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Q Fever Serological Survey and Associated Risk Factors in Veterinarians, Southern Belgium, 2013

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Summary

A sero-epidemiological survey was organized among veterinarians working in Southern Belgium to estimate the seroprevalence of Q fever and the risk factors associated with exposure. A total of 108 veterinarians took part to this cross-sectional study, with a majority practicing with livestock animals. The overall seroprevalence was 45.4%, but it increased to 58.3% among veterinarians having contact with livestock. Three main serological profiles were detected (relatively recent, past and potentially chronic infections). The contact with manure during the prior month was the risk factor associated with seropositivity after multivariate logistic regression analysis. Classification and regression tree analysis identified the age as the most predictive variable to exclude potentially chronic infection in apparently healthy seropositive veterinarians. In conclusion, livestock veterinarians practicing in Southern Belgium are highly exposed to Q fever, a neglected zoonosis for which serological and medical examinations should be envisaged in at risk groups.

Introduction

Q fever is a zoonosis caused by *Coxiella burnetii*, an obligate intracellular gram-negative bacterium first described in 1937 (Derrick, 1937; Davis and Cox, 1938). Ruminants are considered the primary source of infection for humans (Maurin and Raoult, 1999), and inhalation of contaminated aerosols represents the main route of transmission (Maurin and Raoult, 1999; Schimmer et al., 2009). Q fever is an occupational disease, with livestock farmers, veterinarians and abattoir workers being the most at risk of contact with infected animal products (CDC, 1986; Whitney et al., 2009; Schimmer et al., 2012, 2014; Van den Brom et al., 2013). However, the Q fever epidemic that occurred in the Netherlands between 2007 and 2010 was characterized by an unexpectedly high exposure of the general population, living in the surroundings of infected herds (Dijkstra et al., 2012).

In humans, Q fever is mainly asymptomatic or presents as a non-specific flu-like illness (Raoult et al., 2005). However, acute Q fever can be characterized by severe clinical conditions, such as atypical pneumonia and hepatitis in 2–5% of cases (Raoult et al., 2005). Despite the fact that *C. burnetii* infection is usually a self-limiting disease, immunocompromised persons, patients with valvulopathy and, to a lesser extent, pregnant women may develop chronic Q fever (Raoult et al., 2005).

In Belgium, Q fever is a notifiable disease in humans and animals. The disease is enzootic in the domestic ruminant population (Czaplicki et al., 2012; Vangeel et al., 2012;

Boarbi et al., 2014), while sporadic cases are detected in humans (M. Van Esbroeck, unpublished data; Wattiau et al., 2011). Indeed, a retrospective study showed that for the period from January 2003 to November 2010, a minimum of 8 to a maximum of 60 confirmed or possible acute Q fever infections were registered by the Belgian Q fever Reference Laboratory (Institute of Tropical Medicine) per year (Naesens et al., 2012). Unfortunately, potential risk factors associated with these cases were not investigated, and therefore, the association of those registered Q fever cases with existent epidemiological conditions and potential risk factors could not be established.

In the current work, a serological survey has been performed among veterinarians, to evaluate the seroprevalence against *C. burnetii*, as well as to characterize the serological responses. In parallel, all participants were asked to complete a questionnaire allowing the investigation of associated risk factors.

Materials and Methods

Samples and data collection

This study was approved by the Medical Ethical Committee of Liege University (reference number B707201215222). Written informed consent was obtained from all participants.

A cross-sectional study was conducted in November 2013 among veterinarians attending an annual professional event in the Walloon Region (Southern part of Belgium). A single blood sample was collected by a professional nurse. The tubes were centrifuged and serum samples were stored at −20°C until testing.

Each participant filled in a questionnaire allowing to collect information on potential risk factors associated with the serological condition of the veterinarians (Table 1).

A letter containing the individual serological results as well as the contact details of a physician who could provide complementary medical information was sent to each participant.

Laboratory analyses and interpretation

Serum samples were tested using a Q fever indirect immunofluorescence assay (IFA) (Focus Diagnostics, Cypress, CA, USA) for the detection and semi-quantitation of IgM and IgG antibody response to phase I and phase II *C. burnetii* antigens. IFA was performed following manufacturer's recommendations and an IgM and an IgG phase I and/or phase II titre ≥1 : 32 and 1 : 64 were defined as positive, respectively. In case of a positive result, an end-point titration of serial two-fold dilutions of the serum was performed. In the interpretation of the Q fever serology, a serum sample was defined as positive in the presence of an antibody response equal to or above

Table 1. Description of variables considered as potential risk factors of exposure of veterinarians to *Coxiella burnetii*, Southern Belgium, November 2013

Variables	Description
Demography	
Gender	Female, male
Age	In years (y)
Residence	Urban, peri-urban, rural area
Proximity to a bovine farm	Adjacent, <2 km, >2 km
Proximity to a caprine farm	Adjacent, <2 km, >2 km
Proximity to an ovine farm	Adjacent, <2 km, >2 km
Veterinary occupation	
Years of practice	Number of years of veterinarian activity (<1, ≥1 < 5, ≥5 < 10, ≥10 years)
Type of veterinary occupation	Practicing (yes, no) and/or other occupations (such as administrative or research activity)
Contact with animals	Different species were listed: bovine, caprine, ovine, swine, horses, dogs, cats
Frequency of contact with animals	For each animal species (never, rare, frequent, very frequent contact)
Contact with birth products	Contact with foetal membranes, products of abortion, realization of caesarean sections with the aforementioned animal species
Frequency of contact with birth products	For each animal species (never, rare, frequent, very frequent contact)
Contact with manure	Yes, no
Last contact with manure	In months when was the last contact (<1, ≥1 < 6, ≥ 6 < 12, ≥12)
Frequency of contact with manure	Never, rare, frequent, very frequent contact
Use of personnel protective equipment	Overall, gloves, boots, mask (yes, no)
Non-occupational related	
Tick bite	Yes, no
Frequency of tick bites	Never, rare, frequent, very frequent
Last tick bite	In months when was the last tick bite (<1, ≥1 < 6, ≥6 < 12, ≥12)
Delay of tick removal	Approximately delay in hours between an at risk activity and the tick removal (<12, ≥12 < 24, ≥24 < 48, ≥48)
Consumption of raw milk products	Yes, no
Frequency of consumption	Never, rare, frequent, very frequent consumption
General health conditions	The presence of clinical signs compatible with febrile illness, hepatitis, pneumonia was questioned
Known history of Q fever infection	The presence of a previous laboratory confirmed diagnosis of Q fever was asked

the cut-off levels. A solitary IgM phase II response was considered as a recent infection. The presence of IgM and IgG antibody was interpreted as compatible with a relatively recent infection, while a solitary IgG response was defined as a past infection. A potential chronic infection was defined by an IgG phase I titre ≥1 : 1024 (van der Hoek et al., 2011). This cut-off was used to facilitate the comparison with a recent Dutch study performed among veterinarians with possible chronic infection (Wielders et al., 2015).

Statistical analyses

To identify possible risk factors associated with the seropositivity to *C. burnetii*, the answers to the questionnaire were encoded and merged with the serological results (positive, negative) of each veterinarian. First, a univariate analyses was conducted and odds ratio's (OR) with 95% confidence intervals (CIs 95%) were attributed to each variable. Then, a multivariate logistic regression analysis was performed using variables with P values <0.05. In the presence of correlation between two variables, only one (with the highest biological relevance) was included in the multivariate analysis. The use of the Firth logit method allowed inference of ORs and CIs when complete separation (zero cells) occurred (Heinze and Schemper, 2002). Backward stepwise logistic regression was used to exclude progressively variables having the highest P value. This was realized only if the variation of the ORs of the remaining variables was lower than 20%.

Classification and regression tree (CART) analysis was performed using the explanatory variables used in the multivariate analysis. CART was developed by Breiman et al. (1984) and has been applied in epidemiology and clinical settings (Marshall, 2001; Saegerman et al., 2011). The serological results to *C. burnetii* were used as outcome variables. In particular, seronegative and seropositive results were used, and within the seropositive, the three main serological profiles (recent, past and potentially chronic infections) were included. The aim of this CART analysis was to identify variables which could be used for their predictive value for the given outcomes.

Results

A total of 108 veterinarians participated to the survey, with 74 males (68.5%) and 34 females (31.5%). Participants' age ranged from 23 to 68 years, with an average of 38 years. Participants had mostly a rural residence (65.7%), followed by a peri-urban (19.4%) and an urban residence (13.9%).

Of the participants, 96 (88.9%) had a professional activity in the Walloon Region or in the Brussels-Capital Region. The other participants exercised their profession in the Grand Duchy of Luxembourg (n = 2) and in the north of France (n = 10), at the border with the southern part of Belgium.

Concerning their professional activity, 10 veterinarians (9.3%) currently were not practicing and exercised administrative and research functions within different Belgian federal institutions, veterinary research institutions and laboratories. Fifty-three (49.1%) were livestock veterinarians (their range of activity included domestic ruminants and swine), 16 (14.8%) were in contact only with companion animals (dogs, cats and horses), and 29 (26.8%) had a mixed activity.

Q fever seroprevalence and serologic profiles

The overall seroprevalence was 45.4% (95% CI: 35.8–55.2; 49/108), but it increased to 58.5% (95% CI: 47.1–69.3; 48/82) among veterinarians having contact with livestock. On the contrary, the seroprevalence was significantly reduced (6.25% with 95% CI: 0.16–30.2; 1/16) among veterinarians dealing only with companion animals.

The most frequent serological profile among the positive veterinarians was the past infection (n = 28), followed by the relatively recent infections (n = 14). Only one veterinarian had a solitary IgM phase 2 response (1 : 128), corresponding to a recent infection. Six veterinarians (representing the 12.2% of the seropositive veterinarians) had a serological profile suggesting a chronic Q fever infection.

Since the observation of these three main serological profiles (recent, past and potentially chronic), three sub-populations were created among the seropositive veterinarians. These three sub-groups were identified as miscellaneous sub-populations on the basis of the different age distribution (Kruskal–Wallis equality-of-populations rank test, P = 0.0003). Indeed, veterinarians with a recent infection were the youngest, with a mean age of 31.3 years (SD = 8.1); those with past infection had a mean age of 43.3 years (SD = 12.4); lastly, veterinarians with a potential chronic infection were the oldest, with a mean age of 53.5 years (SD = 9.5).

Risk factors associated with Q fever seropositivity

In the univariate analysis, different variables were associated with a higher exposure to *C. burnetii*. These variables, with a P value <0.05, are presented in Table 2.

Veterinarians' age was associated with Q fever exposure. First, the mean age was significantly higher among seropositive (40.8 years; SD = 12.9) than seronegative veterinarians (35.1 years; SD = 10.2) (two-sample Mann–Whitney test, P = 0.02). Second, the participants with an age category ranging from 48 to 68 years were found to be significantly more frequently seropositive during the univariate

Table 2. Univariate logistic regression model for risk factors associated with the seropositive results of veterinarians against *Coxiella burnetii*, Southern Belgium, November 2013

Variable	Seropositive no. (%)	OR (95% CI)	*P* value
Gender			
Female	7 (20.6)	Reference	–
Male	42 (56.8)	5.06 (1.96–13.09)	0.001
Age, years			
23–29	10 (30.3)	Reference	–
30–33	11 (45.8)	1.77 (0.60–5.20)	0.30
34–47	12 (48)	1.93 (0.67–5.59)	0.23
48–68	15 (60)	3.14 (1.07–9.19)	0.037
Residence			
Urban	2 (13.3)	Reference	–
Peri-urban	7 (33.3)	3.25 (0.57–18.58)	0.19
Rural	40 (56.3)	8.39 (1.76–39.95)	0.008
Practicing			
No	0 (0)	Reference	–
Yes	49 (50)	21 (2–368)	0.037
Animal contacts during profession			
Bovine			
Never	1 (5.6)	Reference	–
Rare	2 (20)	4.25 (0.33–54.07)	0.27
Frequent	1 (12.5)	2.43 (0.13–44.50)	0.55
Very frequent	45 (62.5)	28.33 (3.57–225.09)	0.002
Caprine and ovine			
Never	4 (18.2)	Reference	–
Rare	1 (7.1)	0.35 (0.04–3.47)	0.37
Frequent	15 (57.7)	6.14 (1.62–23.29)	0.008
Very frequent	29 (63)	7.67 (2.23–26.47)	0.001
Swine			
Never	13 (28.3)	Reference	–
Rare	10 (43.5)	1.95 (0.69–5.55)	0.21
Frequent	19 (63.3)	4.38 (1.64–11.70)	0.003
Very frequent	5 (71.4)	6.35 (1.09–36.92)	0.04
Horses			
Never	8 (25)	Reference	–
Rare	8 (47)	2.67 (0.77–9.25)	0.12
Frequent	13 (52)	3.25 (1.06–9.97)	0.04
Very frequent	20 (58.8)	4.29 (1.50–12.27)	0.007
Contacts with birth products[a]			
Bovine			
Never	1 (11.1)	Reference	–
Rare	1 (5.9)	0.50 (0.03–9.08)	0.64
Frequent	1 (25)	2.67 (0.12–57.62)	0.53
Very frequent	46 (59)	11.50 (1.37–96.50)	0.02
Caprine and ovine			
Never	9 (32.1)	Reference	–
Rare	8 (30.8)	0.94 (0.30–2.96)	0.91
Frequent	11 (57.9)	2.90 (0.87–9.71)	0.08
Very frequent	21 (60)	3.17 (1.12–8.98)	0.03
Contacts with manure			
Last contact, months			
<1	42 (57.5)	6.77 (1.80–25.45)	0.005
≥1 < 6	3 (25)	1.67 (0.28–10.09)	0.58
≥6 < 12		n.d.	
≥12	3 (16.7)	Reference	–

OR, odds ratio; CI, confidence interval; n.d. not determined.
Only variable with *P* value <0.05 are shown.
[a]Birth products include fetal membranes, products of abortion, caesarean sections.

analysis (Table 2). Males were significantly more often seropositive than females, and living in a rural area was significantly associated with exposure. A significantly higher OR was found for practicing compared to non-practicing veterinarians. In particular, the very frequent contacts with bovine, as well as the frequent and very frequent contacts with caprine, ovine, swine or horses during the professional activity, were associated to a significantly higher exposure to *C. burnetii*. Interestingly, when the univariate analysis concerned the contacts with birth products (foetal membranes, aborted foetuses, caesarians), an association was found only with the very frequent contact for bovine, caprine and ovine. Lastly, a contact with manure during the prior month was significantly associated with exposure.

Questions concerning the use of personal protective equipment were included in the questionnaire submitted to the veterinarians. The univariate analysis did not show their role in the exposure of veterinarians to Q fever. Similarly, questions related to non-occupational factors such as tick bites and consumption of raw milk products did not show any association with the exposure to *C. burnetii*.

Explanatory variables with a *P* value <0.05 in the univariate logistic regression were included in the multivariate model. However, the contacts with an animal species and with the birth products of the same species were found to be correlated. Therefore, only the contact with birth products was included into the model. Initially, seven variables were included into the multivariate logistic regression analysis. After backward stepwise procedure, only four variables were left into the model, and among those, only the contact with manure during the prior month was a risk factor for Q fever seropositivity (OR 4.18; 95% CI: 1.01–17.25; $P = 0.048$) (Table 3). The other three variables were left into the final model regardless their significance because of their biological importance.

CART analysis

Classification and regression tree analysis was performed using the following exposure variables: gender, age, residence, practicing, contact with birth products (bovine, caprine, ovine) and manure. The outcome variables were the

Table 3. Multivariate logistic regression model for variables associated with the seropositive results of veterinarians against *Coxiella burnetii*, Southern Belgium, November 2013

Variable	OR (95% CI)	*P* value
Age, years		
23–29	Reference	
30–33	1.58 (0.47–5.35)	0.458
34–47	1.65 (0.49–5.57)	0.418
48–68	2.23 (0.65–7.64)	0.203
Contacts with birth products		
Bovine		
Never	Reference	
Rare	0.29 (0.02–4.18)	0.362
Frequent	1.56 (0.07–34)	0.776
Very frequent	2.8 (0.29–26.54)	0.370
Contacts with birth products		
Caprine, ovine		
Never	Reference	
Rare	0.45 (0.09–2.24)	0.334
Frequent	0.71 (0.14–3.55)	0.682
Very frequent	0.76 (0.18–3.37)	0.735
Contacts with manure		
Last contact, months		
<1	4.18 (1.01–17.25)	0.048
≥1 < 6	1.96 (0.29–9.95)	0.560
≥ 6 < 12	n.d.	
≥12	Reference	

OR, odds ratio; CI, confidence interval; n.d. not determined.

following: seronegative, seropositive with recent, past or potentially chronic infection. The population was split into categories based on the risk factors introduced into the model (Fig. 1). The variable age allowed identifying two main sub-populations. Veterinarians below 34 years constituted the first sub-population, in which seronegative (36/59), recent (12/15) and past infections (10/28) were gathered. In the second sub-population (veterinarians above 34 years), seronegative (23/59), recent (3/15), past (18/28) and potentially chronic infections (6/6) were clustered together.

Among the first sub-population, CART used the contact with manure to distinguish two groups: one constituted primarily by seronegative veterinarians (17/59), but also recent (1/15) and past infections (1/28). This cluster was characterized by a contact with manure occurred more than 1 month ago. On the contrary, the other group, characterized by a contact with manure during the prior month, was composed by most of the recent infections (11/15), past infections (9/28) and seronegative veterinarians (19/59). Considering the sub-population of veterinarians older than 34 years, the very frequent contacts with bovine birth products allowed gathering all chronic infections (6/6), as well as past infections (18/28), recent infections (2/15) and a minority of negative cases (13/59). All the other frequencies in the contacts with bovine birth products (from absence to frequent) allowed to obtain a cluster constituted

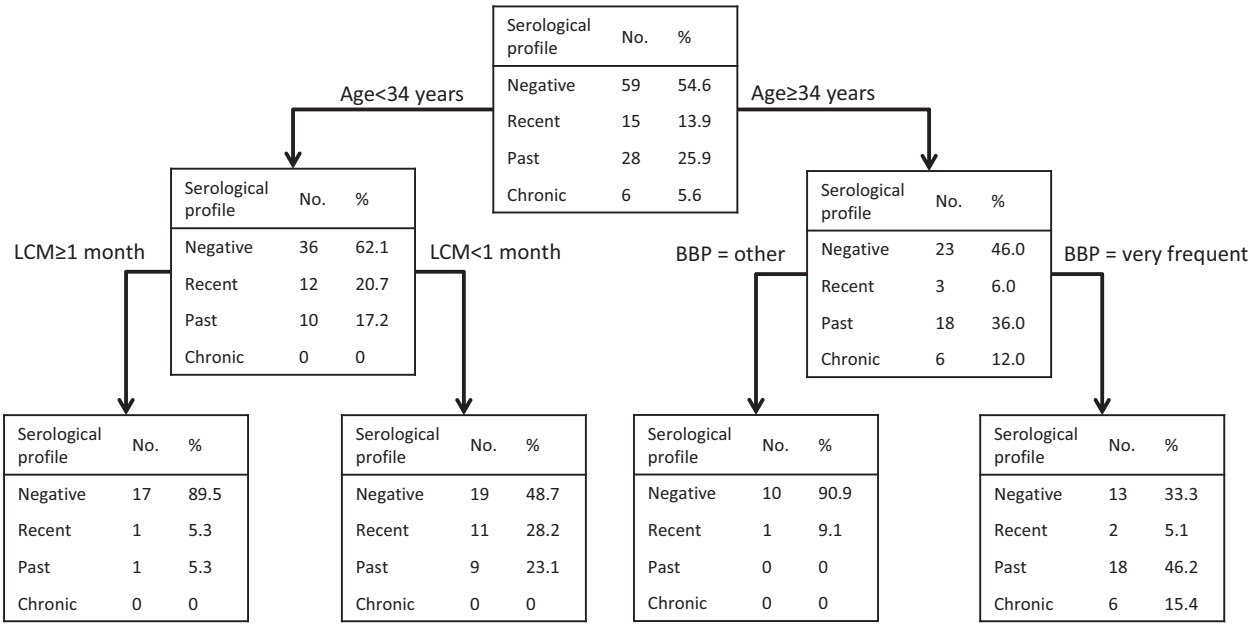

Fig. 1. Classification and regression tree obtained by including into the model the serological profiles (negative, relatively recent, past, potentially chronic infection) and the variables selected after univariate analysis. The first predictor variable used was the age, which allowed splitting the total population into two sub-populations. On the left side of the figure, the second predictor variable used was the last contact with manure (LCM). On the right side of the figure, the second predictor variable used was the frequency of contacts with bovine birth products (BBP). Within each node, the number of cases responding to the predictor variable is presented, together with the relative proportion in % of each outcome variable.

by seronegative veterinarians (10/59) and veterinarians with recent infection (1/15).

Discussion

In this work, a cross-sectional study was performed among veterinarians mainly working in Southern Belgium. We found an overall seroprevalence of 45.4%, with a higher exposure among livestock veterinarians (seroprevalence of 58.5%). Among the potential risk factors analysed using logistic regression models, only the contact with manure during the prior month proved to be significantly associated with a higher risk of seropositivity to *C. burnetii*. Furthermore, the use of CART analysis allowed identifying the age as a predicting variable to exclude potentially chronic infection in apparently healthy seropositive veterinarians.

Previous studies realized on veterinarians in European and non-European countries showed several risk factors associated with Q fever exposure. Some risk factors were related to the profession, such as the occupational exposure to bovine (Bernard et al., 2012; Whitney et al., 2009), ovine (Bernard et al., 2012), swine (Whitney et al., 2009; Van den Brom et al., 2013), wildlife (Whitney et al., 2009), practicing and the years of practice (Van den Brom et al., 2013). Some other risk factors were independent from the profession, such as the male sex (Bernard et al., 2012), the increasing age (Bernard et al., 2012), living in a sub-urban and rural area (Van den Brom et al., 2013) and routine contact with pond water (Whitney et al., 2009). To the authors' knowledge, this is the first study showing the contact with manure during the prior month as a risk factor of exposure to *C. burnetii* in veterinarians. This risk factor is related to the profession because livestock veterinarians practicing in a rural environment are the most in contact with farming animal products, such as manure. However, the contact with manure is not exclusive of the veterinarian profession, and it could be extended to farmers as well. Although in the questionnaire submitted to the participants, the origin of the manure (animal species) was not specified, we could assume that it could derive from ruminants and swine, because the majority of the responding veterinarians had an activity including these livestock animals (75.9%). Several studies suggested the role of caprine and ovine manure in the transmission of *C. burnetii* to humans (Berri et al., 2003; Gyuranecz et al., 2014; Hermans et al., 2014), especially as a source of contamination within the farm and during the spreading on the fields. However, the role of land-applied goat manure in the transmission of *C. burnetii* to humans during the epidemic in the Netherlands was recently reconsidered (Van den Brom et al., 2015). Because the contact with manure was identified as the risk factor of exposure to *C. burnetii* for veterinarians, it would be worth studying the role of the different sources of manure in the transmission of *C. burnetii*, taking into consideration the farm practices and the proportion of the different livestock population in Southern Belgium.

Our survey was organized in an epidemiological context characterized by a sporadic detection of human Q fever cases and an enzootic situation in the domestic ruminant population (Czaplicki et al., 2012; Vangeel et al., 2012; Boarbi et al., 2014). Remarkably, during the Q fever epidemic occurred in the Netherlands between 2007 and 2010 (van der Hoek et al., 2012), the aforementioned condition in humans and ruminants in Belgium did not change. In this scenario, the seroprevalence found among the veterinarians was similar to the data published by two recent studies in the Netherlands and in Germany. In the Netherlands, an overall seroprevalence of 65.1% was found among livestock veterinarians (Van den Brom et al., 2013). Contemporarily, in Bavaria the occupational exposure of veterinarians to cattle, sheep, goats, pigs and horses was associated with a seroprevalence ranging from 51% to 63% (Bernard et al., 2012). It is interesting to notice that the seroprevalence among livestock veterinarians in the Netherlands was about 7% higher than in the South of Belgium and 2–14% higher than in Bavaria.

It should be stressed that the comparison of Q fever estimates in the literature is difficult because of the use of different tests and cut-off values. Indeed, IFA is considered the reference diagnostic test especially for seroprevalence studies (Dupont et al., 1994), but a general consensus does not exist over the cut-off values and the interpretation of the positive results. The combined use of other laboratory methods (such as PCR) could be more appropriate during epidemic settings to allow classification of confirmed, probable and possible acute Q fever cases (Jaramillo-Gutierrez et al., 2013). Concerning chronic Q fever, the diagnostic criteria are still under debate and the proposed cut-offs for IgG phase I of 1 : 1024 and/or 1 : 1600 showed low specificity (Frankel et al., 2011; van der Hoek et al., 2011). Despite this, the cut-off 1 : 1024 is the one currently used in the Netherlands, and it has been recently used in a follow-up study among seropositive veterinarians (Wielders et al., 2015). During the 3-year observation period, the authors found that IgG phase I titres remained constant or slightly increased and they formulated the hypothesis that the continuous exposure to *C. burnetii* and the consequent boostering effect on the immune system could be at the origin of the persistently high antibody levels. Despite the high IgG phase I titres, the veterinarians did not show any other signs of chronic infection (Wielders et al., 2015). Similarly to what was described in the Dutch study, here we found also a group of six healthy veterinarians with IgG phase I titres $\geq$1 : 1024. However difficult to interpret, the presence in healthy veterinarians of an immunological response

which could be associated with a pathological condition (chronic Q fever), has to be carefully considered and would require further investigations and monitoring.

An interesting finding of our serosurvey was the identification of three main serological profiles among the seropositive veterinarians. To find possible predictive variables associated with these serological profiles, a CART analysis was performed. Veterinarians with a serological profile of a relatively recent *C. burnetii* infection were mostly under 34 years old and had a contact with manure during the prior month (73.3% of the relatively recent infections). However, also a proportion of veterinarians with past infections (32.1%) shared the same features, indicating that those variables could not sufficiently discriminate between relatively recent and past infections. Past infections occurred also in veterinarians older than 34 years. In this case, past infections shared the same predictive variable with the potentially chronic Q fever infections, namely having very frequent contacts with bovine birth products. Based on these findings, in our population, only the age was found to be a determinant predictive variable to exclude potentially chronic Q fever infection in seropositive veterinarians below 34 years old. On the contrary, CART analysis did not identify predictive variables for discriminating recent from past infections, as well as past from potentially chronic Q fever infections. Although the six veterinarians having a potentially chronic infection constituted a separate group with a higher mean age compared to the other two groups of veterinarians, their serological profile could not be explained by other variables of epidemiological relevance.

Conclusion

Veterinarians and especially livestock veterinarians practicing in the South of Belgium are highly exposed to Q fever. Disease is mostly not recognized because of the asymptomatic and the benign evolution of the infections. However, among the seropositive veterinarians, 6 male livestock veterinarians with a mean age of 53.5 years showed a serological profile compatible with chronic Q fever. They were all apparently healthy, but a serological follow-up for Q fever as well as a medical follow-up would be recommended. Moreover, it should be encouraged to increase the awareness of the potential consequences associated with undiagnosed chronic Q fever infections and recommend periodical screening of zoonotic infections such as Q fever, in high risk groups.

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