N., Surveillance for West Nile virus in American white pelicans, Montana, USA, 2006-2007. *Emerg. Infect. Dis.*, 2010, **16**, 406-411.

JOUBERT L., OUDAR J., HANNOUN C., CHIPPAUX M. Reproduction expérimentale de la méningo-encéphalomyélite du cheval par l'arbovirus West Nile. III. Relations entre la virologie, la sérologie et l'évolution anatomo-clinique. Conséquences épidémiologiques et prophylactiques. *Bull. Acad. Vet.*, 1971, **44**, 159-167.

JOURDAIN E., GAUTHIER-CLERC M., SABATIER P., GREGE O., GREENLAND T., LEBLOND A., LAFAYE M., ZELLER H.G. Magpies as hosts for West Nile virus, Southern France. *Emerg. Infect. Dis.*, 2008, **14**, 158-160.

JUVET F., LAPPIN M.R., BRENNAN S., MOONEY C.T. Prevalence of selected infectious agents in cats in Ireland. *J. Feline Med. Surg.*, 2010, **12**, 476-482.

K

KANTSO B., SVENDSEN C.B., JENSEN P.M., VENNESTROM J., KROGFELT K.A., Seasonal and habitat variation in the prevalence of *Rickettsia helvetica* in *Ixodes ricinus* ticks from Denemark. *Ticks and tick-borne diseases*, 2006, **1**,101-103.

KAPLAN B., RABINERSON D., BEN-ARI S., NERI A., MERLOB P. An isolated case of Q fever during pregnancy. *Acta Obstet. Gynecol. Scand.*, 1995, **74**, 848-849.

KAPLAN N.A., MOORE B.R. Case report: *Streptococcus equi* endocarditis, meningitis and panophtalmitis in a mature horse. *Equine Vet. Educ.*, 1996, **8**, 313–316.

KARAGIANNIS I., SCHIMMER B., VAN LIER A., TIMEN A., SCHNEEBERGER P., VAN ROTTERDAM B., DE BRUIN A., WIJKMANS C., RIETVELD A., VAN DUYNHOVEN Y. Investigation of a Q fever outbreak in a rural area of The Netherlands. *Epidemiol. Infect.*, 2009, **137**, 1283-1294.

KARAKOUSIS P.C., TRUCKSIS M., DUMLER J.S. Chronic Q fever in the United States. *J. Clin. Microbiol.*, 2006, **44**, 2283-2287.

KARZENSKI S., CRISMAN M.V., ROBERTSON J. Endocarditis in a stallion with a history of testicular torsion. *Equine Pract.*, 1997, **19**, 24–27.

KAZAR J., BREZINA R., PALANOVA A., TVRDA B., SCHRAMEK S. Immunogenicity and reactogenicity of a Q fever chemovaccine in persons professionally exposed to Q fever in Czechoslovakia. *Bull. World Health Organ.*, 1982, **60**, 389-394.

KAZAR J., SCHRAMEK S., LISAK V., BREZINA R. Antigenicity of a chloroform-methanol-treated *Coxiella burentii* preparation. *Acta Virol.*, 1987, **31**, 158-167.

KAZAR J., LESNY M., PROPPER P., VALKOV D., BREZINA R. Comparaison of virulence for guinea pigs and mice of different *Coxiella burnetii* phase 1 strains. *Acta Virol.*, 1993, **37**, 437-448.

KEESING F., BRUNNER J., DUERR S., KILLILEA M., LOGIUDICE K., SCHMIDT K., VUONG H., OSTFELD R.S. Hosts as ecological traps for the vector of Lyme disease. *Proc. R. Soc. Lond. B. Biol. Sci.*, 2009, **276**, 3911-3919.

KEESING F., BELDEN L.K., DASZAK P., DOBSON A., HARVELL C.D., HOLT R.D., HUDSON P., JOLLES A., JONES K.E., MITCHELL C.E., MYERS S.S., BOGICH T., OSTFELD R.S., Impacts of biodiversity on the emergence and transmission of infectious diseaseas. *Nature*, 2010, **468**, 647-652.

KELLEY T.W., PRAYSON R.A., RIUZ A.I., ISADA C.M., GORDON S.M., The neuropathology of West Nile virus meningoencephalitis. A report of two cases and review of the literature. *Am. J. Clin. Pathol.*, 2003, **119**, 749-753.

KHAVKIN T. N. Pathologo-anatomic and experimental study of the morphology of Q fever. *Arkh. Patol.*, 1977, **39**, 75-84.

KILPATRICK A.M., KRAMER L.D., JONES M.J., MARRA P.P., DASZAK P. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior, *PLoS Biol.*, 2006, **4**, e82.

KIM S. G., KIM E.H., LAFFERTY C.J., DUBOVI E. *Coxiella burnetii* in bulk tank milk samples, United States. *Emerg. Infect. Dis.*, 2005, **11**, 619-621.

KLAASSEN C. H. W., NABUURS-FRANSEN M.H., TILLBURG J.J.H.C., HAMANS M.A.W.M., HORREVORTS A.M. Multigenotype Q fever outbreak, The Netherlands. *Emerg. Infect. Dis.*, 2009, **15**, 613-614.

KLEE A.L., MAIDIN B., EDWIN B., POSHNI I., MOSTASHARI F., FINE A., LAYTON M., NASH D. Long-term prognosis for clinical West Nile virus infection. *Emerg. Infect. Dis.*, 2004, **10**, 1405-1411.

KNOCKAERT D.C., VANDERSCHUEREN S., BLOCKMANS D. Fever of unknown origin in adults: 40 years on. *J. Intern. Med.*, 2003, **253**, 263–275.

KOMIYA T., SADAMASU K., KANG M.I., TSUBOSHIMA S., FUKUSHI H., HIRAI K. Seroprevalence of *Coxiella burnetii* infections among cats in different living environments. *J. Vet. Med. Sci.*, 2003, **65**, 1047-1048.

KOPLAN J. CDC's strategic plan for bioterrorism preparedness and response. *Public Health Rep.*, 2001, **116**, 9.

KOSATSKY T. Household outbreak of Q-fever pneumonia related to a parturient cat. *Lancet*, 1984, **2**, 1447-1449.

KOSOY M.Y., REGNERY R.L., TZIANABOS T., MARSTON E.L., JONES D.C., GREEN D., MAUPIN G.O., OLSON J.G., CHILDS J.E. Distribution, diversity, and host specificity of *Bartonella* in rodents from Southeastern United States. *Am. J. Trop. Med. Hyg.*, 1997, **57**, 578-588.

KOSTER F.T., WILLIAMS J.C., GOODWIN J.S. Cellular immunity in Q fever: specific lymphocyte unresponsiveness in Q fever endocarditis. *J. Infect. Dis.*, 1985a, **152**, 1283-1289.

KOSTER F.T., WILLIAMS J.C., GOODWIN J.S. Cellular immunity in Q fever – modulation of responsiveness by a suppressor T cell monocyte circuit. *J. Immunol.*, 1985b, **135**, 1067-1072.

KOULLA-SHIRO S., KUABAN C., BELEC L. Microbial etiology of acute community-acquired pneumonia in adult hospitalized patients in Yaounde-Cameroon. *Clin. Microbiol. Infect.*, 1997, **3**, 180–186.

KRAMER M., OBERMAJER N., MATIJASIC B.B., ROGELJ I., KMETEC V. Quantification of live and dead probiotic bacteria in lyophilized products by real-time PCR and by flow cytometry. *Appl. Microbiol. Biotechnol.*, 2009, **84**, 1137-1147.

KRT B. The influence of *Coxiella burnetti* phase I and phase II antigens on the serological diagnosis of Q fever in cattle. *Slov. Vet. Research*, 2003, **40**, 203-207.

KRUMBIEGEL E.R., WISNIEWSKI H.J. Q fever in the Milwaukee area II. Consumption of infected raw milk by human volunteers. *Arch. Environ. Health*, 1970, **21**, 63–65.

KRUSE H., KIRKEMO A.M., HANDELAND K. Wildlife as source of zoonotic infections. *Emerg. Infect. Dis.*, 2004, **10**, 2067-2072.

KRUSZEWSKA D., LEMBOWICZ K., TYLEWSKA-WIERZBANOWSKA S. Possible sexual transmission of Q fever among humans. *Clin. Infect. Dis.*, 1996, **22**, 1087-1088.

KUIKEN T., LEIGHTON F.A., FOUCHIER R.A., LEDUC J.W., PEIRIS J.S., SCHUDEL A., STOHR K., OSTERHAUS A.D., Public health: pathogen surveillance in animals. *Science*, 2005, **309**, 1680-1681.

KUROIWA Y., OYANAGI R., FUSE S., MORI T., UENO H., TSUTSUMI H. Persistent hepatitis and repeated wheezing in an infant. Q fever. *Pediatr. Infect. Dis. J.*, 2007, **26**, 763, 768-769.

L

LAMBIN E.F., TRAN A., VANWANBEKE S.O., LINARD C., SOTI V. Pathogenic landscapes: interactions between land, people, disease vectors, and their animal hosts. *Int. J. Health Geogr*., 2010, **9**, 54

LANCIOTTI R.S., ROEHRIG J.T., DEUBEL V., SMITH J., PARKER M., STEELE K., et al., Origin of West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science*, 1999, **286**, 2333-2337.

LANG G.H. Coxiellosis (Q fever) in animals. In: Marrie T.J (Ed), Q fever. The disease. CRC Press: Boca raton, Florida, 1990, vol 1, 23-48.

LANGLEY J. M., MARRIE T.J., LEBLANC J.C., ALMUDEVAR A., RESCH L., RAOULT D. *Coxiella burnetii* seropositivity in parturient women is associated with adverse pregnancy outcomes. *Am. J. Obstet. Gynecol.*, 2003,**189**, 228-232.

LA SCOLA B., RAOULT D. Serological cross-reactions between *Bartonella quintana*, *Bartonella henselae*, and *Coxiella burnetii*. *J. Clin. Microbiol.*, 1996, **34**, 2270-2274.

LA SCOLA B., LEPIDI H., RAOULT D. Pathologic changes during acute Q fever: influence of route of infection and inoculum size in infected guinea pigs. *Infect. Immun.*, 1997, **65**, 2443-2447.

LA SCOLA B., LEPIDI H., MAURIN M., RAOULT D. A guinea pig model for Q fever endocarditis. *J. Infect. Dis.*, 1998, **178**, 278-281.

LEBLOND A., HENDRIKX P., SABATIER P. West Nile outbreak detection using syndromic monitoring in horses. *Vector Borne Zoonotic Dis.*, 2007a, **7**, 403-410.

LEBLOND A., SANDOZ A., LEFEBVRE G., ZELLER H., BICOUT D.J. Remote sensing based identification of environmental risk factors associated with West Nile disease in horses in Camargue, France. *Prev. Vet. Med.*, 2007b, **79**, 20-31.

LEONE M., HONSTETTRE A., LEPIDI H., CAPO C., BAYARD F., RAOULT D., MEGE J.L. Effect of sex on *Coxiella burnetii* infection: protective role of 17beta-estradiol. *J. Infect. Dis.*, 2004, **189**, 339-345.

LEPIDI H., GOURIET F., RAOULT D. Immunohistochemical detection of *Coxiella burnetii* in chronic Q fever hepatitis. *Clin. Microbiol. Inf.*, 2009, **15**: Suppl 2, 169-170.

LEWIS R.J. An introduction to classification and regression tree (CART) analysis. In: The 2000 Annual Meeting of the Society for Academic Emergency Medicine in San Francisco, California, USA. 25 May 2000, 2000, 1-14.

LI Q., NIU D., WEN B., CHEN M., QIU L., ZHANG J. Protective imunity against Q fever induced with a recombinant P1 antigen fused with HspB of *Coxiella burnetii*. *Ann. N.Y. Acad. Sci.*, 2005, **1063**, 130-142.

LIEBERMAN D., SCHLEAFFER F., BOLDUR I., LIEBERMAN D., HOROWITZ S., FRIEDMAN M.G., LEIONONEN M., HOROVITZ O., MANOR E., PORATH A. Multiple pathogens in adult patients admitted with community acquired pneumonia: a one-year prospective study of 346 consecutive patients. *Thorax*, 1996, **51**, 179–184.

LIEBERMAN D., LIEBERMAN D., BEN-YAAKOV M., LAZAROVICH Z., HOFFMAN S., OHANA B., FRIEDEMAN M.G., DVOSKIN B., LEINONEN M., BOLDUR I. Infectious etiologies in acute exacerbation of COPD. *Diagn. Microbiol. Infect. Dis.*, 2001, **40**, 95–102.

LIM J.K., LISCO A., MCDERMOTT D.H., HUYNH L., WARD J.M., JOHNSON H., PAPER J., FOSTER G.A., KRYSZTOF D., FOLLMANN D., STRAMER S.L., MARGOLIS L.B., MURPHY P.M. Genetic variation in OAS1 is a risk factor for initial infection with West Nile virus in man. *PLoS Pathog.*, 2009, **5**, e1000321.

LIM J.K., MCDERMOTT D.H., LISCO A., FOSTER G.A., KRYSZTOF D., FOOLMANN D., STRAMER S.L., MURPHY P.M. CCR5 deficiency is a risk factor for early clinical manifestations of West Nile virus but not for viral transmission. *J. Infect. Dis.*, 2010, **201**,178-185.

LOGUIDICE K., DUERR S.T., NEWHOUSE M.J., SCHMIDT K.A., KILLILEA M.E., OSTFELD R.S. Impact of host community composition on Lyme disease risk. *Ecology*, 2008, **89**, 2841-2849.

LOZANO F., TORRE-CISNEROS T., BASCUNANA A., POLO J., VICIANA P., GARCIA-ORDONEZ M.A., HERNANDEZ-QUERO J., MARQUEZ M., VERGARA A., DIEZ F., PUJOL E., TORRES-TORTOSA M., PASQUAU J., HERNANDEZ-BURRUEZO J.J., SUAREZ I. Prospective evaluation of fever of unknown origin in patients infected with the human immunodeficiency virus. *Eur. J. Clin. Microbiol. Infect. Dis.*, 1996, **15**, 705–711 (Grupo Andaluz para el Estudio de las Enfermedades Infecciosas.).

LUDLAM H., WREGHITT T.G., THORNTON S., THOMSON B.J., BISHOP N.J., COOMBER S., CUNNIFFE J.Q fever in pregnancy. *J. Infect.*, 1997, **34**, 75-78.

LUDWIG A., BIGRAS-POULIN M., MICHEL P., BELANGER D. Risk factors associated with West Nile virus mortality in American crow populations in Southern Quebec. *J. Wild. Dis.*, 2010, **46**, 195-208.

LVOV D.K., BUTENKO A.M., GROMASHEVSKY V.L., KOVTUNOV A.I., PRILIPOV A.G., KINNEY R., ARISTOVA V.A., DZHARKENOV A.F., SAMOKHVALOV E.I., SAVAGE H.M., SHCHELKANOV M.Y., GALKINA I.V., DERYABIN P.G., GUBLER D.J., KULIKOVA L.N., ALKHOVSKY S.K., MOSKVINA T.M., ZLOBINA L.V., SADYKOVA G.K., SHATALOV A.G., LVOV D.N., USACHEV V.E., VORONINA A.G. West Nile virus and other zoonotic viruses in Russia: examples of emerging-reemerging situations. *Arch. Virol.*, 2004, **Suppl 18**, 85-96.

M

MAHARA F. Rickettsioses in Japan and the Far East. *Ann. N.Y. Acad.*, 2006, **1078**, 60–73.

MALLAVIA L. P. Genetic of *rickettsiae*. *Eur. J. Epidemiol.*, 1991, **7**, 213-221.

MALTEZOU H.C., RAOULT D. Q fever in children. *Lancet Infect. Dis.*, 2002, **2**, 689-691.

MANTOVANI A., BENAZZI P. The isolation of *Coxiella burnetii* from *Rhipicephalus sanguineus* on naturally infected dogs. *J. Am. Vet. Med. Asso.*, 1953, **122**, 117-120.

MARFIN A.A., GUBLER J.G. West Nile Encephalitis: an emerging disease in the United States. *Clin. Infect. Dis.*, 2001, **33**, 1713-1719.

MARMION B.P., STOKER M.G.P. Q fever in Great Britain: epidemiology of an outbreak. *Lancet*, 1950, **2**, 611-616.

MARMION B.P. Q fever II. Natural history and epidemiology of Q fever in man. *Trans. R. Soc. Trop. Med. Hyg.*, 1954, **48**, 197-207.

MARMION B.P., STOKER M.G. The epidemiology of Q fever in Great Britain; an analysis of the findings and some conclusions. *Br. Med. J.*, 1958, **2**, 809–816.

MARMION B.P. Development of Q-fever vaccines 1937 to 1967. *Med. J. Aust.*, 1967, **2**, 1074-1078.

MARMION B.P., ORMSBEE R.A., KYRKOU M., WRIGHT J., WORSWICK D.A., IZZO A.A., ESTERMAN A., FEERY B., SHAPIRO R.A. Vaccine prophylaxis of abattoir-associated Q fever: 8 years' experience in Australian abattoirs. *Epidemiol. Infect.*, 1990, **104**, 275-287.

MARMION B.P., SUKOCHEVA O., STORM P.A., LOCKHART M., TURRA M., KOK T., AYRES J., H. ROUTLEDGE H., GRAVES S. Q fever: persistence of antigenic non-viable cell residues of *Coxiella burnetii* in the host – implications for post Q fever infection fatigue syndrome and other chronic sequelae. *QJM*, 2009, **102**, 673-684.

MARRIE T.J., VAN BUREN J., FRASER J., HALDANE E.V., FAULKENER R.S., WILLIAMS J.C., KWAN C. Seroepidemiology of Q fever among domestic animals in Nova Scotia. *Am. J. Public Health*, 1985, **75**, 763-766.

MARRIE T.J., SCHLECH W.F., WILLIAMS J.C., YATES L. Q fever pneumonia associated with exposure to wild rabbits. *Lancet*, 1986, **1**, 427-429.

MARRIE T.J. Q fever, 1979-1987 –Nova Scotia. *Can. Dis. Weekly Rep.*, 1988a, **14**, 69-70.

MARRIE T.J., MACDONALD A., DURANT H., YATES L., MCCORMICK L. An outbreak of Q fever probably due to contact with a parturient cat. *Chest*, 1988b, **93**, 98-103.

MARRIE T.J., DURANT H., WILLAIMS J.C., MINTZ E., WAAG D.M. Exposure to parturient cats: a risk factor for acquisition of Q fever in Maritime Canada. *J. Infect. Dis.*, 1988c, **158**, 101-108.

MARRIE T.J., LANGILLE D., PAPUKNA V., YATES L. Truckin pneumonia – an outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with newborn kittens. *Epidemiol. Infect.*, 1989, **102**, 119-127.

MARRIE T.J. Acute Q Fever. In: Marrie T.J. (ed), Q Fever. The Disease. CRC Press: Boca Raton, Florida, 1990, 125–160.

MARRIE T.J., STEIN A., JANIGAN D., RAOULT D. Route of infection determines the clinical manifestations of acute Q fever. *J. Infect. Dis.*, 1996a, **173**, 484-487.

MARRIE T.J., PEELING R.W., FINE M.J., SINGER D.E., COLEY C.M., KAPOOR W.N. Ambulatory patients with community-acquired pneumonia the frequency of atypical agents and clinical course. *Am. J. Med.*, 1996b, **101**, 508–515.

MARRIE T.J., RAOULT D. Q fever-a review and issues for the next century. *Int. J. Antimicrob. Agents*, 1997, **8**, 145-161.

MASUZAWA T., SAWAKI K., NAGAOKA H., AKIYAMA M., HIRAI K., YANAGIHARA Y. Determination of *rickettsiae* isolated in Japan as *Coxiella burnetii* by 16S rRNA sequencing. *Int. J. Syst. Bacteriol.*, 1997a, **47**, 883-884.

MASUZAWA T., SAWAKI K., NAGAOKA H., AKIYAMA M., HIRAI K., YANAGIHARA Y. Relationship between pathogenicity of *Coxiella burnetii* isolates and gene sequences of the macrophage infectivity potentiator (Cmip) and sensor-like protein (qrsA). *FEMS Microbiol. Lett.*, 1997b, **154**, 201-205.

MATTEWMAN L., KELLY P., HAYTER D., DOWNIE S., WRAY K., BRYSON N., RYCROFT A., RAOULT D. Exposure of cats in southern Africa to *Coxiella burnetii*, the agent of Q fever, *Eur. J. Epidemiol.*, 1997, **13**, 477-479.

MAURIN M., RAOULT D. Q fever. *Clin. Microbiol. Rev.*, 1999, **12**, 518-553.

MAXSON A.D., REEF V.B. Bacterial endocarditis in horses: Ten cases (1984–1995). *Equine Vet. J.*, 1997, **29**, 394–399.

MC CAUGHEY C., MC KENNA J., MC KENNA C., COYLE P.V., O'NEILL H.J., WYATT D.E. Human seroprevalence to *Coxiella burnetii* (Q fever) in Northern Ireland. *Zoonoses Public Health*, 2008, **55**, 189-194.

MC CAUL T.F., WILLIAMS J.C. Developmental cycle of *Coxiella burnetii*: structure and morphogenesis of vegetative and sporogenic differentiations. *J. Bacteriol.*, 1981, **147**, 1063-1076.

MC CAUL T.F., HACKSTADT T., WILLIAMS J.C. Ultrastructural and biological aspects of *Coxiella burnetii* under physical disruptions. In: Burdorfer W., Anacker R.L. (ed.), *Rickettsiae* and rickettsial diseases. Academic Press Inc: New York, USA, 1981, 267-280.

MC CAUL T.F., WILLIAMS J.C., THOMPSON H.A. Electron microscopy of *Coxiella burnetii* in tissue culture. Induction of cell types as products of developmental cycle. *Acta Virol.*, 1991, **35**, 545-556.

MC CORMICK B.S., PEET R.L., DOWNES K. *Erysipelothrix rhusiopathiae* vegetative endocarditis in a horse. *Aust. Vet. J.*, 1985, **62**, 392.

MC QUISTON J. H., CHILDS J.E. Q fever in humans and animals in the United States. *Vector Borne Zoonotic Dis.*, 2002, **2**, 179-191.

MICELI M.H., VERYSER A.K., ANDERSON A.D., HOFINGER D., LEE S.A., TANCIK C. A case of person-to-person transmission of Q fever from an active duty serviceman to his spouse. *Vector Borne Zoonotic Dis.*, 2010, **10**, 539-541.

MILAZZO A., HALL R., STROM P.A., HARRIS R.J., WINSLOW W., MARMION B.P. Sexually transmitted Q fever. *Clin. Infect. Dis.*, 2001, **33**, 399-402.

MILLION M., LEPIDI H., RAOULT D. Q fever: current diagnosis and treatment options. *Med. Mal. Infect.*, 2009, **39**, 82-94.

MITSCHERCH E., MARTH E.H (Eds), Microbial Survival in the Environment, Springer-Verlag: New York, USA, 1984, 148-156.

MO YY., CIANCIOTTO N.P., MALLAVIA L.P. Molecular cloning of a *Coxiella burnetii* gene encoding a macrophage infectivity potentiator (Mip) analog. *Microbiol.*, 1995, **141**, 2861-2871.

MONTEJO BARANDA M., CORRAL CARRANCEJA J., AGUIRRE ERRASTI C. Q fever in the Basque Country: 1981-1984. *Rev. Infect. Dis.*, 1985, **7**, 700-701.

MOODIE C. E., THOMPON H.A., MELTZER M.I., SWERDLOW D.L. Prophylaxis after exposure to *Coxiella burnetii*. *Emerg. Infect. Dis.*, 2008, **14**, 1558-1566.

MOORE T.R., LONGO J., LEOPOLD G.R., CASOLA G., GOSINK B.B. The reliability and predictive value of an amniotic fluid scoring system in severe second-trimester oligohydramnios. *Obstet. Gynecol.*, 1989, **73**, 739-742.

MOOS A., HACKSTADT T. Comparative virulence of intra- and interstrain lipopolysaccharide variants of *Coxiella burnetii* in the guinea pig model. *Infect. Immun.*, 1987, **55**, 1144-1150.

MORITA C., KATSUYAMA J., YANASE T., UENO H., MURAMATSU Y., HONDATSU T., KOYAMA H. Seroepidemiological survey of *Coxiella burnetii* in domestic cats in Japan. *Microbiol. Immunol.*, 1994, **38**, 1001–1003.

MORISAWA Y., WAKIGUCHI H., TAKECHI T., KURASHIGE T., NAGAOKA H. Intractable Q fever treated with recombinant gamma interferon. *Pediatr. Infect. Dis. J.*, 2001, **20**, 546-547.

MORSE S.S. Factors and determinants of disease emergence. *Rev. Sci. Tech.*, 2004, **23**, 443-451.

MUMCUOGLU K.Y., BANET-NOACH C., MALKINSON M., SHALOM U., GALUN R., Argasid ticks as possible vectors of West Nile virus in Israel. *Vector Borne Zoonotic Dis.*, 2005, **1**, 65-71.

MURAMATSU Y., HUKUTA K., SATOH S., MURAMATSU M., NISHIMURA M., NAGAHATA H., UENO H., MORITA C., TAMURA Y. Seroepidemiologic survey of *Coxiella burnetii* and attempt to detect *Coxiella* DNA in aged non-laying chickens in a prefecture of Japan where poultry farming prospers. *J. Vet. Med. Sci.*, 2006, **68**, 1007–1008.

MURGUE B., MURRI S., ZIENTARA S., DURAND B., DURAND J.P., ZELLER H. West Nile outbreak in horses in Southern France, 2000: the return after 35 years. *Emerg. Infect. Dis.*, 2001, **7**, 692-696.

MUSSO D., RAOULT D. Serological cross-reaction between *Coxiella burnetii* and *Legionella micdadei*. *Clin. Diagn. Lab. Immunol.*, 1997, **4**, 208-212.

N

NAGAOKA H., AKIYAMA M., SUGIEDA M., NISHO T., AKAHANE S., HATTORI H., HO T., FUSHI H., HIRAI K. Isolation of *Coxiella burnetii* from children with influenza-like symptoms in Japan. Microbiol. Immunol., 1996, **40**, 147–151.

NAGAOKA H., SUGIEDA M., AKIYAMA M., NISHINA T., AKAHANE S., FUJIWARA K. Isolation of *Coxiella burnetii* from the vagina of feline clients at veterinary clinics. *J. Vet. Med. Sci.*, 1998, **60**, 251–252.

NASH D., MOSTASHARI F., FINE A., MILLER J., O'LEARY D., MURRAY K., HUANG A., ROSENBERG A., GREENBERG A., SHERMAN M., WONG S., LAYTON M. Outbreak of West Nile virus infection, New York City area in 1999. *N. Engl. J. Med.*, 2001, **344**, 1807-1814.

NGUYEN S.A.V., TO H., MINAMOTO N., OGAWA M., YAMAGUCHI T., FUKUSHI H., HIRAI K. Evaluation of high-density agglutination test for *Coxiella burnetii* antibodies in animals. *Clin. Diagn. Lab. Immunol.*, 1997, **4**, 679–680.

NHS. Complement Fixation Tests. VSOP18. Standards Unit, Evaluations and Standards Laboratory. [on line] (2006) URL Address: http://www.hpa-standardmethods.org.uk Accessed on 17 July 09.

NIELSEN C.F., REISEN W.K., ARMIJOS M.V., MACLACHLAN N.J., SCOTT T.W. High subclinical West Nile virus incidence among non vaccinated horses in northern California associated with low vector abundance and infection. *Am. J. Trop. Med. Hyg.*, 2008, **78**, 45-52.

NILSFORD L., LOMBARD C.W., WECKNER D., KVART C. Case report: Diagnosis of pulmonary valve endocarditis in a horse. *Equine Vet. J.*, 1991, **23**, 479–482.

NOVAK M., BREZINA R., KAZAR J. Immunoblot analysis of antibody response in mice infected with *Coxiella burentii* phase I. *Acta Virol.*, 1992, **36**, 39-44.

NUMAZAKI K., UENO H., YOKOO K., MURAMATSU Y., CHIBA S., MORITA C. Detection of serum antibodies to *Bartonella henselae* and *Coxiella burnetii* from Japanese children and pregnant women. *Microbes Infect.*, 2000, **2**, 1431–1434.

O

ODA H., YOSHIIE K. Isolation of *Coxiella burnetii* strain that has low virulence for mice from a patient with acute Q fever. *Microbiol. Immunol.*, 1989, **33**, 969–973.

OKIMOTO N., ASAOKA N., OSAKI K., KURIHARA T., YAMATO K., SUNAGAWA T., FUJITA K., OHBA H., NAKAMURA J., NAKADA K. Clinical features of Q fever pneumonia. *Respirology*, 2004, **9**, 278–282.

OKIMOTO N., KIBAYASHI T., MIMURA K., YAMATO K., KURIHARA T., HONDA Y., OSAKI K., ASAOKA N., OHBA H. *Coxiella burnetii* and acute exacerbations/infections in patients with chronic lung disease. *Respirology*, 2007, **12**, 619–621.

OMSLAND A., COCKRELL D.C., HOWE D., FISCHER E.R., VIRTANEVA K., D.E. STURDEVANT D.E., PORCELLA S.F., HEINZEN R.A. Host cell-free growth of the Q fever bacterium *Coxiella burnetii*. *Proc. Natl. Acad. Sci. USA*, 2009, **106**, 4430-4434.

ORMSBEE R., PEACOCK M., GERLOFF R., TALLENT G., WIKE D. Limits of rickettsial infectivity. *Infect. Immun.*, 1978, **19**, 239-245.

OSTLUND E.N., AANDRESEN J.E., ANDRESEN M. West Nile encephalitis. *Vet. Clin. North Am. Equine Pract.*, 2000, **16**, 427-441.

OU A.C., RATARD R.C. One-year sequelae in patients with West Nile virus encephalitis and meningitis in Louisiana. *J. La State Med. Soc.*, 2005, **157**, 42-46.

P

PACE L.W., WIRTH N.R., FOSS R.R., FALES W.H. Endocarditis and pulmonary aspergillosis in a horse. *J. Vet. Diagn. Invest.*, 1994, **6**, 506–508.

PALMER N.C., KIERSTEAD M., KEY D.W., WILLIAMS J.C., PEACOCK M.G., VELLEND H. Placentitis and abortion in goats and sheep in Ontario caused by *Coxiella burnetii*. *Can. Vet. J.*, 1983, **24**, 60–61.

PANAIOTOV S., CICCOZZI M., BRANKOVA N., LEVTEROVA V., MITOVA-TIHOLOVA M., AMICOSANTE M., REZZA G., KANTARDJIEV T. An outbreak of Q fever in Bulgaria. *Ann. Ist. Super. Sanita*, 2009, **45**, 83-86.

PAPE M., MANDRAVELI K., ARVANITIDOU-VAGIONA M., NIKOLAIDIS P., ALEXIOU-DANIEL S. Q fever in northern Greece: epidemiological and clinical data from 58 acute and chronic cases. *Clin. Microbiol. Infect.*, 2009, **15**: Suppl 2, 150-151.

PARKER R.R., DAVIS G.E. A filter-passing infectious agent isolated from ticks. II. Transmission by *Dermacentor andersoni*. *Public Health Rep.*, 1938, **53**, 2267-2269.

PAROLA P., RAOULT D. Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. *Clin. Infect. Dis.*, 2001, **32**, 897-928.

PATNAIK J.L., HARMON H., VOGT R.L. Follow-up of 2003 human West Nile virus infections, Denver, Colorado. *Emerg. Infect. Dis.*, 2006, **12**, 1129-1131.

PATTESON M.W., Endocarditis In: Patteson M.W. (Ed), Equine Cardiology. Blackwell Science Ltd: UK, 1996, 150–154.

PATTESON M.W. Equine cardiology. [on line] (2002) Adresse URL: http://www.provet.co.uk./equinecardiology/5a66646.htm. Accessed 5/11/2006.

PEIRIS J.S.M., AMERASINGHE F.P. West Nile fever. In: Beran G.W., J.H. Steele (Eds), Handbook of Zoonoses Section B: Viral. CRC Press: Boca Raton, Florida, USA, 1994, 139-148.

PEPPERELL C., RAU N., KRAJDEN S., KERN R., HUMAR A., MEDERSKI B., SIMOR A., LOW D.E., MCGEER A., MAZZULLI T., BURTON J., JAIGOBIN C., FEARON M., ARTSOB H., DREBOT M.A., HALLIDAY W., BRUNTON J. West Nile virus infection in 2002: morbidity and mortality among patients admitted to hospital in southcentral Ontario. *Can. Med. Assoc. J.*, 2003, **168**, 1399-1405.

PERELMAN A., STERN J. Acute pancreatitis in West Nile fever. *Am. J. Trop. Med. Hyg.*, 1974, **23**, 1150-1152.

PETER O., DUPUIS G., BURGDORFER W., PEACOCK M. Evaluation of the complement fixation and indirect immunofluorescence tests in the early diagnosis of primary Q fever. *Eur. J. Clin. Microbiol.*, 1985, **4**, 394-396.

PETER O., DUPUIS G., PEACOCK M.G., BURGDORFRJ W. Comparison of enzyme-linked immunosorbent assay and complement fixation and indirect fluorescent-antibody tests for detection of *Coxiella burnetii* antibody. *J. Clin. Microbiol.*, 1987, **25**, 1063–1067.

PETERSEN L.R., ROEHRIG J.T. West Nile Virus: A reemerging global pathogen. *Emerg. Infect. Dis.*, 2001, **7**, 611-614.

PETRIE A., WATSON P. Statistics for veterinary and animal science. Blackwell Science Ltd.: Oxford, UK, 2006, 299.

PHILIP C.B. Observations on experimental Q fever. *J. Parasitol.*, 1948, **34**, 457-464.

PINSKY R.L., FISHBEIN D.B., GREENE C.R., GENSHEIMER K.F. An outbreak of cat associated Q fever in the United States. *J. Infect. Dis.*, 1991, **164**, 202-204.

PLOMMET M., CAPPONI M., GESTIN J., RENOUX G. Fièvre Q expérimentale des bovins. *Ann. Rech. Vet.*, 1973, **4**, 325-346.

PLUTA S., HARTELT K., OEHME R., MACKENSTEDT U., KIMMIG P. Prevalence of *Coxiella burnetii* and *Rickettsia* spp. in ticks and rodents in southern Germany. *Ticks and Tick-borne Dis.*, 2010, **1**, 145-147.

POGODINA V.V., FROLOVA M.P., MALENKO G.V., FOKINA G.I., KORESHKOVA G.V., KISELEVA L.L., BOCHKOVA N.G., RALPH N.M. Study on West Nile virus persistence in monkeys. *Arch. Virol.*, 1983, **75**, 71-86.

POLYDOROU K. Q fever in Cyprus: a short review. *Br. Vet. J.*, 1981, **137**, 470-477.

POPE J. H., SCOTT W., DWEYER R. *Coxiella burnetii* in kangaroos and kangorro-ticks in Western Queenland. *Aust. Ann. Med.*, 1960, **9,** 214-216.

PRADIER S., LEBLOND A., DURAND B. Land cover, landscape structure, and West Nile virus circulation in Southern France. *Vector Borne Zoonotic Dis.*, 2008, **8**, 253-263.

R

RAMZAN P.H.L. Case report: Vegetative bacterial endocarditis associated with septic tenosynovitis of the digital sheath in a Thoroughbred racehorse. *Equine Vet. Educ.*, 2000, **12**, 120–123.

RANSOM S.E., HUEBNER R.J. Studies on the resistance of *Coxiella burnetii* to physical and chemical agents. *Am. J. Hyg.*, 1951, **53**, 110-119.

RAOULT D., DRANCOURT M., VESTRIS G. Bactericidal effect of doxycycline associated with lysosomotropic agents on *Coxiella burnetii* in P388D1 cells. *Antimicrob. Agents Chemother.*, 1990, **34**, 1512-1514.

RAOULT D. Treatment of Q fever. *Antimicrob. Agents Chemother.*, 1993, **37**, 301-302.

RAOULT D., STEIN A. Q fever during pregnancy, a risk for women, fetuses and obstetricians. *N. Engl. J. Med.*, 1994, **330**, 371.

RAOULT D., LAURENT J.C., MUTILLOD M. Monoclonal antibodies to *Coxiella burnetii* for antigenic detection in cell culture and in paraffin-embedded tissues. *Am. J. Clin. Pathol.*, 1994, **101**, 318-320.

RAOULT D., TISSOT-DUPONT H., FOUCAULT C., GOUVERNET J., FOURNIER P.E., BERNIT E., STEIN A., NESRI M., HARLE J.R., WEILLER P.J. Q fever 1985-1998 : Clinical and epidemiological features of 1,383 infections. *Medicine (Baltimore)*, 2000, **79**, 109-123.

RAOULT D., FENNOLAR F., STEIN A. Q fever during pregnancy: diagnosis, treatment and follow-up. *Arch. Int. Med.*, 2002, **162**, 701-704.

RAOULT D., MARRIE T., MEGE J. Natural history and pathophysiology of Q fever. *Lancet Infect. Dis.*, 2005, **5**, 219-226.

REEF V.B. Mitral valvular insufficiency associated with ruptured chordae tendineae in three foals. *J. Am. Vet. Med. Assoc.*, 1987, **191**, 329–331.

REEF V.B., SPENCER P. Echocardiographic evaluation of equine aortic insufficiency. *Am. J. Vet. Res.*, 1987, **48**, 904–909.

RHYAN J.C., SPRAKER T.R. Emergence of diseases from wildlife reservoirs. *Vet. Pathol.*, 2010, **47**, 34-39.

RIEMANN H.P., THOMPSON R.A., BEHYMER D.E., WIJAYASINGHE C. Toxoplasmosis and Q fever antibodies among wild carnivores in California. *J. Wildl. Manag.*, 1978, **42**, 198-202.

ROBY K.A., REEF V.B., SHAW D.P., SWEENEY C.R. Rupture of an aortic sinus aneurysm in a 15-year-old broodmare. *J. Am. Vet. Med. Assoc.*, 1986, **189**, 305–308.

RODOLAKIS A., BERRI M., HECHARD C., CAUDRON C., SOURIAU A., BODIER C.C., BLANCHARD B., CAMUSET P., DEVILLECHAISE P., NATORP J.C., VADET J.P., ARRICAU-BOUVERY N. Comparison of *Coxiella burnetii* shedding in milk of dairy bovine, caprine, and ovine herds. *J. Dairy Sci.*, 2007, **90**, 5352–5360.

ROEST H.I.J., RUULS R.C., TILBURG J.J.H.C., NABUURS-FRANSSEN M.H., KLAASSEN C.H.W., VELLEMA P., VAN DEN BROM R., DERCKSEN D., WOUDE W., SPIERENBURG M.A.H., VEN DER SPEK A.N., BUIJS R., DE BOER A.G., WILLEMSEN P.Th.J., VAN ZIJDERVELD F.G. Molecular epidemiology of *C. burnetii* from ruminants in Q fever outbreak, the Netherlands. *Emerg. Infect. Dis.*, 2011a, **17**, 668-675.

ROEST H.I., TILBURG J.J., VAN DER HOEK W., VELLEMA P., VAN ZIJDEVELD F.G., KLAASSEN C.H., RAOULT D. The Q fever epidemic in the Netherlands: history, onset, response and reflection. *Epidemiol. Infect.*, 2011b, **139**, 1-12.

ROLAIN J.M., MALLET M.N., RAOULT D. Correlation between serum levels of doxycycline and serology evolution in patients treated for *Coxiella burnetii* endocarditis. *J. Infect. Dis.*, 2003, **9**, 1322-1325.

ROLAIN J.M., BOULOS A., MALLET M.N., RAOULT D. Correlation between ratio of serum doxycycline concentration to MIC and rapid decline of antibody levels during treatment of Q fever endocarditis. *Antimicrob. Agents Chemother.*, 2005a, **49**, 2673-2676.

ROLAIN J.M., LAMBERT F., RAOULT D. Activity of telithromycin against thirteen new isolates of *C. burnetii* including three resistant to doxycycline. *Ann. N.Y. Acad. Sci.*, 2005b, **1063**, 252-256.

ROMAN M.J., CORIZ P.D., BACA O.G. A proposed model to explain persistent infection of host cells with *Coxiella burnetii*. J. Gen. Microbiol., 1986, **132**, 1415-1422.

ROSSI S.L., ROSS T.M., EVANS J.D. West Nile Virus. *Clin. Lab. Med.*, 2010, **30**, 47-65.

ROUSSET E., DUQUESNE V., RUSSO P., THIERY R. Fièvre Q : problématiques et risques sanitaires. *Bull. Acad. Vet. Fr.*, 2007a, **160**, 107-114.

ROUSSET E., DURAND B., BERRI M., DUFOUR P., PRIGENT M., RUSSO P., DELCROIX T., TOURATIER A., RODOLAKIS A., AUBERT M. Comparative diagnostic potential of three serological tests for abortive Q fever in goats herds. *Vet. Microbiol.*, 2007b, **124**, 286-297.

ROUSSET E., BERRI M., DURAND B., DUFOUR P., PRIGENT M., DELCROIX T., TOURATIER A., RODOLAKIS A. *Coxiella burnetii* shedding routes and antibody response after outbreaks of Q fever-induced abortion in dairy goat herds. *Appl. Environ. Microbiol.*, 2009a,**75**, 428-433.

ROUSSET E., DURANT B., CHAMPION J.L., PRIGENT M., DUFOUR P., FORFAIT C., MAROIS M., GASNIER T., DUQUESNE V., THIERY R., AUBERT M.F. Efficiency of a phase 1 vaccine for the reduction of vaginal *Coxiella burnetii* shedding in a clinically affected goat herd. *Clin. Microbiol. Infect.*, 2009b, **15**: Suppl 2, 188-189.

RUIZ M., EWIG S., MARCOS M.A., MARTINEZ J.A., ARANCIBIA F., MENSA J., TORRES A. Etiology of community-acquired pneumonia: impact of age, comorbidity, and severity. *Am. J. Respir. Crit. Care Med.*, 1999, **160**, 397–405.

RUSSEL-LODRIGUE K.E., ZHANG G.Q., MCMURRAY D.N., SAMUEL J.E. Clinical and pathologic changes in a guinea pig aerosol challenge model of acute Q fever. *Infect. Immun.*, 2006, **74**, 6085-6091.

S

SAEGERMAN C., SPEYBROEK N., ROELS S., VANOPDENBOSCH E., THIRY E., BERKVENS D. Decision support tools for clinical diagnosis of disease in cows with suspected bovine spongiform encephalopathy. *J. Clin. Microbiol.*, 2004, **42**, 172-178.

SAEGERMAN C. Epidémiosurveillance des événèments rares chez les bovins en Belgique (Thèse de doctorat). Université de Liège : Liège, Belgique, 2005, 43-50.

SAITO A., KOHNO S., MATSUSHIMA T., WATANABE A., OIZUMI K., YAMAGUCHI K. Prospective multicenter study on the causative organisms of community-acquired pneumonia in adults in Japan. *J. Infect. Chemother.*, 2006, **12**, 63–69.

SAMPERE M., FONT B., FONT J., SANFELIU I., SEGURA F. Q fever in adults: review of 66 clinical cases. *Eur. J. Clin. Microbiol. Infect. Dis.*, 2003, **22**, 108–110.

SAMPSON B.A., AMBROSI C., CHARLOT A., REIBER K., VERESS J.F., ARMBRUSTMACHER V. The pathology of human West Nile infection. *Hum. Pathol.*, 2000, **31**, 527-531.

SAMUEL J.E., FRAZIER M.E., KAHN M.L.E., THOMASHOW L.S., MALLAVIA L.P. Isolation and characterization of a plasmid from phase 1 *Coxiella burnetii*. *Infect. Immun.*, 1983, **41**, 448-493.

SAMUEL J.E. Developmental cycle of *Coxiella burnetii*. In: Brun, Y. V., Shimkets L.J. (eds), Procaryotic development. ASM Press: Washington DC, USA, 2000, 427-440.

SAMUEL M.A., DIAMOND M.S. Alpha/beta interferon protects against lethal West Nile virus infection by restricting cellular tropism and enhancing neuronal survival. *J. Virol.*, 2005, **79**, 13350-13361.

SANCHEZ J., SOURIAU A., BUENDIA A.J., ARRICAU-BOUVERY N., MARTINEZ C.M., SALINAS J., RODOLAKIS A., NAVARRO J.A. Experimental *Coxiella burnetii* infection in pregnant goats: a histopathological and immunohistochemical study. *J. Comp. Pathol.*, 2006, **135**, 108-115.

SANZO J.M., GARCIA-CALABUIG M.A., AUDICANA A., DEHESA V. Q fever: prevalence of antibodies to *Coxeilla burnetii* in the Basque Country. *Int. J. Epidemiol.*, 1993, **22**, 1183-1187.

SARDELIS M.R., TURELL M.J., DOHM D.J., O'GUINN M.L. Vector competence of selected North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. *Emerg. Infect. Dis.*, 2001, **7**, 1018-1022.

SAVINELLI E.A., MALLAVIA L.P. Comparison of *Coxiella burnetii* plasmids to homologous chromosomal sequences present in a plasmidless endocarditis-causing isolate. *Ann. N.Y. Acad. Sci.*, 1990, **590**, 523-533.

SCHIMMER B., MORROY G., DIJKSTRA F., SCHNEEBERGER P.M., WEERS-POTHOFF G., TIMEN A., WIJKMANS C., VAN DER HOEK W. Large ongoing Q fever outbreak in the south of The Netherlands, 2008. *Euro. Surveill.*, 2008, **13**, 1-3.

SCHMEER N., MULLER P., LANGEL J., KRAUSS H., FROST J.W., WIEDA J. Q fever vaccines for animals. *Zentralbl. Bakteriol. Mikrobiol. Hyg. A.*, 1987, **267**, 79-88.

SCHNEEBERGER P.M., HERMANS M.H.A., VAN HANNEN E.J., SCHELLEKENS J.J.A., LEENDERS A.C.A.P., WEVER P.C. Real-time PCR on serum samples is indispensable for early diagnosis of acute Q-fever. *Clin. Vaccine Immunol.*, 2010, **17**, 286-290.

SCHUIL J., RICHARDUS J.H., BAARSMA G.S., SCHAAP G.J. Q fever as a possible cause of bilateral optic neuritis. *Br. J. Ophthalmol.*, 1985, **69**, 580-583.

SCOT G.H., WILLIAMS J.C., STEPHENSON E.H. Animal models in Q fever: pathological responses of inbred mice to phase I *Coxiella burnetii*. *J. Gen. Microbiol.* 1987, **133**, 691-700.

SCOT G.H., WILLIAMS J.C. Susceptibility of *Coxiella burnetii* to chemical disinfectants. *Ann. N.Y. Acad. Sci.*, 1990, **590**, 291-296.

SEBASTAIN M.M., STEWART I., WILLIAMS N.M., POONACHA K.B., SELLS S.F., VICKERS M.L., HARRISON L.R. Pathological, entomological, avian and meteorological investigation of a West Nile epidemic in a horse farm. *Transbound. Emerg. Dis.*, 2008, **55**, 134-139.

SEJVAR J.J., BODE A.V., MARFIN A.A., CAMPBELL G.L., EWING D., MAZOWIECKI M., PAVOT P.V., SCHMITT J., PAPE J., BIGGERSTAFF B.J., PETERSEN L.R. West Nile virus-associated flaccid paralysis. *Emerg. Infect. Dis.*, 2005, **11**, 1021-1027.

SEJVAR J.J. The long-term outcomes of human West Nile virus infection. *Clin. Infect. Dis.*, 2007, **44**, 1617-1624.

SESHADRI R., PAULSEN I.T., EISEN J.A., READ T.D., NELSON K.E., NELSON W.C., WARD N.L., TETTELIN H., DAVIDSEN T.M., BEANAN M.J., DEBOY R.T., DAUGHERTY S.C., BRINKAC L.M., MADUPU R., DODSON R.J., KHOURI H.M., LEE K.H., CARTY H.A., SCANLAN D., HEINZEN R.A., THOMPSON H.A., SAMUEL J.E., FRASER C.M., HEIDELBERG J.F. Complete genome sequence of Q-fever pathogen *Coxiella burnetii*. *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 5455-5460.

SETIYONO A., OGAWA M., CAI Y., SHIGA S., KISHIMOTO T., KURANE I. New criteria for immunofluorescence assay for Q fever diagnosis in Japan. *J. Clin. Microbiol.*, 2005, **43**, 5555–5559.

SHAKED Y., SAMRA Y. Q fever meningoencephalitis associated with bilateral abducens nerve paralysis, bilateral optic neuritis and abnormal cerebrospinal fluid findings. *Infection*, 1989, **17**, 394-395.

SHANNON J.G., COCKRELL D.C., TAKAHASHI K., STAHL G.L., HEINZEN R.A. Antibody-mediated immunity to the obligate intracellular bacterial pathogen *Coxiella burnetii* is Fc receptor- and complement-independent. *BMC Immunol.*, 2009, **10**, 26.

SHIEH W.J., GUARNER J., LAYTON M., FINE A., MILLER J., NASH D., CAMPBELL G.L., ROEHRIG J.T., GUBLER D.J., ZAKI S.R. The role of pathology in an investigation of an outbreak of West Nile encephalitis in New York, 1999. *Emerg. Infect. Dis.*, 2000, **6**, 370-372.

SKYKES J.E. Immunodeficiencies caused by infectious diseases. *Vet Clin. North Am. Small Anim. Pract.*, 2010, **40**, 409-423.

SLAVIN G. 'Q' fever: the domestic animal as a source of infection for man. *Vet. Rec.*, 1952, **64**, 743.

SMITH D.J.W. Studies on the epidemiology of Q fever. III. The transmission of Q fever by the tick *Haemaphysalis humerosa*. *Aust. J. Exp. Biol. Med. Sci.*, 1940, **18**, 103-106.

SMITH D.J.W., DERRICK E.H. Studies on the epidemiology of Q fever. I. Isolation of six strains of *Rickettsia burnetii* from the tick *Haemaphysalis humerosa*. *Aust. J. Exp. Biol. Med. Sci.*, 1940, **18**, 1-5.

SMITH D.J.W. Studies on the epidemiology of Q fever. VIII. The transmission of Q fever by the tick *Rhipicephalus sanguineus*. *Aust. J. Exp. Biol. Med. Sci.*, 1941, **19**, 119-122.

SMITH H.L., MONATH T.P., PARAZOLES P., ROTHMAN A.L., CASEY D.M., TERAJIMA M., ENNIS F.A., GUIRAKHOO F., GREEN, S. Development of antigen-specific meomory CD8+ T cells following live-attenuated chimeric West Nile virus vaccination. *J. Infect. Dis.*, 2011, **203**, 513-522.

SMITHBURN K.C., HUGHES T.P., BURKE A.W., PAUL J.H. A neurotropic virus isolated from the blood of a native of Uganda. *Am. J. Trop. Med.*, 1940, **20**, 471-492.

SOPENA N., SABRIA-LEAL M., PEDRO-BOTET M.L., PADILLA E., DOMINGUEZ J., MORERA J., TUDELA P. Comparative study of the clinical presentation of *Legionella pneumonia* and other community-acquired pneumonias. *Chest*, 1998, **113**, 1195–1200.

SOSIN D.M. Draft framework for evaluating syndromic surveillance systems. *J. Urban Health*, 2003, **80**, i8-i13.

SOVEROW J.E., WELLENIUS G.A., FISMAN D.N., MITTLEMAN M.A. Infectious diseases in a warming world: how weather influenced West Nile virus in the United States (2001-2005). *Envir. Health Perspectives*, 2009, **117**, 1049-1052.

SPELMAN D.W. Q fever: a study of 111 consecutive cases. *Med. J. Aust.*, 1982, **1**, 547-553.

SPEYBROECK N., BERKVENS D., MFOUKOU-NTSAKALA A., AERTS M., HENS N., VAN HUYLENBROECK G., THYS E. Classification trees versus multinomial models in the analysis of urban farming systems in Central Africa. *Agric. Syst.*, 2004, **80**, 133-149.

SPONSELLER B.T., WARE W.A. Case report: Successful treatment of staphylococcal endocarditis in a horse. *Equine Vet. Educ.*, 2001, **13**, 298–302.

STATSOFT INC. Electronic Statistics Textbook. Classification trees. [On line] (2011) URL Address: http://www.statsoft.com/textbook/stneunet.html. Accessed on the 01/07/08.

STEIN A., RAOULT D. Detection of *Coxiella burnetii* by DNA amplification using polymerase chain reaction. *J. Clin. Microbiol.*, 1992, **30**, 2462-2466.

STEIN A., RAOULT D. Q fever during pregnancy: a public health problem in Southern France. *Clin. Infect. Dis.*, 1998, **27**, 592-596.

STOCKER M.G., MARMION B.P. The spread of Q fever from animals to man; the natural history of a rickettsial disease. *Bull. World Health Organ.*, 1955, **13**, 781-806.

STOCKER M.G., MARMION B.P. Q fever in Britain: isolation of *Rickettsia burneti* from the tick *Haemaphysalis punctata*. *J. Hyg. (Lond)*, 1955, **53**, 322-327.

STOENNER H. G., LACKMAN D.B. The biologic properties of *Coxiella burnetii* isolated from rodents collected in Utah. *Am. J. Hyg.*, 1960, **71**, 45-51.

SUMILO D., BORMANE A., ASOKLIENE L., VASILENKO V., GOLOVLJOVA I, AVSIC-ZUPANC T., HUBALEK Z, RANDOLPH S.E. Socio-economic factors in the differential upsurge if tick-borne encephalitis in Central and Eastern Europe. *Rev. Med. Virol.*, 2008, **18**, 81-95.

SUZAN G., MARCE E., GIERMAKOWSKI J.T., MILLS J.N., CEBALLOS G., OSTFELD R.S., ARMIEN B., PASCALE J.M., YATES T.L. Experimental evidence for reduced mammalian diversity causing increased hantavirus prevalence. *PloS ONE* , 2009, **4**, e5461.

SVRAKA S., TOMAN R., SKULTETY L., SLABA K., HOMAN W.L. Establishment of a genotyping scheme for *Coxiella burnetii*. *FEMS Microbiol. Lett.*, 2006, **254**, 268-274.

SYRUCEK L., RASKA K. Q fever in domestic and wild birds. *Bull. World Health Organ.*, 1956, **15**, 329-337.

T

TAKAHASHI H., TOKUE Y., KIKUCHI T., KOBAYASHI T., GOMI K., GOTO I., SHIRAISHI H., FUKUSHI H., HIRAI K., NUKIWA T., WATANABE A. Prevalence of community-acquired respiratory tract infections associated with Q fever in Japan. *Diagn. Microbiol. Infect. Dis.*, 2004, **48**, 247–252.

TARDEI G., RUTA S., CHITU V., ROSSI C., TSAI T.F., CERNESCU C. Evaluation of immunoglobin M (IgM) and IgG enzyme immunoassays in serologic diagnosis of West Nile infection. *J. Clin. Microbiol.*, 2000, **38**, 2232-2239.

TATSUMI N., BAUMGARTNER A., QIAO Y., YAMAMOTO I., YAMAGUCHI K. Detection of *Coxiella burnetii* in market chicken eggs and mayonnaise. *Ann. N.Y. Acad. Sci.*, 2006, **1078**, 502–505.

TAYLOR L.H., LATHAM S.M., WOOLHOUSE M.E. Risk factors for human disease emergence. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 2001, **356**, 983-989.

TELLEZ A., SAINZ C., ECHEVARRIA C., DE CARLOS S., FERNANDEZ M.V., LEON P., BREZINA R. Q fever in Spain: acute and chronic cases 1981-1985. *Rev. Infect. Dis.*, 1988, **10**, 198-202.

THANG N.D., ERHART A., SPEYBROEK N., HUNG LE X., THUAN LE K., HUNG C.T., KY P.V., COOSEMANS M., D'ALESSANDRO U. Malaria in central Vietnam: Analysis of risk factors by multivariate analysis and classification tree models, *Malar. J.*, 2008, **7**, 28.

THERMO FISHER SCIENTIFIC INC. Comparison of Protein A, G, A/G and L. [on line] (2011) URL Address:http://www.piercenet.com/Objects/View.cfm?type=Page&ID=75D6C066-AB3E-4B74-87F2-C38A85F0DBC4. Accessed 20 January 2011.

THIELE D., WILLEMS H., KOPF G., KRAUSS H. Polymorphism in DNA restriction patterns of *Coxiella burnetii* isolates investigated by pulsed field gel electrophoresis and image analysis. *Eur. J. Epidemiol.*, 1993, **9**, 419-425.

THOMAS D.R., SALMON R.L., KENCH S.M., MEADOWS D., COLEMAN T.J., MORGAN-CAPNER P., MORGAN K.L. Zoonotic illness: determining risks and measuring effects of the association between current animal exposure and a history of illness in a well characterized rural population. *J. Epidemiol. Community Health*, 1994, **48**, 151-155.

THOMAS D.R., TREWEEK L., SALMON R.L., KENCH S.M., COLEMAN T.J., MEADOWS D., MORGAN-CAPNER P., CAUL E.O. The risk of acquiring Q fever on farms: a seroepidemiological study. *Occup. Environ. Med.*, 1995, **52**, 644-647.

THOMPSON H.A., BOLT C.R., HOOVER T., WILLIAMS J.C. Induction of heat-shock proteins in *Coxiella burnetii*. *N.Y. Acad. Sci.*, 1990, **590**, 127-135.

THOMPSON H. A., HOOVER T.A., VODKIN M.H., SHAW E.I. Do chromosomal deletions in the lipopolysaccharide biosynthetic regions explain all cases of phase variation in *Coxiella burnetii* strains? *Ann. N.Y. Acad. Sci.*, 2003, **990**, 664-670.

THRUSFIELD M., ORTEGA C., DE BLAS I., NOORDHUIZEN J.P., FRANKENA K., WIN EPISCOPE 20: Improved epidemiological software for veterinary medicine. *Vet. Rec.*, 2001, **148**, 567–572.

TISSOT-DUPONT H., RAOULT D., BROUQUI P., JANBON F., PEYRAMOND D., WEILLER P.J., CHICHEPORTICHE C., NEZRI M., POIRIER R. Epidemiologic features and clinical presentation of acute Q fever in hospitalized patients: 323 French cases. *Am. J. Med.*, 1992, **93**, 427–434.

TISSOT-DUPONT H., TORRES S., NEZRI M., RAOULT D. Hyperendemic focus of Q fever related to sheep and wind. *Am. J. Epidemiol.*, 1999, **150**, 67-74.

TISSOT-DUPONT H., RAOULT D. Clinical aspects, diagnosis and treatment of Q fever. In: Raoult D., Parola P. (eds), Rickettsial diseases, Informa Healthcare USA: New York, USA, 2007, 291-301.

TISSOT-DUPONT H., VAILLANT V., REY S., RAOULT D. Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. *Clin. Infect. Dis.*, 2007, **44**, 232-237.

TO H., HTWE K.K., YAMASAKI N., ZHANG G.Q., OGAWA M., YAMAGICHI T., FUKUSHI H., HIRAI K. Isolation of *Coxiella burnetii* from dairy cattle and ticks, and some characteristics of the isolates in Japan. *Microbiol. Immunol.*, 1995, **39**, 663–671.

TO H., KAKO N., ZHANG G.Q., OTSUKA H., OGAWA M., OCHIAI O., NGUYNE S.V., YAMAGUCHI T., FUKUSHI H., NAGAOKA N., AKIYAMA M., AMANO K., HIRAI K. Q fever pneumonia in children in Japan. *J. Clin. Microbiol.*, 1996, **34**, 647–651.

TO H., HTWE K.K., KAKO N., KIM H.J., YAMAGUCHI T., FUKUSHI H., HIRAI K. Prevalence of *Coxiella burnetii* infection in dairy cattle with reproductive disorders. *J. Vet. Med. Sci.*, 1998a, **60**, 859–861.

TO H., SAKAI R., SHIROTA K., KANO C., ABE S., SUGIMOTO T., TAKEHARA K., MORITA C., TAKASHIMA I., MARUYAMA T., YAMAGUCHI T., FUKUSHI H., HIRAI K. Coxiellosis in domestic and wild birds from Japan. *J. Wildl. Dis.*, 1998b, **34**, 310–316.

TOMA B., DUFOUR B., SANAA M., BENET J.J., SHAW A., MOUTOU F., LOUZA A., Epidémiologie appliquée à la lutte collective contre les maladies animales transmissibles majeures. Ed Association pour l'Etude de L'Epidémiologie des maladies Animales : Maisons-Alfort, France, 2001, 696p.

TORRES A., DORCA J., ZALACAIN R., BELLO S., EL-ELBIARY M., MOLINOS L., AREVALO M., BLANQUER J., CELIS R., IRIBERRI M., PRATS E., FERNANDEZ R., IRIGARAY R., SERRA J. Community-acquired pneumonia in chronic obstructive pulmonary disease: a Spanish multicenter study. *Am. J. Respir. Crit. Care Med.*, 1996, **154**, 1456–1461.

TRAVER C.W., VAN DEN BERG J.S. *Pseudomonas spp.* associated vegetative endocarditis in two horses. *J. S. Afr. Vet. Assoc.*, 1995, **66**, 172–176.

TROCK S.C., MEADE B.J., GLASER A.L., OSTLUND E.N., LANCIOTTI R.S., CROPP B.C., KULASEKERA V., KRAMER L.D., KOMAR N. West Nile outbreak among horses in New York State, 1999 and 2000. *Emerg. Infect. Dis.*, 2001, **7**, 745-747.

TURELL M.J., O'GUINN M., OLIVER J. Potential for New York mosquitoes to transmit West Nile virus. *Am. J. Trop. Med. Hyg.*, 2000, **62**, 413-414.

TYCZKA J., EBERLING S., BALFER G. Immunization experiments with recombinant *Coxiella burnetii* proteins in a murine infection model. *Ann. N.Y. Acad. Sci.*, 2005, **1063**, 143-148.

U

UGHETTO E., GOURIET F., RAOULT D., ROLAIN J.M. Three years experience of real-time PCR for the diagnosis of Q fever. *Clin. Microbiol. Infect.*, 2009, **15**, 200–201.

UNITED STATES ARMY MEDICAL RESEARCH INSTITUTE FOR INFECTIOUS DISEASES Medical management of biological casualities handbook. 5th ed. Capt. Darling R.G., Lt. Col. Woods: Institute of Infectious Diseases, Fort Detrick, Maryland, USA, 2004, 182p.

V

VAIDYA V.M., MALIK S.V.S., SIMRANPREET K., SATISH K., BARBUDDHE S.B. Comparison of PCR, immunofluorescence assay, and pathogen isolation for diagnosis of Q fever in humans with spontaneous abortions. *J. Clin. Microbiol.*, 2008, **46**, 2038–2044.

VAIDYA V. M., MALIK S.V.S., BHILEGAONKAR K.N., RATHORE R.S., KAUR S., BARBUDDHE S.B. Prevalence of Q fever in domestic animals with reproductive disorders. *Comp. Immunol. Microbiol. Infect. Dis.*, 2010, **33**, 307-321.

VALKOVA D., KAZAR J. A new plasmid (QpDV) common to *Coxiella burnetii* isolates associated with acute and chronic Q fever. *FEMS Microbiol. Lett.*, 1995, **125**, 275-280.

VAN LIERDE H. Vademecum biologique du vétérinaire. Prodim: Belgium, 1988, 612p.

VARDI M., PETERSIL N., KEYSARY A., RZOTKIEWICZ S., LAOR A., BITTERMAN H. Immunological arousal during acute Q fever infection. *Eur. J. Clin. Microbiol. Infect. Dis.*, 2011, DO: 10.1007/s10096-011-1255-5.

VAZQUEZ A., SANCHEZ-SECO M.P., RUIZ S., MOLERO F., MAGALLANES A., TEJEDOR C.G., TENORIO A. Putative new lineage of West Nile virus, Spain. *Emerg. Infect. Dis.*, 2010, **16**, 549-552.

VENTER M., HUMAN S., ZAAYMAN D., GERDES G.H., WILLIAMS J., STEYL J., LEMAN P.A., PAWESKA J.T., SETZKORN H., ROUS G., MURRAY S., PARKER R., DONNELLAN C., SWANEPOEL R. Lineage 2 West Nile virus as cause of fatal neurologic disease in horses, South Africa. *Emerg. Infect. Dis.*, 2009, **15**, 877-884.

VERDEGAAL E.J.M.M., DE HEER N., MEERTENS N.M., MAREE J.T.M., SLOET VAN OLDRUITENBORGH-OOSTERBAAN M.M. Case report: A right-sided bacterial endocarditis of dental origin in a horse. *Equine Vet. Educ.*, 2006, **18**, 191–195.

VERDEGAAL E.J.M.M., SLOET VAN OLDRUITENBORGH-OOSTERBAAN M.M. Clinical commentary: Endocarditis in the horse. *Equine Vet. Educ.*, 2006, **18**, 196–198.

VIGIL A., ORTEGA R., NAKAJIMA-SASAKI R., PABLO J., MOLINA D.M., CHAO C.C., CHEN H.W., CHING W.M., FELGNER P.L. Genome-wide profiling of humoral immune response to *Coxiella burnetii* infection by protein microarray. *Proteomics*, 2010, **10**, 2259-2269.

VODKIN M.H., WILLIAMS J.C., STEPHENSON E.H. Genetic heterogeneity among isolates of *Coxiella burnetii*. *J. Gen. Microbiol.*, 1986, **132**, 455.

W

WAAG D.M., BYRNE W.R., ESTEP J., GIBBS P., PITT M.L.M., BANFIELD C.M. Evaluation of cynomolgus (*Macaca fascicularis*) and rhesus (*Macaca mulatta*) monkeys as experimental models of acute Q fever after aerosol exposure to phase I *Coxiella burnetii*. *Lab. Anim. Sci.*, 1999, **49**, 634-638.

WAAG D. M. *Coxiella burnetii*: host and bacterial responses to infection. *Vaccine*, 2007, **25**, 7288-7295.

WANG T., TOWN T., ALEXOPOULOU L., ANDERSON J.F., FIKRIG E., FLAVELL R.A. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nat. Med.*, 2004, **10**, 1366-1373.

WARD M.P. Epidemic West Nile virus encephalomyelitis: a temperature-dependent, spatial model of disease dynamics. *Prev. Vet. Med.*, 2005, **71**, 253-264.

WATANABE A., TAKAHASHI H. Epidemiology, diagnosis, and treatment of Q fever. *Jpn. J. Chemother.*, 2003, **51**, 461–469 (in Japanese).

WATANABE A., TAKAHASHI H. Diagnosis and treatment of Q fever: attempts to clarify current problems in Japan. *J. Infect. Chemother.*, 2008, **14**, 1-7.

WATSON J.T., PERTEL P.E., JONES R.C., SISTON A.M., PAUL W.S., AUSTIN C.C., GERBER S.I. Clinical characteristics and functional outcomes of West Nile fever. *Ann. Intern. Med.*, 2004, **141**, 360-365.

WEBSTER J.P., LLOYD G., MACDONALD D.W. Q fever (*Coxiella burnetii*) reservoir in wild brown rat (*Rattus norvegicus*) populations in the UK. *Parasitology*, 1995, **110**, 31-35.

WEESE J.S., BAIRD J.D., DELAY J., KENNEY D.G., STAEMPFLI H.R., VIEL L., PARENT J., SMITH-MAXIE L., POMA R. West Nile virus encephalomyelitis in horses in Ontario: 28 cases. *Can. Vet. J.*, 2003, **44**, 469-473.

WELSH H. H., LENNETTE E.H., ABINANTI F.R. Air-borne transmission of Q fever: the role of parturition in the generation of infective aerosols. *Ann. N.Y. Acad. Sci.*, 1957, **70**, 528-540.

WHITNEY E.A., MASSUNG R.F., CANDEE A.J., AILES E.C., MYERS L.M., PATTERSON N.E., BERKELMAN R.L. Seroepidemiologic and occupational risk survey for *Coxiella burnetii* antibodies among US veterinarians. *Clin. Infect. Dis.*, 2009, **48**, 550-557.

WHO (WORLD HEALTH ORGANIZATION), Zoonoses and veterinary public health. Emerging zoonoses. [on line] (2011) URL Address: www.who.int/zoonoses/emerging_zoonoses/en Accessed 21 January 2011.

WILLEBERG P., RUPPANNER R., BEHYMER D.E., HAGHIGHI S., KANEKO J.J., FRANTI C.E. Environmental exposure to *Coxiella burnetii*: a sero-epidemiologic survey among domestic animals. *Am. J. Epidemiol.*, 1980, **111**, 437-443.

WILLEMS H., THIELE D., FROLICH-RITTER R., KRAUSS H. Detection of *Coxiella burnetii* in cow's milk using the polymerase chain reaction (PCR). *Zentralbl. Veterinarmed. B.*, 1994, **41**, 580-587.

WILLEMS H., THIELE D., BURGER C., RITTER M., OSWALD W., KRAUSS H. Molecular biology of *Coxiella burnetii*. In: Kazar, J., Toman R. (eds), *Rickettsiae* and rickettsial diseases, Slovak Academy of Sciences: Bratislava, Slovakia, 1996, 363-378.

WILLIAMS J.C., CANTRELL J.L. Biological and immunological propreties of *Coxiella burnetii* vaccines in C57BL/10ScN endotoxin-non responder mice. *Infect. Immun.*, 1982, **35**, 1091-1102.

WILLIAMS J.C. Infectivity, virulence, and pathogenicity of *Coxiella burnetii* for various hosts. In: Williams J. C., Thompson H.A. (eds), Q fever: the biology of *Coxiella burnetii*. CRC Press: Boca Raton, Florida, 1991, 21-71.

WOOLHOUSE M.E., GOWTAGE-SEQUERIA S. Host range and emerging and reemerging pathogens. *Emerg. Infect. Dis.*, 2005, **11**, 1842-1847.

Y

YOSHIIE K., ODA H., NAGANO N., MATAYOSHI S. Serological evidence that the Q fever agent (*Coxiella burnetii*) has spread widely among dairy cattle. *Microbiol. Immunol.*, 1991, **35**, 577–581.

YU X., RAOULT D. Serotyping *Coxiella burnetii* isolates from acute and chronic Q fever patients by using monoclonal antibodies. *FEMS Microbiol. Lett.*, 1994, **117**, 15-19.

YUASE Y., YOSHIIE K., TAKASAKI T., YOSHIDA H., ODA H. Retrospective survey of chronic Q fever in Japan by using PCR to detect *Coxiella burnetii* DNA in paraffin-embedded clinical samples. *J. Clin. Microbiol.*, 1996, **34**, 824–827.

Z

ZALACAIN R., TALAYERO N., ACHOTEGUI V., CORRAL J., BARRENA I., SOBRADILLO V. Community-acquired pneumonia. Reliability of the criteria for deciding ambulatory treatment. *Arch. Bronconeumol.*, 1997, **33**, 74–79.

ZAMBONI D.S., RABINOVITCH M. Nitric oxide partially controls *Coxiella burnetii* phase II infection in mouse primary macrophages. *Infect. Immun.*, 2003, **71**, 1225-1233.

ZELLER H.G., SCHUFFENECKER I. West Nile Virus: an overview of its spread in Europe and the Mediterranean Bassin in contrast to its spread in Americas. *Eur. J. Clin. Microbiol. Infect. Dis.*, 2004, **23**, 147-156.

ZHANG Y.X., ZHI N., YU S.R., LI Q.J., YU G.Q., ZHANG X. Protective immunity induced by 67-K outer-membrane protein of phase I *Coxiella burnetii* in mice and guinea pigs. *Acta Virol.*, 1994, **38**, 327-332.

ZHANG G., SAMUEL J.E. Identification and cloning potentially protective antigens of *Coxiella burnetii* using sera from mice experimentally infected with Nine Mile phase I. Ann. N.Y. Acad. Sci., 2003, **990**, 510-520.