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Disease detection and management at the wildlife-livestock-human interface

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CONTENT

ABSTRACT

This thesis has explored in a practical manner scientific challenges to prevent, detect, control or manage diseases at the wildlife-livestock-human (W-L-H) interface at different spatial levels through a set of field experimental studies. Traditional research on diseases at the W-L-H interface focuses on the role of wildlife in the epidemiology, transmission, and maintenance of infectious diseases. This is used to develop strategies to reduce pathogen transmission to both domestic animals and human beings. This thesis contributes to applied sciences to understand the pathology, epidemiology, and ecology of some specific diseases endemic in Africa and Arabia. Aligned with the One Health/Ecohealth approaches, this thesis also emphasizes the importance of socio-ecosystemic approaches to health concerns at the animal-human-ecosystem interface and points out the need for interdisciplinary studies and the role of anthropogenic factors in disease emergence and spreading.

From the farm to the global level, human activities seem the most propitious to enhance disease transmission at W-L-H and Wildlife-Human (W-H) interfaces. After investigating biosecurity measures applied in different animal sectors in the United Arab Emirates (UAE), it stood out that human activities and disrespect of basic principles of biosecurity are enhancing disease transmission. This should be the central pillar of disease control and prevention before taking any drastic measures among wildlife populations. At the global level, illegal bushmeat is imported from Africa to Europe for personal consumption and/or as part of a lucrative organized trade amounting to around five tonnes of bushmeat smuggled in personal baggage through Paris Roissy-Charles de Gaulle Airport every week. Such trade, by its nature and scale, is driving unsustainable hunting in source countries and posing public health risks in Europe. While presence of food-borne zoonotic bacteria and high levels of Polycyclic Aromatic Hydrocarbons (PAHs) were detected, the risks to European public heath, agriculture and wild fauna still need to be assessed. The main factors promoting the emergence of zoonosis and disease globalization are directly linked to human activities and demographic growth. This calls for a complex, integrative theory that will be able to explain important disease patterns and have practical consequences.

On the ground, preventing and controlling outbreaks at the W-L-H interface is challenging mainly due to our knowledge gaps and our lack of will to acknowledge them. Collaborative work among different sectors is mandatory in order to understand complex ecological systems and to gather the necessary sound scientific data on disease ecology, vaccine efficacy, epidemiological situation, species sensitivity, host specificity, transmission patterns, vectors or climatic conditions affecting pathogens survival and vector competence. By studying an outbreak of Contagious Caprine Pleuropneumonia (CCPP) in the UAE, new information relevant to disease management at W-L-H interface has been collated. The Arabian Oryx (*Oryx leucoryx*), from the hippotraginae family, not previously considered susceptible to CCPP, was proven to be lethally infected by CCPP. Not all commercial CCPP vaccines that were available locally provided the expected protection to prevent and control a CCPP outbreak in a sand gazelle (*Gazella marica)* population. The basic reproductive number R_0 was estimated at 2.3-2.7, while the final observed mortality rate reached up to 70% in the sand gazelle population. A newly described contamination route involving indirect and distant sources of contamination, through droplets transported more than 80m away, is the most likely explanation for this outbreak. Although this information is crucial in building epidemiological scenarios, each outbreak has its own specificity; knowledge of pathogen, environment and host populations are key to contextualise the studies. Epidemiological functional roles and the basic reproductive number for a pathogen are situation and time-dependent. Indeed, wildlife health information might only be valid at a local level for a specific time, for a portion of the casual chain. Therefore, it is important to bridge the knowledge-to-action gap and translate data into policy when necessary.

The understanding of environmental and climatic conditions, ecological diversity and complexity that can encourage or alter pathogen survival and transmission are as important as describing the pathogenic mechanisms themselves. Our studies on chytridiomycosis (*Batrachochytrium dendrobatidis*) and *Acomys dimidiatus'* fleas stress the importance of the environmental condition and host population to study pathogens and highlight the absolute necessity for trans- and inter-disciplinary work. The ecosystem is like a living body, where each population, like organs, interact with each other and the disruption of this balance will make the entire system dysfunctional.

Our serosurvey and Multiple-Locus Variable number tandem repeat Analysis (MLVA) results point out the need for a comprehensive multi-species (W-L) approach to disease monitoring.

Zoning has been widely used in southern Africa and countries in the Arabian Gulf to segregate wild ungulates from livestock or "contaminated" versus "disease-free" areas. In the UAE, our studies on Q fever and *Brucella melitensis* suggest livestock has contaminated wildlife or vice versa even through walls and fences. In Botswana, the recent increase in trans-frontier wildlife protected areas and associated wildlife corridors make any information on pathogen prevalence and transmission among wildlife species all the more important. Our aim was to investigate the seroprevalence of various viral pathogens among four co-occurring large carnivore species: lion (*Panthera leo)*, spotted hyena (*Crocuta crocuta)*, leopard (*Panthera pardus)* and cheetah (*Acinonyx jubatus)*. The differences in seroprevalence between individuals that did or did not come into contact with human activities were emphasized. Disease and pathogen prevalence and molecular epidemiology should be considered and included in the conservation and management plans for these areas but the usefulness and effectiveness of zoning and segregation should take into consideration their environmental cost.

A fundamental reflection around the aims, methods and philosophy currently driving disease detection and management at the wildlife-livestock-human interface in Europe-Middle East-Africa (EMEA) is carried out in this thesis. Even though it is admitted that diseases at wildlife/livestock interface are bi-directional, current research on wildlife health is focussing on discovering threats, describing their consequences and proposing response options. In doing so, health professionals and authorities should ask themselves the following questions: Whose health are they trying to promote? Are they achieving their goals? The description of diseases and epidemiological scenarios are shaped by the methodological approaches used. Furthermore, research topics and funding priorities are shaped by social history and political concerns and agendas. Achieving global health will require building an epidemiology for all. W-L-H interface is currently seen through the lens of zoonosis. An approach emphasizing specific diseases and exposures might prevent us from grasping the bigger picture. We argue that scientific and political focus should shift from zoonotic disease to 'Human-Induced Disease' (HID) – defining both chronic and infectious disease. The term "Human-Induced Diseases" might have the potential to put the 'human' back into perspective and to bring together concerns and efforts. HID as a the label for diseases (from emerging to chronic) caused by human activities places the emphasis on the role of humans and could encourage the collaboration among scientists, politicians, industrials and laymen in common pursuit. Human-Induced Diseases need to be named in order to be collectively claimed.

RESUME

Cette thèse a exploré de manière concrète les défis scientifiques liés à la prévention, la détection, le contrôle et la gestion des maladies à l'interface Faune sauvage-Bétail-Humain (F-B-H) à différentes échelles spatiales grâce à un ensemble d'études expérimentales. La recherche traditionnelle sur les maladies à l'interface F-B-H met l'accent sur le rôle de la faune dans l'épidémiologie, la transmission et le maintien des maladies infectieuses. Ces informations permettent de développer des stratégies visant à réduire la transmission des agents pathogènes aux animaux domestiques et aux êtres humains. Cette thèse apporte des résultats scientifiques nouveaux améliorant notre connaissance sur la pathologie, l'épidémiologie et l'écologie de certaines maladies endémiques en Afrique et en Arabie. Se situant dans la continuité des approches One Health / Ecohealth, cette thèse souligne également l'importance des approches socio-écosystémiques des problèmes de santé à l'interface animal-humain-écosystème et souligne la nécessité de collaborations interdisciplinaires et le rôle des facteurs anthropiques dans l'émergence et la propagation de la maladie.

De la ferme à l'échelle mondiale, les activités humaines semblent des moteurs favorisant la transmission des maladies aux interfaces F-B-H et Faune sauvage-Humain (F-H). Après avoir étudié les mesures de biosécurité appliquées dans différents types d'exploitations animales aux Émirats Arabes Unis, il est apparu que les activités humaines et le manque de respect des principes fondamentaux de biosécurité représentent des facteurs déterminants dans la transmission des maladies. L'amélioration des pratiques de biosécurité devrait être le pilier central du contrôle et de la prévention des maladies avant de prendre des mesures drastiques à l'encontre des populations sauvages. Au niveau mondial, notre étude sur le trafic de viande de brousse d'Afrique vers l'Europe a révélé qu'environ cinq tonnes de viande de brousse étaient importées illégalement chaque semaine via l'aéroport de Paris Roissy-Charles de Gaulle dans les bagages de passagers pour leur consommation personnelle mais aussi dans le cadre d'un commerce organisé lucratif. Un tel commerce, de par sa nature et son ampleur, conduit à la pratique d'une chasse non-durable dans les pays d'origine et pose des risques pour la santé publique en Europe. Des bactéries zoonotiques d'origine alimentaire et de hauts taux d'hydrocarbures aromatiques polycycliques (HAP) ont été détectés mais les risques pour la santé publique européenne, l'agriculture et la faune sauvage doivent encore être évalués. Les

principaux facteurs favorisant l'émergence de zoonoses et de la globalisation des maladies sont directement liés aux activités humaines et à la croissance démographique. Il semble nécessaire de construire un modèle complexe et holistique qui pourra expliquer l'évolution des maladies importantes et conduire à des interventions pratiques.

Prévenir et contrôler les épidémies à l'interface F-B-H est un défi majeur sur le terrain en raison de nos lacunes en termes de connaissances et notre incapacité à le reconnaître. Une collaboration entre différents domaines de compétence est nécessaire à la bonne compréhension de systèmes écologiques complexes et à la collecte des données scientifiques sur l'écologie des maladies, l'efficacité des vaccins, les situations épidémiologiques, la sensibilité des espèces, la spécificité des hôtes, les modèles de transmission, les conditions vectorielles ou climatiques affectant la survie des agents pathogènes et la compétence des vecteurs. En étudiant une épidémie de Pleuropneumonie contagieuse caprine (CCPP) aux Émirats Arabes Unis, de nouvelles informations pertinentes à la gestion des maladies à l'interface F-B-H ont été rassemblées. L'Oryx d' Arabie (*Oryx leuroyx*), appartenant à famille hippotraginae qui n'était pas considérée comme susceptible à la CCPP, s'est avéré sensible et mortellement infecté par CCPP. Tous les vaccins CCPP commerciaux localement disponibles ne fournissaient pas la protection prévue pour prévenir et contrôler une épidémie de CCPP dans une population de gazelle des sables (*Gazella marica)*. Le taux de reproduction de base R0 a été estimé à 2,3-2,7, tandis que le taux de mortalité observé final a atteint jusqu'à 70% dans la population de gazelle des sables. Une nouvelle voie de contamination, impliquant une source de contamination indirecte et éloignée via des gouttelettes transportées à plus de 80 m, est l'explication la plus probable pour cette épidémie. Bien que ces informations soient cruciales à la construction de scénarios épidémiologiques, chaque épidémie a sa propre spécificité et doit être mise en contexte en fonction des particularités de l'agent pathogène, de l'environnement et des populations d'hôtes. Les rôles épidémiologiques et le taux de reproduction de base pour un agent pathogène sont fonction de la situation et du temps. Les informations sur la santé de la faune ne sont éventuellement valides que localement pour une période spécifique et sur une partie de la chaîne causale. Il est donc important de combler l'écart entre les connaissances et leur mise en pratique et de traduire les données en mesures concrètes lorsque cela est nécessaire.

La compréhension des conditions environnementales et climatiques, de la diversité et de la complexité écologique qui peuvent favoriser ou altérer la survie et la transmission des agents

pathogènes est aussi importante que la description des mécanismes pathogéniques en eux-mêmes. Nos études sur la chytridiomycose (*Batrachochytrium dendrobatidis*) et les puces de *Acomys dimidiatus* soulignent l'importance des conditions environnementales et des populations hôtes lors des études sur les agents pathogènes et démontrent une fois de plus la nécessité absolue d'un travail trans- et interdisciplinaire. L'écosystème est comme un organisme vivant, où différentes populations, telles des organes, interagissent les unes avec les autres et toute perturbation de cet équilibre entraîne un dysfonctionnement de l'ensemble du système.

Nos résultats d'enquêtes sérologiques et d'analyses MLVA (*Multiple-Locus Variable number tandem repeat Analysis)* indiquent la nécessité d'une approche globale multi-espèces pour la surveillance des maladies. Le zonage a été largement utilisé en Afrique australe et dans les pays du Golfe Arabe pour séparer les ongulés sauvages du bétail ou bien les zones considérées « contaminées » des zones « indemnes ». Aux Émirats Arabes Unis, nos études sur la fièvre Q et *Brucella melitensis* suggèrent que le bétail a contaminé la faune et/ou vice versa même à travers des murs et clôtures. Au Botswana, la création récente d'espaces protégés transfrontaliers protégés et de corridors fauniques associés appelle à la collecte d'informations sur la prévalence et la transmission des agents pathogènes chez les espèces sauvages. Notre objectif était d'étudier la séroprévalence de divers agents pathogènes viraux parmi quatre espèces de grands carnivores coexistant: le lion (*Panthera leo)*, la hyène tachetée (*Crocuta crocuta)*, le léopard (*Panthera pardus)* et le guépard (*Acinonyx jubatus)*. Les différences de séroprévalence entre les individus qui ont été ou n'ont pas été en contact avec les camps humains ont été notées. La prévalence des maladies et des pathogènes et l'épidémiologie moléculaire devraient être incluses dans les plans de conservation et de gestion de ces espaces, mais l'utilité et l'efficacité des méthodes de zonage et de ségrégation devrait aussi prendre en compte leur coût environnemental.

Des réflexions fondamentales autour des objectifs, des méthodes et de la philosophie qui guident actuellement la gestion des maladies à l'interface entre la faune sauvage, le bétail et l'humain en Europe-Moyen-Orient-Afrique (EMEA) sont développées dans cette thèse. Même s'il est admis que les maladies à l'interface entre la faune et le bétail sont bi-directionnelles, les recherches actuelles sur la santé de la faune se concentrent sur la découverte des menaces que représentent les populations sauvages, en décrivant leurs conséquences et en proposant des options pour y répondre. Ce faisant, les professionnels de la santé et les autorités devraient se

poser les questions suivantes : Qui sont les bénéficiaires de cette approche? Quelle santé est elle promue? Atteignent-ils leurs objectifs? La description des maladies et des scénarios épidémiologiques est façonnée par l'approche méthodologique utilisée. En outre, les questions de recherche et les disponibilités de financement sont influencées par l'histoire sociale et les préoccupations et programmes politiques. Une bonne santé mondiale nécessitera la construction d'une épidémiologie pour tous. L'interface F-B-H est actuellement vue à travers le spectre des zoonoses. Une telle approche mettant l'accent sur certaines maladies et leurs risques associés pourrait nous empêcher d'avoir une vision globale. L'attention des scientifiques et des politiques devrait dépasser les seules maladies zoonotiques et s'élargir aux « Maladies Induites par l'Homme » (MIH) - incluant à la fois les maladies chroniques et infectieuses. Le terme « Maladies Induites par l'Homme » pourrait avoir le potentiel de remettre l'« humain » en perspective et d'unifier les préoccupations et les efforts. MIH comme étiquette pour les maladies (émergentes et chroniques) causées par les activités humaines met l'accent sur le rôle de l'humain et pourrait encourager la collaboration entre les scientifiques, les politiques, les industriels et le grand public dans une action commune. Les Maladies Induites par l'Homme doivent être nommées pour être collectivement reconnues et abordées.

LIST OF ACRONYMS

ANSES: *Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail* - French Agency for Food, Environmental and Occupational Health and Safety

Bd: *Batrachochytrium dendrobatidis.*

BSE: Bovine Spongiform Encephalopathy

CCPP: Contagious Caprine Pleuropneumonia

CDC: Center for Disease Control and Prevention

CITES: Convention on International Trade in Endangered Species of Wild Fauna and Flora

EC: European Commission

EMEA: Europe-Middle East-Africa

EMPRES-livestock: Emergency Prevention System-Livestock

EIDs: Emerging Infectious Diseases

EU: European Union

FAO: Food and Agriculture Organization of the United Nations

FMD: Foot-and-Mouth Disease

GDP: Gross Domestic Product

GLEWS: Global Early Warning System

GOARN: Global Outbreak Alert and Response Network

HIV: Human Immunodeficiency Virus

IHR: International Health Regulations

KAZA/TFCA: Kavango Zambezi Trans-Frontier Conservation Area

Mccp: *Mycoplasma capricolum* subsp. *Capripneumoniae*

MLVA: Multi-Locus Variable number of tandem repeat Analysis

NGS: Next-Generation Sequencing

NTD: Neglected Tropical Diseases

OIE: *Organisation Mondiale de la Santé Animale* - World organisation for animal health

PAHs: Polycyclic Aromatic Hydrocarbons

PCR: Polymerase Chain Reaction

UAE: United Arab Emirates

UNESCO: United Nations Organisation for Education, Science and Culture

USA: United States of America

VBD: Vector Borne Disease

WHER: Wildlife Health Event Reporter

WHO: World Health Organization

W-L-H interface: Wildlife-Livestock-Human interface

WTO: World Trade Organisation

GENERAL INTRODUCTION

For most of their 4.000.000 years of evolutionary history, human populations lived in small, sparsely settled groups[1]. The hunter/gatherer communities already suffered from infectious diseases with characteristics which allowed them to persist in small populations and with occurrences in animal reservoirs as well as in humans (such as yellow fever)[1]. Following the Neolithic revolution and the development of agriculture around 11,000 years ago, a dramatic increase in population size and density occurred. Along with a steep rise in the human population and its settling, the domestication of animals provided a steady supply of vectors and greater exposure to zoonotic diseases leading to a substantial increase in infectious diseases[2]. Nowadays, the majority of our zoonoses come from domesticated animals living for the most part in temperate areas, 62% of all human pathogens are classified as zoonoses and 77% of livestock pathogens infect multiple species[3]. From ancient times to nowadays the social, demographic, and environmental changes in a global ecology have been the driving force of the emergence or re-emergence of diseases[4].

The wildlife-livestock-human interface has changed and is constantly evolving. Factors leading to increased contacts and pathogen transmission at the wildlife-livestock-human interface range from ecological disturbances, to new farm practices and industrial production, to legal and illegal international trade allowing for movements of animals and animal products around the world. Human health, animal health, and environmental health are interconnected. Approaching one of them separately without taking the other into consideration makes no sense. The concept of “One Health” or “Ecohealth” tries to hold back the shift towards specialisation, categorisation, disciplinary boundaries, reductionism and epistemological dispersion that characterises Western history of sciences and knowledge. Within human holistic thinking, health and environment intertwine and their fields of action merge. The Greek Antiquity to which we owe Hippocratic medicine and a cosmic view of the world is definitely a reference that is once again up-to-date. One Health/Eco-Health are not concepts but approaches. One Health and Eco-Heath approaches posit that the epidemiological dynamics and stakeholders’ actions that determine the health of animal and human populations need to be studied in their interconnected ecological, socioeconomic, and political contexts. The One Health/ Eco Health approach optimizes interdisciplinarity including strong participatory and citizenship-related components. This discipline does not only promote a

multi- and or cross-sectoral and collaborative approach. but also an "entire society" approach to health hazards. Aligned with the One Health/ Ecohealth approach, this thesis was structured to emphasize the importance of the socio-ecosystemic approach of health concerns at the animal-human-ecosystem interface and to point out the necessity of interdisciplinary collaboration.

This thesis has explored, in a practical manner, the field of disease detection and management at the wildlife-livestock-human interface at the different spatial level from farms to global scale through a set of practical studies. Disease detection and management have different meanings and realities if it applies to a farm, population or individual level, at a regional or national level or at a global level. A fundamental reflection around the methods currently applied to prevent, detect, control or manage diseases at the wildlife-livestock-human interface were developed during this work. There is comparatively less research directed to exclusive animal diseases that impact on livestock and/or wildlife health than on the zoonotic aspects of some of these diseases. Although human health is driving political, media and scientific interest, livestock constitutes globally on average 37% of the agricultural gross domestic product[5] and is one of the most important and rapidly expanding commercial agricultural sectors worldwide[6]. Diseases that are shared between species can affect biodiversity, change behaviour or composition of animal populations, and even relegate species to the fringe of extinction [7,8]. Wildlife diseases represent a potential burden to the whole ecosystem. Many national disease surveillance and monitoring programmes, therefore, include free-ranging or farmed wildlife. Apart from the direct economic, public health and trade implications of the presence of diseases in wildlife, diseases in wildlife may be important indicators of ecological disturbance, introduction of new animal species, introduction of new diseases, climatic or habitat change, or local pollution[9]. Wildlife health surveillance has become an integral component in the identification and management of potential threats to human and animal health[10].

The wildlife-livestock interface defines the interaction between wildlife and livestock. This not only includes direct contact between wildlife and livestock either sharing the same pasture/water or interacting along fences but also indirect contacts through vectors or wind. The wildlife-livestock-human interface encompasses direct contact between wildlife and livestock, or contact between animals (livestock or wildlife) and humans; it also includes the study of the impact of human activities on animal health and well-being. The interface is not only perceived in a physical sense but extended to how human activities affect disease

transmission, and in more general terms animal, environmental and human health. Aligned with this definition, the three elements (W/L/H) are not always present in all studies but we worked on the interface either between humans or human activities and wildlife, or between wildlife and livestock. This thesis doesn't focus only on zoonotic diseases but also concentrates on non-zoonotic wildlife diseases of conservation-related concern.

Similar underlying factors drive disease emergence in both human and wildlife populations. The direct link between zoonosis and human activities and demographic growth is established. Land use modification for urbanisation, food production and agricultural change accounts for around 50% of all zoonotic Emerging Infectious Diseases (EIDs)[11]. Demographic, societal and behavioural changes gave rise to the Human Immunodeficiency Virus (HIV/AIDS)[12] and outbreaks of syphilis [13]. The advent of mass travel exacerbates the historically established dissemination of infectious diseases along migration routes. Examples are bubonic plague (*Yersinia pestis)*, cholera (*Vibrio cholerae)*, seasonal influenza, Severe Acute Respiratory Syndrome (SARS), and malaria (*Plasmodium falciparum)* [14]. The trade in goods and animals is directly linked to outbreaks such as human monkeypox virus in North America [15] or H5N1 in the United Arab Emirates [16]. Anthropogenic environmental change also lead to the emergence of infectious diseases in wildlife [17]. In this thesis we highlight how human activities could be driving disease in wildlife, livestock and human populations.

Working as a veterinary epidemiologist at the W-L-H human interface rimes with identifying the role of wild and domestic animals in the epidemiology, transmission, and maintenance of specific diseases. Aligned with the One Health concept we wanted to have an holistic approach and put back the human factor into perspective as well as understanding how each of the components interact with one another (Figure 1).

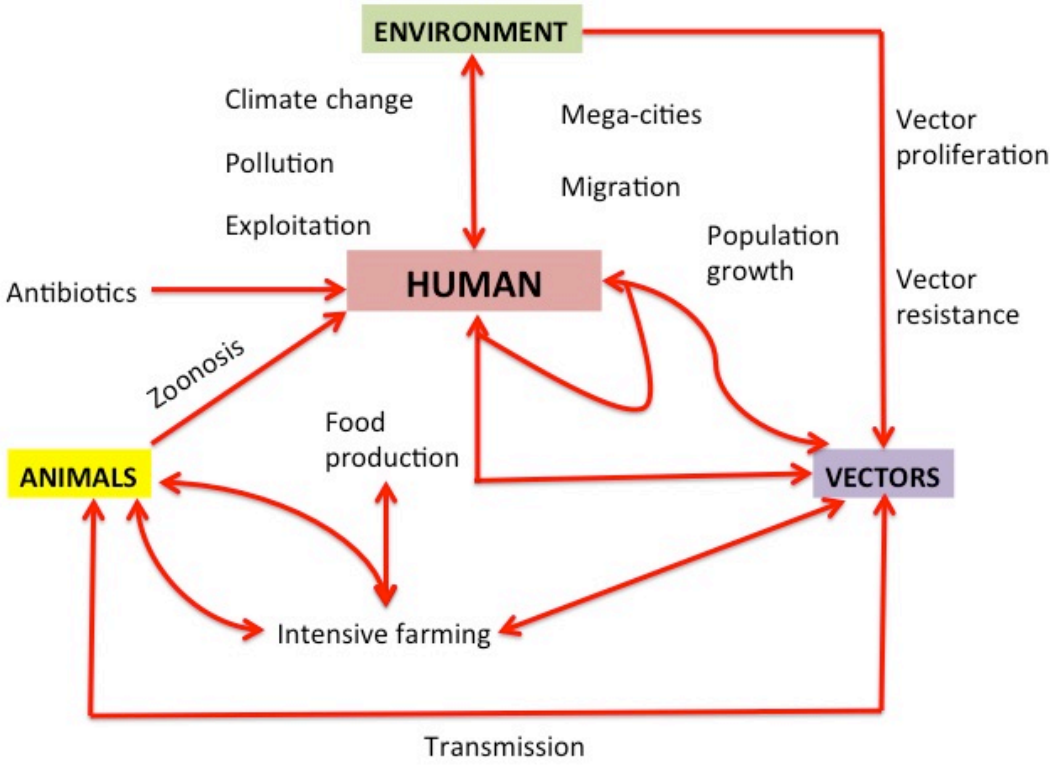

Figure 1: Epidemiological triad emphasizing how human activities affect each one of its elements.

Source: Annals of Community Health (AoCH)

http://annalsofcommunityhealth.in/ojs/images/emrem2.png

Our studies executed in the UAE, Botswana and Europe in ungulates and predators illustrate (Figure 2) that there is a need to:

1) Put the human factor back into perspective if we want to tackle disease transmission and fill the gap between rhetoric and reality;

2) Challenge our assumptions and reflect upon our knowledge gap when managing diseases at the W-L-H interface;

3) Carry out multi- and interdisciplinary studies in order to grasp the complexity of ecological system.

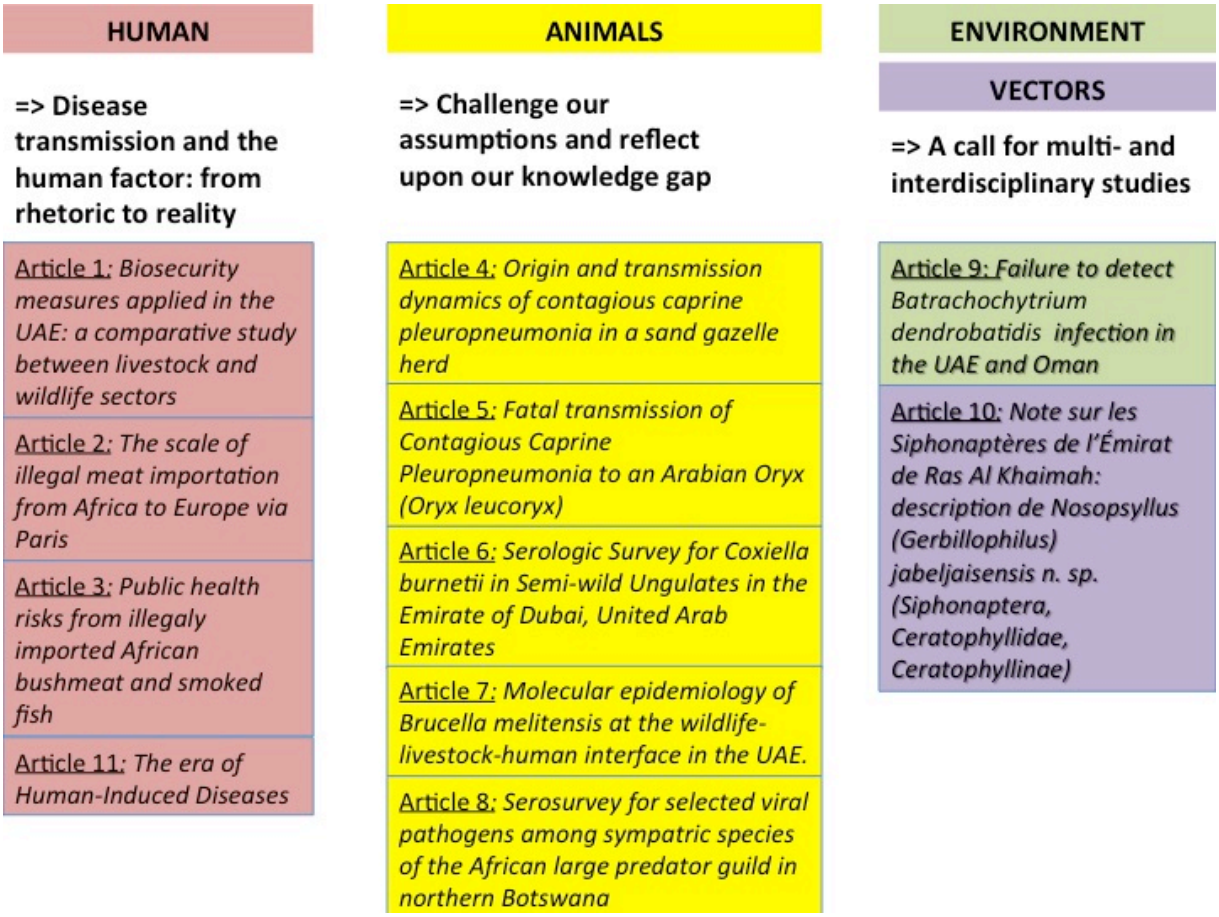

Figure 2: In bold, the conceptual framework of the thesis thereafter associated with the elements of the epidemiological triad studied and the list of articles relating to these elements.

As pathogens circulate freely during widlife-livestock-human contacts, we need to mirror the wildlife-livestock-human interface and build a similar flow of information between ecologists, biologists, entomologist, veterinarians, human doctors, anthropologists and mimic the complexity of the system we are trying to understand**.** In order to be truly holistic, this socio-ecosystemic approach not only needs to integrate the interplay between the social, economic and environmental drivers of health but scientists need to connect with government, industries and the general public in order to bridge the knowledge-to-action gap and translate data into policies whenever necessary.

During this research, we identified some challenges faced by scientists and health authorities to manage diseases at the Wildlife-Livestock-Human (W-L-H) W-H and W-L interfaces and pointed out tools or made recommendations for each of the investigated scenarios. The issue of antimicrobial resistance emergence is not investigated in this thesis.

In order to facilitate the understanding of this material, the literature review and the experimental section follow similar patterns.

PART 1 – LITERATURE REVIEW

Chapter 1: Disease transmission and the human factor: from rhetoric to reality

Animal health is a priority in livestock farming as it is directly linked to productivity. The human population tripled in the last 100 years to reach more than 7 billion today and the consumption of meat by an adult in Europe increased from 229 kcal/person/day in 1961-1965 to 377 kcal/person/day in 2000-2014[18]. Meat demand increased drastically, causing not only an incommensurable environmental impact but also drove major shifts in agricultural practices and traditional livestock production systems. At farm and global levels, the priority is to maintain healthy herds and protect them against pathogens through vaccinations and/or applying biosecurity measures to limit infection from sources.

1) Biosecurity measures applied in the UAE: a comparative study between livestock and wildlife sectors.

The concept of biosecurity has been long recognized by veterinarians but is recently taking great importance in veterinary medicine and public authorities due to disease outbreaks that have threatened to devastate agricultural economies. Biosecurity can be defined as the implementation of measures that reduce the risk of introduction and spread of disease agents. It requires a set of management and physical measures designed to reduce the risk of introduction, establishment and spread of animal diseases, infections or infestations to, from and within an animal population[19]. Biosecurity greatly relies on the respect of the "Five Bs": bio-exclusion (to limit the risk of introduction), bio-compartmentation (to limit the spread of the pathogen within the same facility, e.g. by isolating excreting animals), bio-containment (to limit the spread of the disease agent outside the facility), bio-contamination (to prevent the risk of human bio-contamination) and bio-prevention (to prevent any environmental bio-contamination and persistence of the pathogen)[20]. The Five B concept can also be simplified into all measures related to the prevention of introduction of disease into a herd (i.e. external biosecurity) and all measures to prevent the spread of a disease within a herd (i.e. internal

biosecurity)[21]. It is becoming increasingly evident that there is a need to re-orientate farmers and veterinarians towards preventive rather than curative medicine[22]. The implementation of biosecurity measures is an important way to contribute towards such re-orientation. Our study « Biosecurity measures applied in the United Arab Emirates: a comparative study between livestock and wildlife sectors » (Article 1) aimed primarily to describe the biosecurity measures currently applied in UAE ungulate facilities for different wildlife and livestock sectors. A secondary objective was to use the output from this biosecurity survey to investigate which sector could be categorized into risk groups for disease introduction and spread.

Preventive measures are often based on what is known or assumed, it is important to take into consideration that much uncertainty can remain regarding host specificity, transmission patterns or vaccine efficacy. Protection against some pathogens can rely on vaccination programs in livestock and/or wildlife populations.

2) International bushmeat trade: a threat to both conservation and public health

Understanding and containing emerging infectious diseases, especially those crossing the wildlife, human and domestic animal interface, is a global health imperative[23]. Major drivers of human-animal contact allowing pathogen exchange; including animal domestication for companionship and food production, anthropocentric alteration of the environment and global movement of animals and goods. Economic behaviour is known to play a key role in disease transmission. Throughout history, new pathogens have emerged with the opening of new markets or trade routes[24]. No country is capable of ensuring 100% security of its borders by imposing quarantine measures or import bans on animals and animal products. Humans and commodities, including live animals, may illegally enter any country in the world[25]. Previously, disease spillover events were likely to remain local – even undetected due to natural, cultural, or geographic barriers – but the unprecedented amount of movement of humans, animals, and animal products between countries linked to modern transportation, allows emerging diseases to spread along various globally connected networks in a matter of a day. Local events can now quickly develop into an international dimension. Trade and travel affect the likelihood that pathogens are spread internationally by altering not only the number but also the variety of infectious-susceptible contacts[26–28]. In the so-called ‘global village’,

detecting and controlling emerging or re-emerging diseases presents a challenge not only to individual countries but to the international community as a whole. Different countries have different priorities, varying amounts of financial resources and contrasting levels of infrastructure that can impact effective management on understanding disease emergence and persistence processes [23] and weaken international collaboration.

Several early warning systems have been put in place by international organisations:

The OIE manages the international animal disease reporting system for the main animal diseases, including zoonoses. This system, which started in the early 1980s, is based on official animal disease information that the veterinary authorities of OIE Member Countries have an obligation to report to and is divided into two components: the international early warning system and the international monitoring system.

The Food and Agriculture Organization of the United Nations (FAO) Emergency Prevention System-Livestock (EMPRES-livestock) program promotes the containment and control of transboundary animal diseases, and their progressive elimination on a regional, and ultimately a global basis[25]. FAO is encouraging the use of unofficial wildlife morbidity and mortality events reporting system the Wildlife Health Event Reporter (WHER) that is linked to the FAO EMPRES-i system to increase global early warning capacities using wildlife disease surveillance information.

For diseases of public health concern, the International Health Regulations (IHR) of the United Nations World Health Organization (WHO) require Member Countries to notify the WHO of any human cases of three infectious diseases, namely cholera, plague and yellow fever. In April 2000, the WHO launched the Global Outbreak Alert and Response Network (GOARN)[29], the objective of which is to gather epidemic intelligence from informal sources. In order to improve the efficiency of their early warning systems for the benefit of the international community, the FAO, the OIE and the WHO have embarked on the development of a Global Early Warning System (GLEWS)[29]. A GLEWS 'event' is a health event of potential international concern affecting domestic or wild animal populations, humans or the food chain. Health threats monitored by GLEWS+ include pathogens with high impact – including those that are zoonotic, have jumped species barriers, are of increased virulence or have invaded new geographical areas – and food hazards that threaten the food chain and international trade. The *Manual on the Preparation of National Animal Disease Emergency Preparedness Plans* of the FAO[30], stipulates that each country should, as part of its early warning system, carry out risk analyses to determine the likelihood of exotic

diseases being introduced[25]. No data are available on informal trade between countries. Illegal trade of live exotic species and bushmeat is not fully recorded, controlled or assessed in terms of disease introduction and pathogen pollution. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is a multilateral agreement that regulates wildlife trade through an intergovernmental process, combining wildlife and trade themes within a legally binding instrument, achieving conservation and sustainable use objectives. CITES has a record of 13 million trade transactions (submitted by Parties) but the value of illegal international trade by CITES is evaluated to be between 5 to 20 billion U.S. $/year excluding marine and timber products[31]. Bushmeat or the hunting of wild animals for food is an important food and livelihood resource for many people in tropical areas, including West and Central Africa[32,33]. In some rural areas, bushmeat is the most affordable protein source and may cost only a third of the price of any of the alternative protein sources. However, in large cities, bushmeat is increasingly considered a luxury food item, the consumption of which is linked to cultural traditions in urban environments[34]. The bushmeat trade may also provide significant income to local people[35]. The commercial illegal bushmeat trade has intensified due to increased urban population, modernized hunting methods, road development associated with the logging industry, and increasing international travel[34]. Very little is known or published about intercontinental bushmeat trade, but it is thought that bushmeat is imported into Europe for commercial and cultural reasons[36].

No studies were published so far which evaluate the public health and agricultural risks associated with the international bushmeat trade, but there is evidence of serious health concerns associated at the local level[37].

Data on the international bushmeat trade are extremely scarce. During this study, the author accompanied Customs officers at Roissy-Charles de Gaulle airport (CDG; Paris, France) monitoring flights arriving from West and Central Africa. During a month, bushmeat seized from passengers on these flights was collected and analysed to assess which species were being transported to France, and to evaluate any associated sanitary risks. African bushmeat seized by Customs officers at Toulouse-Blagnac airport (Toulouse, France) was also included in the analyses. Specifically, our two studies: "The scale of illegal meat importation from Africa to Europe via Paris » (Article 2) and « Public health risks from illegally imported african bushmeat and smoked fish » (Article 3) give initial indicators for the following research questions:

- What is the scope of the illegal bushmeat traffic from West and Central Africa to France?
- Which species are involved?
- What is the sanitary risk linked to this trade?

Chapter 2: Challenging our assumptions and reflecting upon our knowledge gap: Disease management at the wildlife/livestock interface.

1) CCPP in Arabian Oryx and Sand gazelles

a) From outbreak management to vaccine assessment

Contagious caprine pleuropneumonia (CCPP), caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) was long considered a goat-specific disease. The disease was first described by Thomas in Algeria in 1873 and then by Hutcheon, who related its introduction in South Africa to the shipment of goats from Turkey[38]. Goats showed signs of respiratory distress, easily explained by the observed massive unilateral pleuropneumonia lesions[39]. The affected lung was hepatized and reddish, but did not display any enlargement of interlobular septa. The lesions extended progressively from the center, which had a grayish appearance due to early stages of necrosis, to the periphery, which was dark purple. Pleurisy could be observed opposite to the lung lesions and resulted in pleural exudate accumulating in the pleural cavity, where it progressively clotted if the animal survived long enough. CCPP was thought to be transmitted via direct contact (nose to nose transmission) and commercial vaccines available in the UAE are used to protect wildlife and livestock collections.

The strict host specificity of Mccp for goats has been increasingly challenged. In our second study, Origin and transmission dynamics of contagious caprine pleuropneumonia in a sand gazelle herd (Article 4), we deal with a CCPP outbreak that occurred in a large collection of sand gazelles (*Gazella marica*) held in the United Arab Emirates. The course of the disease was precisely recorded and its spread within the various pens could be deducted from observations made during the outbreak. These observations were also used to validate the parameters of an in-silico transmission model, which allowed for an evaluation of the basic reproductive number R_0 of CCPP in those herds. Finally, the analysis of the various control measures that were implemented (antibiotic treatments and vaccinations) is used to issue some general recommendations for an early response in case of an outbreak and for its prevention.

b) New sensitive species and undiscovered modes of transmission

As CCPP has never been documented in grazing antelopes (subfamily hippotraginae), they were not considered susceptible. UAE is home to large captive and a semi-wild population of Arabian Oryx (*Oryx leucoryx,* subfamily hippotraginae). Arabian oryx was considered extinct in the wild in 1972[40]. Intensive breeding programs followed by re-introduction programs in the Middle East allowed this emblematic species to recover part of its territories with a total reintroduced population of over 1,000 animals. Re-introduction of animals into the wild relies heavily on captive stocks where genetic and veterinary management are crucial. CCPP is endemic in the Middle East[41]. In naïve flocks of goats, morbidity and mortality may reach 80% and 100%, respectively. Wildlife species are also susceptible and outbreaks of CCPP have been reported in caprinae subfamily: Nubian ibex (*Capra ibex nubiana*), Laristan mouflon (*Ovis orientalis laristanica*)[42], Tibetan antelopes (*Pantholops hodgsonii*)[43] and in antilopinae family: Gerenuk (*Litocranius walleri)* [42]*,* Sand gazelles (*Gazella subgutturosa marica*)[44,45]. In 2004, Ariz *et al*. reported the first outbreak of CCPP in a wildlife collection with a mortality rate reaching 83% in the wild goat and 58% in the Nubian ibex populations. CCPP has not been documented in the hippotraginae family hence was not considered susceptible. We investigated a Fatal transmission of Contagious Caprine Pleuropneumonia to an Arabian Oryx (Oryx leucoryx) (Article 5) and describe CCPP clinical picture in an Arabian oryx (*Oryx leucoryx*) infected at a distance by neighbouring sand gazelles (*Gazella subgutturosa marica*). This case shows for the first time that members of the hippotraginae family, here the Arabian Oryx, can be affected by CCPP from a distance, which was not described previously.

Research efforts are mandatory in getting the necessary information to limit disease transmission between farms and to protect wildlife and livestock herds. In addition, knowledge of the epidemiological situation of the region in which those animal populations live is crucial in adopting the necessary biosecurity measures.

There is now unanimous agreement on the importance of having an efficient national surveillance and monitoring system for animal diseases and zoonoses in domestic and wild animals, capable of generating reliable information on the disease situation within the country and rapidly detecting diseases introduced accidentally or deliberately[25].

OIE *Terrestrial Animal Health Code* (5) states that: 'The Veterinary Services shall have at their disposal effective systems for animal disease surveillance and for notification of disease problems wherever they occur, in accordance with the provisions of the *Terrestrial Code*. Adequate coverage of animal populations should also be demonstrated. They shall at all times endeavour to improve their performance in terms of animal health information systems and animal disease control'.

Nevertheless, in developing countries including rich developing countries, disease surveillance and monitoring on the ground with sometimes few trained epidemiologists, field veterinarians and laboratories remain difficult.

Problems can stem from a lack of veterinary infrastructure, poor communication between governmental institutions and private veterinarians; no understanding of the pathogenic agent itself or its complexity, lack of awareness of the endemic diseases that exist in the livestock or wild animal populations, poor understanding of disease dynamics especially in multiple host systems, few ecological data available and maybe more importantly the difficulty to carry out research and fight against assumptions. *It is now widely recognised that countries that conduct disease surveillance of their wild animal populations are more likely to understand the epizootiology of specific infectious diseases and zoonotic infections within their territorial borders and are therefore better prepared to protect wildlife, domestic animals, and human populations*[46]. In the Office International des Epizooties (OIE: World organisation for animal health) Report of the Working Group on Wildlife Diseases presented at the 67th General Session of the International Committee, it was noted: « *that the presence or absence of an infection in the wild cannot be declared by a country, or a local sub-national authority, unless sampling has been carried out and the results subjected to the appropriate statistical analyses. Clearly, the notion of 'absence of evidence' rather than the 'evidence of absence' is just as relevant to free-ranging animal populations as it is to domesticated animal population* ». Active surveillance of known diseases of economic, public health importance or conservation interest amongst wildlife is particularly beneficial to the national interest.

In our study areas (UAE and Botswana): there were no systems in place to collect, store and necropsy dead wildlife so there was no information regarding the occurrence of important disease processes in wild animal populations, disease record and non-statistical and non-random sampling database that normally provide some insight into the epizootiology.

Mortality events in free-ranging wildlife are not recorded, and thus their significance not assessed. The development of national wildlife disease networks and training modules for wildlife investigators are still needed and my working frame could not rely on this passive

information. An active surveillance program is sometimes a preferred approach. Such programs aim to collect a certain number of samples from a target population (either live and/or dead animal) to determine the point prevalence of certain pathogens using antigen or specific antibody techniques. When surveillance data are unavailable, serological assessment can provide a direct assessment of population immunity derived from natural disease (or immunization in managed populations). A serosurvey is the collection and testing of specimens from a defined population over a specified period of time to determine antibodies against a given etiologic agent[47]. Serological surveys supported by accurate species-specific tests are the most commonly used means to actively assess the extent of an infection within selected free-ranging populations[46]. Assessment of seroprevalence can be instrumental nevertheless logistical, technical challenges and limitations of serosurveys are also highlighted in the following articles.

2) Disease management: Q-fever and *Brucella melitensis* in the UAE

We carried out sero-surveys at wildlife-livestock interface where captive or semi-wild ungulates in the UAE are separated or zoned via walls or fences and questionned the sense of security given by those physical barriers.

a) Serological survey for *Coxiella burnetii* in semi-wild ungulates in the Emirate of Dubai, United Arab Emirates.

Q fever, a highly infectious zoonotic disease caused by *Coxiella burnetii,* has not been officially reported in the United Arab Emirates (UAE). Our study, Serological survey for *Coxiella burnetii* in semi-wild ungulates in the Emirate of Dubai, United Arab Emirates (Article 6), was the first survey of a large group of semi-wild ungulates and livestock in the UAE and indicates that a wide range of ungulates has been exposed to *C. burnetii* in the region. The high resistance of the bacteria in the environment and the inconsistent relationship between serologic status and animals shedding *C.burnetii* make disease management challenging in wild and semi-wild asymptomatic ungulate populations.

b) Molecular epidemiology: study of *Brucella melitensis* strains at the wildlife/livestock/human interface using serology and genomic surveys

Molecular epidemiology allows comparison of pathogen strains circulating among wild

animals, domestic livestock and humans. In this study, Serosurvey and molecular epidemiology of *Brucella melitensis* at the wildlife-livestock-human interface in the United-Arab-Emirates (Article 7), we use genotyping techniques to trace the source of *Brucella melitensis* infection in a herd of Scimitar Horned Oryx (SHO).

The scimitar-horned oryx is a large desert antelope that formerly inhabited large areas of the Sahara and Sahel ranging from Mauritania to Egypt. Due to extensive hunting, habitat loss and competition with domestic cattle, the SHO became extinct in the wild in 2000[48]. Global conservation effort relies heavily on captive stocks for possible re-introduction. Such programs involve conducting wildlife disease risk analysis[49] and the prevention of alien disease introduction to the recipient area, probably the single most important responsibility for decision-makers [50]. Brucellosis is recognized as a major cause of heavy economic loss to the livestock industry and poses a serious human health hazard[51]. It remains one of the most common zoonotic diseases worldwide with more than 500,000 human cases reported annually[52]. The disease is enzootic in the Middle East where it has been reported in almost all domestic farmed animal species and especially in goats and camels[53]. The situation in the UAE has been documented in the human population: both *B.melitensis* biovar 1 and 3 were reported on human cases from Tawam Hospital, Al Ain (Abu Dhabi emirate) hence were co-circulating in the country in 1996[54]. From 2000 to 2003, 6.5 % of the 998 patients admitted in this same hospital were *Brucella* seropositive[55]. In the livestock industry, 55.1% of the 267 domestic farms sampled in the emirate of Abu Dhabi in 2010 were herd seropositive[56] and the Central Veterinary Research Laboratory in Dubai revealed culturing the bacteria in 12% of the 132 raw cow milk samples received in 2014[57]. The wildlife situation with regard to brucellosis seems poorly studied, *B. melitensis* has been reported only once in Dubai in a Nubian Ibex (*Capra ibex nubiana*)[58]. The UAE holds a large captive population of local and exotic ungulate species, with some collections counting more than 30,000 heads in one location. In this study, the *brucella* seroprevalence was investigated within a large population of scimitar-horned oryx (SHO) (*Oryx dammah)* located in Abu Dhabi Emirate and try to trace the source of infection using genotyping techniques. Multi-Locus Variable number of tandem repeat Analysis (MLVA) is a genotyping method that has been extensively used to understand genetic relatedness between isolates, study transmission routes, and determine infection sources. The MLVA assay is rapid, highly discriminatory, and reproducible within human *Brucella* isolates. MLVA can significantly contribute to an epidemiological trace-back

analysis of *Brucella* infections and may advance surveillance and control of human brucellosis[59].

3) Serosurveys for selected viral pathogens among sympatric species of the African large predator guild in northern Botswana*:* disease transmission at the wildlife-human activities interface

Studies aiming at gaining basic scientific insight, ecological data and biodiversity surveys are essential in better understanding epidemiological data. Epidemiology is a component of ecology. There is growing interest in understanding disease in multiple host systems[60] and disease dynamics. Both species richness and species composition of the community can influence disease spread, and in fact, their effects on disease dynamics are inherently intertwined[61]. Three journal communities are active under the umbrella « one health »: the ecology community, veterinary medicine community, and a third group consisting of epidemiological and mathematical biology. Nevertheless, KR Manlove *et al.* found that these three communities overlapped in study system, *approaches, objectives, and methodologies*[23]. Our next study, Serosurvey for selected viral pathogens among sympatric species of the African large predator guild in northern Botswana (Article 8), bridges ecology and veterinary medicine: the carnivores sampled during our health investigation in Botswana were monitored via radio-telemetry and satellite-tracking techniques to not only assess their health status or possible clinical symptoms over-time but also to gather ecological and behavioural data and thus putting into perspective any epidemiological results. We emphasized differences in seroprevalence between individuals that were in contact with human activities and individuals that did not.

In large carnivore conservation, the ecology of diseases has mainly focused on the clinical relationship between a pathogen and its host, on disease-mediated extinction events, and on the consequences that human activities and domesticated animals have in the introduction and spreading of diseases into wildlife populations[62–67]. For example, the rapid disappearance of African wild dogs (*Lycaon pictus*) from the Serengeti ecosystem in eastern Africa during the 90's has partially been credited to a wave of rabies and canine distemper introduced by domestic dogs[68–70]. More recently, studies have focused on cross-species transmission, multi-

host pathogens, and infection reservoir dynamics[71,71–75]. Our knowledge remains, however, relatively limited on the ecology of pathogen prevalence and transmission in complex, large trans-boundary ecosystems, where differential ecological and climatic conditions may further confound the epidemiological scenario[71].

In recent years, the creation of large trans-boundary parks and wildlife corridors between ecosystems has become an integrative part of the present and future conservation action plans[76–78]. A comprehensive understanding of the pathological state of single sub-populations that will become connected through such initiatives is fundamental for the management of species nationally and internationally. One such initiative to natural resources and wildlife management is the Kavango Zambezi Trans-Frontier Conservation Area (KAZA/TFCA) in southern Africa, spanning five countries and 444'000 km^2, potentially the largest trans-boundary conservation area in the world (http://www.kavangozambezi.org). Despite its unique wildlife and ecosystems and the central role that Botswana's Okavango Delta plays within the KAZA/TFCA scenario, relatively little is known about pathogen transmission and prevalence among its large carnivore species. While some information is available for lion (*Panthera leo*) and African wild dog[62,67], no information is available for the three other large carnivores resident in this area; spotted hyena (*Crocuta crocuta*), cheetah (*Acinonyx jubatus*) and leopard (*P. pardus*).

Pathogen transmission and prevalence among these carnivore species are intimately linked with their ecology, frequent direct contact and contact with human activities, for example at kill sites, and are exacerbated by interactions with non-vaccinated domestic dogs[66,67,71]. For instance, evidence suggested that an epidemic of canine distemper spread from domestic dogs to lions and spotted hyenas and subsequently to other sympatric carnivores[66,79]. The wide-ranging behaviour of species such as African wild dogs and cheetahs further increases contact with domestic reservoir populations. Pathogens may, however, be maintained within a system by infected individuals with no evidence of mortality[72] as argued for rabies virus and spotted hyenas[64]. In this study we aimed to investigate the prevalence of several viral pathogens among four co-occurring species of the large African predator guild: lion, spotted hyena, leopard and cheetah. Furthermore, we emphasized differences in seroprevalence between individuals that were in contact with human activities and individuals that did not. All samples were collected in the Okavango Delta in northern Botswana, a region currently part of the KAZA/TFCA trans-boundary initiative.

Chapter 3: Complexity of ecological system – A call for multi- and interdisciplinary studies

1) Failure to detect Batrachochytrium dendrobatidis infection in the UAE and Oman

Preliminary surveys fail to detect *Batrachochytrium dendrobatidis* infection in the United Arab Emirates and Oman (Article 9).

The Global Amphibian Assessment (GAA) revealed that approximately one-third (32%) of the 6,187 recognized amphibian species[80] are threatened with extinction and a further 23% are classified as data deficient[81]. This compares to 12% of birds and 23% of mammals threatened with extinction. At least 43% of amphibian species are experiencing population declines and 165 have gone extinct since 1980[81].

Of the 435 species with documented rapid declines from 1980-2004, 54% were considered to be due to overexploitation or habitat loss, with the remaining declines declared as 'enigmatic' as they occurred primarily in areas where suitable and even pristine habitat was still available and no obvious cause was detected[82]. Evidence-based reasoning by Skerratt *et al.* (2007) identified death due to the disease amphibian chytridiomycosis, caused by the fungal pathogen (*Batrachochytrium dendrobatidis, Bd*), as the most parsimonious explanation of such enigmatic declines[83]. This is supported by the results of several other studies[84–86].

Amphibian chytridiomycosis, first described by Berger and Speare in 1998[84], is an emerging infectious disease affecting all classes of amphibians worldwide[87]. *Batrachochytrium dendrobatidis, has* two life stages: an intracellular zoosporangium and a motile, uniflagellated aquatic zoospore. Amphibians can be experimentally infected by zoospores cultured *in vitro*[88] or by contact with skin harvested from infected animals[84].

There is a paucity of data concerning both the presence or absence of *B. dendrobatidis* in the Middle East and the susceptibility of the amphibians in the region. Soorae *et al*. (2012) skin-swabbed 16 Arabian toads (*Duttaphrynus arabicus*) and 2 Dhofar toads (*Duttaphrynus dhufarensis*) across five sites in the United Arab Emirates (UAE) and found no evidence for the presence of *Bd* using a commercial, pathogen-specific standard PCR[89]. Whilst it is not clear how many animals were skin-swabbed at each site, the sample size is small and would, at best, require a *Bd* infection prevalence of at least 25% (and probably higher, depending on

how many animals were sampled at each site and the total size of each sampled population) for the pathogen to be detected. Here, we expand on the work of Soorae et al. (2012)[90] in the UAE by examining a larger number of wild animals in the region for the presence of *Bd* infection. We screened wild Arabian toads (*Amietophrynus arabicus)* from four different locations in the UAE and Oman for the amphibian fungal pathogen, *Batrachochytrium dendrobatidis* (*Bd*), using a targeted genomic pathogen research method: *Bd*-specific real-time polymerase chain reaction. All of the 127 samples collected were negative for this pathogen. Possible reasons underlying these results are that *Bd* has not yet reached the sampled populations or that the regional climatic conditions do not support infection with this fungus.
PCR-based methods are targeted methods and useful to confirm or inform the presence of genetic material of a targeted pathogen. Non-targeted methods such as the Next Generation Sequencing (NGS) are now considered powerful techniques to identify known and unknown agents in virtually any type of specimen. The combination of targeted and non-targeted genomic pathogen survey is being increasingly used in wildlife pathogen surveys and in the field of pathogen discovery. This emerging method of pathogen detection will be discussed in the final part of this thesis.

Trying to research and understand pathogens' transmission and distribution will make no sense without knowing the host's and vector's biology and ecology. The interdisciplinary approach pioneered by Ecohealth/One Health is mandatory but ecologists and veterinarians are often working in distinct systems with different research questions. The ecologist mainly focuses on ecosystems and a veterinarian on animals or animal population health. Epidemiologists, studying distribution and determinants of health-related states or events (including disease) have to consider the differences (aims, methods and study subjects) that exist between various scientific communities. Our next study on a rodent community in the Hajar Montains is a collaborative project between an ecologist, a veterinarian and an entomologist.

2) Example of a collaborative study between an ecologist, an entomologist and a veterinarian leading to the discovery of a new flea species.

Note on the Siphonaptera of the Emirate of Ras Al Khaimah: description of *Nosopsyllus (Gerbillophilus) jabeljaisensis* n. sp. *(Siphonaptera, Ceratophyllidae, Ceratophyllinae)* (Article 10).

Few studies of the siphonaptera of the Arabian Peninsula are published[91–93] whereas these insects are of medical interest, e.g. vectors of Plague at Yersinia pestis, among others. Working on wildlife and fleas affecting wildlife in a harsh environment is a difficult task that can prevent gathering reliable ecological data. Nevertheless, outbreaks of plague in India remind us that Plague may have retreated over the past decades, but it has not gone away and that there is a need to maintain a core of skills in infectious diseases and the public health infrastructure to detect, monitor, and combat a wide range of disease agents[94]. Working on parasites affecting unstudied rodent communities in extreme environments is both challenging and rewarding. An ecologist, a veterinarian, and an entomologist teamed up to study a rodent community and their vectors in the Hajar mountains in areas where human encroachment due to new construction sites will occur in the next few years. While the ecologist focuses on rodent ecological community description, the entomologist and veterinarian concentrate on this unstudied community and their vectors of public heath interest.

Ecological communities are defined as groups of trophically similar species that either occur or may potentially occur in a specific area and are likely to compete for similar resources [95,96]. We studied two species in the same community Acomys dimidiatus and Gerbillus dasyurus. The composition of a community is conventionally measured by using presence / non-detection data or abundance data [96,97]. One of the major aims in community ecology is to determine which mechanisms influence the structure and dynamics of ecological communities [96]. Ecological communities all occur on gradients of the physical and structural attributes of their environment, in addition, these communities exist in a biotic context (interaction milieu) [98]. It is essential that investigators take both of these influences on community composition into account. Organisms in arid environments are subjected to extreme and heterogeneous environmental conditions. In desert environments, in addition to extreme temperatures, organisms are subjected to temporal and spatial unpredictability of resources, competition and the threat of predation [99,100]. Deserts tend to have broad daily and seasonal temperature ranges, minimal primary productivity, and sporadic and unpredictable rainfall [101]. Despite this, deserts tend to support surprisingly rich faunal communities [102,103]. Twenty-two fleas

were collected from Acomys dimidiatus and Gerbillus dasyurus whilst trapping in the montains of Ras Al Khaimah in the United Arab Emirates and identified as belonging to two species. A new taxon is described in the genus Nosopsyllus that is a known vector of Plague.

Chapter 4: Objectives of the thesis

This thesis has explored, in a practical manner, scientific challenges to prevent, detect, control or manage diseases at the wildlife-livestock-human interface at different spatial levels from farms to the global scale through a set of practical studies. Strengths and limitations of disease detection and management tools currently used at a farm, population or individual level, at a regional or national level, and at a global level were highlighted. The objectives of this thesis are to contribute to applied sciences by:

- Understanding the pathology, epidemiology, and ecology of some specific diseases endemic in Africa and Arabia, and,
- Identifying challenges to prevent, detect, manage diseases at the W-L-H interface

The aim was also to reflect upon methods and philosophy currently driving disease detection and management at the W-L-H interface in Europe-Middle East-Africa (EMEA).

PART 2: EXPERIMENTAL SECTION

Chapter 5: Disease transmission and the human factor: from rhetoric to reality

Farming looks mighty easy when your plow is a pencil, and you're a thousand miles from the corn field.
Dwight D. Eisenhower

Do human activities and the non-observance of laws and recommendations enhance disease emergence and transmission? The following studies illustrate how human conduct, at local and global scale, could drive disease transmission in animal and human populations.

Article 1: Biosecurity measures applied in the United Arab Emirates: a comparative study between livestock and wildlife sectors.

Original article - *Transboundary and Emerging Diseases* – 2016

Preamble: The concept of biosecurity has been long recognized by veterinarians but has recently gained greater importance within veterinary medicine and public authorities due to disease outbreaks that have threatened to devastate agricultural economies. Although the Abu Dhabi Food Control Authority (ADFCA) disseminates guidelines and recommendations regarding biosecurity measures to be applied in Abu Dhabi, there is no data on how farms implement those measures. In addition, there are no federal guidelines and ADFCA guidelines are not necessarily communicated to the farms from the six other emirates. Our study, carried out in the seven emirates, aimed to determine if biosecurity measures are implemented, and if so, to understand which types of farms/animal collections are pro-actively working towards prevention of disease transmission within and between farms.

Transboundary and Emerging Diseases

ORIGINAL ARTICLE

Biosecurity Measures Applied in the United Arab Emirates – a Comparative Study Between Livestock and Wildlife Sectors

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Summary

In 2013, the livestock population in the UAE exceeded 4.3 million heads with sheep and goats accounting for 90% of this. The overall number of captive wild ungulates (gazelle types) is difficult to assess as there is no registration system in place or enforced in the UAE with regard to the possession of wildlife. Those animal collections, mainly owned by high-ranking families, are therefore not registered and kept far from public viewing. Nonetheless, some collections are housing more than 30 000 ungulates in one location. The primary objective of this study was to describe the biosecurity measures currently applied in UAE ungulate facilities for different wildlife and livestock sectors. A secondary objective was to use the output from this biosecurity survey to investigate which sector could be categorized into risk groups for disease introduction and spread. Between October 2014 and May 2015, biosecurity questionnaire data were collected in the Emirates of Abu Dhabi, Dubai, Ras Al Khaimah, Fujeirah, Ajman, Umm al Quwain and Sharjah from 14 wildlife collections, 30 livestock farms and 15 mixed (wildlife and livestock farms). These investigations through questionnaires allowed us to quantify and assess statistically biosecurity practices and levels for both livestock and wildlife sectors. In both sectors, biosecurity measures could be improved and only a few facilities had high biosecurity scores. The group of small unregistered farms (Ezba) represented the highest risk of disease transmission to other animals due to their lack of biosecurity awareness.

Introduction

The concept of biosecurity has recently taken great importance in veterinary medicine. Biosecurity can be defined as the implementation of measures that reduce the risk of introduction and spread of disease agents. It requires a set of management and physical measures designed to reduce the risk of introduction, establishment and spread of animal diseases, infections or infestations to, from and within an animal population (OIE, 2010). Biosecurity greatly relies on the respect of the 'Five Bs': bioexclusion (to limit the risk of introduction), biocompartmentation (to limit the spread of the pathogen within the same facility, e.g. by isolating excreting animals), biocontainment (to limit the spread of the disease agent outside the facility), biocontamination (to prevent the risk of human biocontamination) and bioprevention (to prevent any environmental biocontamination and persistence of the pathogen) (Saegerman et al., 2012).

This Five B concept can also be simplified into all measures related to the prevention of introduction of disease into a herd (i.e. external biosecurity) and all measures to prevent the spread of a disease within a herd (i.e. internal biosecurity) (OIE, World Organisation for Animal Health, 2010).

It is becoming increasingly evident that there is a need to reorientate farmers and veterinarians towards preventive rather than curative medicine (Saegerman et al., 2009). The implementation of biosecurity measures is an important way to contribute towards such reorientation.

The implementation of biosecurity measures in the management of facilities holding wild ungulates and/or domestic livestock in the UAE has not been studied so far. This study aims to determine whether biosecurity measures are implemented in the UAE and if so, which types of farms/animal collections are proactively working towards prevention of disease transmission within and between farms.

Material and Methods

Farms' selection

Data were collected from all seven emirates where farms holding domestic or wild ungulates were willing to complete the questionnaire. To encourage transparency and willingness to share information, respondents were guaranteed anonymity. As most of the wildlife collections are not officially referenced, the questionnaire was given opportunistically during the 2014 Sharjah Conference on Biodiversity Conservation in the Arabian Peninsula and completed on the spot by animals' collection representatives (manager, veterinarian or owner). Questionnaires were completed during visits to randomly selected farms after being translated into Arabic and explained if necessary. Visits to farms, industrial or familial, permitted the inclusion of small unregistered farms (locally called Ezba) in this study. Any facilities holding ungulates could participate to the study. All answers were treated confidentially.

Questionnaire

The questionnaire was designed to collect basic information on farms typology, biosecurity awareness, disease prevention measures implemented and proximity to other farms. The questionnaire consisted of 12 questions and was intentionally short to increase the response rate. As much as possible, closed questions with multiple choices were used to ensure consistency in the information gathered. The questionnaire (in Arabic or English) can be obtained upon request.

Data processing

Data were encoded numerically to assist analysis, entered into a database worksheet program (Microsoft Excel, 2010) and recorded into categorical data (nominal or ordinal level) for further analysis.

Data analysis

Biosecurity scoring

A binary biosecurity scoring system was created following Van Steenwinkel method (Van Steenwinkel et al., 2011) (value of 1 for biosecurity measure present and value of 0 for biosecurity measures absent). The variables were indicators aiming at covering internal and external biosecurity measures.

Internal biosecurity was assessed via the following questions: Is the farm representative aware of the notion of biosecurity and can he define it? Is the food stored in a closed container or building? Is a pest control plan in place? Are sick animals isolated?

The external actions recorded included: Is the wheel bath filled? Are new animals coming to the collection? Are incoming animals quarantined? Is there a disease-screening programme in place?

The presence or absence of buffer zone, fencing system as well as the distance to other farms was also noted.

Analysis

Groups were defined as livestock (farms holding domestic ungulates such as sheep, goats cows and camels), wildlife collection (with at least one wild ungulate species) and both where wild and domestic species were in the same location. Encoded data were summed up per group and each variable tested for significance with Fisher's exact test. A *P*-value of 0.05 was considered as significant. The variables that had a *P*-value less than 0.05 were aggregated by scoring (i.e. score is the sum of the seven biosecurity measures that were present) and each score (count variable) was analysed, in first instance, by a Poisson regression model and finally using a negative binomial regression due to extra-binomial variability (Dohoo et al., 2010). In addition, the seven significant variables in univariate analysis were also entered into a classification and regression tree (CART) analysis model to identify significant exploratory variables (Saegerman et al., 2011). A CART analysis is a nonlinear and nonparametric model that is fitted by binary recursive partitioning of multidimensional covariate space (Breiman et al., 1984). Using Salford Predictive Modeler software (Salford Systems, San Diego, CA, USA), the analysis successively splits the data set into increasingly homogeneous subsets until it is stratified to meet 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. CART performs cross-validation by growing maximal trees on subsets of data 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. livestock farms, wildlife collections and both livestock farms and wildlife collection). CART 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 combina-

tions 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 subset. The results of the 10 minitests 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 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. Further details about CART are presented in previously published articles (e.g. Porter et al., 2011; Saegerman et al., 2011, 2014, 2015; Zanella et al., 2013).

Results

Seven variables linked to biosecurity measures were found to be statistically different in each sector (i.e. awareness of biosecurity, pest control plan, isolation of sick animals, disease-screening programme, fencing system, buffer zone and distance to other farm for at least 500 m). In addition, the calculation of odds ratio permitted us to identify farms holding only livestock with a value less than 1 for these seven biosecurity measures present in comparison with collections holding wild ungulates. Results are summarized in Table 1.

Biosecurity scores were significantly lower in farms holding only livestock (mean = 0.80; SD = 1.73) in comparison with collections holding wild ungulates (mean = 4.29; SD = 1.20) (negative binomial regression; *P*-value <0.001). However, no significant difference was observed in scores between farms with both livestock and wild ungulates (mean = 4.07; SD = 1.79) in comparison with collections holding wild ungulates. In addition, unlike the livestock farms, wildlife collections biosecurity scores describe a normal distribution defining a Gaussian curve (Shapiro test, *P*-value = 0.39).

CART analysis revealed that 'presence of a disease-screening programme', 'presence of a perimeter fence' and 'distance to other farms' were the three main predictors of divergence between the three groups (Table 2; Fig. 1).

Discussion

Few farms obtained an overall high biosecurity level. Distribution of scores in the livestock sector is skewed towards 0 with few professional farms reaching the highest scores. The farms implementing biosecurity measures were registered as commercial farms breeding sheep and goats intensively, with herds of more than 3000 heads. Most of the

Table 1. Description of farms holding animals in wildlife collections, livestock farms and farms with both wildlife and livestock animals regarding to biosecurity measures

	Animals held						Fischer's exact test	Domestic versus wild		Both versus wild	
	Wild		Domestic		Both						
Variables	Score 0	Score 1	Score 0	Score 1	Score 0	Score 1	(*P*-value)	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Internal biosecurity											
1) Aware of the notion of biosecurity	4	10	24	6	5	10	0.001	0.10 (0.02–0.43)	0.002	0.80 (0.16–3.88)	0.78
2) Able to define biosecurity	10	4	26	4	11	4	0.41	0.38 (0.08–1.84)	0.23	0.91 (0.18–4.64)	0.91
3) Food stored in a closed container or building	6	8	21	9	8	7	0.20	0.32 (0.09–1.20)	0.09	0.75 (0.17–3.33)	0.71
4) Pest control plan in place	5	9	27	3	6	9	< 0.001	0.62 (0.01–0.31)	0.001	0.83 (0.19–3.75)	0.81
5) Sick animals are isolated	7	7	25	5	5	10	0.002	0.20 (0.05–0.83)	0.026	2.00 (0.45–8.96)	0.37
External biosecurity											
6) Rotoluve is filled	11	3	27	3	13	2	0.55	0.41 (0.07–2.34	0.31	0.56 (0.08–4.01)	0.57
7) New animals are coming	13	1	30	0	14	1	0.24	0.15 (0.006–3.86)	0.25	0.93 (0.09–10.07)	0.95
8) Incoming animals are quarantined	10	4	23	7	12	3	0.85	0.09 (0.009–0.87)	0.037	0.91 (0.18–4.64)	0.91
9) Disease-screening programme is in place	1	13	26	4	3	12	<0.001	0.02 (0.003–0.13)	<0.001	1.00 (0.16–6.08)	1.00
10) Presence of a buffer zone	10	4	29	1	11	4	0.02	0.15 (0.03–0.73)	0.019	0.67 (0.15–3.01)	0.60
11) Presence of a fencing system	3	11	28	2	4	11	<0.001	0.01 (0.001–0.12)	<0.001	0.31 (0.03–3.38)	0.34
12) Distance to other farm (at least 500 m)	8	6	27	3	10	5	0.03	0.76 (0.18–3.20)	0.71	0.63 (0.11–3.48)	0.59

Table 2. Relative importance of the different biosecurity measures (splitters) obtained after classification and regression tree (CART) analysis (maximum relative importance = 100)

Biosecurity measure	Relative importance
Presence of a fencing system	100
Disease-screening programme is in place	92.68
Pest control plan in place	47.55
Aware of the notion of biosecurity	39.46
Sick animals are isolated	32.44
Distance to other farm (at least 500 m)	4.76
Presence of a buffer zone	0.71

sheep and goats are bred in Abu Dhabi, respectively, 85% and 65% of the country flock (United Arab Emirates National Bureau of Statistics, 2014), where herd *Brucella* seroprevalence is of 55.1% (Mohammed et al., 2013). Some diseases are known to be enzootic and/or epizootic in the country such as contagious caprine pleuropneumonia (Chaber et al., 2014), foot-and-mouth disease (Lignereux and Al Kharusi, 2013), Q fever (Chaber et al., 2012), lumpy skin disease (Tuppurainen and Oura, 2012), bovine tuberculosis (Wernery et al., 2007). Other diseases seem to be directly related to international animal trade (livestock or wildlife), such as Crimean–Congo haemorrhagic fever

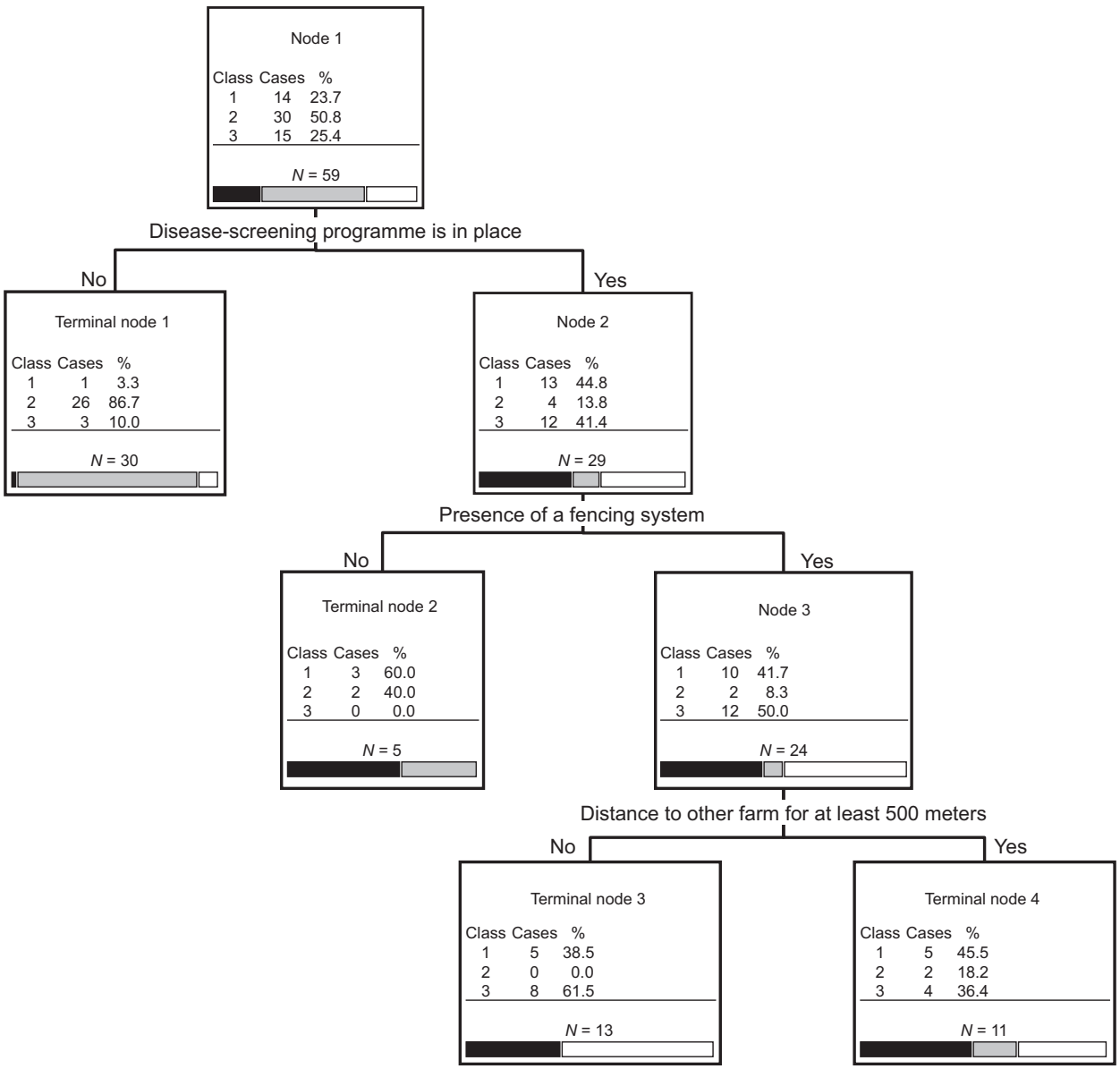

Fig. 1. Classification and regression tree analysis for main biosecurity measures applied in three types of farms (wildlife collections [class 1; in black], livestock farms [class 2; in grey] and farms with both wildlife and livestock animals [class 3; in white]).

(Rodriguez et al., 1997) and Rift Valley fever (Boshra et al. 2015). In this environment, it is essential and understandable that professional farms holding large herds apply the strictest biosecurity measures as a breach in those measures may have catastrophic economic consequences. In the UAE, the livestock population grew from 0.4 million in 1970 (Thomson et al., 2000) to 4.3 million registered heads today (United Arab Emirates National Bureau of Statistics, 2014). The expansion of this industry has major ecological and epidemiological consequences that have a national impact on disease security. On one hand, livestock industry has a very limited effect on the gross national product with agriculture representing 0.8% of the GDP and disease security is costly, from agricultural advisory services to veterinary research and investigation laboratories to veterinary field services. On the other hand, relying on importation for major food products is a national risk in case of conflicts in the region. Control of the major livestock diseases is a necessary step in improving livestock productivity on a national scale.

The majority of the investigated farms, called locally 'Ezba', were not officially registered and managed by families with the help of poorly qualified workers. 'Ezba' can be found in small numbers in isolated secluded desert areas or in hundreds aggregated in designated areas much closer to other type of animal holding facilities. One 'Ezba' is generally overseeing 50–200 small ruminants and is implementing few to no biosecurity measures. Animals are transported to local markets for sale during festivities and religious events. This group represents a very high risk of spreading diseases to any other farms in their vicinity.

Most of the wildlife collections are owned by high-ranking families and managed by qualified professionals who are aware of the importance of biosecurity. Few wildlife collections indicated the number and species of wild ungulates held in their facility as those questions could be politically sensitive.

Seven biosecurity variables were significantly different (Fisher exact test, P-value <0.05) between the wildlife and livestock sectors.

Internal biosecurity measures were generally better enforced in the wildlife sector as representatives of wildlife collections were more conscious and better able to define biosecurity than livestock farmers. This is linked to staff education levels and ultimately to salaries offered in this sector. Sick animals were isolated only in 16% of the livestock farms and in 58% of the wildlife collections. Isolation of sick animals requires competent staff to identify sickness and ability to seclude potentially contagious animals. Farm design and space allocations can be an obstacle in small-scale farms. In addition, pest control plans were rarely implemented in 'Ezba', not only hampering livestock health safety in their farms but also any adjacent farms as cat, dog, raven and rodent populations are likely to travel from farm to farm and disseminate contaminated material (Sumner and Buck, 2007).

External biosecurity measures were also more thoroughly followed in wildlife collections than on livestock farms. Indeed, disease screening of incoming animals was performed by 86% of the collections holding wildlife versus 13% of the livestock sector, as the livestock sectors surveyed were largely unregistered and therefore not falling into groups screened by governmental agencies. It is interesting to note that animal health authorities focus their pathogen screening on *Brucella* and foot-and-mouth disease only. Livestock traded within the country or with adjacent countries harbouring similar epidemiological situations might not expose the UAE herd to new diseases; nevertheless, the trade between the Horn of Africa and the Middle East has been estimated to be around US$0.6 billion per year, and is, therefore, ten times greater than intraregional trade (Admassu, 2009). Wildlife is being brought from all over the world and might also be the source of new and emerging diseases. In addition, the list of diseases screened and methods employed in wildlife collections remained at the discretion of the veterinarian or collection managers.

Pathogen introduction is also prevented via a proper fencing system, the presence of wide buffer zones and farm-to-farm distances. The perimeter fence was only a single fence for 93% of the surveyed farms, but a double fence or a wall for up to 76% of the wildlife collections. This is largely explained by the nature of the wildlife collections, which are mainly private collections surrounding palaces and therefore benefit from the security measures set to protect goods and people. In addition, 27% of the wildlife collections had a buffer zone that was not the case for the livestock sector. Similarly and linked to the social rank of the owner, the farm-to-farm distance was less than 500 m for 98% of the livestock farms surveyed against 37.9% of the collections holding wild ungulates.

Nevertheless, both wildlife and livestock sectors had poor scores regarding some internal and external biosecurity indicators. New animals were coming to all the facilities, and quarantine was rarely properly enforced. Even if quarantine enclosures are present in the facilities, they are not used to avoid contact with the already established animal collections.

Wheel dips, when present, were also filled and used in only 17% and 10% of the livestock and wildlife farms, respectively. Although wheel dip is a requirement of all farms that have to be registered by the Abu Dhabi Food Control Authority, wheel dips are actually not at all effective in preventing entry of pathogens. Firstly, wheels that rotate at high speed at a high temperature over generally dry surfaces (particularly so in the UAE) do not provide a good habitat for pathogens. Secondly, if the wheels have

solid material like dung in their grooves, the wheel bath will not remove it and the disinfectant would not reach any pathogens lodged in it. Thirdly, pathogens in vehicles are most likely to be present in the loading space and in the cab of the vehicle, where contaminated animals or materials and contaminated footwear provide a rich potential source of pathogens. Washing and disinfection of vehicles are nevertheless important in some intensive livestock farming operations where vehicles from outside are excluded altogether from entry by having delivery and loading points on the periphery of the farm.

The animal feed was stored in a closed container or building in 30% of the livestock farms and in 51.7% of the wildlife collections. Food in the open attracts and will increase the presence of potentially harmful pests, such as insects, rodents and wild birds that can act as pathogen vectors.

Although free roaming wildlife is often seen as the source of contagious diseases, human activities and disrespect of biosecurity measures are enhancing disease transmission and thus should be the central pillar of actions regarding disease control and prevention. The 'Five Bs' of biosecurity measures have to be addressed specifically as the disrespect of only one might render null and void all efforts to control disease and will thus jeopardize the entire farm or national security. In addition, implementation of biosecurity programmes helps in preventing the introduction of all pathogens and is not limited to specific pathogens.

Zoonotic disease might also be transmitted from humans to animals, and the government has identified tuberculosis as an important disease with 179 reported positive TB cases (55 goats, 51 sheep, 71 camels) in 2010 by Abu Dhabi Food Control Authority. Serious losses of valuable wildlife as well as economic losses on livestock farms due to abattoir condemnations have been recorded as a result of infection of animals with human tuberculosis by workers or visitors. Although locally circulating TB strains remain unknown, national measures are in place to prevent transmission of human tuberculosis caused by *Mycobacterium tuberculosis* to wildlife or livestock. UAE ministerial decree No 28 of 2010 and Federal Law No 7 of 2008 state that all newcomers found to have active or old pulmonary TB in a chest X-ray will be denied a fitness certificate and thus UAE resident visa. After initial entry, all workers legally employed are tested every 3 years upon renewal of their visa.

It is commonly assumed by veterinarians in the country that atmospheric conditions in such an arid climate with temperature reaching up to 50°C under the shade during summer time reduces disease burden, especially infection through environmental contamination. Although it is true that UV and heat are not favourable to the survival of most bacteria and viruses, recontamination of the environment is problematic at animal reservoirs; or when asymptomatic shedders have not been cleared. In addition, some bacteria have the ability to sporulate such as *Coxiella burnetti* and can thus persist in the environment for up to 5 months (Welsh et al., 1958).

Conclusion

Before any drastic measures are taken in the country to control animal infectious diseases, such as culling of animals that tested positive for specific diseases, it seems important to define and implement biosecurity measures from the local farms to the national level. Education on the importance of understanding and respecting biosecurity measures should be encouraged for all stakeholders involved in animal health.

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Article 2: The scale of illegal meat importation from Africa to Europe via Paris.

Original article in *Conservation Letters journal* – 2010 - *3*(5), pp.317-321.

Preamble: In remote rural areas in Central and West Africa bushmeat is a very important and essential part of the diet. In Africa, food availability is a primary concern. Food security is defined as "physical and economic access to food for all people at all times"[104]. In African villages, bushmeat contributes substantially to the total animal protein intake.
Studies show that bushmeat is an important source of protein among poor households and is not only a luxury product eaten by the rich. In Europe, the African population chooses to eat bushmeat due to cultural origin and taste preferences.
In addition to its nutritional importance, the bushmeat trade is significant in providing economic benefits to different groups of people in the commodity chain. Many rural people have turned to commercial hunting and trading of bushmeat due to limited off-farm jobs available[105]. The estimated annual value of the bushmeat trade in West and Central Africa could exceed 1 billion US dollars. In the northern region of Korup National Park, on the border with Nigeria, 80% of meat obtained is destined for commercial markets[106]. This illustrates the economic importance of the trade on a regional level, therefore one would expect that on a national level there would be a significant contribution to the economy.

Bushmeat marketing is well developed in West Africa and has been well documented[107–110]. In Kumasi, Ghana, bushmeat trade is organised involving a chain of bushmeat traders (mainly women), wholesalers and retailers[111]. Bowen-Jones reported in 1998 that all major urban centres in Central and West Africa are foci for the growing trade in wild animal meat[112]. The demand is constant, and when transport between rural areas to larger towns and cities, of any form, becomes available, bushmeat traders inevitably use them[112]. Logging trucks facilitate the transport of meat to market centres: in southeast Cameroon, 85% of the meat taken by poachers is conveyed out on logging trucks to fuel the commercial trade[112]. Meat can also be transported from the forest to the African markets by plane, boat, and train[113,114].

International infringements are not restricted to the African continent but may extend to Europe. This extension can be linked to the increasing number of flights between Africa and France. As the global economy is becoming more and more connected, the aviation industry is one of the fastest transportation sectors. Commercial airlines carried more than 3.5 billion passengers and generated a global revenue of 518 billion U.S. dollars in 2015. According to the latest statistics from OAG (Official Airline Guide), the world's authority on flight information, the number of flights scheduled for May 2017 has increased more than 5% compared with the same month last year.

Between 1999 and 2004, 180,000 immigrants from sub-Saharan Africa arrived in France, which represent an increase of 45% in the immigration rate since 1999. The total of Sub-Saharan African immigrants in France is 570,000 people (Institut National de la Statistique et des Etudes Economiques)[115]. Seven out of ten African immigrants come from former French colonies[116].

International laws on wild meat trade are based on the sanitary risk posed by the introduction of wild meat and the species status of the animal with regards to the Convention on International Trade in Endangered Species (CITES). Most of the countries from West and Central Africa adhered voluntarily to this international agreement. Angola (Central Africa) is the only country from these regions that did not ratify the convention. Under this Convention, the export of endangered species is either banned or controlled through the allocation of quotas. Exports under quotas are controlled by the national Scientific Authority which issues a certificate of origin and certifies that a shipment is within the quota limit allowed by CITES. CITES quotas are routinely exceeded. CITES prosecutions are rare as illegal meat is destroyed immediately after the confiscation due to the health risk. No testing is done to identify the species. Thus, the proportion of endangered species in bushmeat imports is unknown.

We decided to investigate illegal trade and especially bushmeat trade from Africa to Europe to assess the volume of the trade and expose some challenges of disease control at the wildlife-human interface and try to formulate adequate answers to be used by decision-makers. Here, the results are reported of a systematic survey of customs seizures of bushmeat, livestock meat and fish carried by passengers arriving from sub-Saharan Africa at Paris Roissy-Charles de Gaulle airport, from which the total volumes being carried was estimated. We then focus particularly on the bushmeat component of imports, describing the species

involved, and investigating how the meat is traded after it leaves the airport. Finally, we highlight further research needs stemming from our findings and discuss their implications for policy options to alleviate the problem.

LETTER

The scale of illegal meat importation from Africa to Europe via Paris

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Abstract

Concerns have been raised about the illegal import of bushmeat from Africa into Europe, particularly regarding the health risks posed to people and livestock. The role of international trade in driving unsustainable hunting in source countries is unknown, but generally assumed to be limited. Here, we present the first systematic study of the scale and nature of this international trade. We estimate that around five tonnes of bushmeat per week is smuggled in personal baggage through Paris Roissy-Charles de Gaulle airport. Bushmeat is not only imported for personal consumption but is part of a lucrative organized trade, with high prices indicating luxury status. A wide range of species is carried, many of which are CITES-listed. Based on these findings, we suggest ways in which customs, airlines, and airport authorities could reduce imports, focussing on raising awareness of regulations, and improving surveillance and deterrence, particularly where CITES-listed species are concerned.

Introduction

Bushmeat (the meat of wild animals) is an important component of trade, diet, and culture in many parts of the world (Milner-Gulland *et al.* 2003). However, unsustainable hunting for bushmeat is a leading threat to many of the species concerned, and there is a need to reduce the pressure that hunting imposes on tropical wildlife populations (Bennett *et al.* 2002). While there is anecdotal evidence of international trade in bushmeat, including seizures of African bushmeat at airports, and the occasional prosecution of traders in European cities, it is a neglected aspect of the issue. International trade is of concern for two primary reasons. First, it might be contributing to unsustainable demand, exacerbating the overexploitation of source populations. Second, the international movement of animal products, including bushmeat, is likely to pose a threat to human and animal health through the introduction of pathogens. Bushmeat is of particular concern here, since it is illegal and therefore falls entirely outside the normal regulatory procedures.

The international carriage of uncertified meat and fish products is illegal for sanitary reasons under national, European Union, and International Air Transport Association regulations. For example, the European Union prohibits any personal consignment of meat, or meat products, from entering the Union unless specifically authorized and certified as being eligible for import (EC Regulation 745/2004 of 16 April 2004). In addition, international trade in many wild species and their products is prohibited or regulated for conservation reasons under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Many species used as bushmeat, including all primates, are subject to CITES regulations (www.cites.org). Despite this

regulatory framework, enforcement of the rules is patchy due to the difficulty of detection, and it is likely that substantial volumes of bushmeat and other animal products continue to enter Europe undetected. However, there have been few attempts to establish how this international trade operates, and there is currently no published research estimating the amounts of illegally transported meat and fish.

Here, we report the results of a systematic survey of customs seizures of bushmeat, livestock meat, and fish carried by passengers arriving from sub-Saharan Africa at Paris Roissy-Charles de Gaulle airport, from which we estimate the total volumes being carried. We then focus particularly on the bushmeat component of imports, describing the species involved, and investigating how the meat is traded after it leaves the airport. Finally, we highlight further research needs stemming from our findings, and discuss their implications for policy options to alleviate the problem.

Methods

Airport data collection and estimation of total imports

Permission and support were requested from the French customs and veterinary authorities to participate in sanitary inspections at terminal 2E, Roissy-Charles de Gaulle airport, Paris, France, to identify species and quantify volumes of import. Between the 3 and 20 June 2008, 29 Air France flights arriving at Roissy-Charles de Gaulle airport from Central and West Africa were checked between 0500 and 1100 hours, when most African flights land. Passengers carrying iceboxes were targeted for inspection, while those carrying only standard luggage were randomly selected, and their baggage thoroughly searched. Information recorded at the airport was, for the ith port of origin, flights scheduled per week (f_i), flight capacity (c_i), number of passengers checked (n_i), and the weight of fish, livestock, and bushmeat carried by the jth passenger (k_{ij}). No information was available on the numbers of passengers travelling per flight; however, these flights usually travel at or near capacity, and we therefore assume that flights have an average proportional fill (p) of 0.9. The total estimated weight of fish or meat imported per week for a given country is given by

$$K_i = pf_i c_i \frac{\sum k_{ij}}{n_i}$$

and the total weight imported across all of the routes searched is the sum of the country-specific weights. Confidence intervals for total weights were estimated from 10,000 stratified bootstrap samples, resampling k_{ij} values with replacement, and keeping sample sizes from each country equal to the actual numbers checked.

Species determination

Bushmeat arrived dressed and often smoked. Some species in this state could be identified by superficial examination, including pangolins, porcupines, and cane rats. However, other species required skeletal examination. Specimens were prepared by the manual removal of soft tissues followed by boiling the carcasses for about 1.5 hours. Bones were degreased using 5% sodium perborate and 5% Argosol® (Argos society, Algiers) for 10 minutes, and bleached by immersion in 30% hydrogen peroxide for 14 hours. The skeletons were then reassembled and species were determined by comparison with collections from the Muséum d'Histoire Naturelle in Toulouse. When confirmation was necessary, measurements and pictures were sent to H. Obermaier at the Institut für Palaeoanatomie und Geschichte der Tiermedizin (Munich University).

Paris market prices and structure

Informal discussions were held with three bushmeat traders in the market near Château Rouge station (Rue des Poissonniers, XVIII^{e} arrondissement, Paris), covering the price of bushmeat and how it could be bought in Paris. Traders were approached informally as a potential customer, and appeared open about their services. Customers were observed being charged prices in line with those quoted by traders, suggesting that these were accurate.

Results

Nature of seizures

Fish and smaller quantities of livestock meat were carried in iceboxes, but bushmeat and larger livestock, such as entire sheep and calves, were wrapped in plastic and placed in casual holdalls. Travellers reported slaughtering the livestock just before boarding and, consistent with this, most livestock meat was fresh. About half of the travellers carrying foodstuff presented sanitary certificates apparently issued by the veterinary authorities from their country of origin. These papers listed the foodstuff carried, such as *viande de chasse* (bushmeat) or *divers* (miscellaneous), and certified that they were fit for human consumption, but were not in fact legally valid (see Discussion).

Frequency and quantity of seizures

A total of 134 passengers arriving on 29 flights from 14 west and central African countries were searched at

Table 1 Summary of flight frequencies, passenger numbers, and numbers of passengers checked for carriage of meat or fish arriving from African departure points at Paris Roissy-Charles de Gaulle airport

Country (ports) of origin	Flights per week	Flight capacity	Maximum passengers per week	Flights checked	Passengers checked
Senegal (Dakar)	15	270	4,050	4	13
Mali (Bamako)	7	215	1,505	2	9
Burkina Faso (Ouagadougou)	4	215	860	1	5
Niger (Niamey)	4	252	1,008	1	4
Chad (N'Djamena)	6	140	840	1	3
Guinea (Conakry)	4	215	860	3	10
Ivory Coast (Abidjan)	2	270	540	2	10
Benin (Cotonou)	5	215	1,075	1	8
Cameroon (Douala, Yaounde)	12	252	3,024	4	20
Gabon (Libreville)	4	310	1,240	1	3
C. African Republic (Bangui)	1	215	215	3	30
Republic of Congo (Brazzaville)	3	215	645	3	13
D. R. Congo (Kinshasa)	4	215	860	2	4
Angola (Luanda)	1	310	310	1	2
Total	72		17,032	29	134

Roissy-Charles de Gaulle airport (Table 1), and almost half of these were found to be carrying meat or fish (Table 2). Fish was found in the greatest overall quantity (446 kg). Amounts of livestock and bushmeat were lower and comparable to one another (131 and 188 kg, respectively), but bushmeat tended to be carried by fewer passengers in larger consignments: 7% of those searched were found to be carrying bushmeat, compared with 25% and 37% for livestock and fish, respectively, but average individual consignments were over 20 kg for bushmeat,

Table 2 Summary of the rates of carriage of fish, livestock meat and bushmeat, and estimated total rates of import, arriving from African departure points at Paris Roissy-Charles de Gaulle airport

Country of origin	Number (proportion) carrying			Total kg seized			Mean kg per carrier			Estimated tonnes imported per week (95% confidence interval)		
	Fish	Livestock	Bushmeat	Fish	Livestock	Bushmeat	Fish	Livestock	Bushmeat	Fish	Livestock	Bushmeat
Senegal	3 (0.23)	3 (0.23)	0	42	1	0	14	0.3	–	11.78	0.28	0
Mali	3 (0.33)	3 (0.33)	0	23	14	0	7.7	4.7	–	3.46	2.11	0
Burkina Faso	0	1 (0.2)	0	0	3	0	–	3	–	0	0.46	0
Niger	0	2 (0.5)	0	0	30	0	–	15	–	0	6.8	0
Chad	0	0	0	0	0	0	–	–	–	0	0	0
Guinea	4 (0.4)	5 (0.5)	0	27	10	0	6.8	2	–	2.1	0.77	0
Ivory Coast	4 (0.4)	0	1 (0.1)	18	0	1	4.5	–	1	0.87	0	0.05
Benin	4 (0.5)	4 (0.5)	0	35	10	0	8.8	2.5	–	4.23	1.21	0
Cameroon	8 (0.4)	0	2 (0.1)	74	0	27	9.3	–	13.5	10.07	0	3.67
Gabon	1 (0.33)	0	0	11	0	0	11	–	–	4.09	0	0
C. African Republic	14 (0.47)	14 (0.47)	5 (0.17)	116	53	147	8.3	3.8	29.4	0.75	0.34	0.95
Republic of Congo	7 (0.54)	0	1 (0.08)	72	0	13	10.3	–	13	3.22	0	0.58
D. R. Congo	2 (0.5)	0	0	28	0	0	14	–	–	5.42	0	0
Angola	0	1 (0.5)	0	0	10	0	–	10	–	0	1.4	0
Total	50 (0.37)	33 (0.37)	9 (0.07)	446	131	188	8.9	4	20. 9	45.98 (30.11–63.15)	11.98 (5.69–22.85)	5.25 (0.97–11.76)

Table 3 Summary of bushmeat species (numbers of carcasses) arriving from African departure points at Paris Roissy-Charles de Gaulle airport

Species	C. African Republic	Republic of Congo	Cameroon	Ivory Coast	Species total (proportion)
Crested porcupine					
Hystrix cristata	34	6	0	0	40 (0.42)
Blue Duiker					
Philantomba monticola	19	2	1	0	22 (0.23)
Brush-tailed porcupine					
Atherurus africanus	6	3	0	0	9 (0.09)
Cane Rat					
Thryonomys swinderianus	8	0	0	1	9 (0.09)
Guenon species					
Cercopithecus sp.	2	0	3	0	5 (0.05)
Long-tailed pangolin					
Uromanis tetradactyla	5	0	0	0	5 (0.05)
Mangabey species					
Cercocebus sp.	1	0	1	0	2 (0.02)
Red river hog					
Potamochoerus porcus	0	0	1	0	1 (0.01)
Nile Crocodile					
Crocodylus niloticus	1	0	0	0	1 (0.01)
Slender-snouted crocodile					
Crocodylus cataphractus	0	0	1	0	1 (0.01)
Giant pangolin					
Smutsia gigantea	0	0	1	0	1 (0.01)
Total	76	11	8	1	96

compared with 4 and 9 kg for livestock and fish. The largest individual consignment of bushmeat was 51 kg carried by a passenger with no other baggage. In total, we estimate that 63.2 tonnes of meat and fish were imported per week on the Air France routes checked, of which 8% (5.25 tonnes) was bushmeat (Table 2). Assuming that these rates are representative of the average weekly rate over the year, this equates to 3,287 tonnes of meat and fish imported per year on these flights, of which 273 tonnes is bushmeat.

Sample sizes for individual countries were low, making any comparisons tentative. However, it appears that Central African Republic, Cameroon, and Republic of Congo are the main sources of bushmeat, with a small amount from Ivory Coast, and none from any other country. However, the Democratic Republic of Congo and Gabon in particular have small sample sizes, and both are countries that might be expected to be large bushmeat sources on the basis of known trade in country (Fa *et al.* 2003; Wilkie *et al.* 2005). Fish and livestock came from a wider range of countries, although little fish and no bushmeat came from the Muslim majority Sahelian nations (Mali, Niger, Chad, and Burkina Faso). Only Chad yielded no records of any meat or fish import, although, again, this needs to be treated with caution given the small sample size (only three passengers checked).

Bushmeat species

Eleven bushmeat species were found, including two primate, two ungulate, three rodent, two crocodile, and two pangolin, with the rodents and blue duiker making up 75% of the total number of carcasses found (Table 3). In addition, one piece of elephant trunk (*Loxondonta* sp.) was found during a lower intensity survey conducted simultaneously in Toulouse airport. The elephant and crocodiles are all listed in CITES Appendix 1 (trade banned), while the pangolins and blue duiker are in CITES Appendix 2 (trade restricted). The primates could not be identified to species, but could be placed in one of two genera (guenons *Cercopithecus* sp. or mangabeys *Cercocebus* sp.), all species of which are listed in one of the top two CITES Appendices. Overall, 39% of the bushmeat carcasses were CITES-listed species. None of the species identified are currently listed as threatened by the IUCN (www.iucnredlist.org), although giant pangolin and elephant are considered Near Threatened, and slender-snouted crocodile is considered Data Deficient. The IUCN also lists a number of mangabey and guenon species as threatened to varying degrees (see Discussion), although, due to the generic identification of primates, we do not know whether any of these species were present in seizures.

Paris market operation and prices

Three bushmeat traders were spoken to, all middle-aged women. They trade from the streets and by telephone, taking advance orders and arranging deliveries. Prices quoted were between €20 and €30 per kg across a wide range of meats, including primate, crocodile, cane rat, porcupine and smoked fish. This compares to an average price of around €15 per kg for domestic meat sold in French supermarkets.

Discussion

Scale and implications of meat and fish import

While it has long been known that meat and fish are imported illegally from Africa to Europe, the scale of this trade has so far remained unquantified; as far as we are aware, this investigation provides the first systematic evaluation of the volumes and nature of illegal imports. Although the estimated amount of bushmeat imported is a tiny proportion of the total estimated harvest (thought to be upward of one million tonnes per year in the Congo basin, Wilkie & Carpenter 1999), and is clearly not an important current driver of hunting in Africa, the volume and nature of import and trade suggests the emergence of a luxury market for African bushmeat in Europe. Imports are supplying an organized system of trade and are not solely being brought for personal consumption. This is indicated by the large size of many individual bushmeat consignments, and the presence of traders within Paris who are able to supply bushmeat to order. Furthermore, consumers in Paris are willing to pay high prices for the meat, with costs at the top end of premium meat prices. The bushmeat trade in Paris thus appears to be at the extreme end of a spectrum, from rural source areas in Africa through urban areas of increasing size and distance from the source, along which bushmeat becomes increasingly expensive (Starkey 2004; East *et al.* 2005). The development of a luxury market, linked to increasing affluence of the consumer population, is of particular concern because of the potential for demand to remain high even as supply dwindles and prices rise, potentially driving the extinction of even relatively resilient target species (Hall *et al.* 2008).

A more immediate conservation concern is the presence of CITES-listed species among those imported. During its 11th meeting, the Conference of the CITES Parties concluded that most of the trade in bushmeat is probably domestic and therefore not directly relevant to CITES. However, while international trade is small relative to in-country trade, the significant volumes reported here, coupled with the presence of species listed in both Appendices 1 and 2, suggest that the issue should be of immediate concern to CITES.

Drivers of import

There are a number of factors facilitating the illegal import of meat and fish. First, detecting and seizing these products is not a priority for customs officials. Officers reported that searches for meat are time consuming, not cost-effective, unpleasant, and potentially dangerous, and that seizures do not qualify for bonuses as do other illegal merchandise, such as counterfeits and drugs. As a result, they do not generally target meat. Adding to this, officers generally change shifts at around 0700 hours, resulting in a one-to-two hour period of reduced surveillance at the time when arrivals from Africa peak.

Second, penalties for importing illegal meat or fish are low and rarely imposed. The maximum penalty under French law is confiscation of the goods and a €450 fine, or €150 if less than 15 kg are carried. While meat and fish were always confiscated when found, only one passenger carrying bushmeat and none of the 55 people carrying fish or livestock were actually required to pay a fine. While French law implementing CITES is robust, in practice prosecutions under these regulations are rare. Such prosecutions require evidence of the species involved, but no protocols exist for the identification of seizures, which are instead sent immediately for incineration.

Third, the rewards from transporting bushmeat are potentially high. A 4 kg monkey will cost approximately €100 in France, 20 times more than the same monkey bought for around €5 in Cameroon (Willcox & Nambu 2007). While flight costs are substantial, three of the nine passengers carrying bushmeat were flying on discounted Air France tickets, which entitle family members of employees to discounts of up to 90%.

Finally, illegal meat and fish imports are encouraged by a lack of information on, and knowledge of, regulations. Most of the passengers searched claimed to be unaware of any restrictions and stated that no prohibition signs were displayed at their ports of departure. While some of these claims may not have been truthful, we believe that the availability of information and levels of knowledge are genuinely low. This is further supported by the fact that many of the people searched carried veterinary certificates, apparently signed by veterinary authorities in the countries of origin, which they believed entitled them to import the goods carried. In fact, these certificates did not follow European regulations for the certification of animal products and held no legal value.

Data quality and assumptions

Because the survey was short, and sample sizes consequently low, the precision of our estimates of import volume is also low. There are also two key potential sources of bias in our data. First, it may be that the dates on which we carried out the survey were unrepresentative of patterns over the rest of the year. Bushmeat supply in source countries can be seasonal to some extent (de Merode *et al.* 2004; Cowlishaw *et al.* 2005). However, we have no clear evidence of how this might have affected our results. Second, it may be that targeting of iceboxes by customs officials could lead us to overestimate the volumes of fish and livestock meat, since these are usually transported in iceboxes. However, bushmeat imports should not be sensitive to this bias since no bushmeat was found in iceboxes during this study.

Research and policy implications

The relatively short-time scale and restricted geographical coverage of this study leaves a number of important questions. A study operating over a larger scale and longer period is now required to extend geographical coverage, improve the precision of estimates, and reduce potential sources of bias. This should include coverage of other potential supply routes, particularly freight, which may be a major under-recorded channel for illegal imports (e.g., in 2008, a consignment of 340 kg of bushmeat was found in boat cargo arriving at Tilbury in the United Kingdom). Expanded monitoring will provide crucial information to improve the assessment of risks posed by this trade, and to prioritize actions for tackling it effectively.

On the basis of the results presented here, we suggest a number of policy options for reducing illegal imports. In the case of customs authorities, detection rates could be improved by incentivising officers to search for imports and by increasing the use of meat-detection dogs. Furthermore, when imports are detected, the appropriate fines should be imposed as a matter of course; without these two actions, there is effectively no deterrent. The efficiency of surveillance might also be improved by the introduction of effective checks at ports of departure. Improved ability to prosecute importers, particularly those importing large quantities and/or CITES-listed species, would also improve deterrence. This would require a change in regulations to allow the safe processing and storage of seizures as evidence. Training of customs officers to distinguish key bushmeat taxa visually could also help. There is a need to improve the information available to passengers on the illegality of carrying unregulated meat or fish, making clear why it is illegal and the risk of prosecution and substantial penalties. For example, in the United Kingdom, it appears that the amount of illegal meat imports via personal baggage has declined recently, possibly as a result of increasing awareness of the regulations following a targeted publicity campaign (DEFRA 2008). Developing common strategies across Europe based on effective practice such as this could help greatly. Airlines could also help, for example, by imposing penalties, perhaps including the threat of dismissal, on the staff members through whom the tickets were obtained when holders of discounted tickets available to staff are found to be carrying meat. While implementing all these suggestions might be difficult in practice, the large scale of current imports makes it important to consider all options for reducing the flow of illegal meat and fish in general and of bushmeat in particular.

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Article 3: Public health risks from illegally imported African bushmeat and smoked fish.

Short communication in *EcoHealth journal* – 2016 - *13*(1), pp.135-138.

Preamble: The European Union has laid down common rules for the import of foodstuffs of animal or plant origin, aiming to protect consumer health and the territory of the Union from the introduction of devastating animal or plant diseases.

The European Commission Decision on personal imports (EC) No 745/2004 of 16 April 2004 laid down measures with regards to imports of products of animal origin for personal consumption and prohibits "permanently all personal consignments of meat, meat products, milk and milk products from entering the EU, including packages sent to private persons, unless specifically authorized and certified as being eligible for EU entry" (EC No 745/2004 of 16 April 2004). Annexe 1 of the EC Decision No 745/2004 of 16 April 2004 lists products which are exempted from veterinary checks: "Meat and meat products and milk and milk products which are subject to the derogation from systematic veterinary checks when carried by travellers entering the Community: Powdered infant milk, infant food and special foods required for medical reasons, under the conditions that these products do not require refrigeration before opening, that they are packaged proprietary brand products for direct sale to the final consumer, and that the packaging is unbroken unless in current use."

The Commission Decision of 7 July 1997 drew up provisional lists of third country establishments from which the member states (of the EU) can authorize imports of wild game meat (97/468/EC). On this list, some establishments in South Africa and Namibia are allowed under strict rules to export wild meat to member states (Annex 1). No Central and West African countries are present on this list.

Our study aimed at assessing public health risks from illegally imported African bushmeat and smoked fish linked to the consumption of meat contaminated by known pathogens. Microbial pathogens of current concern that need to be identified in fresh meat include *Salmonella*, *Campylobacter*, enterohaemorrhagic *E.coli* including serotype O157:H7, as well

as other enteric pathogens[117]. *Listeria monocytogenes* with its ubiquitous presence, contaminating products after processing, and its ability to multiply even at low temperatures[118] is the number one target when controlling ready-to-eat meat. Major meat safety issues include the need to control traditional as well as new, emerging, or evolving pathogenic microorganisms[117].

The United States Center for Disease Control and Prevention (CDC) published summary statistics for food borne outbreaks in 2006, revealing that 28% of the outbreaks and 16% of the cases of reported food borne illnesses remain unresolved and the etiologic agent unknown[119]. It is to be expected that undiscovered, emerging, evolving and/or exotic pathogens are associated with food borne illnesses. Detection of emerging pathogens and new pathogen discovery will be emphasized in the discussion part of the thesis.

In addition to the microbial hazard, the risk associated with the consumption of dangerously treated meat was investigated. Most of the meat and fish is smoked, and this processing method is a great matter of concern, with the possible accumulation of toxic polycyclic aromatic hydrocarbons (PAHs). PAHs are produced as by-products of burning fuel (whether fossil fuel or biomass). As a pollutant, they are of concern because some compounds have been identified as carcinogenic, mutagenic and teratogenic. In addition to their presence in fossil fuels (and thus fuel spills), they are also formed by incomplete combustion of carbon-containing fuels such as wood, coal, diesel, fat, tobacco, etc[120]. In Europe, the maximum levels for benzopyrene in fish and meat products is 5 micrograms/kg, in oils and fats 2 micrograms and in children's foods 1 microgram/kg[121].

EcoHealth
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Short Communication

Public Health Risks from Illegally Imported African Bushmeat and Smoked Fish

Public Health Risks from African Bushmeat and Smoked Fish

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Abstract: Large-scale importation of bushmeat from West and Central Africa into Europe was reported in 2010. We sampled 18 illegal African bushmeat consignments seized at Charles de Gaulle airport, Paris, France and tested for the presence of bacteria. Additionally, five smuggled smoked fish were analysed for polycyclic aromatic hydrocarbons, which are known carcinogens. All bushmeat samples had viable counts of aerobic bacteria above levels considered safe for human consumption. We also identified zoonotic bacterial pathogens in bushmeat and unsafe levels of carcinogens in fish. The illegal importation of meat is a potential risk for the introduction of pathogens.

Keywords: public health, illegal trade, bushmeat, Europe

Introduction

Bushmeat hunting has been identified as a source of zoonotic disease transmission, including the emergence of novel human pathogens (Smith et al. 2012; Wolfe et al. 2004).The international bushmeat trade from West and Central Africa into Europe (Falk et al. 2013; Chaber et al. 2010) might pose human or animal health risks due to infectious agents. In addition, bushmeat and fish are often smoked and this method of preservation has been identified as a source of exposure to polycyclic aromatic hydrocarbons (PAH), which are known carcinogens for consumers (Akpambang et al. 2009). During a study carried out at Roissy-Charles-de-Gaulle airport (CDG), France in 2008, we sampled illegally imported bushmeat and fish that had been seized from passengers by customs officers. Here, we report the findings of our analyses of potential public health risks associated with this trade.

Material and Methods

Twenty-nine Central and West African Air France flights were checked from the 3rd to 20th of June 2008. Customs inspections were run opportunistically based on custom officer availability and passenger arrival at the gate. Passengers' luggage was opened for manual inspection and bushmeat was seized when present. Such seizures almost always comprised whole carcasses which were either

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smoked (80 % of the bushmeat seized) or fresh (especially crocodiles and pangolins).

One 30 gram sample was excised from each seized bushmeat carcass, from the skin to deep in the muscle. Each sample was placed in sealed triple plastic bags, labelled, frozen at -20°C and stored for up to one month until analysed. Ninety samples were collected in this way, 18 of which were selected at random and submitted to a commercial laboratory (MC Labo, Saint-Sever, France) for bacteriological analyses. Samples were thawed at 4°C for 24–36 h and were analysed for Aerobic Viable Count (AVC), *Enterobacteriaceae*, *Listeria monocytogenes* and *Salmonella* spp. For AVC and *Enterobacteriaceae* counts, the respective procedures were followed: ISO 4833 (2004) and AFNOR NF V08-054 (1999). The isolation of *Salmonella* spp. was carried out in accordance with ISO 6579 (1993). For the detection of *L. monocytogenes*, the procedure described in ISO 11290-1 (1996) was followed. Bacterial isolates characteristic of *Staphylococcus* spp. or *Streptococcus* spp., were further identified using protocols ISO 6888-1 (1999) for *Staphylococcus* spp. identification and colonial appearance, Gram stain, catalase test, Lancefield grouping and optochin sensitivity for *Streptococcus* spp. identification.

One smoked fish was randomly selected from each of five different flights (two from Central African Republic, and one each from Angola, Guinea and Cameroon), and sent to the French National Reference Laboratory (LABERCA, Nantes, France) for PAH analysis. The samples were analysed for 15 PAH using an accredited method (Veyrand et al. 2007).

Results

All samples taken deep in the muscle were bacteriologically sterile, but a range of bacteria was cultured from the surface of each sample submitted. Not all bacteria cultured were identified due to financial restrictions. The bacteriology results are presented in Table 1. Briefly, mesophilic bacteria were abundant in all 18 samples tested, with AVCs ranging

Table 1. Bacteriology Results from Illegally Imported Bushmeat Skin Samples Seized at Charles de Gaulle Airport.

Species sampled	AVC[a]	Totals coliforms[a]	*Listeria* spp.[a]	Other
Pangolin	4.52×10^7	0[b]	4.85×10^4 *L. monocytogenes*	*Staphylococcus aureus Streptococcus* sp.
Pangolin	1.01×10^8	0[b]	7.23×10^4 *L. ivanovii*	Not detected
Pangolin	9.3×10^6	0[b]	2.23×10^3 unidentified species	*Klebsiella oxytoca Enterobacter* sp. *Staphylococcus* sp.
Porcupine	8.18×10^8	0[b]	0[b]	Not specified
Porcupine	Not countable[c]	0[b]	8711 *L. ivanovii*	*Streptococcus* sp.
Porcupine	3.51×10^7	0[b]	3.34×10^7 *L. grayi*	Not specified
Primate	1.31×10^7	0[b]	1.49×10^6 unidentified species	*Staphylococcus* sp.
Primate	3.89×10^6	0[b]	1.25×10^5 unidentified species	*Staphylococcus* sp.
Crocodile	2.62×10^6	0[b]	2.77×10^5 unidentified species	*Staphylococcus* sp.
Crocodile	1.50×10^7	0[b]	2.00×10^3 *L. grayi*	*Citrobacter freundii Staphylococcus* sp.
Crocodile	4.5×10^6	0[b]	1.15×10^5 *L. welshimeri*	*Staphylococcus* sp.
Duiker	Not countable[c]	1.89×10^3	Not countable[c] *L. grayi*	*Citrobacter freundii*
Duiker	3.15×10^8	1.69×10^5	6.64×10^7 unidentified species	*Staphylococcus* sp. *Staphylococcus aureus*
Duiker	Not countable[c]	0[b]	Not countable[c] *L. grayi*	Not specified
Unknown	1.18×10^8	0[b]	0[b]	Not specified
Great cane rat	Not countable[c]	0[b]	8.07×10^4 *L. ivanovii*	Not detected
Red hog	1.4×10^6	0[b]	1.32×10^5 *L. monocytogenes*	*Staphylococcus aureus*
Mix fish/meat	6.7×10^6	0[b]	3.35×10^5 unidentified species	*Staphylococcus aureus–Klebsiella ozonae* Bacillus sp. (non *anthracis*)

[a]Colony forming units per gram of bushmeat.
[b]Below the detectable limit of 30 colony forming units per plate.
[c]Above the maximum limit of 300 colonies forming units per plate.

Table 2. Concentrations of the Sum of 15 Polycyclic Aromatic Hydrocarbons and of Benzo[a]pyrene in Illegally Imported Smoked Fish Seized at Charles de Gaulle Airport.

Sample	∑PAHs µg/kg of fresh weight	Concentration in benzo(a)pyrene (BaP) µg/kg of fresh weight (±SD)
1	133.12	17.35 ± 2.72
2	209.27	20.40 ± 3.19
3	406.43	45.02 ± 7.07
4	134.66	15.27 ± 2.40
5	190.91	22.43 ± 3.52

from 1.4×10^6 to 8.18×10^8. No *Salmonella* spp. were isolated and *E. coli* was cultured from only two samples. *Listeria* spp. (including the human pathogen, *Listeria monocytogenes*) were cultured from 10 samples. *Streptococcus* spp. and *Staphylococcus* spp. were identified in nine of the samples analysed. Potentially zoonotic pathogenic biotypes of these Gram-positive bacteria, such as *S. aureus*, were detected in four samples (Table 1). PAH values in the five fish samples tested ranged from 133.12 to 406.43 µg/kg of fresh weight (Table 2).

Discussion

The high abundance of the total mesophilic bacterial flora was probably due to unhygienic handling of the meat prior to seizure and sampling. Most of the bacteria found and identified were environmental and few were known pathogens, but the sample size was small and a wider range of zoonotic pathogens (e.g. *Salmonella* spp.) might be found if a larger sample size was tested. The absence of bacterial growth from samples taken deep in the muscle indicates that bacterial contamination was superficial. Bacterial identification revealed various biotypes of which *Listeria monocytogenes*, *L. grayi* and *S. aureus* are associated with food-borne illnesses (Scallan et al., 2011).

All of the bushmeat analysed, had AVC above the 5 log cfu/cm^2 limit set by the European Regulation (EC) No. 1441/2007 (2007,7,12) and would therefore be considered unfit for human consumption in Europe. Although zoonotic pathogens, such as *L. monocytogenes*, were isolated from the bushmeat, the risks from these hazards can be adequately controlled through basic kitchen hygiene, which includes taking steps to avoid cross contamination, and thorough cooking. Most traditional African meat dishes involve long periods of stewing which should kill any bacteria present.

The 15 PAH tested for have been identified as a priority for food hygiene measures because they show clear evidence of mutagenicity and genotoxicity in somatic cells, both in vivo and in experimental animals (European Commission 2005). PAH are produced by natural and anthropogenic processes, principally pyrogenic (incomplete combustion of coal, wood or other organic substances) and petrogenic (incomplete combustion of petroleum products) inputs (Hodgeson 1990). PAH contamination of fish (and bushmeat) is linked to the smoking process. Our results showed extremely high levels of PAH contamination. Cancer risk estimates for oral uptake of PAH are based on those for benzo[a]pyrene (BaP). In Europe, the maximum allowable levels for BaP in fish and meat products is 5 micrograms/kg and only 1 microgram/kg in children's food (JECFA 2005). BaP values in the five fish we tested were three to $>$ 5 times higher than the maximum allowed in the European Community (Table 2). Alonge (1988) implicated food-borne PAH from smoked dried meat in the high incidence of primary liver and stomach cancer reported in Nigeria.

Conclusion

Although our sample size was low, we identified unsafe levels of bacteria and zoonotic bacterial pathogens in bushmeat and unsafe levels of carcinogens in fish illegally imported to Europe. The illegal importation of fresh meat should not be overlooked as a potential risk for the introduction of pathogens. A larger sample size and the inclusion of virological analyses could identify additional public or livestock health threats from this illegal trade. Further research is needed to fully understand the scale of, and risks associated with, the international illegal meat trade.

Acknowledgments

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Chapter 6: Challenging our assumptions and reflecting upon our knowledge gap: Disease management at the wildlife/livestock interface.

Great works are performed not by strength, but by perseverance.
Samuel Johnson

Preamble: On the ground, preventing and controlling outbreaks at the W-L-H interface is challenging mainly due to our knowledge gaps. Collaborative work among different sectors is mandatory in order to understand complex ecological systems and to gather the necessary sound scientific data on disease ecology, vaccine efficacy, epidemiological situation, species sensitivity, host specificity, transmission patterns, vectors or climatic conditions affecting pathogens survival and vector competence. By studying an outbreak of Contagious Caprine Pleuropneumonia (CCPP) in the UAE, new information relevant to disease management including on the efficacy of the inactivated vaccines commercially available in the UAE at W-L-H interface has been collated.

Article 4: Origin and transmission dynamics of contagious caprine pleuropneumonia in a sand gazelle herd.

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Title page

Origin and transmission dynamics of contagious caprine pleuropneumonia in a sand gazelle herd

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Abstract

Contagious caprine pleuropneumonia (CCPP), caused by *Mycoplasma capricolum* subsp. *capripneumoniae*, has long been considered a goat-specific disease. Since 2007 there has been growing evidence that this disease can affect wild ungulates either kept in captivity or in the wild. In 2013, a large collection of sand gazelles (*Gazella marica*) held in the United Arab Emirates suffered heavy losses due to a CCPP epizootic confirmed by PCR and isolation. Animals displayed typical lesions, namely unilateral pneumonia and profuse pleurisy. An initial antibiotic treatment consisting in tylosin administered in the drinking water did not improve the animals' condition and vaccination failed to stop the spread to contiguous pens. A treatment consisting in tetracycline mixed in feed pellets finally succeeded to stop the evolution of the disease. A subsequent vaccine trial, performed on naïve animals, showed that only a reference CCPP vaccine produced according to OIE standards induced a sero-conversion by CCPP competition ELISA, while the commercially available vaccines did not. A SEIRD compartment transmission model was developed to better understand the dynamics of the disease. The parameters were initially set as per expert opinion and then adjusted to fit the observed mortality data.

The basic reproductive number R_0 was estimated between 2.3-2.7, while the final mortality rate reached up to 70% in some pens. Transmission of infectious droplets from an external source, through a distance of at least the 50m separating the pens from the perimeter fence, remains the most plausible explanation for the contamination of this stock of gazelles.

Keywords

Contagious caprine pleuropneumonia

Sand gazelle

SEIRD model

Basic reproductive number

Introduction

Contagious caprine pleuropneumonia (CCPP), caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp), induces lesions of pneumonia and pleurisy that result in respiratory distress and often death in acute cases [1]. The disease was first described by Thomas in Algeria [2] and then by Hutcheon, who reported its introduction in South Africa through a shipment of goats from Turkey [3]. Long considered restricted to Africa and the Middle-East, CCPP is now known to occur in China [4] and Tajikistan [5]. Hence, CCPP must be present in a region spanning from Tunisia-Niger to China and, consequently, represents a threat to hundreds of millions of goats. The distribution of CCPP outside this area, notably west of Niger, north of Turkey-Tajikistan and south of Tanzania, is still uncertain.

CCPP was long considered a goat-specific disease, though this strict host specificity was put in question when Mccp was isolated from sheep in Kenya [6] and Uganda [7] [8]. However, sheep were less susceptible than goats and it remains unclear whether they may play a role in the epidemiology of CCPP, as asymptomatic carriers, or if they only become infected when in contact with infected goats. Since 2007 there has been a growing number of reports of CCPP in wild ungulates, either held in captivity or free ranging. The number of affected species is expanding progressively: wild goat (*Capra aegagrus*), Laristan mouflon (*Ovis orientalis laristanica*), Nubian ibex (*Capra ibex nubiana*), gerenuk (*Litocranius walleri*) [9], Tibetan antelope (*Pantholops hodgsonii*) [10]and Arabian oryx (*Oryx leucoryx*) [11].

We report here a CCPP outbreak that occurred in a large collection of sand gazelles (*Gazella marica*) held in the United Arab Emirates. The course of the disease was precisely recorded and its spread through the various pens could be deduced from observations made during this outbreak. These observations were also used to validate the parameters of an *in-silico* transmission model, which allowed us to estimate the basic reproductive number R_0 and the case fatality rate of CCPP in those herds. Finally, the analysis of the various control measures that were implemented, consisting in antibiotic treatments and vaccination, was used to issue some general recommendations, both for the early response to an outbreak and for its prevention. It was shown, notably, that CCPP could be transmitted in the absence

of close animal contact, at a distance of at least 80m, and that the commercial CCPP vaccines that were locally available did not provide the expected protection.

MATERIAL AND METHODS

Housing facilities and population affected by the outbreak

At the beginning of the outbreak, the herd consisted of 3355 sand gazelles (*Gazella marica*) held in a compound as described in Figure 1. There were four groups of pens (numbered I to IV), separated from each other by a distance of 50 to 80m. In each group, each pen (numbered .1 to .4) had a size of 100x200m and contained up to five 10x15m shade structures, where animals could shelter. Feeders and water troughs were located away from the fence. All the pens were contiguous to at least another pen, separated by a wire fence. A perimeter fence around the whole compound delimited a buffer zone of 50m between all the groups of pens and the outside.

Most of the pens initially housed large numbers of animals, ranging from 250 to 400, except for pen I.1 and pen I.2, which housed 39 and 19 animals respectively. Sexes were separated and no births were recorded. Pens IV.3 and IV.4 housed Arabian oryx. About 70 days after the onset of the outbreak, for vaccination management purposes, the herd in pen II.1 was merged with that in pen II.4 and, similarly, II.2 with II.3, III.1 with III.4 and III.2 with III.3. No animals had been introduced in the herd within the 12 months preceding the outbreak. A traditional sheep and goat farm, locally called "Ezba" [12], housing 50 animals, was situated 270 meters south-east of the compound, with the closest pen being II.1. Wild gazelle's tracks and free roaming camels could be seen occasionally along the perimeter fence.

Daily climatic data

Wind direction and speed, temperature and humidity data were recorded by automated surface observing systems (ASOS) at two local airports: Al Ain airport, located 61 km to the east, and Al Dafrah airport, located 54 km to the west. These data are accessible online: https://mesonet.agron.iastate.edu/sites/dyn_windrose.phtml?station=OMAL&network=AE__ASOS.

Clinical and pathological data

Mortality was recorded daily, including an indication of the possible cause of death, and 70 animals were randomly selected for necropsy during the outbreak, which made it possible to follow the course of the disease.

Mccp culture, isolation and genotyping

Lung samples were collected aseptically for pathogen identification. Frozen samples stored at -20°C were shipped to CIRAD-ASTRE (Montpellier, France), OIE/FAO reference laboratory for CCPP. DNA was extracted from lung samples with the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and analyzed by real-time PCR [13]. Cultivation of mycoplasmas was performed using modified Hayflick's medium supplemented with horse serum (25%) and sodium pyruvate (0.2%) [14]. Plates were incubated at 37°C under anaerobic conditions. Mycoplasma colonies were cloned to ensure purity and then grown in liquid medium for identification of Mccp by real-time PCR. One of these clones was then typed by multi-locus sequence analysis (MLSA), based on the concatenation of 8 locus sequences according to [15].

Antibiotics and vaccines

The sequence of antibiotic treatments and vaccinations is displayed in Table suppl. 1. Tylosin tartrate powder (ADWIA/Egypt) was administered at a dose of 10mg/kg of body weight (BW) single in day (SID) for 14 days dissolved in drinking water. Oxytetracyclin-HCL (Oxivet 20%®, Centrovet Ltd/Chile) was given at 10mg/kg BW, SID for 14 days mixed with the feed (pellets). Two commercially available CCPP vaccines were used during the outbreak. Pulmovac® (VETAL Animal Health Products, Adiyaman, Turkey) batches 12/PU/11 and 13/PU/03 were initially used, then Caprivax® batch 18/013 (KEVEVAPI, Nairobi, Kenya). All the animals in each pen were vaccinated to obtain 100% immunization coverage.

Serological assessment of CCPP vaccines

One hundred unaffected male sand gazelles in a pen situated in the northern part of the facility (Figure 1) were divided in five groups of 20 animals (Table suppl. 2). Group A did not receive any treatment,

and was used as negative control. Group B was vaccinated first with "Pulmovac" batch 13/PU/03, and three months later with Jovaplasm C® batch JP1812 (JOVAC, Jordan), a third commercially available vaccine. Group C, used as positive control, received one dose of a reference vaccine produced at CIRAD according to the OIE guidelines (ref. OIE terrestrial manual), containing 0.15 mg of purified Mccp antigen and 3mg saponin (Sigma S4521) per dose. Group D received an injection of "Caprivax" batch 18/013 and a triple dose of the same vaccine three months later. Finally, group E received two injections of "Caprivax" one month apart. It must be noted that the number of gazelles decreased over time due to traumatic injuries following fights.

Blood samples were harvested monthly from all animals, coded blindly, and sent to CIRAD for analysis. Serology was performed using the CCPP competition ELISA (cELISA) kit (IDEXX, Montpellier, France). The test was performed according to the manufacturer's instructions and under ISO17025 accreditation by the French committee of accreditation (N° 1-2207 rev. 9). The validity conditions, notably homogeneity of variances and covariance matrixes, were first tested [16] and a two-factor ANOVA with repeated measures on one factor was used to compare the seroconversion kinetics [17]. In addition, an in-house indirect ELISA was performed to evaluate and compare the global quantity of Mccp-recognizing antibodies one month after vaccination. Shortly, Greiner plates ref. 655061 coated overnight at +4°C with an inactivated, purified Mccp antigen (0.03µg/ml) were incubated one hour at 37°C with goat sera diluted 1/1300, then 30 minutes in DAKO™ P0160 conjugate (1/1500), and finally 20 minutes in tetramethylbenzidine substrate. After adding the stop solution, the optical density (OD) readings were performed at 450nm. Each step was followed by plate washes. All diluents and solutions were obtained from the IDEXX cELISA kit. The mean OD between groups of animals was assessed using a two-sample t test with unequal variances (Welch's test) [18]. For all tests, P values < 0.05 were considered significant.

Development of a compartmental model

A compartmental model was developed to estimate the basic reproductive number (R_0), as a measure of the contagiousness of the disease, and the case fatality rate (p). The pen II.2 was chosen for

these estimations because it was the first where cases were recorded. Consequently, the gazelles in this pen could be considered a naïve population and the disease spread through direct contact within the pen. As the disease progressed to other pens the dynamics certainly became more complicated, involving interactions among animals in different pens. The within-pen spread of CCPP was described using a "SEIRD" model, a modified version of the SEIR [19] [20] [21], where S, E, I, R and D are non-overlapping epidemiological compartments representing the number of susceptible (S), exposed (E, infected but not infectious yet), infectious (I), recovered (R) and infection related dead (D) animals respectively. A pictorial representation of the model is shown in Figure 2.

The temporal evolution of the disease spread is described through the set of equations:

$$\frac{dS}{dt} = -\beta \frac{SI}{N}$$

$$\frac{dE}{dt} = \beta \frac{SI}{N} - \frac{1}{\tau_E} E$$

$$\frac{dI}{dt} = \frac{1}{\tau_E} E - \frac{1}{\tau_I} I$$

$$\frac{dR}{dt} = (1-p) \frac{1}{\tau_I} I$$

$$\frac{dD}{dt} = p \frac{1}{\tau_I} I$$

$$N = S + E + I + R$$

Animals in the infectious stage can infect susceptible ones with a probability β per unit of time. The probability β, or transmission contact rate, takes into consideration many factors related to the transmission of the mycoplasma in the local environment. Exposed animals spend τ_E days in a latent stage before becoming infectious and then remain τ_I days in this stage. At the end of the infection stage animals either recover, with a probability "1-p", or die, with a probability "p", which represents the case

fatality rate. A time step of one day was used and the disease progression was simulated for 100 days. The population was considered constant except for infection-induced deaths. This assumption was supported by the fact that during the first 80 days of the infection, the mortality rate in pen II.2 (first infected) was almost 15 times higher than that in pen IV.I (last infected).

The basic reproductive number R_0 was then evaluated using two approaches:

- As the largest eigenvalue of the Next Generation matrix $R_0 = (\beta\tau_I)$. [22]
- Using the final size relation: $R_0 = -\frac{\ln(s_f)-\ln(s_0)}{s_f-s_0}$ [23], where s_0, s_f, indicate the fraction of susceptible animals at the beginning (s_0) and at the end (s_f) of the epizootic respectively, with the final fraction of susceptible animals estimated as $s_f = 1 - \frac{Total\ Number\ of Deaths}{p*N}$

This model depends on four parameters: the contact rate β, the latent τ_E and infectious τ_I periods, , and the case fatality rate p. All of them, except the latent period τ_E , whose value was fixed at 7 days as per expert opinion, were estimated through the calibration of the model. We used Monte Carlo Markov Chains (MCMC) methods [24] [25], with a Poissonian likelihood, to fit the model to the daily number of deaths in pen II.2. Metropolis-Hastings sampling was used to explore the parameters space, checking convergence by using 100 chains of 1,000 iterations (after a 100 burn in period), starting from several different initial values of the parameters set. The calibration was performed using the R-package FitR (https://github.com/sbfnk/fitR) developed by Funk and Camacho at the London School of Hygiene and Tropical Medicine.

A single infectious period was assumed in our model, thus excluding differences in contagiousness between asymptomatic and symptomatic phases. To strengthen the results from our model, two other models were considered. In one model (called 'SEIIRD') the infectious stage and its period τ_I were partitioned in two infectious stages, asymptomatic and symptomatic, with different transmission probabilities ($\beta_A < \beta_S$). In the other model (called 'SEIRD with constant mortality'), a constant mortality rate was considered during the infectious stage, indicating that animals could die at any

moment. The two models were calibrated with the same set of data and R_0 was evaluated accordingly. The lower Deviance Information Criterion (DIC) [24] [25] was used as a measure of model adequacy, to compare the three models.

RESULTS

Atmospheric conditions preceding and during the outbreak The climate in the UAE was arid and from April to October air temperature varied from 20°C at night to over 48°C during daytime, while the average humidity ranged from 20% during the day to 75% at night. Most of the animals tended to congregate under shade structures at daytime, thus favoring close animal contacts. Some rain was recorded between the 22nd and the 29th of April 2013 and no precipitations were observed for 198 consecutive days from May 2nd to November 15th. After the rainfall in April, a pungent ammoniac smell was detected in the enclosures due to manure degradation. A strong prevailing wind, locally called "Alkaus", which blows from the South-South East, was recorded at over 12m/sec on April 5th and 6th 2013.

Onset of a CCPP outbreak in the sand gazelle herd

The 27th of April 2013, "salivating gazelles" and a sudden mortality increase were observed in pen II.2. Two days later, the first three necropsies presenting CCPP-compatible lesions were performed in this same pen and initiated a closer follow-up of the pens. Biosecurity measures were immediately implemented. These notably included the disinfection of boots and wheels of vehicles with 1/200 diluted Virkon-S (DuPont). Healthy pens were visited first and contaminated pens last. Gazelle carcasses were disposed of in individual plastic bags and incinerated. Nonetheless, CCPP spread to adjacent pens (II.1 and II.3) and then to groups of pens I and III (Figure suppl. 1).

Initially, animals presented good body condition, with or without sero-hemorrhagic epistaxis and salivation, and did not show overt disease signs. At a later stage, animals were reluctant to move and their breathing was labored and painful. Typical CCPP lesions, confined to the thoracic cavity, were observed at post-mortem examinations.

227 The administration of tylosin in the water was not followed by any improvement in the animal's health
228 and the initial vaccination with "pulmovac" did not prevent the spread of CCPP to neighboring pens.
229 Only the treatment with oxytetracyclin-HCL in the feed yielded some improvement and induced a
230 marked bending of the mortality curve, notably in pen III.3 after day 179. At that date the cumulative
231 mortality was reaching 40% and it did not increase much after that date. However a second treatment
232 was given on day 205 as mortality seemed to resume in that pen. The overall evolution of the disease
233 mortality is illustrated in Figure 3.

234 **Laboratory diagnosis and molecular typing.**

235 Out of four samples tested at CIRAD, only one yielded a positive result by direct Mccp-specific real-
236 time PCR following DNA extraction. However, given the strict specificity of this test, this was sufficient
237 to confirm the presence of Mccp. Isolation trials were positive for two of the samples and the identity
238 of the mycoplasma cultures was confirmed by PCR. The molecular typing of these isolates by MLSA
239 resulted in the identification of a new type, only differing from previously defined MLSA types by a
240 single nucleotide polymorphism.

241 **Experimental assessment of the seroconversion induced by CCPP vaccines.**

242 As clinical observations had cast a reasonable doubt on the efficacy of the commercially available CCPP
243 vaccines, a comparative efficacy study was performed in the northern pens of the compound, where
244 gazelles had stayed unaffected. The outcome of the vaccination was monitored by serology. Before
245 vaccination, all gazelles displayed low percentage of inhibition (PI) values (PI<35) by the cELISA test.
246 After vaccination, none of the locally available CCPP vaccines induced any seroconversion (Figure 4A).
247 By contrast, the reference vaccine batch, produced according to the OIE manual of standards, induced
248 a sharp seroconversion, which started to decline only after two months. This seroconversion differed
249 significantly from that of the other vaccines (ANOVA *P*-value = 0.01). After five months, 11 gazelles
250 out of 15 remaining in this group had titers that were above the cut-off, which is set at 55 PI for this test
251 in goats. These results were confirmed by an in-house indirect ELISA, which measured the global
252 quantity of antibodies recognizing the Mccp antigens coated on the plate. One month after vaccination,

253 only the OIE reference vaccine induced a sharp seroconversion (mean OD= 2.1). This value was
254 significantly higher than the value obtained before vaccination (Welch's test; *P*-value < 0.0001). By
255 comparison, only group B vaccinated gazelles, vaccinated with both "Pulmovac" and "Jovaplasm",
256 showed a significant (Welch' test; *P*-value = 0.002) but very slight seroconversion (mean OD= 0.128).
257 The other protocols using commercial vaccines did not induce a significant OD increase (Welch's test;
258 *P*-value ≥ 0.13). (Figure 4B).

259 **R_0 estimates from SEIRD model outcomes.**

260 The total population at the beginning of the epizootic in pen II.2 consisted of 277 gazelles, of which
261 around 7% were considered in the latent phase. The latent time τ_E was fixed to 7 days, as from expert
262 opinion. The other parameters were calibrated by MCMC procedure using a Poissonian Likelihood for
263 the data. Results are shown in Figure 5, where black dots correspond to data and red shades correspond
264 to the average and confidence intervals of the model. Table Suppl. 3 shows the estimated parameters of
265 the model that yield the best agreement between model and data. The case fatality rate "p" was estimated
266 at around 59% (95% CI: 54 to70). Using the estimated parameter values, R_0 was estimated to have an
267 average value of 2.32 (95% CI: 1.86 to2.79), when evaluated through the Next Generation matrix [22],
268 and a slightly higher value, 2.68 (95% CI: 2.07 to 3.43), when evaluated using the final size relation
269 [23]. The comparison of the three models Table Suppl. 4 through the estimation of the DIC, showed that
270 the simplest SEIRD model was the most adequate to describe the disease propagation in pen II.2.

271 # DISCUSSION

272 The susceptibility of *Gazella marica* to CCPP infection has been confirmed, with animals showing
273 typical symptoms and lesions and final cumulated mortality rates reaching 70% in the absence of
274 antibiotic treatment. This is very similar to what is observed in goats [1] [26].

275 The onset of a CCPP outbreak in the sand gazelle stock was quite a surprise as this herd was supposedly
276 well isolated from surrounding susceptible animals. No ungulates were introduced in the compound
277 since the arrival of Arabian oryx a year before. These oryx had been kept for two years in enclosures

where no history of disease had been reported. It is therefore unlikely that these animals were the source of the infection. A contamination through fodder is also very unlikely, as alfalfa hay was imported from CCPP-free countries.

Transmission from an external source, through a distance of at least the 50m separating the pens from the perimeter fence, remains the most plausible explanation. CCPP is enzootic in the UAE, and more generally in the Arabic Peninsula [27]. The first pen that became contaminated was located about 270m away, leeward a goat farm located south from the gazelles. Unfortunately, it was not possible to obtain epidemiological data to determine if there had been an incidence of CCPP in this farm just before the outbreak in the gazelle herd. Although the daytime atmospheric conditions were very harsh, the nighttime conditions (20°C and 75% relative humidity) were propitious to mycoplasma survival, which may have allowed the dissemination of cough droplets by the wind over relatively long distances. Such indirect transmission had been strongly suspected for contagious bovine pleuropneumonia, another pulmonary mycoplasma disease affecting cattle [28]. Much longer aerosol transmissions, 5-10 km, were experimentally recorded for *Mycoplasma hyopneumoniae* [29]. In addition, the rain that occurred just before the outbreak had induced a peak of ammonia production from the dampened dejections, which may have acted as predisposing factor. Climatic factors have often been associated to the onset of CCPP outbreaks. In North Africa, CCPP appeared more often in the winter [30][31]; in Oman, CCPP outbreaks seemed to be more frequent in January, when the lowest temperatures and highest pluviometry are recorded, and in July, when the highest temperatures are registered [32]. A long distance transmission was confirmed during this outbreak, since CCPP spread from one group of pens to another, separated by up to 80m, in spite of the biosecurity measures implemented. However, the pens located in the Northern part of the compound (400m away from the infected pens) were never affected.

The MLSA type of the Mccp strain isolated from the gazelles was closely related to the genetic types of strains isolated in Qatar and East Africa [15]. Unfortunately, no information regarding Mccp strains circulating in neighboring herds was available to establish a possible epidemiological link.

303 The use of tylosin in drinking water had apparently no effect on the course of the disease, though this
304 and other macrolides are considered active against mycoplasmas. Tylosin is usually administered
305 parenterally, which was considered completely impractical for a collection of wild ungulates, but it is
306 also administered orally to calves at a dose of 10-20 mg/kg BW twice daily for 7 to 14 days
307 (www.biovet.com/products). Many factors could explain the failure of this initial treatment. First, the
308 quality of the antibiotics commercially available is not routinely and independently controlled by a
309 regulatory body of inspection. The way the tylosin powder was dissolved into the 5000 liter water tanks
310 may not have ensured a homogeneous distribution of to all animals. Besides, tylosin tartrate is a weak
311 base with a pKa value of 7.1 [33]. The low pH adult ruminant stomach may not favor its biodisponibility,
312 which has been shown to be quite low (25%) in poultry [34]. All these factors put together, it is very
313 unlikely that the animals received an appropriate dose ensuring a clinical cure. On the other hand,
314 oxytetracyclin mixed with the food pellets yielded better results, as the mortality decreased very rapidly
315 following this treatment. In some pens the treatment had to be repeated after 30-40 days, which indicated
316 that Mccp was still circulating in the gazelle herd after the initial treatment.

317 None of the CCPP vaccines that were commercially available in the UAE at the time of the
318 outbreak seemed to be effective. In fact, they were used in an emergency situation that was far from
319 ideal and the clinical observations were not sufficient to prove their lack of immunogenicity. However,
320 the suspicion that they were not really protective was confirmed by serological analyses: the three
321 commercial CCPP vaccines did not induce any detectable seroconversion by cELISA, while the
322 reference control vaccine did, as expected [35]. The absence of seroconversion using a monoclonal
323 antibody (MAb)-based assay may have been attributed to a modification of the MAb-recognized epitope
324 during the vaccine production process. This possibility was ruled out by the use of an indirect ELISA,
325 which confirmed the absence of seroconversion induced by the commercial vaccines. Such results are
326 of great concern for the protection of CCPP susceptible wildlife, but also for the subsistence of
327 commercial goat herds, which use vaccines that are neither cheap nor effective.

328 To our knowledge, this is the first time that a SEIRD mathematical model is developed to describe the
329 CCPP transmission dynamics and that model parameters are calibrated with observed data. The basic

330 reproductive number R_0 was established between 2.3 and 2.7, with an average case fatality rate of around
331 60%. These values pertain to the epidemiological situation of the gazelle herd under study and they
332 represent some kind of "maximum value" for CCPP, as the animals were permanently in very close
333 contact. In practical terms, it means that CCPP is not very contagious but that an infected animal has a
334 high probability to dy in the absence of antibiotic treatment. By comparison, R_0 values are much higher
335 for viral diseases such as foot and mouth disease (R_0 around 4.5 [36]) or peste des petits ruminants (Rt
336 certainly superior to 4 [37]). Determining R_0 makes it possible to define the percentage of animals (q)
337 that should be protected to prevent the dissemination of the disease by using the relation q=1- (1/R0). In
338 the case of CCPP, q should be around 64% to stop the epizootic spread, if we assume that the CCPP
339 vaccines are 100% efficient. Obviously, the commercial vaccines tested here did not induce such level
340 of protection. Another efficient way to stop disease transmission is to reduce the contact rate β . This
341 may be achieved by various means including the culling of symptomatic animals, their isolation in
342 quarantine, or even their treatment with appropriate antibiotics to reduce mycoplasma shedding. All
343 these measures can naturally complement the effect of vaccination and lead to a $R_0<1$, which would lead
344 to the extinction of the epizootic disease situation.

345 In conclusion, it has been shown that CCPP may be transmitted at a distance of more than 50-80m and
346 that swift action must be taken to prevent dramatic losses, especially in flocks of endangered species
347 kept in captivity. The most efficient strategy may be to vaccinate the animals with quality-assured CCPP
348 vaccines injected simultaneously with an appropriate effective antibiotic treatment, as previously shown
349 [38]. We strongly recommend that CCPP vaccine batches be validated by cELISA showing a clear
350 seroconversion in vaccinated animals. Affected groups of animals can quite easily be treated with
351 antibiotics by mixing tetracyclin to feed pellets. However such treatments may lead to the emergence of
352 antimicrobial resistance, not only in the mycoplasmas but also in the rest of the bacterial flora.
353 Preventive vaccination should always be preferred to antibiotic treatment, which should be restricted to
354 emergency situations. This can only be advocated when good quality vaccines are available
355 commercially, which did not seem to be the case in 2013.

From a practical point of view CCPP-free countries should pay a close attention to animals imported in zoos, as these may represent a risk of CCPP introduction. Further studies may be needed to establish if the increased detection of CCPP in wildlife results from raised awareness and improved diagnostic capabilities or if it is the result of an evolution of Mccp strains and a host switch, similar to what has been observed for *M. gallisepticum* in finches [39].

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LL performed the field work, designed the study and experiments, LMS, AP, FT performed laboratory analysis, LL and FT analyzed the data, CS performed the statistical analysis, AA develop the model, LL, ALC and FT wrote the manuscript draft. All authors contributed to the manuscript. All authors read and approved the final manuscript.

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Ethics approval

The herd of gazelles belongs to the governmental entity Environment Agency-Abu Dhabi.

379 Figures and tables legend

380

381 **Figure 1: Map of the compound**

382 Each pen has a size of 100x200m. Groups of 4 pens are separated from each other and from the perimeter
383 fence by at least 50m. A private sheep and goat farm is located 270m away from the compound in a
384 South-Southeast direction, coinciding with the predominant wind direction.

385 **Figure 2: SEIRD compartmental model for CCPP**

386 The model comprises 5 compartments, namely, susceptible (S), exposed (E= infected but not infectious),
387 infectious (I), recovered (R) and infection-related dead animals (D). Bêta (β) represents the probability,
388 by unit of time, that a susceptible animal becomes infected and switches to the exposed compartment.
389 Exposed animals then spend τ_E days in a latent stage before becoming infectious, and remain τ_I days in
390 this stage. At the end of the infection stage, animals either recover, with a probability "1-p", or die, with
391 probability "p", which represents the case fatality rate.

392 **Figure 3: Cumulative death rate and interventions in the various pens**

393 This graph represents the cumulated death rate in each pen according to the number of days after the
394 initial identification of CCPP losses. All causes of death contribute and the data from a pen unaffected
395 by CCPP provides a reference for this background death rate. For convenience,, the data from the pens
396 that were grouped at day 70 were cumulated. The date and type of interventions in the pens are indicated
397 by arrows pointing towards the respective curves.

398 **Figure 4: serological results after vaccination of naïve sand gazelles**

399 A: Mean percentage of inhibition (PI) values obtained by CCPP competition ELISA on gazelles
400 vaccinated either with commercial vaccines or with a reference vaccine. Bars indicate the minimum and
401 maximum values obtained. The PI obtained on the unvaccinated, negative control remained very low
402 (<35) throughout the assay and did not significantly differ from the results obtained with the commercial

403 vaccine. The PI values obtained on the positive control group rose sharply after a single injection and
404 remained above the 55 PI cut-off (threshold established for goats) for more than 3 months.

405 B: Indirect CCPP ELISA results, expressed in optical density (OD), with sera collected one month after
406 the first vaccine injection. The names of the vaccines were Pulmovac (PUL); Caprivax (CAP-1 and
407 CAP-2) reference batch produced by CIRAD according to the manual of standards of the OIE (OIE).
408 The reference OIE vaccine batch induced a significant and strong sero-conversion. Pulmovac induced a
409 significant but very slight sero-conversion while Caprivax did not induce any seroconversion.

410 **Figure 5: Comparison of the MCMC model output with observed data.**

411 The cumulative number of deaths in pen II.2 is represented by the black dots. The red line is the mean
412 value predicted by the model, the dashed red line is the median, and the shaded areas correspond to 50%
413 and 95% confidence Intervals.

414

415

416

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506

Additional files

Additional Table 1: Antibiotic treatments and vaccination schedule

Sequence of interventions in the compound: dates, time elapsed since the beginning of the outbreak, type of intervention (vaccine or antibiotic treatment) and corresponding groups and pens.

Additional Table 2: Experimental vaccination trial

The experimental scheme comprised five groups of about 20 animals including an unvaccinated, negative control (A), a positive control vaccinated once with a vaccine produced according to OIE standards (C), and three groups vaccinated with commercial vaccines from VETAL and KEVEVAPI (with booster vaccination either one or three months after the first vaccination either with VETAL, KEVEVAPI or JOVAC).

Additional Table 3: Parameter estimates for the SEIRD model after the calibration procedure and R_0 estimates.

Additional Table 4: Comparison of three "SEIRD" models

Deviance Information Criterion (DIC) was used to compare three "SEIRD" models. A lower DIC indicates the quality of the fit procedure. In this case, the simplest model gave the lower DIC value. R_0 and p are the parameter estimates according to each model.

Additional Figure 1: **graphical representation of the disease spread**

The cumulative mortality is represented by a color evolution from green (<6%) to red (70%). The states of the pens are represented at 3 weeks interval. This representation shows the extension of the disease from pen II.2 towards neighboring pens and, then, to other groups of pens, irrespective of their localization.

Figure 1-Map of the compound

Click here to download Figure Figure 1-V08-Enclosures Map CCPP Gazelles.jpg

Figure 2-SEIRD compartmental model for CCPP

Click here to download Figure Figure 2-V08-SEIR Model.jpg

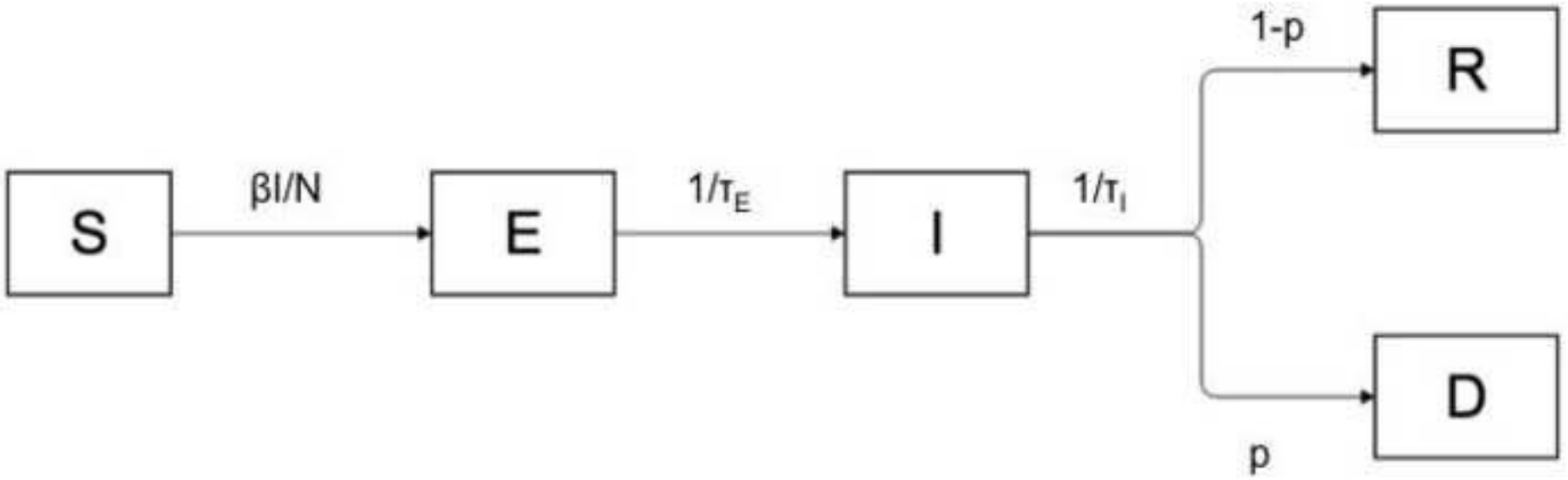


Figure 3-Cumulative death rate and interventions in the various pens

Click here to download Figure Figure 3-V08.2-Percentage mortality and treatments.pdf

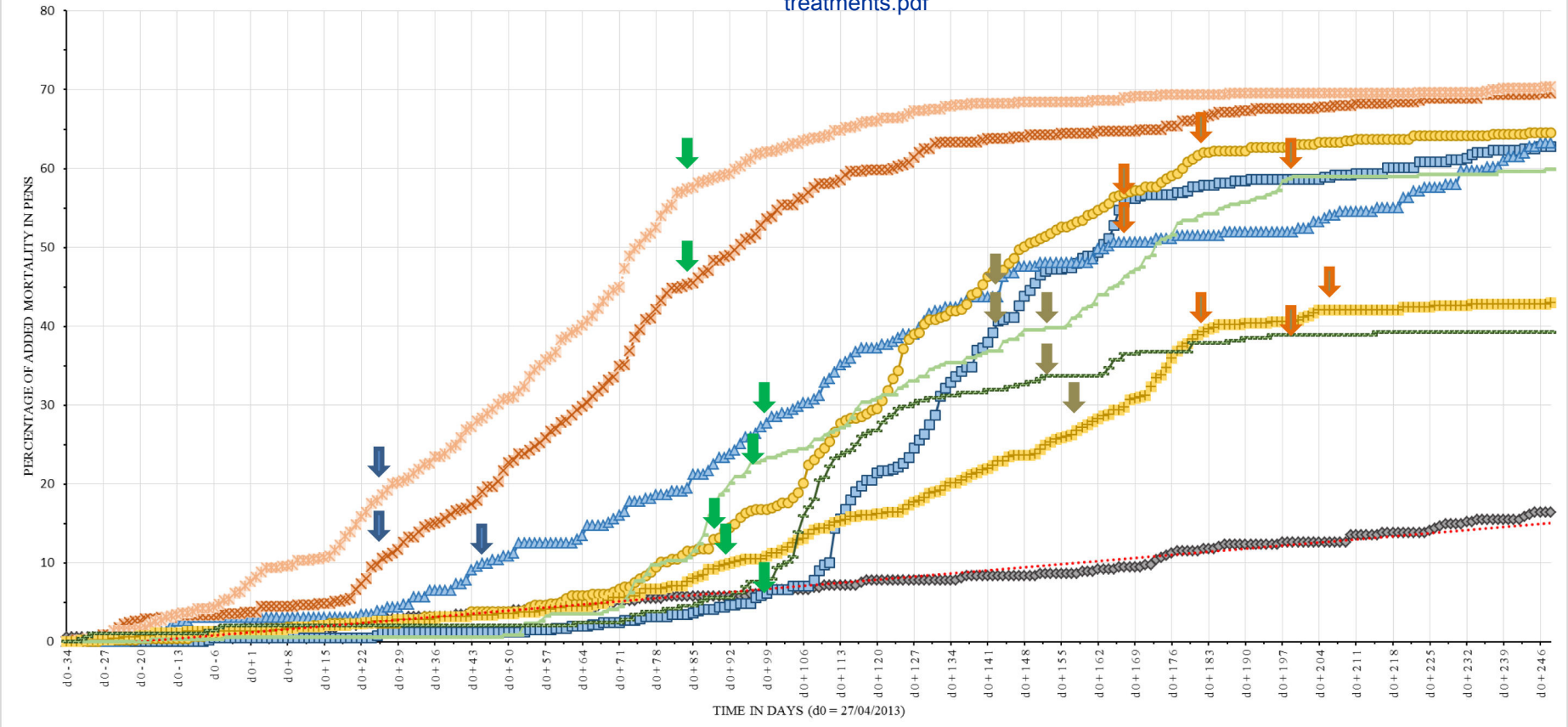
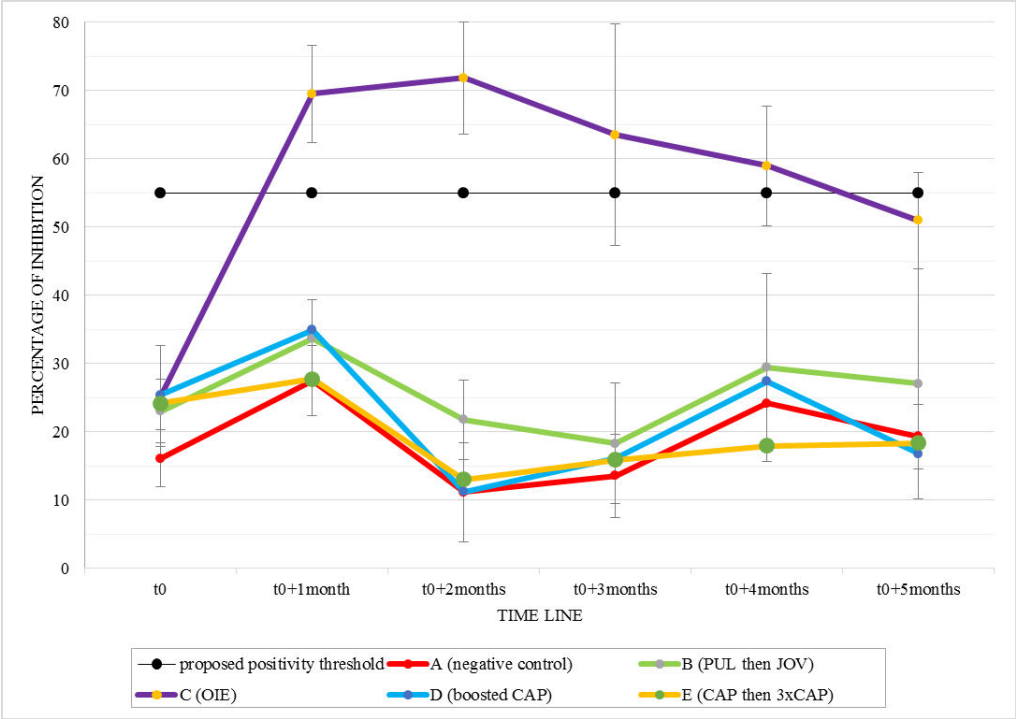


Figure 4A and B-serological results after vaccination of naïve sand gazelles

Click here to download Figure Figure 4-V08-serology.pdf

A



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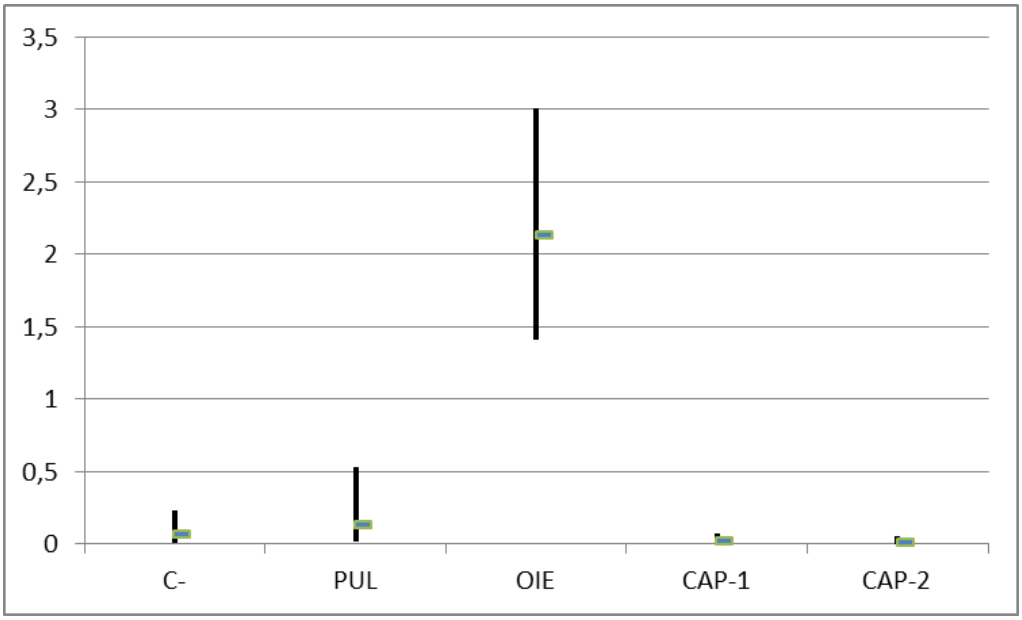

Figure 5- Comparison of the MCMC model output with observed data

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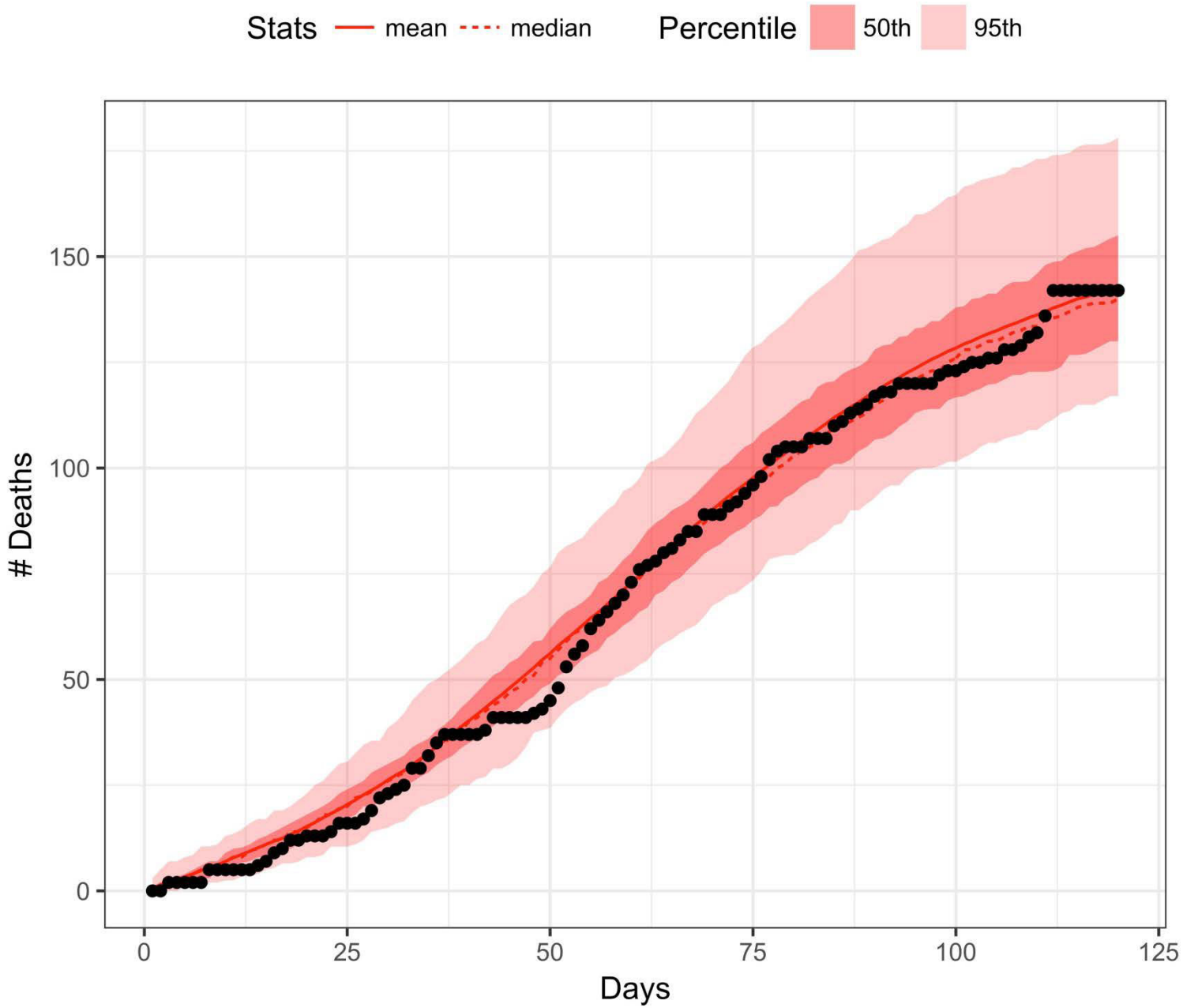

Article 5: Fatal transmission of Contagious Caprine Pleuropneumonia to an Arabian Oryx (*Oryx leucoryx*).

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Short Communication

Fatal transmission of contagious caprine pleuropneumonia to an Arabian oryx (*Oryx leucoryx*)

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ABSTRACT

Contagious caprine pleuropneumonia (CCPP) is an infectious respiratory disease mainly affecting domestic goats. As CCPP has never been documented in grazing antelopes (subfamily hippotraginae), they were not considered susceptible. *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp) was isolated from pleural liquid collected during the necropsy of a severely emaciated Arabian oryx with mild nasal discharge. The Mccp isolate was then genotyped using a multilocus sequence scheme; the sequence type was identical to the Mccp strain previously identified in a sand gazelle from a nearby enclosure. This case shows for the first time that members of the hippotraginae subfamily, here the Arabian oryx, can be affected by CCPP. In addition, genotyping shows that the oryx was most probably infected, at a distance, by sand gazelles.

1. Introduction

Arabian oryx (*Oryx leucoryx*) was considered extinct in the wild in 1972 (Henderson, 1974). This emblematic species recovered part of its territories thanks to re-introduction programs relying heavily on captive stock where veterinary management is crucial. Contagious caprine pleuropneumonia (CCPP) is an infectious respiratory disease mainly affecting domestic goats. CCPP is endemic in the Middle East (World Organization for Animal Health, 2009). In naïve flocks of goats, morbidity and mortality may reach 100% and 80%, respectively (MacOwan and Minette, 1976). The evidence that some wild ungulates are highly susceptible to CCPP is a recent finding (Arif et al., 2007). CCPP has never been documented in the hippotraginae subfamily hence was not considered susceptible. We describe here a fatal case of CCPP in an Arabian oryx (*O. leucoryx*) infected at a distance by neighbouring sand gazelles (*Gazella subgutturosa marica*).

2. Materials and methods

2.1. Study site

A mixed group of 14 Arabian oryx was kept among a large collection of local gazelles in the United Arab Emirates. The studied population was housed within 4 side-by-side enclosures (Fig. 1) that were separated from

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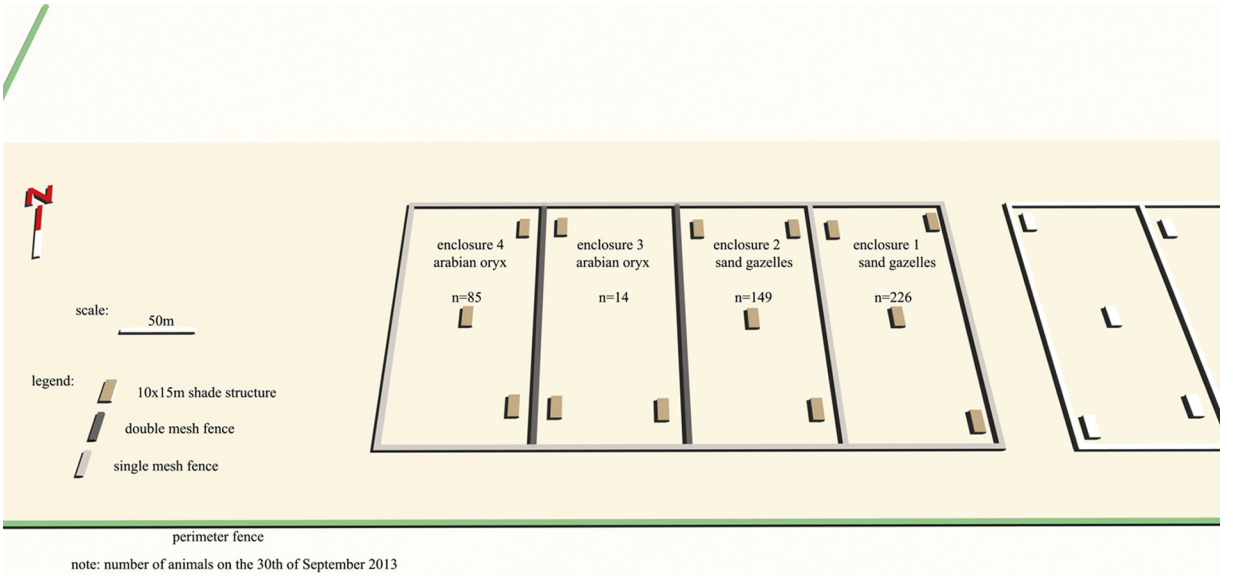

Fig. 1. Details of the animal housing facility.

each other by a double chain link fence with a mesh size of 5 cm. Oryx and gazelles' fences were spaced from one another by 8 cm. No animals were brought in the collection within a year prior to this case.

An outbreak of contagious caprine pleuropneumonia (CCPP) was identified the 15th of June in the adjacent sand gazelle population (enclosure 1), followed by enclosure 2 the 28th of June. The outbreak in the enclosure contiguous to the Oryx (enclosure 2) claimed a total mortality of 34.3%, with a peak the third week of August and a weekly mortality approaching 14.7%. It was controlled only in October after a therapeutical approach initiated months before and involving drastic reduction of gazelle density, mass vaccination and use of oxytetracycline.

2.2. Clinical history

On September the 30th a female adult Arabian oryx showed signs of general depression in enclosure 3. It was emaciated, recumbent and was reluctant to move. A mild sero-haemorrhagic bilateral nasal discharge was observed. Thoracic auscultation revealed unilateral crackling respiratory sounds. No treatments were administered and the animal was euthanized on welfare grounds. No other oryx was affected.

2.3. Post-mortem examination and sample collection

The post-mortem examination revealed unilateral pleuropneumonia characterised by localised pleurisy on both pleurae (Fig. 2A) with profuse pleural fluid, yellowish fibrin deposits and severe consolidation of apical and cardiac lobes of the right lung, associated with pericarditis (Fig. 2A and B). No other gross lesion that may point to concomitant disease was observed. Pleural fluid samples were collected aseptically for pathogen identification.

3. Results

Two samples of pleural fluid from the oryx were initially sent to a local laboratory for bacteriology and *Mycoplasma* genus PCR search, but no pathogens were identified. Due to the ongoing CCPP outbreak in the sand gazelles and to the conspicuous macroscopic lesions, another sample of pleural fluid was sent to CIRAD-CMAEE, OIE/FAO reference laboratory for CCPP. At CIRAD, DNA was extracted using the DNeasy blood and tissue kit (Qiagen) and tested by real-time PCR for the detection of *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) (Lorenzon et al., 2008) providing a positive result. Subsequently, a pure mycoplasma culture was isolated from the same sample after four days of incubation in a modified CCPP medium (World Organization for Animal Health, 2009). The Mccp isolate was then genotyped using a multilocus sequence scheme (Manso-Silván et al., 2011). A new sequence type was identified, which differed by a single nucleotide polymorphism in locus 03 as compared to sequence type 1-010. This sequence type, previously identified in East Africa, but also in Qatar, was identical to the Mccp strain isolated from a sand gazelle in the adjacent pen during the CCPP outbreak giving a very strong indication that CCPP was transmitted from the sand gazelles to the oryx.

4. Discussion

This case shows for the first time that members of the hippotraginae family, here the Arabian oryx, can be affected by CCPP. In addition, genotyping shows that the oryx was most probably contaminated, at a distance, by sand gazelles housed in an adjacent pen separated by a double chain linked fence.

Some wild ungulates have recently been reported to be highly susceptible to CCPP (Arif et al., 2007). It was

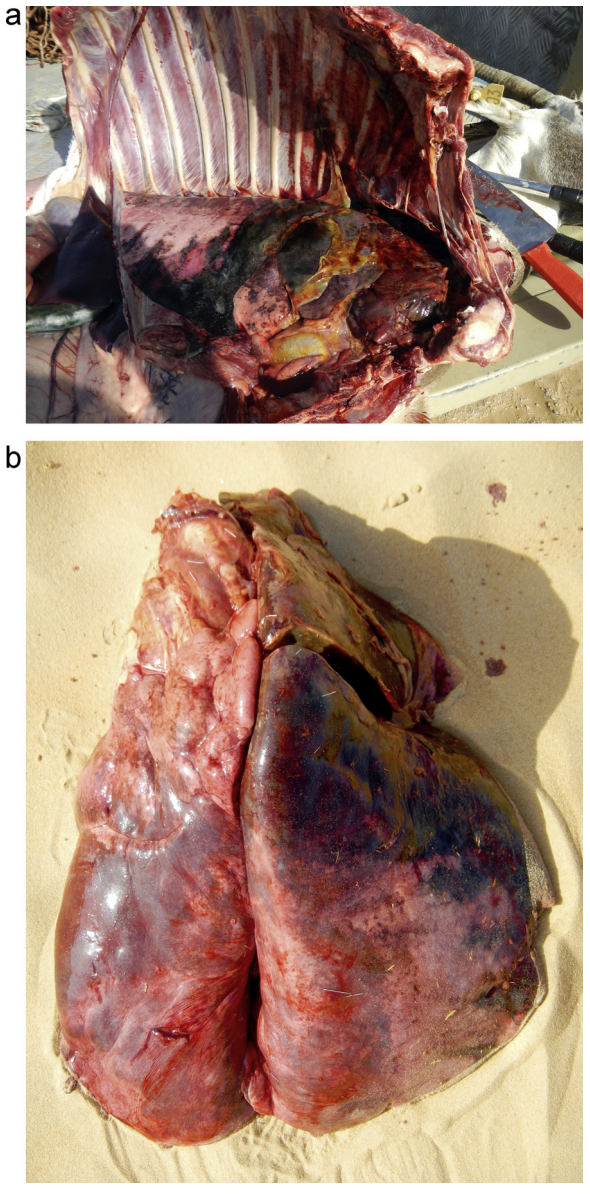

Fig. 2. (A) Thoracic cavity of an Arabian oryx affected by CCPP. (B) Lungs of an Arabian oryx affected by CCPP.

discovered in the caprinae subfamily: Nubian ibex (*Capra ibex nubiana*), Laristan mouflon (*Ovis orientalis laristanica*) (Arif et al., 2007) and Tibetan antelopes (*Pantholops hodgsonii*) (Yu et al., 2013) and was also suspected in the markhors (*Capra falconeri*) (Ostrowski et al., 2011) but could not be confirmed in that species yet. CCPP was also detected in gerenuk (*Litocranius walleri)* (Arif et al., 2007) and in sand gazelles (*Gazella subgutturosa marica*) (Nicholas et al., 2008) both from the antilopinae subfamily. The Arabian oryx must be added to the list of CCPP-susceptible species. Further studies may be needed to determine precisely which species/families are CCPP-sensitive and to understand whether this susceptibility may be caused by a recent evolution of the infectious agent or more likely by increased exposure between infected and naïve, susceptible animals but what is now certain is that CCPP should be considered a real threat to wild ungulates, both in their natural habitat and in captivity. Intensive breeding programs followed by re-introduction plans in the Middle East allowed this emblematic species to recover part of its territories with a total reintroduced population over 1000 animals (International Union for Conservation of Nature and Natural Resources, 2011). Re-introduction of animals into the wild relies heavily on captive stocks where genetic and veterinary management are crucial. The last Middle East Arabian oryx disease survey (Lignereux and Al Kharusi, 2013) revealed that 94% of the Arabian oryx collections surveyed in the region are in direct contact with other ungulate species and may therefore be threatened by CCPP. For animals kept in captivity, the risk may be linked to animal movements between zoos or reserves. Such movements may even cause an introduction into CCPP-free countries. This risk calls for renewed efforts directed to a better detection and control of CCPP.

Although mycoplasmas are theoretically very fragile wall-less bacteria, this CCPP case shows that "at a distance" transmission is possible even in the Emirates' environment. In September air temperature ranges from 28 °C at night to 42 °C during the day. No precipitation was recorded in September 2013 Although gazelles and oryx, especially males, tend to rub their horns on the mesh and will occasionally travel along the fence, nose-to-nose contact was impossible due to the behavioural habits of these species and, most importantly, to the presence of the double fence. During this case, CCPP did not affect the 13 other oryx that were housed in the same pen and no clinical signs were detected within the seven months following this case. The low animal density in the enclosure may have reduced the transmission risk, notably since the affected animal was reluctant to move and did not mix with the others.

Since Mccp isolation is very fastidious (Nicholas and Churchward, 2011) PCR and real-time PCR methods are the preferred detection techniques being both rapid and specific. Local diagnostic laboratories should have the capability to perform these tests and should be structured as part of an emergency prevention system for CCPP in the region.

Prevention of CCPP introduction in ungulate collections must rely on the existence of buffer zones around pens housing susceptible animals and on the application of strict biosafety measures. These must include quarantine procedures prior to introducing any domestic or wild ruminants to a given population (bio-exclusion) (Saegerman et al., 2012). In addition, vaccination of susceptible animals must be considered, as it may prove the best strategy to reduce the contamination risk. CCPP vaccines are currently based on saponin-adjuvanted inactivated Mccp antigen (Rurangirwa et al., 1991). Correctly vaccinated animals will develop an antibody rise that can be monitored using a specific competition ELISA (Peyraud et al., 2014) hence verifying the vaccine was appropriate and the animal responded adequately.

Increased surveillance and control strategies will be required to limit the expansion of the disease in wild

ungulate species, preventing its introduction to CCPP-free countries and avoiding losses in endangered species.

Disclaimer

The content of this article is the sole responsibility of the authors. The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official position of any agency, group or organization.

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Preamble: Zoning has been widely used in southern Africa and countries in the Arabian Gulf to segregate wild ungulates from livestock or "contaminated" versus "disease-free" areas. In the UAE, our studies on Q fever and *Brucella melitensis* suggest livestock has contaminated wildlife or vice versa even through walls and fences. Our serosurvey and Multiple-Locus Variable number tandem repeat Analysis (MLVA) results point out the need for a comprehensive multi-species (W-L) approach to disease monitoring. In Botswana, the recent increase in trans-frontier wildlife protected areas and associated wildlife corridors make any information on pathogen prevalence and transmission among wildlife species all the more important. Our aim was to investigate the seroprevalence of various viral pathogens among four co-occurring large carnivore species: lion (*Panthera leo)*, spotted hyena (*Crocuta crocuta)*, leopard (*Panthera pardus)* and cheetah (*Acinonyx jubatus)*. The differences in seroprevalence between individuals that did or did not come into contact with human activities were emphasized. Disease and pathogen prevalence and molecular epidemiology should be considered and included in the conservation and management plans for these areas but the usefulness and effectiveness of zoning and segregation should take into consideration their effectiveness and their environmental cost.

Article 6: A Serological Survey of Q-fever in Semi-Wild Ungulates in the Emirate of Dubai, United Arab Emirates (UAE).

Original article – *Journal of Wildlife Diseases* – 2012 - *48*(1), pp.220-222

Journal of Wildlife Diseases, 48(1), 2012, pp. 220–222

A Serologic Survey for *Coxiella burnetii* in Semi-wild Ungulates in the Emirate of Dubai, United Arab Emirates

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ABSTRACT: Q fever, a highly infectious zoonotic disease caused by *Coxiella burnetii*, has not been officially reported in the United Arab Emirates (UAE). This first serosurvey of a large group of semi-free-ranging animals in the UAE indicates that a wide range of ungulates have been exposed *C. burnettii* in the region.

Q fever, for query fever, is a highly infectious disease caused by the obligate intracellular bacterium *Coxiella burnetii.* Exposure to a single organism is sufficient for transmission (McQuiston et al., 2002). The bacterium is stable in the environment for long periods. Aerosols remaining infective for up to 2 wk, and soil can be contaminated for up to 5 months (Welsh et al., 1958). *Coxiella burnetii* is a ubiquitous organism and a range of hosts are infected asymptomatically (Babudieri, 1959) becoming potential reservoirs. We assessed the prevalence of *C. burnetii* in semi-free-ranging Bovidae of 10 species (Table 1) in Dubai, 2005–2008.

From February 2005 to December 2008, 333 semi-free-ranging exotic ungulates maintained in five private zoological collections in the United Arab Emirates (UAE) were randomly selected and blood sampled. Serologic tests were used to detect antibodies to *C. burnetii.* Blood samples were randomly collected from every third animal physically restrained during annual health checks at the collections and at the two adjacent farms that were <2 km from the wild animal collections perimeter. No samples were taken from animals with signs of sickness. Analysis of serum was performed at the Central Veterinary Research Laboratory (CVRL), Dubai. As recommended for screening by the World Organization for Animal Health (OIE), the CHEKIT Q-Fever enzyme-linked immunosorbent assay (ELISA) indirect test kit (CHEKIT; IDEXX Laboratories, Bern, Switzerland) was used to detect serum antibodies against *C. burnetii*. This technique uses microtiter plates coated with *C. burnetii* antigen and a horseradish peroxidase labeled anti-ruminant IgG conjugate monoclonal antibody. This test is recommended by the manufacturer for use in nondomestic ruminants. This CHEKIT assay has not been tested on all of the species sampled during this survey, but has a general specificity for ruminants. These ready-to-use kits are commercially available and can detect anti-phase II antibodies or both anti-phase I and II antibodies. For commercial kits, interpretations and values are provided with the kit. Results were expressed as a percentage of the optical density of the test sample (value). Sera were considered to be ELISA-positive if they had a value of ≥40%, suspect if the value was 30% –40%, and negative if the value was <30%.

Twenty of 333 (6%) semi-free-ranging wild ruminants tested for antibodies for *C. burnetii* were ELISA-positive and 0.9% (three out 333) had borderline values (Table 1). Seventy percent of the livestock tested in the vicinity of two of the sites were antibody-positive (46 of 65 sheep [*Ovis aries*] and goats [*Capra aegagrus*]). Virtually all species tested had at least one positive individual, and positive animals were distributed over all five study sites. This is the first published study on the Q-

TABLE 1. *Coxiella Burnetii* antibody results using the CHEKIT Q-Fever enzyme linked immunosorbent assay (ELISA) indirect test kit (CHEKIT; IDEXX Laboratories, Bern, Switzerland) on 333 serum samples from 10 species of semi-free-ranging wild ungulates in the Emirate of Dubai.

Species	Total sera	Suspect sera	Positive sera
Arabian oryx (*Oryx leucoryx*)	170	2	7
Sand gazelle (*Gazella leptoceros*)	6	0	2
Impala (*Aepyceros melampus*)	7	1	0
Black buck (*Antilope cervicapra*)	36	0	2
Laristan sheep (*Ovis laristanica*)	3	0	0
Speke's gazelle (*Gazella spekei*)	70	0	6
Dorcas gazelle (*Gazella dorcas*)	3	0	1
Mountain gazelle (*Gazella gazella*)	3	0	0
Grant's gazelle (*Nanger granti*)	15	0	1
Lesser kudu (*Tragelaphus imberbis*)	20	0	1
TOTAL	333	3	20

fever status of a large group of semi-free-ranging ruminants in the Middle East. Our positive results indicate that these semi-wild populations have been exposed to *C. burnetii*. Our positive results contrast with the absence of reported Q fever in the farm animal population in Dubai (WAHID, 2008). Nevertheless, outbreaks may not have been reported.

Seroconversion is an indirect indicator of infection or immunization and indicates exposure to *C. burnetii*. With the exception of one clinical report (Lloyd et al., 2010) in dama gazelles (*Gazella dama*), clinical signs have not been reported in the non-domestic ungulates. There is an inconsistent relationship between serologic status and *C. burnetii* shedding in nondomestic and domestic species. Seroconversion only proves that the pathogen is present in the herd. Both shedding routes and patterns vary. As part of the disease risk management, it is highly recommended to make every attempt to detect potential shedders, especially for animals from collections who are part of exchange and reintroduction programs. Potential shedders in wild semi-free-ranging populations under field conditions could theoretically be detected through intense monitoring by collecting freshly voided feces and using polymerase chain reaction (PCR) or real time PCR assay on these samples (Guatteo, 2006).

In these collections, no tests have been implemented to screen animals and detect potential asymptomatic carriers. Given the extremely high resistance of the pathogen in the environment, the difficulty of finding positive animals and the potential presence of asymptomatic carriers, once established in a semi-free-living population, it may be impossible to eradicate a *C. burnetii* infection.

The route of transmission of *C. burnetii* to these Arabian oryx (*Oryx leucoryx*), sand gazelle (*Gazella leptoceros*), impala (*Aepyceros melampus*), black buck (*Antilope cervicapra*), Laristan sheep (*Ovis laristanica*), Speke's gazelle (*Gazella spekei*), Dorcas gazelle (*Gazella dorcas*), mountain gazelle (*Gazella gazella*), Grant's gazelle (*Nanger granti*) and lesser kudu (*Tragelaphus imberbis*) herds remains unclear. The food given to the animals may represent a potential vector for spore-like "small cell variant" of the pathogen. Additional wildlife and domestic animals are often imported into the UAE from neighboring countries. Many animals bypass veterinary inspections and quarantine and it is possible that Q fever could be introduced with these animals. Camel (*Camelus dromedarius*) movement through the parks is another possible source of transmission. The CVRL, the reference laboratory in the UAE, estimates that up to 40% of camels are antibody-positive in the country (U. Wernery, pers. comm.). The

pathogen is shed by infected individuals in body fluids (especially aborted fetuses, placenta and fetal fluids), spread over ground and vegetation and might then be moved by vectors such as ticks, rodents, cats or humans. Temperatures in the region range from 12.7 C to 48.4 C (O'Donovan, 2005) but will not affect the persistence of the *C. burnetii* which is able to form spore-like "small cell variants". Winds are constant throughout the year with an average speed of 5.9 km/hr in 2008 (O'Donovan, pers. comm.) and are likely to spread this pathogen (Tissot-Dupont, 1999). It is highly possible that the agent is transmitted by herds of sheep and goats housed close to all the areas where wildlife is kept.

Specific seroconversion of domestic and nondomestic ruminants tested during this study indicates exposure to the *C. burnetii*. It is not possible to prove that *C. burnetii* has a deleterious effect on these populations. Nevertheless, transfer of animals between collections should be undertaken with care. Clinicians working with nondomestic ungulates should be aware that clinical cases of Q fever have been reported within the UAE in dama gazelles (Loyd et al., 2010) and should remain vigilant to this disease which is probably under-diagnosed and under-reported in the region.

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Article 7*: Brucella melitensis* at the wildlife-livestock-human interface in the UAE.

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Molecular epidemiology of *Brucella melitensis* at the wildlife-livestock-human interface in the United-Arab-Emirates.

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Key words:

Brucella melitensis, wildlife-livestock-human interface, MLVA

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Abstract

Once roaming free in the immense Sahara and Sahel, the scimitar-horned oryx (SHO) (*Oryx dammah*) is now extinct in the wild and the survival of this emblematic large desert antelope through conservation programmes relies heavily on captive stocks disseminated around the globe for possible future re-introductions. Several thousand SHO are bred in

animal collections across the UAE where brucellosis is a widespread enzooty and a public health concern with numerous reported human cases. The situation in wildlife species is close to unknown and little epidemiological data are available on *Brucella* species and biovars circulating in this country.

In this study we investigate the *brucella* seroprevalence within a large population of SHO and try to trace the source of infection using Multi Locus Variability Analysis (MLVA).

Introduction

The scimitar-horned oryx is a large desert antelope that formerly inhabited large areas of the Sahara and Sahel ranging from Mauritania to Egypt. Due to extensive hunting, habitat loss and competition with domestic cattle, the SHO became extinct in the wild in 2000 ([1]). Global conservation effort relies heavily on captive stocks for possible re-introduction. Such programs involve conducting wildlife disease risk analysis ([2]) and the prevention of alien disease introduction to the recipient area is probably the single most important responsibility for decision-makers ([3]).

Brucellosis is recognized as a major cause of heavy economic losses to the livestock industry and poses serious human health hazard ([4]). It remains one of the most common zoonotic diseases worldwide with more than 500,000 human cases reported annually ([5]). The disease is enzootic in the Middle East where it has been reported in almost all domestic farmed animal species and especially in goats and camels ([6]).

The situation in the UAE has been documented in the human population: both *B.melitensis* biovar 1 and 3 were reported on human cases from Tawam Hospital, Al Ain (Abu Dhabi emirate) hence were co-circulating in the country in 1996 ([7]). From 2000 to 2003, 6.5 % of the 998 patients admitted in this same hospital were *Brucella* seropositive ([8]). In the livestock industry, 55.1% of the 267 domestic farms sampled in the emirate of Abu Dhabi

in 2010 were seropositive ([9]) and the Central Veterinary Research Laboratory in Dubai revealed culturing the bacteria in 12% of the 132 raw cow milk samples received in 2014 ([10]). The wildlife situation with regard to brucellosis seems poorly studied, *B. melitensis* has been reported only once in Dubai in a Nubian Ibex (*Capra ibex nubiana*) ([11]). The UAE hold large captive population of local and exotic ungulate species, with some collections counting more than 30 000 heads in one location.

In this study we investigate the *brucella* seroprevalence within a large population of scimitar horned oryx (SHO) (*Oryx dammah)* located in Abu Dhabi Emirate and try to trace the source of infection using genotyping techniques.

Material and methods

Studied Population

In 2008, an isolated and fully secluded captive collection of 15 000 wild ungulates has been transferred to a new location. Although veterinary reports from that time are not available, those animal populations were said to be free of brucellosis or with low prevalence. From the 15 000 wild ungulates, 4,000 SHO are housed in pens and 2 km away from a group of 400 sand gazelles.

Serology

SHO were individually captured and physically restrained using a chute system (Tamer®, Fauna Reseach Inc. USA), allowing to draw blood and put a visual identification system (ear tags). Gazelles were captured using a net system. Rose Bengal Test (RBT)

(Bengatest® Synbiotics, Lyon, France) was performed as described in the OIE manual ([14]). In one occasion, serum obtained from cardiac blood from 2 aborted foetus was also screened using the same RBT.

In addition, i-ELISA tests were also performed. ELISA plates (650101, Greiner Bio-One, Austria) were coated with 100µl s-LPS from *B. abortus w99* at a final concentration of 1µg/ml in GS buffer (1M glycine, 1.7M NaCl pH9.2) for 3 h at 37°C and overnight at 4°C. Samples were diluted 1/50 in GS-EDTA-TW buffer (GS buffer 1/10, 50mM EDTA, 0.1% Tween80, pH 9.2) and incubated for 1h at room temperature. After a washing cycle, the antibody-antigen interaction was detected by the protein G-horseradish peroxidase conjugate (Biorad, Belgium). After an incubation of 1h at room temperature and a washing cycle, the peroxidase activity was visualized by a citrate-phosphate buffer containing 0.4% of o-phenylenediamine and 2mM of H2O2. Optical densities were measured by the difference of 490nm and 620nm absorbance on a iMark Microplate Absorbance Reader (Biorad, Belgium).

A standard curve of 6 dilutions from 1/270 to 1/8640 of the national reference sera 1121 in GS-EDTA-TW buffer (GS buffer 1/10, 50mM EDTA, 0.1% Tween80, pH 9.2) auditioned with 2% foetal calf serum were applied in each plates. The cut-off was determined as the mean of 1/8640 dilutions of the standard curve.

Bacteriology

Fourteen randomly selected samples collected from animals in different pens and consisting in different organs: oryx foetal spleen, lung, or stomach content, or gazelles articular fluid were sent under controlled temperature for culture and genotyping using Multiple-Locus Variability Analysis (MLVA) at CODA-CERVA- Belgium ([15]).The

inoculated plates were incubated at 37°C aerobically in an atmosphere of 5 to 10 % carbon dioxide, and examined after seven days for *Brucella*-like colonies. A subculture from plates with colonies typical of *Brucella* was made on glucose agar prepared from 40 mg heart infusion agar and 20 mg D-glucose. Plates without any evident growth were discarded. The colonies were identified and biotyped using the classical procedures described by Alton et al. ([16]) and based onoxidase and catalase production, agglutination with monospecific anti-A and anti-M sera, carbon dioxide requirement, hydrogen sulphide and urease production, and dye sensitivity by culture in the presence of thionin, basic fuchsin and safranin.

Genotyping

Genotyping analysis was performed by MLVA using 15 variable number tandem repeats (VNTRs) described by Le Flèche et al. ([17]) and Bricker et al. ([18]). Fifteen VNTRs divided in three groups or panels were investigated. Panel 1 groups eight minisatellites (bruce06, bruce08, bruce11, bruce12, bruce42, bruce43, bruce45, bruce55), panel 2A groups two microsatellites (bruce18, bruce21) and panel 2B groups five microsatellites (bruce04, bruce07, bruce09, bruce16, bruce30). The 15 markers were amplified for one isolate per animal, as previously described ([17]). Band sizes of tandem repeat units longer than 600pb (bruce06, bruce11, bruce42 and bruce55) were analyzed on a 2% agarose gel. The remaining PCR products were analyzed by capillary electrophoresis in a CEQ 8000 automatic DNA Analysis System (Beckman-Coulter) using a commercial kit (GenomeLab™ DTCS-Quick Start Kit, Beckman-Coulter) according to the manufacturer' instructions. Band size and peak numbers were converted to number of units using the *Brucella melitensis* 16M reference strain (ATCC 23456) typed by Le Flèche et al. ([17]).

Allele profiles were compared in the public data base MLVABank (http://mlva.u-psud.fr/mlvav4/genotyping/index.php).

Results

Clinical examination

External examination might reveal enlarged testicles in male oryx (Figure 1) while gazelles species present mainly hygroma at the tibio-tarsal and metacarpo/tarso-phalangeal joints (Figure 2), leading to reluctance to move, loss of body condition and subsequent death. Accurate data on reproductive parameters and new-born mortality is extremely difficult to obtain in such large collection of animals. It can only be reported that the population is currently growing with apparent limited number of abortions and neonatal deaths. The predominant species in this collection is the Indian Blackbuck (*Antelope cervicapra*) (n=7000). While next to no disease screening has been conducted on this species, no external clinical signs are observed.

Serology

Among the 480 juveniles/sub-adults SHO and the 400 adults SHO tested in different enclosures, 75% (95% CI: 70.9-78.8) and 95% (95% CI: 92.4-96.9) of them were *Brucella* seropositive respectively based on both single RBT and ELISA. Cardiac blood from the two aborted SHO foetus screened was also RBT positive. The 15 gazelles were all seropositive.

Bacteriology

One sample of oryx foetal stomach content and one sample of metacarpal fluid from a gazelle allowed culture and *B.melitensis* biovar 1 was isolated. These two animals were

151 also seropositive.

152 The MLVA profile of this two new isolated strains are identical and fits to three strains
153 previously isolated in 1998 from goats in Dubai (UAE). The MLVA genetic tree shows
154 that strain isolated cluster also with strains isolated from livestock and human in the
155 Middle East ([17,19,20]) (Figure 3 and Table).

156

157 **Discussion**

158

159 Although the specificity and the sensitivity of RBT are yet unknown in SHO, this test is
160 recommended for the screening of sheep and goats for *B.melitensis* infection and for small
161 and large ruminants ([14]). Clinical signs (orchitis, hygromas) and bacterial culture were
162 associated with positive RBT and ELISA. No vaccination was performed in the past and
163 the false positivity due to common lipopolysasaccharide (LPS) from other bacteria species
164 is most likely insignificant. In addition, RBT has proven itself useful at determining the
165 level of infection at a herd level. The extremely high prevalence in the herd of SHO
166 studied here corroborates with previous findings on brucellosis epidemiology: at herd level
167 large flock with high density and cohabitation with other ruminants were significantly
168 associated with a higher odds of being seropositive ([21]). Anthropogenic factors could
169 therefore account with the high observed seroprevalence in captive collections.

170

171 It is interesting to note that the cardiac blood from the aborted SHO foetus screened was
172 also positive which tend to confirm vertical transmission of *Brucella abortus* discussed by
173 Plommet et al. ([22]). Congenital infection is of major epidemiological significance:
174 brucellosis can also be transmitted to the newborn calf immediately after birth ([23]) or when
175 passing through the birth canal, or by suckling colostrum or milk from infected cows. A

proportion of heifers, born from *Brucella*-infected dams, may only reveal their infection at the time of the first calving, often after a very long serologically negative period. Based on standardised experimental infections of unvaccinated animals, this percentage has been estimated at 3.5% (95%, CI: 0.76-10.32%) ([24]). Such animals pose a serious threat to brucellosis control and eradication.

The livestock management system in the UAE is in favour of *Brucella* spreading with the coexistence of several livestock species and possible direct contact between free roaming livestock and wild ungulates. In addition, some traditional farms (called "Ezba") discard carcasses, placentas or aborted foetuses of recently calved livestock in the desert or in the farms' vicinity. Even though climatic factors present in this area (desiccation and exposure to sunlight) work against the survival of *Brucella* organisms ([25]), recent distant transmission via biological (dogs, foxes, raven) ([26,27]) and mechanical vectors such as insects ([28]) is possible.

The MLVA analysis indicates that the origin of the strain isolated in this study was related to the strain isolated previously. The phylogenetic tree shows a link between strains from wildlife to livestock and human (Figure 3). Movement of animals and the creation of new interfaces between livestock and wildlife due to human activity is the most important factor in disease transmission ([29]). "The identification and molecular characterization of prevailing *Brucella* species are a cornerstone to understand the epidemiology of the disease in a region and implement adequate strategies to control this important zoonosis" ([24,30]). In addition MLVA profiles are known to be stable over time and stick to specific areas unless infected animals are being moved from one country to another. Regarding collection of wild animal species, MLVA profile will thus help determining source of infection and can give epidemiological indication to legal or illegal animal movement. Other *brucella* strains

should be isolated and genotyped in the country to allow a better understanding pathogen transmission at the wildlife-livestock-human interface.

Supposedly, oryx and/or gazelles initially acquired brucellosis infection from livestock, and only later did it start circulating among the wildlife collection. Moreover, *Brucella* spp. that has not been genotyped has clinically contaminated 3 ungulate keepers handling contaminated materials in this same collection. All were cured following the World Health Organisation treatment recommendations. Although in most countries brucellosis is a nationally notifiable disease and reportable to the local health authority, it is under reported and official numbers constitute only a fraction of true incidence of the disease ([31]). Thus the true incidence of human brucellosis is unknown and the estimated burden of the disease varies widely, from <0.03 to >160 per 100,000 population ([32,33]).

Cost effective control measures for brucellosis are known, yet there is either a lack of funds and/or political "know how" to implement the measures in many countries. A sensible intersectional collaboration between public health and veterinary sectors based on the concept of 'one medicine' ([34]) would greatly help improving the health status both in animals (wild and domestic) and humans. Control or eradication strategies of brucellosis should begin by establishing the different epidemiological contexts within a country or region.

In heavily infected countries, testing of all susceptible species (wild and domestic) with appropriate techniques and introduction of rational vaccination schemes are key to raise herd/flock immunity and reduce abortion rates. Application of test-and-slaughter programmes could become feasible if and only if flock prevalence levels are lower than 1% ([24]), and that movement control within and between countries is implemented. While

dealing with species extinct into the wild such as SHO, one has to remember that the genetic value of the animal has to be taken into account prior taking any drastic decision.

Introduction of pathogens into previously unexposed wild populations can seriously challenge conservation efforts ([35]). In addition the presence of brucellosis in free-ranging wild ruminant populations is a major health management problem in several countries because of the risk of transmission to livestock species ([36]). Extreme care should thus be taken to reintroduce only animals determined to be free of exotic pathogens.

Conclusion

Brucella melitensis infection is recognized as a significant public health challenge, with a major economic impact and drastic consequences on wildlife either direct or indirect through their unsuitability for release or conservation programmes.

A revised brucellosis control programme should be proposed in the UAE. Given the high baseline prevalence, it should be based on vaccination accompanied by measures to promote hygiene and husbandry practices that minimize the risk of introduction and maintenance of *Brucella* spp., and thereby the risk of human infection.

MLVA typing could be a useful tool to improve brucellosis surveillance and control programs. *Brucella* species are highly monomorphic, with minimal genetic variation among species, hindering the development of reliable subtyping tools for epidemiologic and phylogenetic analyses.

Holistic and ecosystem based approach should be used to tackle *Brucella* transmission and maintenance in heavily contaminated environment. Efforts in identifying species and subtyping of *Brucella* isolates are paramount for any preventive (awareness campaign) and

epidemiologic surveillance-control programme in *Brucella*-endemic area. Active surveillance of susceptible animals and occupational health screening of the workers will give a more accurate picture of the *Brucella* foci in the country and will help defining the appropriate control strategy. In heavily infected countries, mass vaccination programmes seem inevitable but require proficient veterinary services. Implementation of a strategic approach of the enzooty on the scimitar horned oryx will require preliminary studies on diagnosis tests, host/pathogen/environment interactions, specific vaccine assessment and appropriate veterinary protocols.

Legends for figures

Figure 1: Orchitis in scimitar-horned oryx (*Oryx dammah)*.

Figure 2: Hygroma at the tibio-tarsal and metacarpo/tarso-phalangeal joints in gazelle species.

Figure 3: Minimum spanning tree analysis of MLVA profiles from Brucella strains. 15-locus MLVA profiles were determined for 22 strains. Clustering by minimum spanning tree was performed with Bionumerics (MLVA profiles derived from publicly available data). Numbers on the connecting lines refer to the number of markers differing between samples.

Table: MLVA data from *Brucella melitensis* strains.

Figure 1: Orchitis in scimitar-horned oryx (*Oryx dammah)*.

Figure 2: Hygroma at the tibio-tarsal and metacarpo/tarso-phalangeal joints in gazelle species.

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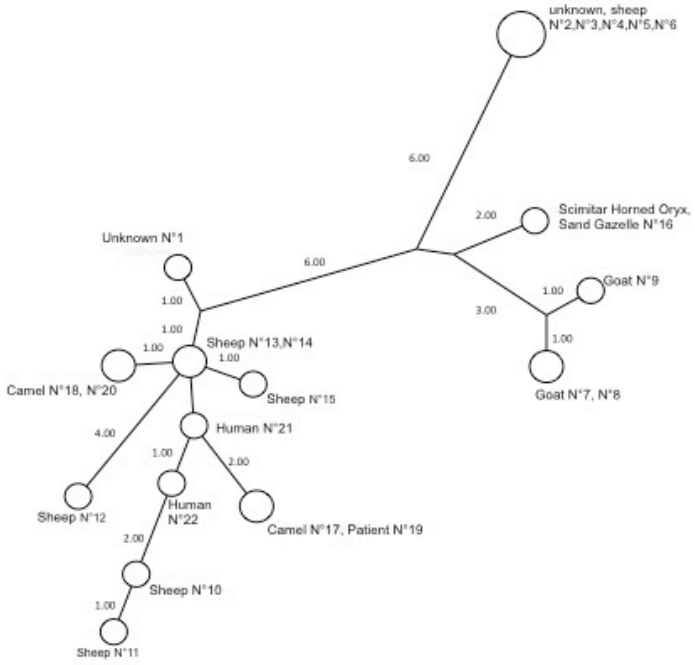

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279 Figure 3: Minimum spanning tree analysis of MLVA profiles from Brucella strains. 15-locus MLVA profiles were determined for 22 strains.

280 Clustering by minimum spanning tree was performed with Bionumerics (MLVA profiles derived from publicly available data). Numbers on

281 the connecting lines refer to the number of markers differing between samples

Biovar	Strain	Reference	ID	Year	Host	Country	bruce06	bruce08	bruce11	bruce12	bruce42	bruce43	bruce45	bruce55	bruce04	bruce07	bruce09	bruce16	bruce18	bruce21	bruce30
b1	BCCN 75-478	14	1	1975	unknown	Israel	1	5	3	13	3	2	3	2	5	5	3	5	4	8	4
b1	BCCN 96-22	14	2	1996	sheep	Israel	3	4	2	13	4	2	3	3	2	4	6	3	8	6	6
b1	BCCN 96-24	14	3	1996	unknown	Israel	3	4	2	13	4	2	3	3	2	4	6	3	8	6	6
b1	BCCN 96-27	14	4	1996	sheep	Israel	3	4	2	13	4	2	3	3	2	4	6	3	8	6	6
b1	BCCN 96-28	14	5	1996	sheep	Israel	3	4	2	13	4	2	3	3	2	4	6	3	8	6	6
b1	BCCN 96-29	14	6	1996	sheep	Israel	3	4	2	13	4	2	3	3	2	4	6	3	8	6	6
b1	BfR23	14	7	2006	goat	U.A.E	3	5	3	12	4	2	3	5	5	4	7	6	7	8	5
b1	BfR25	14	9	2006	goat	U.A.E	3	5	3	12	4	2	3	5	4	4	6	6	7	8	5
b3	BCCN 96-31	14	10	1996	sheep	Israel	1	4	3	13	3	2	3	2	5	4	3	4	4	8	5
b3	BCCN 96-32	14	11	1996	sheep	Israel	1	4	3	13	3	2	3	2	6	4	3	4	4	8	5
b2	BfR50	14	12	1990	sheep	Syria (Aleppo)	1	5	3	13	3	2	3	2	8	4	8	7	4	8	3
b2	BfR49	14	13	1991	sheep	Syria	1	5	3	13	3	2	3	2	4	4	3	5	4	8	4
b2	BfR51	14	14	1990	sheep	Syria (Meskane)	1	5	3	13	3	2	3	2	4	4	3	5	4	8	4
b2	BfR48	14	15	1993	sheep	Syria	1	5	3	13	3	2	3	2	4	4	3	6	4	8	4
b2	Patient	16	17	2009	human	U.A.E	1	5	3	13	2	2	3	2	6	4	3	4	4	8	4
b1	Camel 1	16	18	2009	camel	U.A.E	1	5	3	13	2	2	3	2	4	4	3	5	4	8	4
b2	Camel 2	16	19	2009	camel	U.A.E	1	5	3	13	2	2	3	2	6	4	3	4	4	8	4
b1	Camel 3	16	20	2009	camel	U.A.E	1	5	3	13	2	2	3	2	4	4	3	5	4	8	4
unknown	H13944	17	21	2011	human	Kurdistan	1	5	3	13	3	2	3	2	4	4	3	4	4	8	4
b3	BfR62		22	2007	human	Iraq	1	5	3	13	3	2	3	2	4	4	3	4	4	8	5
b1		this work	16	2013	SHO	U.A.E	3	5	3	12	4	2	3	5	6	4	4	5	8	8	6
b1		this work		2013	Gazelle	U.A.E	3	5	3	12	4	2	3	5	6	4	4	5	8	8	6

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283 Table: MLVA data from *Brucella melitensis* strains.

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Article 8: Serosurvey for selected viral pathogens among sympatric species of the African large predator guild in northern Botswana.

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Serosurvey for Selected Viral Pathogens among Sympatric Species of the African Large Predator Guild in Northern Botswana

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ABSTRACT: The recent increase in the creation of transboundary protected areas and wildlife corridors between them lends importance to information on pathogen prevalence and transmission among wildlife species that will become connected. One such initiative is the Kavango Zambezi Transfrontier Conservation Area of which Botswana's Okavango Delta constitutes a major contribution for wildlife and ecosystems. Between 2008 and 2011, we collected serum samples from 14 lions (*Panthera leo*), four leopards (*Panthera pardus*), 19 spotted hyenas (*Crocuta crocuta*), and six cheetahs (*Acinonyx jubatus*) in the Okavango. Samples were tested for antibodies against canine distemper virus (CDV), feline panleukopenia virus, enteric coronavirus, feline calicivirus, feline herpesvirus (FHV-1), and feline immunodeficiency virus (FIV). Evidence of exposure to all of these pathogens was found, to varying degrees, in at least one of the species sampled. High antibody prevalence (>90%) was only found to FHV-1 and FIV in lions. Only hyenas (26%, 5/19) were positive for CDV antibody. Except for one case, all individuals displayed physical conditions consistent with normal health for ≥12 mo following sampling. Our results emphasize the need for a comprehensive, multispecies approach to disease monitoring and the development of coordinated management strategies for subpopulations likely to be connected in transboundary initiatives.

Key words: Carnivores, conservation and management, Kavango Zambezi Transfrontier Conservation Area, Okavango Delta, pathogen prevalence, transboundary wildlife areas.

In large carnivore conservation, disease ecology has mainly focused on clinical host-pathogen relationships, disease-mediated extinction, and the consequences of human activities and domesticated animals on the introduction and spread of diseases into wildlife populations (Woodroffe 1999; Cleaveland et al. 2007; Alexander and McNutt 2010). Recent studies have focused on cross-species transmission, multihost pathogens, and reservoir infection dynamics (Lembo et al. 2008; Alexander et al. 2010). Our knowledge, however, remains limited on the ecology of pathogen prevalence and transmission in complex, large transboundary ecosystems, where differential ecologic and climatic conditions may further confound epidemiologic scenarios.

The creation of large transboundary parks and wildlife corridors between ecosystems has recently become an integral part of conservation plans (Silveira et al. 2014). A comprehensive understanding of the health of subpopulations that become connected through such initiatives is fundamental for the management of species nationally and internationally. One such initiative is the Kavango Zambezi TransFrontier Conservation Area (KAZA/TFCA) in southern Africa. Despite its unique wildlife and ecosystems and the central role that Botswana's Okavango Delta plays within the KAZA/TFCA scenario, relatively little is known about pathogen transmission and prevalence in its large carnivore species.

We investigated the prevalence of antibody to various viral pathogens among four co-occurring large carnivore species: lion (*Panthera leo*), spotted hyena (*Crocuta crocuta*), leopard (*Panthera pardus*), and cheetah (*Acinonyx jubatus*). We emphasize differences in antibody prevalence between individuals that did or did not come into contact with human activities.

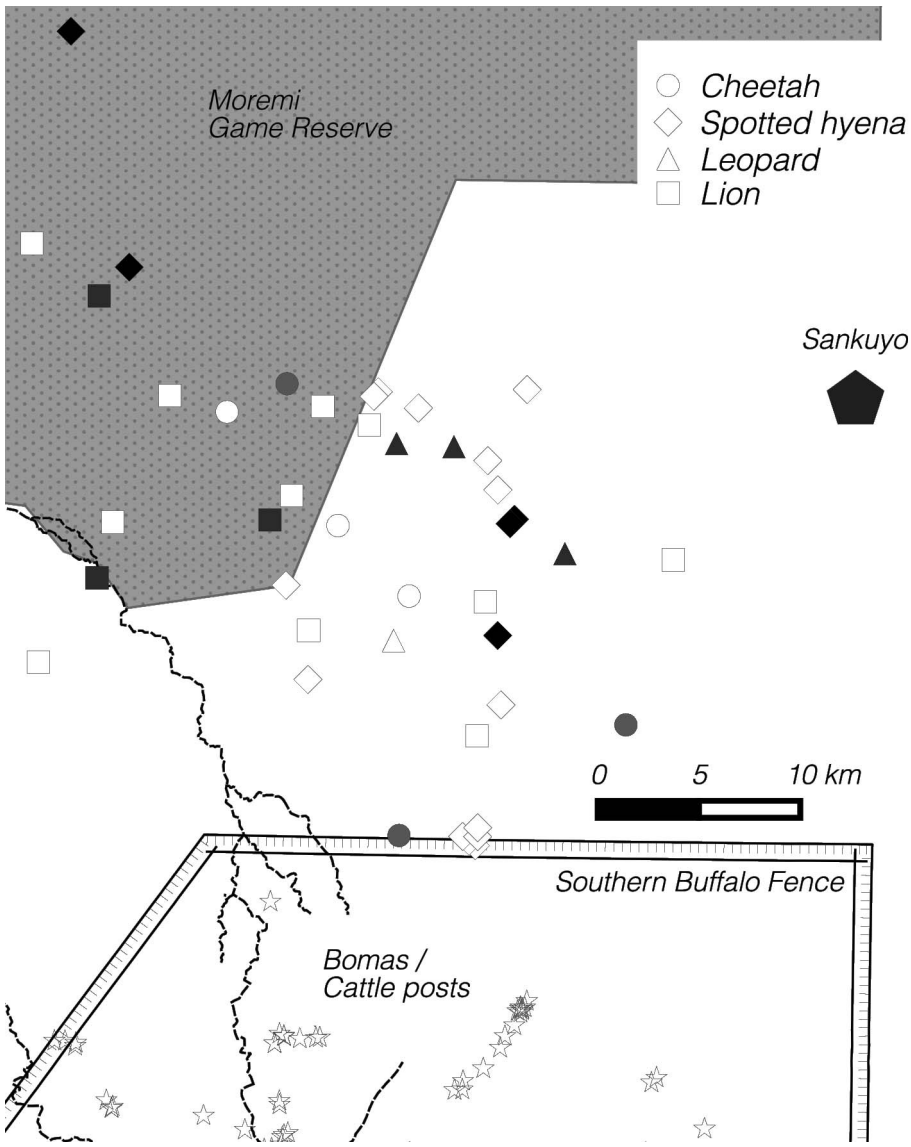

FIGURE 1. The study area in the Okavango Delta, northern Botswana. Dashed lines: rivers. Sampling locations are shown for four species of predators. Black symbols: canine distemper virus positive; dark grey: feline calicivirus positive; light grey: feline panleukopenia virus or enteric corona virus positive; white: negative serologic result; stars: cattle posts or bomas.

This study was conducted in an area of 2,000 km^2 in the Okavango Delta in northern Botswana, between 2008 and 2011 (Fig. 1). The only permitted human activities were photographic and trophy-hunting tourism. With the exception of Sankuyo Village, there are no settlements in the study area (Fig 1). The southern boundary of the study area was delimited by the Southern Buffalo Fence (SBF). Subsistence pastoralism was common south of the fence (Fig. 1). Most households south of the fence and in Sankuyo had domestic dogs (*Canis familiaris*) and occasionally cats (*Felis catus*). Farmers and domestic dogs rarely accompanied free-ranging livestock, and contact between dogs and wildlife far from settlements is, therefore, limited (Alexander and McNutt 2010).

Animals were anesthetized by a qualified veterinarian as part of ongoing carnivore research. During immobilization, animals were clinically examined and thoroughly checked for symptoms related to viral infections. Samples were collected from 14 lions, 19 hyenas, four leopards, and six cheetahs. Blood samples from the medial saphenous vein were collected in dry tubes (BD Vacutainer®, Johannesburg, South Africa). Samples were centrifuged at 2,795 × G for 10 min within 6 h of collection. Serum was collected and stored at –18 C until tested.

Serum samples were tested at the Department of Veterinary Tropical Diseases, University of Pretoria, for antibody to six pathogens of concern to the species we examined canine distemper virus (CDV; Onderstepoort strain), feline panleukopenia virus (FPV), feline enteric coronavirus (FCoV), feline calicivirus (FCV), feline herpesvirus (FHV-1), and feline immunodeficiency virus (FIV). An enzyme-linked immunosorbent assay with a puma lentivirus–derived synthetic peptide as coating antigen was used to test for antibody to FIV (Van Vuuren et al. 2003). For the three felid species, antibodies to CDV, FPV, FCoV, FCV, and FHV-1 were assessed by indirect fluorescent antibody assay by using in-house prepared slides. Sera were screened at a 1:20 dilution. The conjugate used was fluorescein-labeled antifeline immunoglobulin G antibody diluted in 0.05% Evans blue counterstain. Slides were viewed by using a microscope with fluorescence function and examined for cytoplasmic, nuclear, whole cell, and inclusion body fluorescence. For hyenas, CDV antibody analyses were carried out by using a serum neutralization test with the Onderstepoort virus strain. Subjects whose serum showed virus neutralization at dilutions $\geq$1:8 were considered likely exposed to CDV (Appel and Robson 1973). We tested hyenas only for CDV because the other tests used antifeline conjugates.

The majority of sampled individuals were fitted with global positioning system collars to allow monitoring of their movements (Cozzi et al. 2013). When no collar was deployed, the possibility that an animal would have crossed the SBF and moved into pastoral land (Fig. 1 and Table1) was estimated, based on the long-term knowledge of its movements and the dynamics of the group to which it belonged.

Antibodies to all six pathogens tested for were present in the study population (Table 1). Cheetahs, leopards, and lions were CDV antibody negative, while 26% (5/19) of the hyenas were positive. No positive hyenas showed obvious signs of disease at capture, nor did any of the individuals that were regularly monitored. Antibodies to FPV and FCoV were detected in one (17%) and two (33%), respectively, of the six sampled cheetahs. In contrast, antibodies to FCV were found in lions (21%, 3/14) and leopards (75%, 3/4) but not cheetahs. Only one leopard was FIV antibody positive, and the same individual was FCV antibody positive. All 14 lions were positive for FIV antibody, and all but one (92%) were positive for FHV-1 antibody. All lions were in good condition at capture and throughout the study.

We confirmed the presence of multihost pathogens in four species of the African large carnivore guild in the Okavango Delta. To varying degrees, all pathogens tested for were present in the study population. Nevertheless, individuals were in healthy condition when sampled and throughout the study.

Of possible concern is the detection of CDV antibody only among hyenas in this ecosystem. The five CDV antibody–positive hyenas belonged to three clans whose collective territories spanned from the SBF well into Moremi Game Reserve (Fig. 1). Members of these clans interact on occasion. Hyenas in the study population regularly cross the SBF (Cozzi et al. 2013), increasing their chances of exposure to CDV due to interactions with domestic dogs (Alexander and McNutt 2010). However, recent studies suggested that domestic dogs are not the sole driver of CDV infection in wildlife populations (Harrison et al. 2004). It is not known whether CDV is persistently present in the Okavango ecosystem, whether hyenas act as a potential reservoir species for the virus, or whether they encounter the virus periodically

TABLE 1. Serologic results conducted on blood samples collected 2008–11 in the Okavango Delta, Botswana.[a,b]

Species	Sex[b]	Age	Sampled	Group	Crossed	CDV	FPV	FEC	FCV	FHV	FIV
Cheetah (*Acinonyx jubatus*)	F	Adult	10/2009	NA	No	0	0	0	0	0	0
	F	Adult	11/2010	NA	No	0	0	0	0	0	0
	F	Adult	07/2010	NA	No	0	0	**≥1:20**	0	0	0
	F	Adult	05/2011	NA	Yes	0	**≥1:20**	0	0	0	0
	M	Adult	12/2010	NA	No	0	0	0	0	0	0
	M	Adult	07/2010	NA	Possible	0	0	**≥1:20**	0	0	0
Lion (*Panthera leo*)	F	Adult	06/2009	Mogoge	Unlikely	0	0	0	0	**≥1:20**	**≥1:20**
	M	Adult	11/2008	Gomoti	Yes	0	0	0	**≥1:20**	**≥1:20**	**≥1:20**
	M	Adult	01/2009	Flycamp	Unlikely	0	0	0	0	**≥1:20**	**≥1:20**
	F	Adult	10/2010	Flycamp	Yes	0	0	0	0	**≥1:20**	**≥1:20**
	M	Adult	03/2009	Xini	No	0	0	0	**1:20**	**≥1:20**	**≥1:20**
	F	Adult	05/2011	Xini	No	0	0	0	0	**≥1:20**	**≥1:20**
	F	Adult	05/2011	Xini	No	0	0	0	0	**≥1:20**	**≥1:20**
	F	Adult	11/2008	Clare	No	0	0	0	0	**≥1:20**	**≥1:20**
	F	Adult	01/2010	Clare	No	0	0	0	0	**≥1:20**	**≥1:20**
	F	Adult	11/2010	Kazikini	Possible	0	0	0	0	**≥1:20**	**≥1:20**
	M	Adult	05/2011	Chitabe	Possible	0	0	0	0	0	**≥1:20**
	F	Adult	09/2009	Santaw	No	0	0	0	**≥1:20**	**≥1:20**	**≥1:20**
	F	Adult	09/2010	Santaw	No	0	0	0	0	**≥1:20**	**≥1:20**
	M	Adult	05/2011	Santaw	No	0	0	0	0	**≥1:20**	**≥1:20**
Leopard (*Panthera pardus*)	M	Adult	08/2009	NA	No	0	0	0	**≥1:20**	0	**1**
	M	Adult	02/2009	NA	No	0	0	0	**≥1:20**	0	0
	F	Adult	10/2009	NA	No	0	0	0	0	0	0
	F	Adult	09/2009	NA	No	0	0	0	**≥1:20**	**≥1:20**	0
Spotted hyena (*Crocuta crocuta*)	F	Adult	06/2009	Ginger	No	0	NA	NA	NA	NA	NA
	F	Adult	08/2009	Ginger	No	0	NA	NA	NA	NA	NA
	F	Adult	08/2009	Giner	No	0	NA	NA	NA	NA	NA
	Unkn	12–18 mo	08/2009	Ginger	No	0	NA	NA	NA	NA	NA
	Unkn	12 mo	08/2009	Ginger	No	0	NA	NA	NA	NA	NA
	F	Adult	10/2010	Ginger	Unlikely	0	NA	NA	NA	NA	NA
	F	Adult	11/2010	Fly	Unlikely	0	NA	NA	NA	NA	NA
	Unkn	Unkn	08/2009	Fly	Possible	**1:20**	NA	NA	NA	NA	NA
	M	12–18 mo	08/2009	Fly	Unlikely	**1:10**	NA	NA	NA	NA	NA
	M	18–24 mo	08/2009	Fly	Unlikely	**1:28**	NA	NA	NA	NA	NA
	F	12–18 mo	08/2009	Tori	Likely	0	NA	NA	NA	NA	NA
	M	Adult	08/2009	Tori	Likely	0	NA	NA	NA	NA	NA
	M	Unkn	08/2009	Tori	Likely	0	NA	NA	NA	NA	NA
	F	Adult	08/2009	Tori	Yes	0	NA	NA	NA	NA	NA
	M	18 mo	08/2009	Tori	Likely	0	NA	NA	NA	NA	NA
	F	Adult	01/2009	Vera	Likely	**1:10**	NA	NA	NA	NA	NA
	F	Adult	08/2009	Xini	No	**1:10**	NA	NA	NA	NA	NA
	F	Adult	09/2009	Athena	Possible	0	NA	NA	NA	NA	NA
	M	Adult	05/2011	Athena	Likely	0	NA	NA	NA	NA	NA

[a] 0 = negative; bold cells = positive (dilutions are indicated). The sex, age, and identity of the group each individual belonged to and whether or not it crossed the buffalo fence (Fig. 1) are shown.

[b] F = female; M = male; Unkn = unknown; NA = not applicable; CDV = canine distemper virus; FPV = feline panleukopenia virus; FEC = enteric corona virus; FCV = feline calicivirus; FHV = feline herpesvirus; FIV = feline immunodeficiency virus.

from other wild and domestic sources (Harrison et al. 2004). This may suggest episodic CDV exposure: all positive samples were collected in 2009 and two of the five positive individuals were approximately 18 mo old.

We found high antibody prevalence only to FIV and FHV-1 in lions. High prevalence of both antibodies have been reported in other free-ranging lion populations, but negative demographic impacts or manifestations of disease linked to such exposure are rare or nonexistent (Packer et al. 1999; Ramsauer et al. 2007). Epidemiologic models predict that a high contact rate within social groups increases the prevalence of directly transmitted infections (May and Anderson 1979). The highly cohesive social structure of lions may explain the 100% FIV antibody prevalence. Transmission of FIV between lions and leopards is theoretically possible, but Troyer et al. (2008) demonstrated that most species for which FIV is endemic harbor monophyletic, genetically distinct, species-specific FIV strains, suggesting that FIV transfer between felid species is infrequent. The leopard with FIV antibody also had antibody to FCV. It was found dead 7 mo after sample collection, following a constant decline in physical condition. All cheetahs were FIV antibody negative.

The cheetah with parvovirus antibody was the only cheetah that frequently traveled across the SBF (Cozzi et al. 2013), where it may have come into contact with unvaccinated domestic cats and dogs, which can transfer viral antigens to cheetahs (Thalwitzer et al. 2010; Avendaño et al. 2016). Cross reactions are possible with related viruses that share group-specific antigens, including canine parvoviruses that can also infect felids. The solitary nature of the cheetah, however, provides limited opportunity for viral transmission between wild cheetahs during active infection, thus reducing contamination within the population (Munson et al. 2004). Both FCoV and FHV-1 are assumed to have minimal impact on the general health of wild felids (Packer et al. 1999; Ramsauer et al. 2007) and are of minor concern.

Due to limited sample size, we could not test for differences between sexes, ages, social status, and group membership. Additional samples should be collected in the future. Because our antibody tests are subject to possible cross reactivity with other antigens, results should ideally be validated by non–species-specific tests. Nevertheless, this study lays the groundwork for future studies. In general, the wide-ranging behavior of these large carnivore species increases exposure to, and likely transmission of, pathogens within and between species. The current emphasis on large landscape management of wildlife species, therefore, lends importance to a more holistic, community-wide approach to wildlife disease management.

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Chapter 7: Complexity of ecological system – A call for multi- and interdisciplinary studies

Preamble: The understanding of environmental and climatic conditions, ecological diversity and complexity that can encourage or alter pathogen survival and transmission are as important as describing the pathogenic mechanisms themselves. Our studies on chytridiomycosis (*Batrachochytrium dendrobatidis*) and *Acomys dimidiatus'* fleas stress the importance of the environmental condition and host population to study pathogens and highlight the absolute necessity for trans- and inter-disciplinary work.

Article 9: Failure to detect Batrachochytrium dendrobatidis infection in the United Arab Emirates and Oman.

Original article in the *Herpetological review* – 2016 - *47*(3), pp.403-404.

Preamble: A. Cunningham defined pathogen pollution as being the anthropogenic introduction and movement of parasites outside their natural geographic or host species range. Bd appears to be globally widespread (Figure 2) and the potential for anthropogenic introduction is supported by the finding of *Batrachochytrium dendrobatidis* (*Bd*) infected animals in the international trade of amphibians for pet stores, ornamental pond-stocking, zoos, laboratories, consumption as food by humans and in amphibian species introduced into Australia and North and South America[122,123]. A human mediated transfer of *Bd* between different sites visited by tourists or even researchers is also a risk after *Bd* is first introduced in a country. Nervetheless, survival of *Bd* depends of the local environmental and climate conditions that have to be taken into account while studying presence or abscence of chytrid. Our study on *Bd* in the UAE and Oman discuss anthropogenic and climatic factors that encourage or alter pathogen survival and transmission and give the first results of a *Bd* survey targeting wild toads in the UAE and Oman.

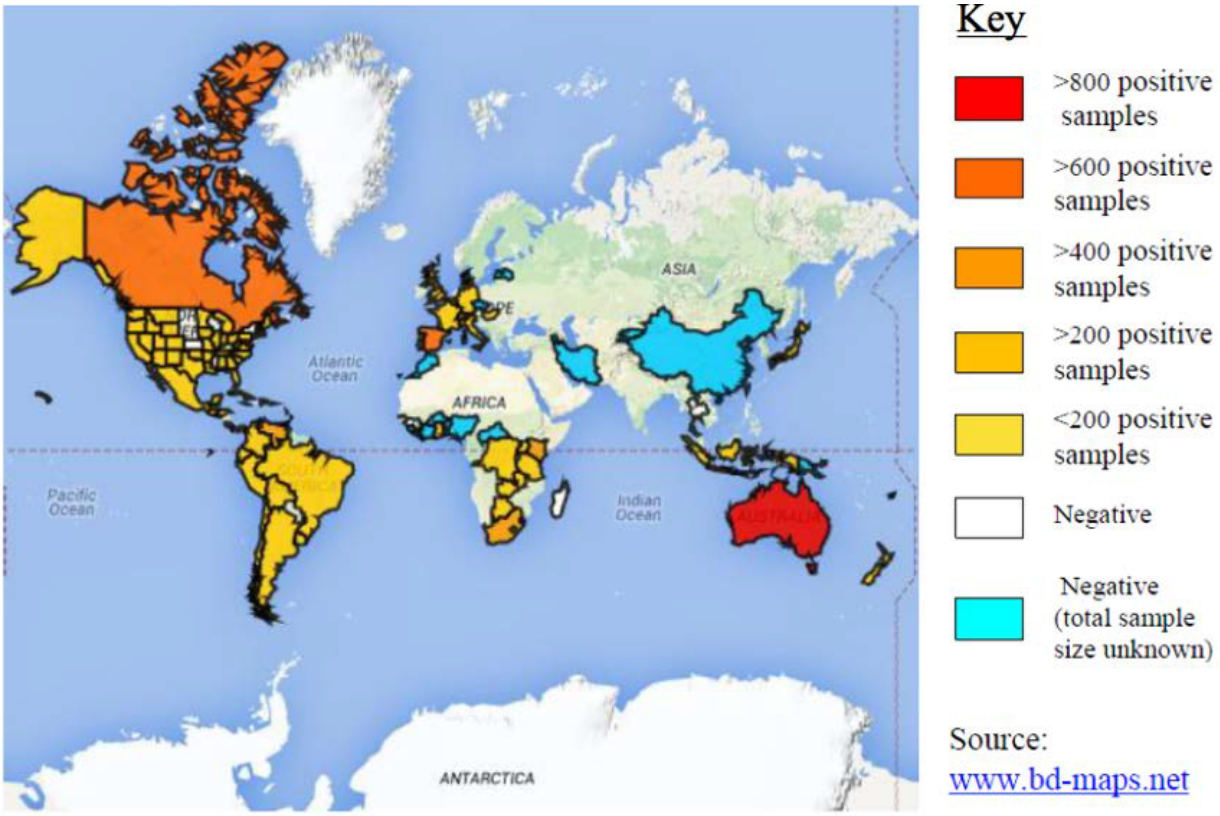

Figure 3 : The map shows the countries where samples have been collected and the colours show the number range of *Bd*-positive samples per country.

Source : www.bd-maps.net

Herpetological Review, 2016, 47(3), 403–404.

Preliminary Surveys Fail to Detect *Batrachochytrium dendrobatidis* Infection in the United Arab Emirates and Oman

There is a paucity of data concerning either the presence or absence of *Batrachochytrium dendrobatidis* (*Bd*) in the Middle East and the susceptibility of amphibians in the region to this pathogen. Soorae et al. (2012) skin-swabbed 16 Arabian Toads (*Amietophrynus arabicus*; previously *Bufo orientalis, B. arabicus* and, more recently, *Amietophrynus arabicus*, Portik and Papenfuss 2015) and two Dhofar Toads (*Duttaphrynus dhufarensis*) across five sites in the United Arab Emirates (UAE) and found no evidence for the presence of *Bd* using a commercial, pathogen-specific standard Polymerase Chain Reaction (PCR) analysis. Whereas it is not clear how many animals were skin-swabbed at each site, the sample size was small and would, at best, require a *Bd* infection prevalence of at least 25% (and probably higher, depending on how many animals were sampled per site and the total size of each sampled population) for the pathogen to be detected. Here, we expand on the work of Soorae et al. (2012) in the UAE by examining a larger number of wild animals in the region for the presence of *Bd* infection.

Arabian Toads were sampled from January 2013 to January 2014 at two sites in the UAE and at two sites in Oman (Fig. 1; Table 1). At least 30 toads were sampled per population through the study duration. This sample size was chosen as it would enable the detection of a *Bd* prevalence of 15% with a 99% probability, or a prevalence of 10% with a 95% probability (Cannon and Roe 1982). A population was defined as the same species sharing the same water-body.

Toads were caught by hand and handlers were wearing plastic gloves that were changed between each animal to avoid cross-contamination. Per site, for each animal sampled, the ventral surface of the skin was swabbed with a single dry sterile rayon-tipped swab approximately 35 times. Target areas included the pelvic patch (5 swabs), ventral thighs (5 swabs per thigh), and toe webbing (5 swabs per foot). The swab was air-dried for approximately 5 minutes and then replaced in a plastic sterile sleeve. The swabs were kept at -20°C for up to 6 months before being processed. All the toads screened were in water at the time of sampling and were likely to have been in an aquatic environment for at least one month before being sampled as the site visits took place during the mating season.

DNA was extracted from the swabs and analyzed using a *Bd*-specific real-time PCR assay according to Boyle et al. (2004), modified by the addition of bovine serum albumin to minimize PCR inhibition (Garland et al. 2010). Each sample was tested in duplicate and each PCR plate contained two negative control wells (containing laboratory grade distilled water) and a duplicate set of four positive controls (100, 10, 1, and 0.1 zoospore equivalents).

In total, 67 toads were sampled from the UAE and 60 toads were sampled from Oman. All samples tested were *Bd*-negative. All negative and positive controls gave expected results. Thus, *Bd* is either not present at the sites sampled or is present at a prevalence of less than 10%.

Lack of *Bd* detection could result from several scenarios, including: *Bd* is not native to the area and has not yet reached the sampled amphibian populations; the sampled toads are resistant to *Bd* infection; and the local environmental conditions are not conducive to support the survival of *Bd*. Generally the climate of the UAE and Oman is classified as hyperarid. Limited amounts of fresh water, in combination with extremely high summer temperatures and high evaporation rates, make the Arabian Peninsula a harsh environment for the people, fauna, and vegetation (Böer and Chaudhary 1999). Nevertheless, two of the sites surveyed are known sources of fresh water and sustain aquatic organisms throughout the year. The air temperature in the region ranges from 12°C to more than 49°C and can stay over 30°C for more than 24 h during summer with water temperature in the river-beds over 25°C during summer and is thus higher than the optimal thermal conditions for *Bd*, 17–25°C (Piotrowski et al. 2004). Additionally, *Bd* infection can be "cured" by exposing infected animals to temperatures >25°C for one month (Chatfield and Richards-Zawacki 2011). It is nonetheless important to note that thermal maxima for *Bd* growth are *Bd*-isolate dependent and that the pathogen may exhibit local adaptation (Stevenson et al. 2013).

Although the exact origin of *Bd* has not yet been determined, it has become clear that the global amphibian trade is likely a primary driver for the international spread of *Bd* (Weldon et al. 2004; Schloegel et al. 2010). Dubai Airport is the third busiest cargo hub airport in the world, yet there is no significant amphibian trade in the UAE. Although Soorae et al. (2012) reported finding amphibians for sale in 5 of 16 pet shops visited in the UAE, a 2013 survey of 104 pet shops in the emirates of Dubaï, Sharjah, and Abu Dhabi failed to find any amphibians for

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TABLE 1. Arabian Toad (*Amietophrynus arabicus*) sample sizes per location in the United Arab Emirates (UAE) and Oman for *Batrachochytrium dendrobatidis* analyses.

Site Name	Latitude	Longitude	Elevation (m)	No. samples
Wadi Wurayah – Site 1 (UAE)	25.39583°N	56.23777°E	464	33
Wadi Wurayah – Site 2 (UAE)	25.34805°N	56.24555°E	26	34
Wadi Jazira (Oman)	24.31888°N	56.15000°E	333	30
Wadi Al Hayl Oman)	24.30888°N	56.32916°E	450	30

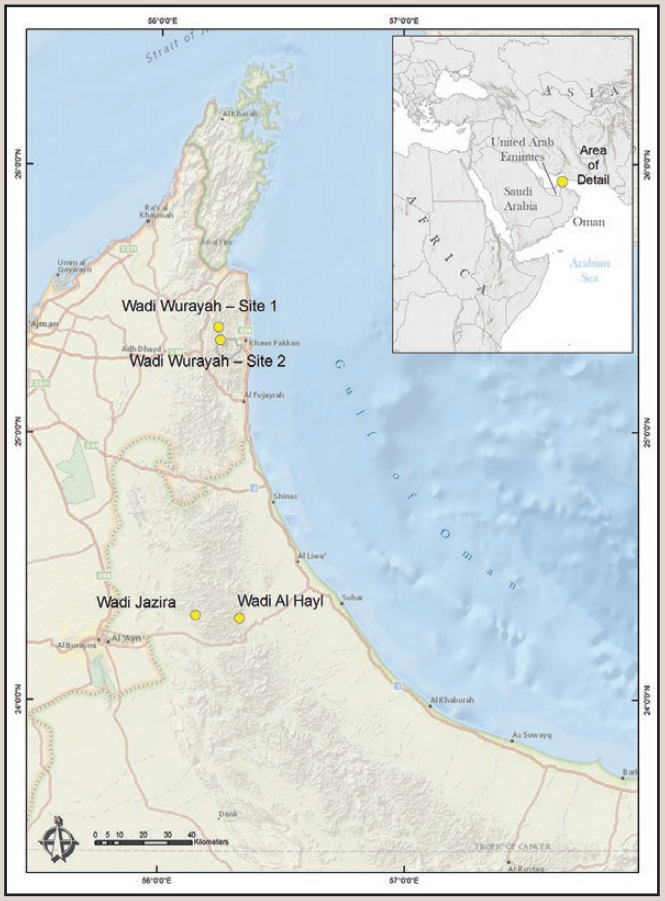

FIG. 1. Sampling locations of Arabian Toads (*Amietophrynus arabicus*) for *Batrachochytrium dendrobatidis* in the United Arab Emirates and Oman.

sale (ALC, unpubl.). However, both public and private amphibian collections are present in the UAE exhibiting species exotic to the region (Soorae et al. 2012). The World Organization for Animal Health's regulations requiring that amphibians be free of *Bd* infection before international shipment to a *Bd*-free country (Schloegel et al. 2010) warrants consideration for any amphibian shipments to the UAE or Oman due to their unique fauna which appears to be *Bd*-free.

In addition, the apparently *Bd*-free native, wild Arabian amphibians suggests that biosecurity of captive animals in the area may be important to consider. It would be prudent to test amphibians currently held in zoological collections in the region for *Bd* infection to address possible pathways of inadvertent environmental contamination. At this time, the susceptibilities to *Bd* of the two amphibian species occurring in the UAE and Oman, *Amietophrynus arabicus* and *Duttaphrynus dhufarensis*, remain unknown. Infection experiments would be useful for informing the degree of risk *Bd* might present native amphibians should it be introduced to the region.

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Article 10: Note sur les Siphonaptères de l'Émirat de Ras Al Khaimah: description de *Nosopsyllus* (*Gerbillophilus*) *jabeljaisensis* n. sp. (*Siphonaptera, Ceratophyllidae, Ceratophyllinae*).

Short Note in *Annales de la Société entomologique de France (NS)* – 2016 - 52(2), pp. 102-106. Taylor & Francis

Preamble: Flea-borne diseases have a wide distribution in the world. Studies on the identity, abundance, distribution and seasonality of the potential vectors of pathogenic agents (e.g. *Yersinia pestis*, *Francisella tularensis*, and *Rickettsia felis*) are necessary tools for controlling and preventing such diseases outbreaks[124]. *Xenopsylla cheopis* is the most prominent vector of plague (*Yersinia pestis)* for humans. Human plague is acquired most often from the bites of infected fleas that leave their rodent hosts. Plague outbreaks are often following seasonal pattern linked to vectors and rodents ecology. Interdisciplinary studies are therefore necessary in order to understand each element of the transmission cycle. In the UAE, human encroachment in wild habitats is giving rise to more human-wildlife contacts. In addition, eating behavior could represent an additional risk in endemic foci as illustrated in the last plague event in Saudi Arabia where four case-patients got infected by *Yersinia pestis* after eating raw camel liver. *Yersinia pestis* was isolated from bone marrow of the camel and from jirds (*Meriones libycus*) and fleas (*Xenopsylla cheopis*) captured at the camel corral[125]. Our next study is an example of a collaborative work between an ecologist, a veterinarian and an entomologist to collect relevant ecological data on rodents and fleas populations in the mountains of Ras Al Khaimah (UAE).

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SHORT NOTE - BRÈVE NOTE

Note sur les Siphonaptères de l'Émirat de Ras Al Khaimah: description de *Nosopsyllus* (*Gerbillophilus*) *jabeljaisensis* n. sp. (Siphonaptera, Ceratophyllidae, Ceratophyllinae)

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(Accepté le 1 avril 2016)

Summary. Note on the Siphonaptera of the Emirate of Ras Al Khaimah: description of *Nosopsyllus (Gerbillophilus) jabeljaisensis* n. sp. (Siphonaptera, Ceratophyllidae, Ceratophyllinae). Twenty two fleas collected during *Acomys dimidiatus* et *Gerbillus dasyurus* trapping in the montains of Ras Al Khaimah in the United Arab Emirates were identified as belonging to two species. A new taxon is descrided in the genus *Nosopsyllus*.

Résumé. Vingt-deux puces collectées lors de captures de *Acomys dimidiatus* et *Gerbillus dasyurus* dans les montagnes de Ras Al Khaimah aux Émirats arabes unis ont été identifiées comme appartenant à deux espèces. Un nouveau taxon est décrit dans le genre *Nosopsyllus*

http://www.zoobank.org/urn:lsid:zoobank.org:pub:ACB8C547-5A7C-4D45-BBC3-2ABD705FE797

Keywords: United Arab Emirates; *Xenopsylla cheopis cheopis*; *Xenopsylla ch. bantorum*; *Nosopsyllus (Gerbillophilus) jabeljaisensis n. sp*; *Acomys dimidiatus*

Mots-clés: Émirat Arabes Unis ; *Xenopsylla cheopis cheopis* ; *Xenopsylla ch. bantorum* ; *Nosopsyllus (Gerbillophilus) jabeljaisensis n. sp*. ; *Acomys dimidiatus*.

L'Émirat de Ras Al Khaimah fait partie des Émirats arabes unis (EAU), enserrés entre le Golfe persique et la mer d'Oman, d'une part, entre l'Arabie Saoudite et Oman d'autre part. Les Émirats arabes unis sont une fédération de sept émirats dont Dubai et Abou Dabi sont les plus connus. Ras Al Khaimah, d'où viennent nos puces, est moins connu et plus « sauvage », et situé juste au sud du détroit d'Ormuz. Peu d'études portant sur les siphonaptères de la Péninsule arabique sont publiées (Lewis 1964, 1982; Lewis & Lewis 1990) bien qu'ils soient reconnus comme des vecteurs potentiels de *Yersinia pestis*, l'agent de la peste. Un seul travail concerne les EAU (Howard et al. 2014) et il ne cite que des Pulicidae.

Dans ce travail nous signalons la présence de deux taxa non répertoriés jusqu'alors dans cet émirat. Ils furent collectés sur une montagne, le Jabel Jaïs qui culmine à 1900 m. Le biotope (photo 1) est rocheux et aride, des ravins creusent de place en place la montagne mais ne laissent que peu de place à la végétation qui souffre aussi du surpâturage par les chèvres domestiques. D'octobre 2014 à mai 2015, des piégeages de petits mammifères ont été effectués mensuellement à différentes altitudes du Jabel Jaïs afin de déterminer les taxa présents et d'estimer leur abondance. Deux espèces seulement de petits mammifères « terrestres » furent capturées: sur les 114 *Acomys dimidiatus* et 27 *Gerbillus dasyurus* examinés lors de ces captures, vingt-deux puces seulement furent collectées et préservées dans de l'éthanol à 70% pour leur identification. L'espèce la mieux représentée dans nos collectes est *Xenopsylla cheopis* (Rothschild, 1903) (Pulicidae, Xenopsyllinae), représentée par *X. cheopis cheopis* (Rothschild, 1903), en mélange avec la variété bantorum Jordan, 1938 (*cf* Lewis 1982; Schwan 1992), parfois considérée comme une sous espèce. Non signalée par Howard et al. (2014), cette dernière était, de loin, la plus abondante dans nos collectes, et en syntopie totale avec *X. ch. cheopis*. La présence de *Xenopsylla cheopis s.l.* était attendue: nous l'avons trouvée sur *Acomys* et *Gerbillus*. La deuxième espèce est un *Nosopsyllus* Jordan, 1933 (Ceratophyllidae, Ceratophyllinae) appartenant au sous-genre *Gerbillophilus* Wagner, 1934, représenté par 10 exemplaires, espèce que nous considérons comme nouvelle.

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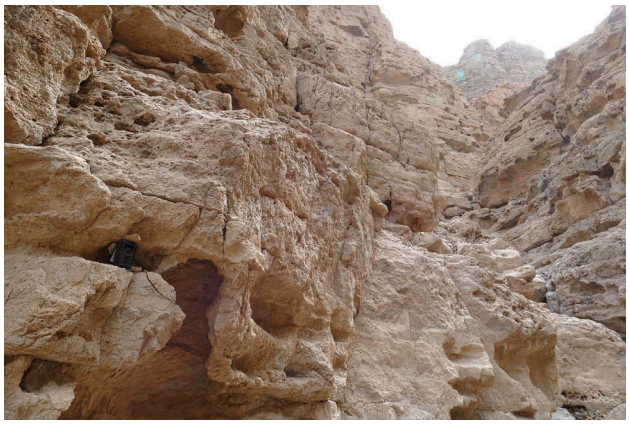


Photo 1. Photographie du biotope rocheux et aride de la montagne du Jebel Jaïs à Ras Al Khaimah, lieu des collectes décrites.

Étonnement, cette puce fut observée uniquement sur des *Acomys* (Rongeurs Muridés), en l'occurrence *A. dimidiatus*, bien que des gerbilles, *Gerbillus dasyurus* (Wagner, 1942) (Rongeurs Gerbillidés), soient présentes, surtout dans les zones les plus élevées de la montagne.

Décrire un taxon nouveau dans le genre *Nosopsyllus*, et particulièrement dans le sous-genre *Gerbillophilus*, est délicat car ces puces sont mal connues, souvent insuffisamment décrites et, pour beaucoup, seulement répertoriées de régions peu étudiées. Traub et al. (1983) admettaient 49 espèces dispersées essentiellement en régions paléarctique et orientale, mais quelques autres furent décrites depuis (Wang & Liu 1981; Beaucournu & Launay 1987/1988; Aktaş 1999; Beaucournu et al. 2012). Smit (1960) à propos de ce genre écrivait « *There is a great need for a revision of the genus* Nosopsyllus »; Lewis (1982) appuyait cette opinion « *The entire genus is in dire need of taxonomic revision* » et cela était redit, en termes identiques, par Traub et al. (1983) « *The genus is in need of revision…* ». Par ailleurs, il semble que des enquêtes de terrain tendent à montrer que plusieurs de ces taxa, sous-espèces mais éventuellement espèces ou considérées comme telles, montrent des clines de variabilité tels qu'ils rendent impossible une scission sub-spécifique, voire spécifique, entre les individus. Nous pensons, toutefois, qu'il est préférable de donner à cette population un nom spécifique car aucune espèce décrite ne semble assez proche, morphologiquement, pour recevoir ce taxon en tant que sous-espèce.

***Nosopsyllus* (*Gerbillophilus*) *jabeljaisensis* Chaber & Beaucournu n. sp.**

Matériel de description: mâle holotype sur *Acomys dimidiatus* (Cretzschmar, 1826) (Rodentia, Muridae), dans le Jabel Jais (lat. 25.939026 N.; long. 56.130855 E.), altitude 1600 m, en janvier 2015, Émirat de Ras Al Khaimah (Émirats arabes unis); femelle allotype, même hôte, mêmes données; 5 mâles, 3 femelles paratypes, tous prélevés sur *Acomys dimidiatus*, dans le Jabel Jaïs, à l'altitude de 1600 m, le 16 janvier 2015.

Dépôt des types: types et paratypes sont déposés dans les collections J.C.B., collections déposées ultérieurement au Muséum d'Histoire naturelle à Paris (MNHN).

Description: il s'agit d'un *Gerbillophilus* typique, de taille relativement faible (*cf infra*).

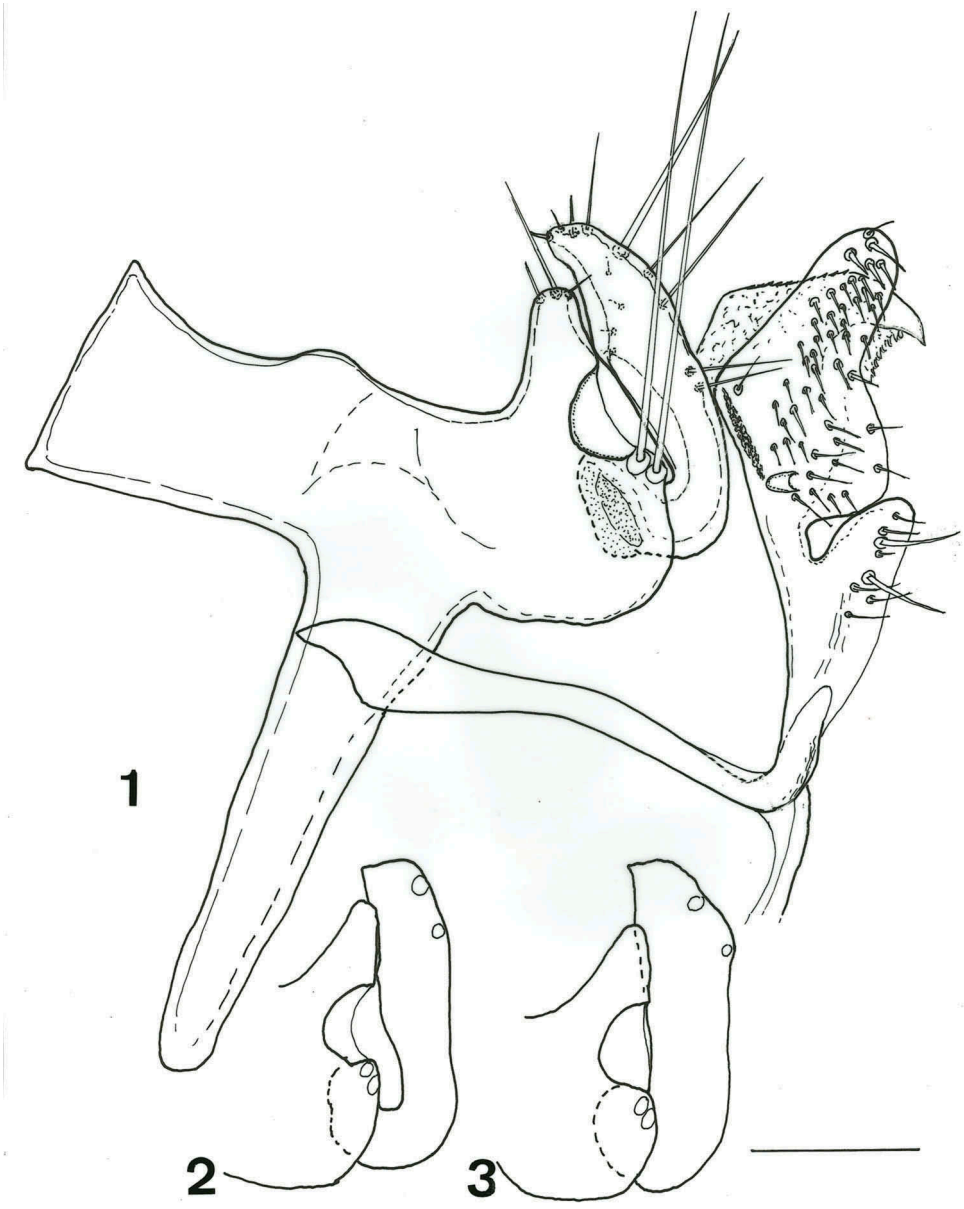
Capsule céphalique montrant la chétotaxie classique; palpe labial atteignant la base ou la moitié du trochanter. Les soies dorsales de la rangée céphalique postérieure sont doucement érigées; il en est de même pour les soies du thorax et de l'abdomen; il s'agit d'un caractère classique chez *Gerbillophilus*.

Thorax. Prothorax montrant une cténidie de 20 à 23 dents chez les mâles, de 22 à 23 chez les femelles, cette cténidie est plus longue que le segment; une rangée précténidiale de 5 soies; mesothorax montrant 4 à 5 *pseudosetae* (généralement 4) chez les mâles, 4 à 6 (généralement 5) chez les femelles; metathorax avec 2 à 3 spinules dans les 2 sexes. Metepimeron portant 7 soies; le stigmate est petit (diamètre identique à celui de la base d'une spinule). Cette petite taille, confirmée par celles des stigmates tergaux (où leur diamètre est inférieur ou égal à celui des spinules), est normale pour une espèce vivant en milieu désertique (Smit 1960, 1972).

Les soies des tarses sont longues, autre caractère de puce « désertique ». Pour le tarse de la 3^{e} paire de pattes, la plus longue soie du 1er segment atteint la base du 3^{e}; les plus longues soies du 2^{e} segment atteignent la base du 5^{e} segment pour l'une, le milieu du 5^{e} pour l'autre; la plus longue soie du 3^{e} segment atteint, elle aussi, la base du 5^{e} segment.

Abdomen (segments non génitaux). Rangées principales de 8 ou 9 soies, l'inférieure se situant au niveau du stigmate, ou très légèrement au-dessous. Soies ante-sensiliales au nombre de 2 ou 3 chez les mâles, l'inférieure étant fortement régressée: pour les 2 soies supérieures, leur rapport est de 1/3, la plus longue étant la médiane; chez les femelles, ce rapport est identique, mais la soie inférieure est bien développée, mesurant environ les neuf dixièmes de la soie médiane.

Abdomen (segments génitaux mâles). Tergite VIII (Figure 4) petit, son bord dorsal perpendiculaire au bord distal qui est convexe; dans son ensemble, ce segment est un peu plus long que haut; généralement 3 soies marginales et 4 latérales. Sternite VIII vestigial ce qui est de règle dans le genre *Nosopsyllus*. Segment IX (Figures 1–3): tergite montrant un manubrium long et relativement étroit, perpendiculaire au segment; basimère montrant un apex triangulaire, saillant, sa partie immédiatement au-dessus des soies acetabulaires étant translucide donnant l'impression à faible grossissement que ce segment est fortement échancré; télomère allongé, à bord antérieur faiblement arqué ou rectiligne, bord postérieur doucement



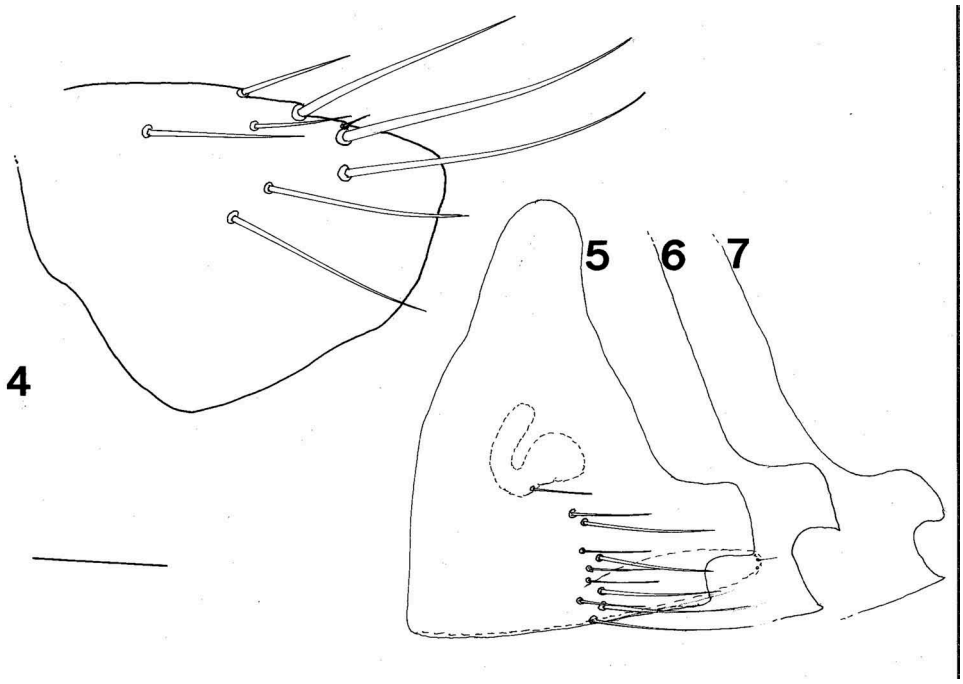
Figures 1–3. *Nosopsyllus (Gerbillophilus) jabeljaisensis* n. sp., **1**, holotype, segment IX et apex du phallosome; **2 et 3**, paratypes, basimère et télomère. Echelle: 200 µm.

convexe avec une petite concavité sub-médiane; 2 soies principales marginales, la supérieure plus épaisse et plus longue que l'inférieure. Sternite IX: bras proximal long, grêle et doucement arqué; bras distal montrant une partie apicale large, son bord dorsal courbé à 80° environ, couvert dans sa moitié inférieure de soies courtes et doucement épaissies, soies devenant sub-spiniformes vers l'apex.

Phallosome (Figure 1): *hamulus* formant un bec acuminé se projetant vers l'arrière. La glande de Wagner est présente, comme il est classique dans ce genre.

Abdomen (segments génitaux femelles). Segment anal: stylet anal allongé, de rapport L/l = 2,7/1; une longue soie terminale et une autre, courte, à mi-segment. Sternite VII (Figures 5–7) de contour général classique pour ce sous-genre: le lobe distal est quadrangulaire avec souvent un « petit bec » ventral. Spermathèque et *ducti* (Figure 8): *ductus bursae* assez long, rectiligne puis faisant une circonvolution à l'apex; spermathèque non distinctive.

Dimensions (insectes montés): mâles, de 1,5 à 2,1 mm (holotype: 1,8 mm); femelles, de 1,9 à 2;6 mm (allotype: 2,6 mm). C'est une espèce plutôt petite pour ce genre.



Figures 4–7. *Nosopsyllus (Gerbillophilus) jabeljaisensis* n. sp., **4**, holotype, tergite VIII; **5**, allotype, sternites VII et VIII; **6 et 7**, paratypes, sternite VII.

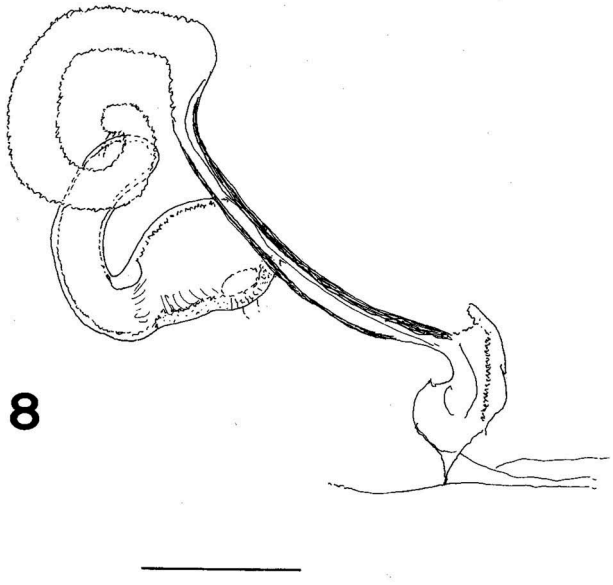


Figure 8. *Nosopsyllus (Gerbillophilus) jabeljaisensis* n. sp., allotype, spermathèque et *ducti*.
Echelle 200 µm, sauf pour les figures 5 à 7, où elle vaut 400 µm.

Derivatio nominis: du Jebel Jaïs, lieu des collectes.

Discussion

Il nous paraît peu crédible que *Acomys dimidiatus* soit l'hôte primaire de *N.* (*G.*) *jabeljaisensis* n. sp., d'autant plus que, nous l'avons dit, des gerbilles sont en sympatrie. *Nosopsyllus* (*Penicus*) *russatus* Traub, 1963 (*Penicus* étant un genre monotypique), semble, lui, lié au genre *Acomys, A. russatus* en l'occurrence et ce, sans modification de ses soies. Il est très bien caractérisé et n'est signalé, pour le moment, que de Jordanie et du Sinaï.

Si l'on se réfère aux espèces du sous-genre *Gerbillophilus* déjà connues de la Péninsule arabique, *N.* (*G.*) *iranus theodori* Smit, 1960 (taxon décrit comme bonne espèce, ce que nous estimons exact) et *N.* (*G.*) *pringlei* Hubbard, 1956 sont les seules citées à ce jour (Lewis & Lewis 1990). Ce sont des parasites de gerbilles, bien que deux paratypes de *theodori* proviennent d'*Acomys russatus* et d'*A. cahirinus* (espèce dont *dimidiatus* fut, un temps, considéré comme synonyme). *N. pringlei* est immédiatement écarté, entre autres, par le contour du sternite VII de la femelle; *N. i. theodori* est très proche de *N. jabeljaisensis* n. sp., mais s'en sépare par l'apex du basimère (plus étroit chez *i. theodori*) et par le télomère également plus étroit et dont les 2 soies marginales principales sont de même épaisseur; l'apex du sternite IX ne comporte pas de soies spiniformes; de plus, chez la femelle, le *ductus bursae* est bien différent.

N. pumilionis Smit, 1960, fut récolté sur gerboises et gerbilles; il n'est connu que d'Iraq, de Jordanie et de Palestine, mais sa ressemblance avec *N. jabeljaisensis* n. sp. est nette: il pourra s 'en séparer toutefois par la forme du tergite VIII, la largeur du manubrium, la taille des 2 soies principales du télomère et, au niveau du bras distal du sternite IX, la forme des 2 grosses soies spiniformes de

la base et la chétotaxie de l'apex du sternite IX qui ne montre, chez *pumilionis*, que des petites soies non spiniformes. En revanche, mais cela est classique, les femelles ne semblent pas séparables.

Les *Acomys*, hôtes apparents de *N.* (*G.*) *jabeljaisensis* n. sp. sont caractérisés, entre autres, par leur fourrure riche en jarres « épineuses ». Parallèlement, certaines de leurs puces spécifiques vont montrer, au niveau des segments abdominaux, des soies épaisses, épineuses, à leur tour: c'est le cas du genre *Parapulex* Wagner, 1910 (Pulicidae, Xenopsyllinae), contenant 2 espèces, *P. chephrenis* (Rothschild, 1903) et *P. echinatus* Smit, 1956; seule la première est signalée de la Péninsule arabique et son absence dans nos collectes est étonnante, même si Howard et al. (2014) ne le signale pas, eux non plus. Rappelons, à ce propos, que des rongeurs appartenant à d'autres familles et présentant le même type de revêtement pileux peuvent, eux aussi, avoir des puces spécifiques « épineuses »: par exemple, en zone neotropicale, les rongeurs du genre *Proechimys* (Rodentia, Echimyidae) sont parasités par des puces du genre *Polygenis* Jordan, 1939, genre « épineux ».

Remerciements

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PART 3 – GENERAL DISCUSSION, CONCLUSION AND PERSPECTIVES

Chapter 8: General discussion

The majority of human and animal infectious pathogens have the ability to infect more than one species (62% of all human pathogens are classified as zoonoses, 77% of livestock pathogens infect multiple species and 91% of domestic carnivore pathogens infect multiple hosts)[126]. Nearly all pathogens that threaten endangered species around the world infect multiple species[127]. Thus, many infectious pathogens may have reservoirs in one species from which they can be transmitted to the species of concern.

Detecting and managing disease at the Wildlife-Livestock-Human (W-L-H) or W-L or W-H interface requires an understanding of which species are reservoir and spillover hosts, what are the pathogen transmission pathways (i.e.: direct, vector born disease, relying on bridge host) and what is the interface and how does it evolve over time. Wild species can be considered as maintenance, spill over or spill back hosts depending on the diseases and the epidemiological context. Knowledge of which animals are involved in the chain of transmission and understanding of their epidemiological role are therefore key in identifying and targeting the weak spot of disease transmission at the W-L-H interface.

Surveillance is '*the systematic on-going collection, collation, and analysis of information related to animal health and the timely dissemination of information to those who need to know so that action can be taken*.' (OIE Terrestrial Animal Health Code)[128]. Surveillance relies on constant investigations and vigilance for pathogens, regular analysis of the data for specific purposes and communication of the results to appropriate agencies and institutions. Surveillance programs in human and livestock populations are often in place in developed countries and being set up in most developing countries. Wildlife surveillance programs are challenging; from the difficulty to find exploitable dead/diseased wild animals, to sample collection and to laboratory identification of pathogens and diseases[129]. In the wild, most samples in wildlife pathogen surveillance are therefore 'convenient-sampling' (non-random) based on what is possible to achieve given the difficulties of securing samples. When a disease of importance (with an impact on public health, agriculture or wildlife conservation) is found in wildlife, monitoring programs are set up to describe and define the epidemiological role(s) of the animal(s) or vector(s) involved in pathogen transmission and maintenance in a specific context[130]. The contextualisation of the study is crucial and one

situation is not similar to another; the host density and the network of interactions in each ecosystem could be different, and therefore unlikely that a species would play the same epidemiological functional role across its range[131]. In addition, the value of 'R0' (that symbolises the basic reproductive number for a pathogen) changes according to the characteristics of the environment and of the host population, and it also can change during the course of a disease occurrence event[132].

Depending on the countries, various institutions public or private can be involved in wildlife surveillance and monitoring programs. As an example, in France, the National Hunting and Wildlife Agency established a wildlife surveillance network relying mainly on hunters (SAGIR). This agency is a public administrative institution under the joint supervision of the ministries in charge of ecology and agriculture[133]. Research institutions and universities can also take part in disease surveillance and monitoring efforts. From the design to the implementation of surveillance activities, research groups play a major role in developing research programs. With various groups working in the wildlife sectors: non-governmental organisation, universities and public administrative institutions, information management and communication of results between those institutions seem crucial but difficult to achieve. As a comparison and to stress the difficulty in channelling health information to authorities, we will just recall that the first professionals that prompted a response from the French government (due to a heat wave in 2003 causing human mortalities) were by overwhelmed funeral companies and not the governmental human health epidemiological surveillance network in place. As a consequence of these events, a major task of adapting information systems in the human sector has been undertaken, relying more on "real-time" morbidity or mortality data. Availability of "real-time" information in livestock and especially in wildlife is even more challenging. In this thesis, we have carried out several studies using different tools targeting specific diseases (and already emphasized advantages and limitations of those techniques), the earliest moments of disease emergence are nevertheless often invisible to surveillance systems. They can be invisible to epidemiologists and microbiologists or virologists. The first one to detect disease occurrence in wildlife might be the local population, layperson, hunters, or nature enthusiast. Social and medical anthropologists working on human-animal socio-ecology are reframing the local vernacular knowledge to help epidemiologists in the identification of the source of the emergence of a zoonotic infection[134–136]. Participatory epidemiology is taking great importance to understand epidemiological scenarios, improve our understanding of the social and cultural contexts that

affect the distribution and dynamics[137] and develop best-bet assessments of health situations[138]. These approaches, based on participatory rural appraisal[139] by professionals, emphasize the need to learn from stakeholders, rather than just extract data.

The global population can also be used as a source of data and more importantly « real-time data » thanks to smartphones that are widely used in developed countries and rising in developing countries with smartphone ownership in countries like Malaysia going from 34% to 65% between 2013 and 2015[140]. Digital Epidemiology can capture digital data such as location, activity data (heart rate, etc), laboratory tests, what did the person eat, how did the person sleep, how does he/she feel, health history, DNA, and genomic data, etc, via mobile applications[141]. In parallel to the globalization of trade and thus pathogens, the information revolution where each user is also an information transmitter could be turned into a useful tool; if this metadata is correctly analysed. Online social media data offers not only increasingly large data volumes but also highly contextual, networked[142] and increasingly hyper-local[141]. Digital data from social media can be used for early detection of disease outbreaks and to monitor disease levels without relying only on official reports. Digital epidemiology can be of particular interest for Emerging Infectious Diseases (EIDs) that can appear unexpectedly, spread very rapidly, be potentially devastating to millions of people, and at a time when individual behaviour is at the centre of disease dynamics and control[141].

EIDs are defined as “diseases that have recently increased in incidence or geographic range, recently moved into new host populations, recently been discovered or are caused by newly-evolved pathogens[122,143,144]”. Today’s globalised and interconnected world enhances several social, political, and economic factors allowing viral and bacterial pathogens rapid and easy access to new environments and populations. Wildlife EIDs, as human EIDs, have parallel major drivers such as anthropogenic environmental change and are a product of the globalization of agriculture, commerce and human travel[122,145–147]. Cunningham and *al.* (2003)[148] used the term "pathogen pollution" to describe the anthropogenic movement of pathogens into new geographic locations. This is a “disease equivalent of anthropogenic biological invasions and introductions”[149]. Wildlife EIDs are responsible for loss of biodiversity due to mass mortalities, local (population) extinctions and global (species) extinctions[147]. Species removal can lead to major environmental modifications that drives the emergence of human, domestic animal and wildlife EIDs[122]. EIDs also have a major impact on domestic animals; without considering the welfare issues related to the introduction of

disease pathogens, the economic impact of zoonotic EIDs may be significant. The Nipah virus disease outbreaks in Malaysia resulted in the slaughter of over one million pigs, a loss of around 60% of Malaysian pig farms, 36,000 jobs and US$ 120 million in exports[150,151].

People with high levels of exposure to wild animals, such as hunters, butchers of wild game, wildlife veterinarians, workers in the wildlife trade, and zoo workers and/or livestock could be used as sentinels in early warning and monitoring systems[152]. Human blood samples can be screened for known viral, bacterial and parasitological agents, with a priori (targeted) bacteria/virus/parasites approach such as PCR, cell-based assays for serology, neutralization tests, antiviral research and isolation techniques. Recent advances in molecular techniques and the development of next-generation sequencing (NGS) and bioinformatics offer new techniques to identify known and unknown agents in virtually any type of specimen[153]. Advances in NGS have allowed significant breakthroughs in microbial ecology studies and could also be of use in disease risk assessment linked to wildlife trade. Disease risk introduction in legal exotic trade currently booming via e-commerce, and in illegal bushmeat and wildlife trade have not been thoroughly assessed. The French Agency for Food, Environmental and Occupational Health and Safety (*Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail* - ANSES) in its April 2016 report on the prioritization of health hazards, exotic or present, in metropolitan France in new pets, zoo, circus and laboratory animals (« *Hiérarchisation des dangers sanitaires, exotiques ou présents, en France métropolitaine chez les nouveaux animaux de compagnie, les animaux de zoo, de cirque et de laboratoire*») points out that this analysis relates exclusively to the health hazards of interest identified at the present state of knowledge and at the time of writing and that the experts did not take into account a possible evolution of the species/taxon concerned (fashion phenomena)[154]. Trade and especially e-commerce of exotic animals remains a large and vast international network, that connects ecosystem interfaces around the world, but the movement of pathogens across this network is entirely unregulated and poses challenges to authorities in charge in detecting and managing diseases at the W-L-H interface. In 2003, an outbreak of 72 confirmed or suspected cases of monkeypox burst in Wisconsin, Illinois, and Indiana (USA)[155]. Monkeypox is a rare viral disease caused by Monkeypox virus, a member of the orthopoxvirus group of viruses. It is endemic to rainforest countries of central and west Africa[156], but has never been detected in North America before 2003. Traceback investigations from infected patients followed the route of introduction to a distributor in Illinois, who had imported legally a shipment of approximately 800 small exotic mammals

[six genera of African rodents: rope squirrels (*Funisciurus spp*.), tree squirrels (*Heliosciurus spp*.), Gambian giant rats (*Cricetomys spp*.), brush-tailed porcupines (*Atherurus spp*.), dormice (*Graphiurus spp*.), and striped mice (*Hybomys spp*.)] into the United States through Texas from Ghana, West Africa[155,157]. According to CITES, 97% of live animals are traded for commercial purposes while 0.07% (~30,000 out of ~43.6 million) are traded for zoological purposes and 0.02% were traded for circuses/traveling exhibitions (*Tom De Meulenaer,* personal communication). Regarding the illegal meat trade, several studies stress the role of bushmeat in the transmission of infectious agents from wildlife and has been demonstrated for many pathogens, including the deadly agents of haemorrhagic fevers, among which the Ebola virus in Western Africa[158]. Several studies addressing the question of the presence of zoonotic viruses in bushmeat samples resulted in a long list of pathogens[158–163]. Simian immunodeficiency virus, human T-cell lymphotrophic virus, simian foamy virus, monkeypox virus, Ebola and Marburg filoviruses, anthrax, herpes viruses, hepatitis viruses, paramyxoviruses and various parasites are pathogens transmissible to humans through bushmeat consumption[164]. The question of the potential introduction of significant pathogens into Europe through the illegal importation of meat products, both bush and domestic, can now be tackled using a complete pipeline of metagenomic analysis offering a high sensitivity in detecting pathogens[165–167].

Although EIDs are causing the most media outcry, neglected tropical diseases NTD (i.e.: *Brucella melitensis* and Q fever) are also occurring at the W-L-H interface. Those diseases are endemic in the tropical belt and often affect poor populations. Detection of those diseases is often possible in both animal and human sectors but the management of NTD zoonosis at W-L-H interface requires a One Health approach and a long-term commitment from the health and political authorities.

Management of disease and health at a global scale implies that there is a consensus between health professionals (veterinarians, human doctors, epidemiologists, etc) and also on a political level (national, inter- and supra-national) on health definition. Is it just an absence of diseases? Whose health is being promoted?

Human Health, be it universal or individual, actually implemented or scientifically theorized, analysed through medicals terms and concepts or popularized, is lost in a maze of definitions and interpretations. Medical sciences define it as an absence of diseases. The World Health Organisation (WHO) makes this idea more positive by de-medicalizing it: “Health is a state of

complete physical, psychological and social well-being and does not only consist in the absence of diseases or disabilities". Even though health asserts itself here above all as an individual issue, the individual is considered being part of an integrated whole, linked to its environment. Human health, animal health, and environmental health are interconnected. Considering one of them without taking the other into consideration makes no sense. In addition and even though the socioeconomic dimension of animal health crises is barely taken into consideration by OIE member countries (apart from Europe, virtually no other OIE Regional Commission measures this dimension)[168]. Growing economic evidence[169,170] and the social, technical, animal and human health, environmental and information benefits[171] of the One Health approach have prompted an important momentum at national and international levels to promote and encourage collaborations between human and animal health professionals and authorities.

The historic and social evolution made human health a public, domestic and international issue. The British Ministry of Public Health was founded in 1848 following the cholera outbreak that affected London and the World Health Organisation was created in 1918 after the Spanish flu pandemic. Likewise, rinderpest that occurred unexpectedly in Belgium in 1920, as a result of zebus, originating from South Asia and destined for Brazil, transiting via the port of Antwerp, has brought up the need to fight animal diseases at a global level and led to the creation of the International Office of Epizootics on January, 25th, 1924. The OIE (or World Organisation for Animal Health since 2003) created the basis of international animal health policy. The OIE is the referent for the World Trade Organisation (WTO).

Health or diseases of humans and animals are domestic and international affairs. However, each society has its own understandings of disease and health that are themselves symbols of wider ideologies of the "world order", of good and bad, these being created and evolving upon the era[172]. Just like Mirko D. Grmek underlined it, the disease itself is an interpretation of life experiences: "*Diseases are concepts with arbitrary outlines which as such do not immediately ensue from our life experiences and vary over space (cultural diversities) and time (historical diversities). Diseases defined by medicine only exist as part of an interpretive system of the reality. They are explanatory models of the reality and not a constituent element of it.*"[173]. To the variations of social and cultural representations of diseases and *in fine* the importance that the populations grant them; countries and governments' different points of

view towards diseases and disease management can be added, and particularly “notifiable diseases” (WHO for human diseases and OIE for animals). In addition, the economic and/or media impact of those declarations interfere with transparency.

In Europa, the foot-and-mouth disease (FMD) is a nice example of a veterinary victory and knowledge allowing the control of a disease but also highlights the countries’ different concerns regarding this virus in accordance to their economic outlook. The foot-and-mouth disease has been recognized as a large epizootic disease threatening cattle production since the 16th century.

Loeffler and Frosch discovered at the end of the 19th century that this disease was caused by a transmissible agent, smaller than every known bacteria. Thus, FMD was the first vertebrate animal virus ever discovered, shortly after the discovery of the tobacco mosaic virus in the tobacco plant. The experimental research allowed the production of efficient vaccines at a large scale. Since the 1950s, Europe initiated immunization programs and succeeded to eradicate the disease in 1989. The 2001 epidemic in England consequently had a large psychological, social, media and economic impact. The European Union decreed a global ban on every British export of cattle, meat, and products of animal origins. 10 million sheep and cattle were slaughtered and destroyed. In Africa, where productivity is less, agricultural issues are different and infected animals are not always euthanized. The slaughter and destruction of healthy animals, only “contaminated”, even slaughtered for preventive purposes, surprised and shocked many. FMD control measures applied in Southern Africa were drastic, with the construction of thousands of kilometres of veterinary cordon fences intending to separate “disease-free livestock” from “infected livestock” and their closest wild relatives. Even though since 1982 it was hypothesized that airborne transmission of FMD was possible under certain conditions (even over a long sea passage) and relative risks of uncontrollable (airborne) spread of FMD by different species was described since 2000. Fences have adverse effects on wild mammals at the individual, population and species level and were considered by conservationists the single most damaging development to Southern African wildlife in history. Although FMD does not affect suitability for human consumption, those fences have been erected in order to meet the conditions set by the OIE for trade and more lucrative international markets with little to no consideration about ecological costs on wildlife and ecosystem. In November 2016, Animal health and wildlife conservation experts from the five-nation Kavango Zambezi Transfrontier Conservation Area (KAZA TFCA) met with an aim towards easing tensions at the livestock-wildlife interface and discussed environmentally-friendly ways to manage trade-sensitive animal diseases like FMD. Unlike rinderpest, the

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independent persistence in buffalo and savannah wildlife might render FMD eradication not feasible or even necessary in Africa[174] This meeting promoted new approaches to the safe trade of beef and beef products based on the meat production process itself ("commodity-based trade"), rather than solely on livestock's geographic origin as delineated by fencing. In some Arabian Gulf States, this disease has had a negligible economic impact on their gross domestic product (GDP), which is not linked to agricultural activities. Because of it, foot-and-mouth disease's epizootics are not consistently reported to domestic authorities or notified internationally due to the negative political and media image that would spread.

Animal health falls within the scope of common market forces and the free movement of goods and services. Animal health law is torn between pressing needs, which are recognised as unequal values: - preservation of public and consumers' health; - preservation of economic interests represented by the activities related to livestock production and use; - but also animal protection. Animal health is a priority in livestock farming as it is directly linked to productivity and on a national level for the ability of trading livestock.
Health and its representations became a politic field of action often digressing from scientific, ethic or philosophical concerns. Public health and environment or animal protection concepts sometimes collide with each other. Wildlife is often referred to as spillover/spillback, maintenance, or dead-end hosts[175,176], overemphasizing the role of wildlife in transmission while neglecting the manifold values of wildlife[177]. The emergence of zoonotic diseases urges one to reflect on our relation with wild animals, persecuted survivors of biological diversity. Do we have the responsibility to protect them or would we take it upon ourselves the power, even the will to destroy them?

The OIE Training Manual on wildlife diseases and surveillance states that there are: « *four strategies that can be applied to the management of pathogens and diseases in wild animals.*

Before health issues arise from wildlife pathogens:

1. Prevent new health problems from arising.

After health issues from wild animal pathogens have emerged:

2. *Take no action or response to the health issue;*
3. *Intervene to control the health issue to some degree;*

4. *Intervene to eradicate the pathogen of concern.*

Decisions on whether or not to attempt to control or eradicate pathogens in wild animal populations should be informed by a complete review of the control methods available and of the rationale and objectives of a control programme. Most often, there is little that can be done to control pathogens in wildlife populations, and the best choice will be to attempt to reduce the impact of such pathogens by actions that target the affected domestic animal or human populations»[178].

Disease control can target the maintenance host to stop pathogen maintenance and circulation in an ecosystem, try to break the transmission pathway that brings the pathogen to the target host or act directly on the target population to protect it from being infected.

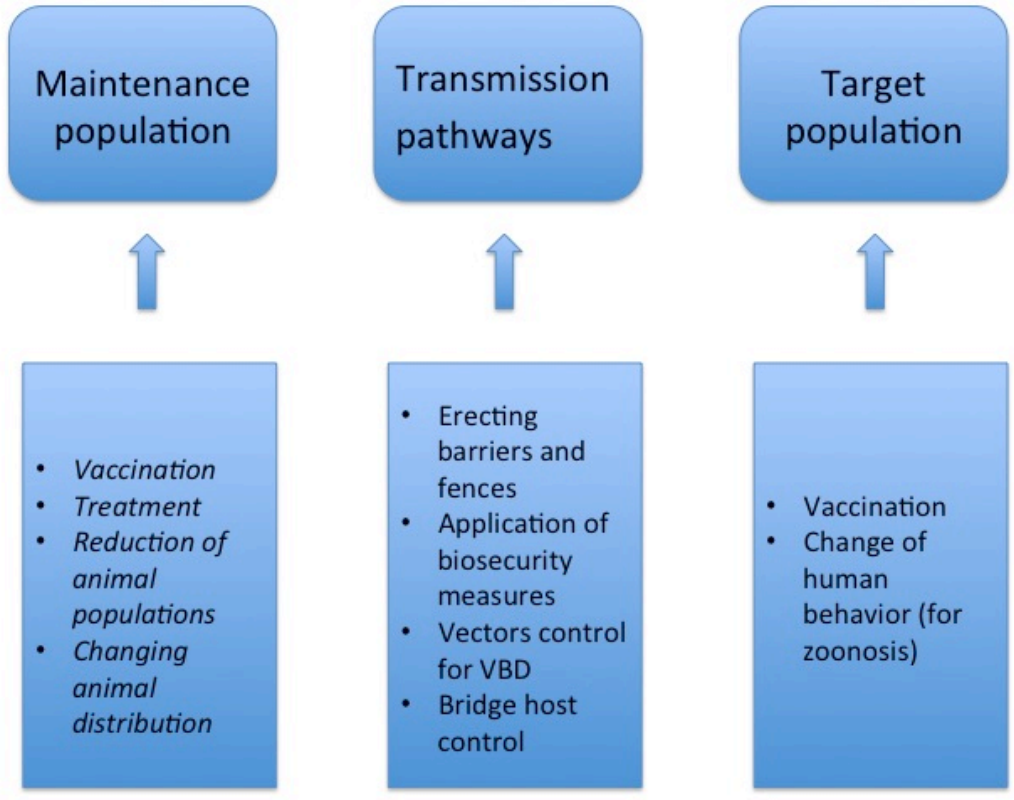


Figure 4: Examples of strategies that can be applied to the management of pathogens and diseases according to the epidemiological role of their host or vector.
VBD stands for Vector Borne Disease

Examples of such disease control measures, including their pros and cons, have been illustrated in this thesis and perfectly captured by Gortazar and collaborators (2015)[130] and by Ryser-Degiorgis (2013)[129].

The scientific and technical difficulties in addressing disease control at the W-L and/or W-L-H interface, such as the mass slaughter of badgers and ibexes for public health reasons in Europe reveal, for instance, the real or simulated confusion that exists, to our leaderships, between causes and consequences. Culling badgers to "control" tuberculosis, and wiping out entire populations of protected Alpine ibexes to "prevent" brucellosis not only show that we might be fighting the wrong battle but also give a clear indication of the value granted to wilderness. Despite the Universal Declaration of Animal Rights announced at UNESCO Headquarters in Paris on October, 15th, 1978 (and revised in 1989) which stated: "*Any action endangering the survival of a wild species, and every decision leading to such act form a genocide, i.e. a crime against the species*" (Article 8)[179]; it is clear that animal protection leans towards being completely annihilated when issues related to human health, even hypothetical, are in question[180].

The arsenal of weapons against diseases in animals is constantly growing: use of antibiotics (leading to antibiotic resistance), mass-immunisation or even eradication of animal "reservoirs". The main factors promoting the emergence of zoonosis are nevertheless directly linked to human activities and demographic growth. For example: land use modifications (deforestation), agricultural and agronomical practices and processes linked to those (i.e. Nipah virus infection in South-East Asia, BSE); demographic, societal and behavioral changes (i.e. Pertussis, HIV, syphilis, *Salmonella* measles); travels and intercontinental human exchanges (i.e. Dengue, seasonal influenza, H5N1, chikungunya virus, malaria); three billion flight passengers per year for an overall population of seven billion; and economic transports of commercial goods and animals (i.e. Monkeypox virus, H5N1, *Salmonella*).

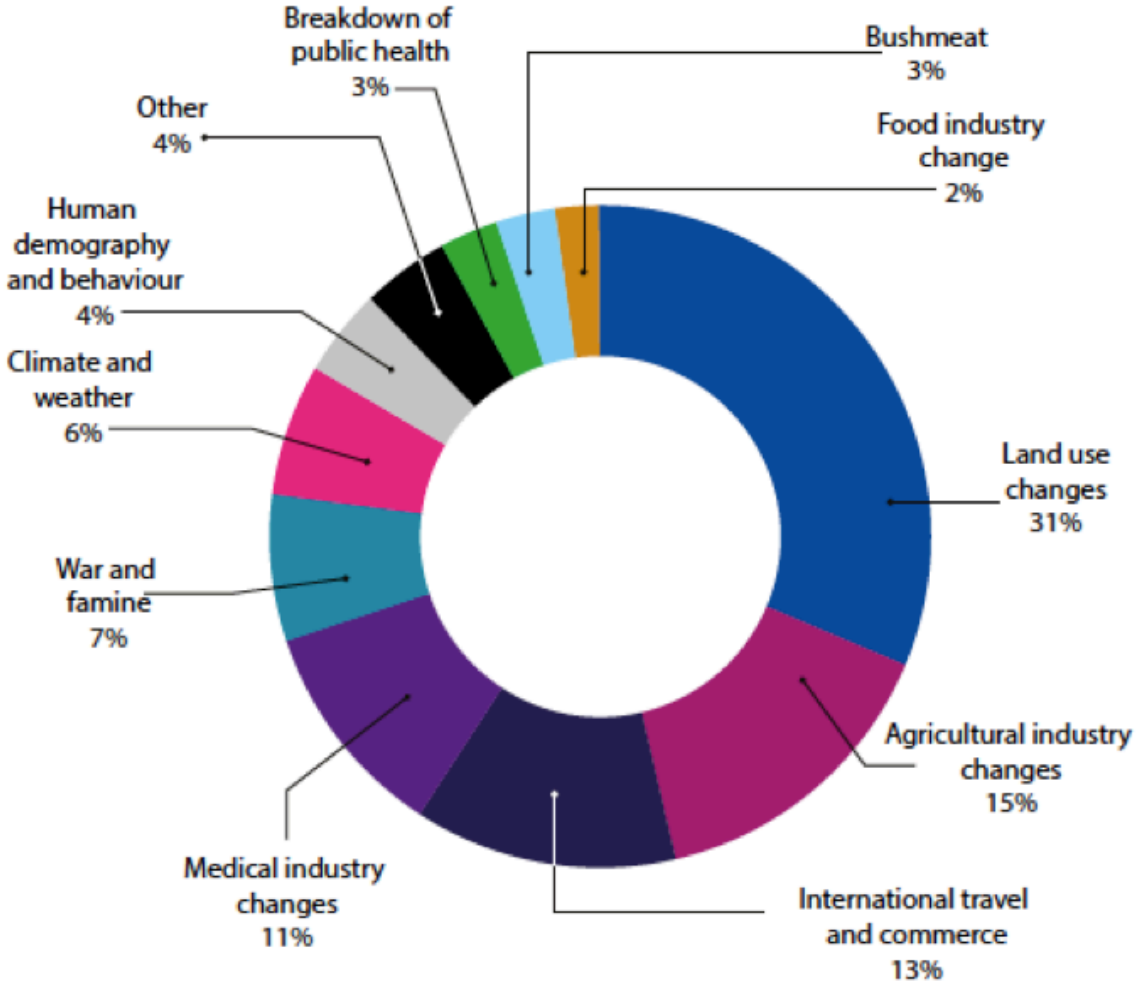


Figure 5: Primary drivers of past disease emergence.
Source: Loh et al. (2015)[181].

The overall effects of industry are rarely seen by studying specific diseases and exposures one at a time and it is argued that current epidemiology protects economic health at the expense of the health of most of the population and of the ecosystem. The description of diseases and epidemiological scenarios are shaped by methodological approaches used. There is no pure science, rather all scientific knowledge is shaped by social history and its production[182]. In 1998, Steve Wing (1998)[182] stated that: "*Constructing health for all requires constructing an epidemiology for all. Such an epidemiology must recognize how it implicitly or explicitly takes sides in global struggles. It will be an epidemiology that attains objectivity through critical self-examination, commitment to fairness, and respect for moral values as well as technical sophistication.*"

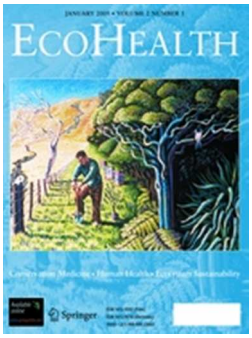
There is now a shift in paradigm from how we previously addressed health issues. One Health is a more inclusive field that integrates both experts and stakeholders (instead of just experts) on local, regional, and global levels, and from areas that were not previously considered as directly relevant to human health such as biodiversity, or ecosystem health. Sarah Whitmee et al. wrote "*we have been mortgaging the health of future generations to realize economic and development gains in the present*"[183]. One Health, Eco-Health and Planetary Health are conceptual scientific movements and political endeavours aiming to include trade-offs between various social, cultural, economic, environmental, and health agendas. Even though the driving forces of One Health, Eco-Health and Planetary Health might be different, those concepts all emphasize the importance of socio-ecosystemic approaches to health concerns at the animal-human-ecosystem interface.

Chapter 9: Perspectives

You never change things by fighting the existing reality. To change something, build a new model that makes the existing model obsolete.
Buckminster Fuller

Article 11: The era of “Human Induced Diseases”

Forum article – Author: Anne-Lise Chaber –Under review in *EcoHealth* journal.



The era of "Human-Induced Diseases"

Journal:	*EcoHealth*
Manuscript ID	ECH-17-0015
Manuscript Type:	Forum
Keywords:	Zoonoses, Chronic diseases, anthropozoonoses, ecohealth, anthropocene, non-infectious disease

The era of "Human-Induced Diseases"

A global political and research infrastructure has grown around the fight against 'zoonoses', or diseases transmitted between vertebrates and humans. Zoonotic disease is a cornerstone of 'One Health' or 'EcoHealth' but focusing on zoonotic disease might be already outdated. This innovation in the integration of human, animal and environmental health and the concomitant critique of the epistemological fragmentation, division and reductionism of the western scientific tradition needs further revision. Scientific and political focus should shift from zoonotic disease to 'Human-Induced Disease' (HID) – defining both infectious and non-infectious disease.

Zoonotic diseases have incurred an estimated $20 billion in direct costs and over $200 in indirect losses in the last 10 years (Bank, 2010). These events generate moral panic, legitimate new spheres of legal activity, and spur new arguments for the funding of research. The arsenal of disease-combatting weapons grows constantly, evidenced by mass-immunization or eradication of animal 'reservoirs'. This dynamic derives from an anthropocentric perception and the psychologically useful imagination of a species barrier.

Most viruses and bacteria are not pathogenic but symbiotic, with humans hosting some 100,000 billion bacteria compared to their own 10 billion cells. Bacteria have a vital role in human bodily function and are integral elements of human cells (mitochondrion), and viruses constitute at least 10% of human DNA. Humans share most of the viruses, bacteria and

fungus with the rest of the animal kingdom and thus it should come as no surprise that zoonotic pathogens were the cause of more than 65% of emergent infectious disease events in the last 60 years, with 75% of these originating in wild fauna (Keusch et al., 2009). The *biologically* relevant question is what allows or prevents infection by microorganisms, whether or not the infected species is human.

The direct link between zoonosis and human activities and demographic growth is established. Land use modification for urbanisation, food production and agricultural change accounts for around 50% of all zoonotic Emerging Infectious Diseases (EIDs) (Keesing et al., 2010). Demographic, societal and behavioural change gave rise to Human Immunodeficiency Virus (HIV/AIDS) (de Sousa et al., 2010) and outbreaks of syphilis (D'Angelo-Scott et al., 2015). Anthropogenic environmental change lead to the emergence of infectious diseases in wildlife (Daszak et al., 2001) The advent of mass travel exacerbates the historically established dissemination of infectious disease along pathways of migration. Examples are bubonic plague (*Yersinia pestis)*, cholera (*Vibrio cholerae)*, seasonal influenza, Severe Acute Respiratory Syndrome (SARS), and malaria (*Plasmodium falciparum)* (Tatem et al., 2006). The trade in goods and animals is directly linked to outbreaks such as human monkeypox virus in North America (Karesh et al. 2005) or H5N1 in the United Arab Emirates (Naguib et al., 2015).

There is a confusion between causes and consequences. It is common that cause is attributed to the animal and not to human behaviour as evidenced, for example, by the mass culling of badgers and the ibex to 'control' tuberculosis or 'prevent' brucellosis in Europe. This also evidences the diminished value to humans of wild animals and comes despite the Universal Declaration of Animal Rights (UNESCO, 1978 and revised, 1989) which states that "action

endangering the survival of a wild species, and every decision leading to such act form a genocide, i.e. a crime against the species" (Article 8). When human health is concerned, even hypothetically, such principles are quickly abandoned in the urge to displace consequence as cause.

Epidemiologists focus on early detection, rapid and accurate etiological identification, rapid response, and effective control (Chua and Gubler, 2013). Yet this is an attempt to reduce impact of zoonoses after transmission has occurred. Is reducing the risk of transmission in the first place really out of our hands?

The impact of neo-liberal economic and industrial growth on the environment and ecosystems becomes a problem when exploitation, pollution, and inefficiency are recognized as a risk to future sustainable development of economy and society. Government and industry typically understand the environment as an exploitable resource or place of waste disposal, and assess the value of ecosystems in terms of their productive utility to human society. Without consensus on definitions, common objectives between beneficiaries, and long-term planning environment health is difficult to protect. Conventions of international environmental law introduced the concept of legal protection for species, sites, habitats and ecosystems. Yet three concepts of nature coexist: it is a Law project (UNESCO, 2016) in which the common heritage dimension is underlined; it is a Legal subject (United Nations, 2016); and, it is a Law purpose (UNEP,2016). The International Criminal Court announced in September 2016 that it will now prioritize crimes that result in the "destruction of the environment", "exploitation of natural resources", and the "illegal dispossession" of land (International Criminal Court,

2016). Yet 'illegality' is contested, and prosecutions will focus only on large-scale environmental damage. Policies intending the liberalization of international trade do not take into account resource-consumption, pollution or negative societal impact in the producing and exporting countries. Traded goods are not priced to incorporate such costs and as such benefits of trade are distorted. A multitude of supranational environmental agencies, commissions, programs and secretariats exist but there is no global authority able to levy an appropriate pollution tax on national government or economic agents within countries (Pearson, 2000).

The World Health Organization (WHO) estimates that ambient air pollution caused 3.7 million deaths throughout the world in 2012(WHO and others, 2014). The Organisation for Economic Co-operation and Development (OECD) claims this death rate increased by 5% in China, 12% in India (OECD, 2014) and 4% worldwide between 2005 and 2010. It estimated that the annual economic cost of illness and premature mortality linked to air pollution is $3,600 billion (OECD, 2014) – a figure that is 85% of the world's annual public budget for human health. The *International Monetary Fund* (IMF) reported the use of fossil energies costs $4,900 billion a year (Parry et al., 2014) in disease, premature death and environmental damage. This figure is 1.2 times more than the combined public health budgets of 193 countries. And yet industries that exploit fossil energies are subsidized directly and indirectly by more than $500 billion annually(iMFdirect - The IMF Blog, 2016).

Environmental degradation is estimated to be the cause of 40% of world mortality (Pimentel et al., 2007). Human activity gives rise to multiple forms of toxic pollution affecting both our health and the environment. For example, production of garments for international markets takes place mainly in developing countries and involves unregulated tannery operations that

generate chromium pollutants known to have carcinogenic effects on both animals and humans. In 2013 industrial plants with poor waste treatment and disposal infrastructure were responsible for the lead pollution linked directly to the death from chronic illness of 853,000 people living mainly in low and middle income countries (Harris and McCartor, 2011). The Institute for Health Metrics and Evaluation also estimated that lead exposure accounted for 9.3% of the global burden of idiopathic intellectual disability, 4% of the global burden of ischaemic heart disease and 6.6% of the global burden of stroke (WHO, 2016). UNESCO's World Water Assessment Programme estimates that industry is responsible for the annual accumulation of 300 to 500 million tons of sludge, heavy metals, and other toxic wastes, and that 70% of untreated industrial waste in developing countries is dumped directly into water systems (United Nations Educational, Scientific and Cultural Organization, 2016).

> "*[For people of] poor countries, resource degradation is the most tragic, forced as they are to overuse natural resources from which depends their survival. They are driven to sacrifice their future to ensure precarious everyday life. That is why in many countries, acting against poverty includes protecting the environment*." (UN Secretary General, 4th June 1992, Rio da Janeiro)

Precarious life conditions make people's lives vulnerable. In 2010 more than 230,000 people died and 300,000 were injured by the earthquake in Haiti in 2010. An 2014 earthquake of similar magnitude in California injured only 100 people and no deaths were reported. The uneven incidence of illness and mortality consequent on environmental degradation within developed countries also follows patterns of relative socio-economic status (Deguen et al., 2015).

It is becoming increasingly apparent to many that solutions and actions cannot be left to government or supranational government agencies. Consumers are boycotting products with negative social, environmental, and health impacts such as slavery in clothing factories, harvesting of exotic timber or methylmercury contamination in seafood.

Environmental citizenship is a recent and theoretically complex concept (Bell, 2005). It is defined by UNEP as an "attempt to make environmental conservation and sustainability an important duty of citizenship that citizens all over the world should be aware of" (UNEP). Many multinational enterprises are now engaged in corporate citizenship programs to promote sustainable development, including the simultaneous consideration of economic growth, environmental protection, and social equity in business planning and decision-making. Yet the willingness and ability of governments to reflect environmental values of their citizens vary greatly among countries (Pearson, 2000).

The individual remains the fundamental element of society. How powerful can one individual be? The internet and its powerful networking effect is beyond control of established institutions. It creates opportunities for horizontal communication, develops new forms of democracy and social participation, and could bring people closer to having impact on issues of common concern such as health, social justice and the environment (Barcena, 1997). Could billions of individuals, scattered through the world but connected via the internet, become the strongest and most active pillar of environmental protection and thus enhance health and improve social justice?

The term "Human-Induced Diseases" might have the potential to put the 'human' back into perspective and unify concerns and efforts. HID as the label for diseases (infectious and non-

infectious) caused by human activities emphasizes the role of human and could serve bringing together scientists, politicians, industrials and laymen in common pursuit.

Human-Induced Diseases need to be named in order to be collectively claimed.

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LIST OF POSTERS and ORAL PRESENTATIONS

- AgraME Conference. Dubaï, UAE. 10-11th April 2017.

  - Reducing cross contamination with secure practices on multispecies farms. **Chaber A.L,** Saegerman C.

- *European OneHealth/EcoHealth workshop.* Belgian Science Policy Office (BelSPO), Brussels, Belgium. 6-7 October 2016.

  - Illegal meat trade: a threat to both biodiversity and public health. **Chaber A.L.**, Lignereux Y., Temmam S, Desnues C., Cunningham AA.

- *Symposium de l'Association d'Epidémiologie et de Santé Animale (AESA),* Liège, Belgium. 7-9 September 2016.

  - Biosecurity measures applied in the United Arab Emirates: a comparative study between livestock and wildlife sectors. **Chaber A.L.**, Saegerman C.
  - Genotyping analysis of Brucella melitensis at the wildlife-livestock-human interface in the Middle East. **Chaber A.L.**, Lignereux L., Fretin D., Saegerman C.

- *One Health for the Real World: zoonoses, ecosystems and wellbeing*, Zoological Society of London, UK.17-18 March 2016.

  - llegal meat trade: a threat to both biodiversity and public health. **Chaber A.L.**, Lignereux Y., Temmam S, Desnues C., Cunningham AA.

- *70th WAZA Annual conference*. Al Ain, UAE. 11-15 October 2015.

- Contagious Caprine Pleuropneumonia to an Arabian Oryx (Oryx leucoryx). **Chaber A.L.**, Lignereux L., Al Qassimi M., Saegerman C., Manso-Silvan L., Dupuy V., Thiaucourt F

- *Brucellosis 2014 International Research Conference.* Berlin. 9-12 Sept 2014.

  - *Brucella melitensis* at the wildlife-livestock-human interface in the Emirate of Abu Dhabi. **Chaber A.L.**, Lignereux L., Fretin D., Saegerman C.

- *16th International Conservation Workshop for Arabia's Biodiversity* ». Sharjah, UAE. 2-4 February 2015.

  - Diseases of the region and their post mortem presentations. **Chaber A.L**
  - Biosecurity, surveillance, propylaxis and diagnostics in the UAE. **Chaber A.L**

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