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REGULAR ARTICLES

Risk factor analysis for antibodies to *Brucella*, *Leptospira* and *C. burnetii* among cattle in the Adamawa Region of Cameroon: a cross-sectional study

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Abstract Brucellosis, leptospirosis and Q fever are important livestock diseases, commonly responsible for significant production losses, yet their epidemiology in sub-Saharan Africa is largely unknown. Animal reservoirs pose the main risk of transmission to humans, where serious disease can occur. In the developing world setting, the flu-like symptoms of the acute stages of these diseases can be misdiagnosed as malaria, which can result in the administration of the wrong treatment, prolonged disease and increase in antibiotic resistance. Multivariable mixed-effects logistic regression models in this study revealed potential risk factors associated with the aforementioned pathogens in cattle in the Adamawa Region of Cameroon, with wildlife, namely, buffaloes, playing a major role in both *Brucella* and *Coxiella burnetii* seropositivity. Cattle mixing with other herds at night and cattle grazing in an area on a route taken by herds on transhumance appear to be positively associated with *Leptospira* seropositivity, while female cows and whether buffaloes are seen during grazing or transhumance are positively associated with *C. burnetii* seropositivity. On the other hand, animals that have been on transhumance in the past year and animals belonging to herdsmen of the Fulbe ethnic group appear to be protected against *Leptospira* and *C. burnetii*, respectively. Cattle of more than 2 years old appear to have increased odds of being seropositive to either pathogen. Further research is needed to confirm these findings and improve the knowledge of the epidemiology of these three pathogens in Africa, taking particular consideration of the wildlife involvement in the disease transmission.

Keywords Brucellosis · Leptospirosis · Q fever · Cattle · Cameroon · Epidemiology · Risk factors

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Introduction

Zoonoses such as leptospirosis, Q fever and brucellosis can cause significant livestock production loses as well as debilitating disease in humans. Such diseases provide a worldwide challenge, which is augmented by the rising population size, globalisation and increased demand for food production.

Brucellosis is a zoonosis caused by Gram-negative bacteria of the genus *Brucella* and commonly affects sheep, goats, cattle and humans. Brucellosis can result in abortion, infertility and reduced milk production in cows and varying degrees of sterility in bulls and is mainly caused by *Brucella abortus* (Corbel 1997; Godfroid et al. 2004). Infected animals excrete *Brucella*

in urine, milk and abortive material, and the organism can survive in the environment for up to 80 days (Doganay and Aygen 2003). In humans, the disease is most commonly caused by *Brucella melitensis* followed by *B. abortus* and invariably involves an undulating fever but has otherwise non-specific symptoms. Although, the mortality rate is low, brucellosis can result in long periods of convalescence and residual disability (Namanda et al. 2009). Brucellosis is considered both a foodborne and an occupational disease, and transmission can occur by contact with infected animal parts, consumption of infected unpasteurised milk products and via the airborne route (Pappas et al. 2006, 2008).

Despite the disease-free status of a number of developed countries around the world, bovine brucellosis still remains one of the most important zoonoses due to its worldwide distribution and great economic and public health impacts (OIE 2009). In sub-Saharan Africa (SSA), brucellosis is considered particularly important and its prevalence ranges from sporadic cases to as high as 41 % in some areas (Domingo 2000; McDermott and Arimi 2002). Risk factors associated with *Brucella* seropositivity suggested by studies carried out in various settings in Africa include high stocking density, common grazing and watering points, older age, herds with multiple livestock species and going on transhumance (McDermott and Arimi 2002; Megersa et al. 2011; Muma et al. 2007; Swai and Schoonman 2010).

Leptospirosis is a neglected disease caused by different species of the spirochete *Leptospira* which has a large variety of carriers and vectors including both wild and domestic animals (Guitián et al. 2001; OIE 2008; Vijayachari et al. 2008). In cattle, it can cause fever, decreased milk production, mastitis or even death and it is also an important cause of abortion and infertility (Hunter 2004; Scolamacchia et al. 2010). Transmission occurs by direct or indirect contact, and excretion is mainly by urine with a variable duration of several weeks to an animal's lifetime (Ellis 1984). The organism may survive in the environment for prolonged periods of time favoured by warm, humid conditions (Levett 2001).

Transmission to humans occurs via exposure to infected animal urine, either directly or through contaminated environment especially water. Leptospirosis is considered an occupational as well as a recreational disease, and it can vary from a subclinical or mild disease to a life-threatening condition called Weil's syndrome. The disease may initially present as a sudden onset fever mimicking other non-specific febrile diseases and can be misdiagnosed. Mortality rates vary depending on the organ systems involved and have been reported to be between 3 and 54 %. In developing countries, mortality rates also depend on diagnostic delays due to lack of clinical suspicion and insufficient infrastructure (Bharti et al. 2003; Esen et al. 2004).

The incidence of leptospirosis in the developed world has decreased considerably, while increasing trends have been described in developing countries (Vijayachari et al. 2008). In Africa, with the exception of South Africa, the status of leptospirosis is largely unknown (Pappas et al. 2008). Recent studies have reported a prevalence of leptospirosis of 19.4 % in KwaZulu-Natal in South Africa (Hesterber et al. 2009) and 10.8 and 30.3 % in Tanga Region of Tanzania (Swai et al. 2005; Schoonman and Swai 2010). Estimates from older studies include 27 % in Zimbabwe (Feresu 1987), 21.4 % in Malawi (Myburgh et al. 1989) and 44.8 % in Bamako, Mali (Niang et al. 1994). Risk factors for bovine leptospirosis, identified mainly in studies outside SSA, include a larger herd size; increased stocking density; access to contaminated water sources; use of an infected bull; co-grazing with infected cattle, sheep or pigs; and older age. Herd type and replacement policy also seem to be important (Alonso-Andicoberry et al. 2001; Lilenbaum 2003; Segura-Correa et al. 2003).

Q fever is a highly infectious re-emerging zoonosis with a worldwide distribution caused by *Coxiella burnetii*, an obligate intracellular Gram-negative bacterium (Maurin and Raoult 1999). It has an extensive range of reservoirs including mammals, birds and ticks. In cattle, the infection is usually asymptomatic but can cause metritis, late-term abortion and stillbirths. In humans, Q fever can present as a flu-like illness, atypical pneumonia or hepatitis in its acute form and as a life-threatening endocarditis or a chronic fatigue syndrome in its chronic form. Transmission to humans mainly occurs via inhalation of contaminated aerosols originating from excretions and abortive material of farm animals such as cattle, sheep and goats as well as pets. The organism is commonly present at high concentrations in the uterus and mammary glands of infected animals; therefore, transmission is associated with abortion of domestic ruminants (Arricau-Bouvery and Rodolakis 2005; Angelakis and Raoult 2010). Control of transmission is particularly tricky due to the exceptionally low infectious dose, the long-term survival of the organism in the environment and the ability of the organism to be transported by strong winds (Oyston and Davies 2011)

Estimates of prevalence of Q fever in cattle in SSA range from 4 % in Chad (Schelling 2003), 14.3 % in the Central African Republic (Nakouné et al. 2004) and 39 % in Zimbabwe (Kelly et al. 1993). Literature on risk factor analysis for Q fever seropositivity is limited but includes factors such as large herd size (Ryan

et al. 2011) and drinking water from a watercourse or a well, while shed disinfection seems to be protective (Czaplicki et al. 2012).

Despite the endemicity of these three zoonoses in SSA, their prevalence is inadequately documented and their epidemiology is poorly understood. The prevalence of the three zoonoses in this study was presented by Scolamacchia et al. (2010) and was estimated to be 30.4 % (95 % confidence interval (CI) 27.6–33.2), 31.2 % (95 % CI 27.3–35.0) and 3.1 % (95 % CI 1.8–4.4 %) for *Leptospira*, *C. burnetii* and *Brucella*, respectively. The current study aims to identify risk factors for seropositivity to the above pathogens and discuss their relevance to previous literature.

Materials and methods

Study background

The current analysis is based on serum samples obtained during a study of foot-and-mouth disease (FMD) in Cameroon. The study was based in the Adamawa Region, which is divided into five administrative divisions and has 88 veterinary centres according to the Ministry of Livestock, Fisheries and Animal Industries. The sampling frame was created according to a rinderpest vaccination database which included 13,006 herds. A stratified, two-stage cluster sampling design resulted in 1,377 sera being collected from 147 herds. Three herds per veterinary centre were randomly selected without replacement, and approximately five adult (more than 24 months of age) and five juvenile (8 to 24 months of age) cattle from each herd were sampled. In-depth information about this study was published by Bronsvoort et al. (2003).

Questionnaire design and data collection

On the day of the visit, a 30–40-min pretested questionnaire and a clinical examination of the animals were used to obtain information on possible risk factors. The questionnaire was carried out in Foulfoulde (the local Fulani dialect) and covered a wide range of topics, including information on housing, grazing, watering, ownership or contact with other animals including wildlife, transhumance routines, purchases from markets, treatments used and more. Additionally, GPS readings were taken in order to match each herd to weather information obtained from the Monitoring Agricultural Resources Unit (http://mars.jrc.ec.europa.eu/mars/About-us/The-MARS-Unit).
Lastly, cattle density in each veterinary centre was estimated based on the total number of herds per veterinary centre, the mean herd size for all sampled herds and the area covered. More details on the cattle density calculation can be found in Handel et al. (2011).

Serology

Serology for the three pathogens was carried out on jugular blood samples obtained on the day of the visit and stored at the FMD World Reference Laboratory, Pirbright at −20 °C. The Linnodee Lepto ELISA kit (Linnodee Animal Care, Ballyclare, Northern Ireland) and the cELISA *Brucella* diagnostic kit (Brucelisa 400, VLA, Weybridge, UK) were used to screen the sera for antibodies to *Leptospira hardjo* and smooth *Brucella* strains (*B. abortus* and *B. melitensis*), respectively. Lastly, CHECKIT Q Fever ELISA kit (Bommeli, IDEXX Laboratories, Broomfield, CO) was used to screen for IgG antibodies to *C. burnetii*. The tests were performed according to the manufacturer's instructions, and more details can be found in Scolamacchia et al. (2010).

Statistical analysis

Statistical analysis was carried out in R-2.12.1 (R Core Development team 2010). Descriptive analysis was performed in order to identify any missing values or data errors and evaluate the distributions of continuous variables. For categorical variables, a Fisher's exact test was performed using the *epicalc* package (Chongsuvivatwong 2011), and for continuous variables, a univariable logistic regression model was fitted (Hosmer and Lemeshow 2000). Any variables with a p value of less than 0.2 were then used to fit a multivariable mixed-effects logistic regression model shown in Eq. 1 using package *lme4* (Bates et al. 2011). The outcome for the model was individual animal (i) seropositivity and herd (j) was used as a random effect. Variables were entered into the model according to their p value, the smallest one entered first, and dropped if the p value was higher than 0.1. The final model was chosen based on the lowest Akaike information criterion (AIC).

$$y_{ij} \sim \alpha + \beta X_{ij} + \mu_j + \varepsilon_{ij} \quad (1)$$

where α is the fixed intercept, β is the fixed effects, X is the covariate, μ_j is the random effect, ε_{ij} is the error, $\mu \sim N(0, \sigma^2_{\text{herd}})$ and $\varepsilon \sim N(0, \sigma^2_{\text{animal}})$.

Table 1 Comparison of mixed-effects logistic regression risk factor models for *Brucella* seropositivity (bruc)

Model	AIC
bruc~1+(1|hcode)	346.6
bruc~age+(1|hcode)	341.5
bruc~age+buffgrz+(1|hcode)	339.1

buffgrz Did you see any buffalo during grazing? *1|hcode* herd code (random effect)

Results

Across the five administrative divisions of the Adamawa Region, 1,377 cattle from 147 herds were included in the analysis. Cattle age ranged from 0.33 to 18 years with a median of 3 years and 412 (30 %) of the cattle were male. 70 % of cattle were Gudali, 21 % White Fulani, 7 % Red Fulani and 1 % Cross bred. Results for variables from the univariable analysis used to fit the multivariable model for each pathogen are shown in tables included in the Electronic Supplementary Material.

Brucella

Table 1 describes the modelling process and shows the final model for *Brucella* seropositivity (last row). The final model was chosen using forward selection based on the lowest AIC and includes cattle age and whether they see buffalo during grazing as shown in Table 2. According to this model, cattle of more than 2 years of age show a positive association with an odds ratio (OR) of 2.76 (95 % CI 2.15–3.55) when compared to cattle 2 years old or younger. Additionally, the odds of being seropositive to *Brucella* are almost ten times higher if farmers see buffalo during grazing (95 % CI 1.12–84.58).

Leptospira

According to the modelling process followed as shown in Table 3, the final model in terms of *Leptospira* seropositivity included variables 'age', 'trns1vr', 'grzrtrn' and 'nitmix'. Cattle of more than 2 years of age show a positive association with an OR of 2.76 (95 % CI 2.15–3.55) when compared to cattle 2 years old or younger. Additionally, the odds of being seropositive increase in cattle that mix with other herds at night (OR 1.48, 95 % CI 1.05–2.07) and cattle that graze in a grazing area on a route taken by herds on transhumance (OR 1.40, 95 % CI 1.04–1.89). On the contrary, there appears to be a protective effect if any of the animals in the herd went on transhumance this year (OR 0.56, 95 % CI 0.42–0.75) (Table 4).

Table 3 Comparison of mixed-effects logistic regression risk factor models for *Leptospira* seropositivity (lept)

Model	AIC
lept~1+(1|hcode)	1,685
lept~age+(1|hcode)	1,621
lept~age+trns1yr+(1|hcode)	1,612
lept~age+trns1yr+grzrtrn+(1|hcode)	1,608
lept~age+trns1yr+grzrtrn+nitmix+(1|hcode)	1,605

trns1yr Did any of this herd you brought here today go on transhumance this years? *grzrtrn* Is the grazing area on a route taken by herds on transhumance? *nitmix* At night, do your cattle mix with other herds? *1|hcode* herd code (random effect)

Q fever

The modelling process described in Tables 5 and 6 shows the final model for *C. burnetii* seropositivity. Similar to the two previous models, cattle being more than 2 years of age show a positive association with an OR of 2.92 (95 % CI 2.20–3.88) when compared to cattle 2 years old or younger. Female cows seem to have increased odds with an OR of 1.55 (95 % CI 1.11–2.15), and the risk also increases if buffaloes are seen during grazing or transhumance (OR 1.89, 95 % CI 1.17–3.05). Lastly, when compared to the herdsman belonging to the Fulbe ethnic group, belonging to Mbororo ethnic group (OR 1.62, 95 % CI 1.00–2.63) and any other

Table 2 Final risk factor model for *Brucella* seropositivity in individual cattle in the Adamawa Region of Cameroon ($n = 1{,}373$; four animals with missing values for buffgrz were dropped)

Variable	Levels	Odds ratio	*p* value	95 % CI
Age (years)	0–2	1		
	>2	2.59	0.019	1.17–5.73
buffgrz	No	1		
	Yes	9.72	0.040	1.12–84.58

Table 4 Final risk factor model for *Leptospira* seropositivity in individual cattle in the Adamawa Region of Cameroon ($n = 1{,}377$)

Variables	Levels	Odds ratio	*p* value	95 % CI
Age (years)	0–2	1		
	>2	2.76	<0.001	2.15–3.55
trans1yr	No	1		
	Yes	0.56	<0.001	0.42–0.75
grazrtrn	No	1		
	Yes	1.40	0.027	1.04–1.89
nitmix	No	1		
	Yes	1.48	0.024	1.05–2.07

Table 5 Comparison of mixed-effects logistic regression risk factor models for *C. burnetii* seropositivity (qfever)

Model	AIC
qfever~1+(1|hcode)	1,611
qfever~age+(1|hcode)	1,535
qfever~age+buffevr+(1|hcode)	1,530
qfever~age+buffevr+sex+(1|hcode)	1,526
qfever~age+buffevr+sex+ethngrp+(1|hcode)	1,524

buffevr Did you see any buffalo during grazing or transhumance? *ethngrp* ethnic group, *1|hcode* herd code (random effect)

Table 6 Final risk factor model for *C. burnetii* seropositivity in individual cattle in the Adamawa Region of Cameroon ($n = 1{,}377$)

Variable	Levels	OR	*p* value	95 % CI
Age	0–2	1		
	>2	2.92	<0.001	2.20–3.88
buffevr	No	1		
	Yes	1.89	0.010	1.17–3.05
Sex	Male	1		
	Female	1.55	0.010	1.11–2.15
ethngrp	Fulbe	1		
	Mbororo	1.62	0.050	1.00–2.63
	Other	2.50	0.023	1.14–5.52

ethnic group (OR 2.50, 95 % CI 1.14–5.52), a positive association with *C. burnetii* seropositivity was shown.

Discussion

The main aim of this study was to identify potential risk factors associated with cattle seropositivity to *Brucella*, *Leptospira* and *C. burnetii*. According to the three multivariable models described above, cattle more than 2 years old have increased odds of being seropositive to either of the three pathogens. This is in accordance to previous studies, and its explanation lies in the fact that the older an animal is, the longer is the potential exposure to the pathogen (Megersa et al. 2011). The only other risk factor identified by this analysis in terms of *Brucella* seropositivity was if farmers see buffalo during grazing (OR 9.72 ,95 % CI 1.12–84.58).

In addition to age, cattle mixing with other herds at night (OR 1.48, 95 % CI 1.05–2.07) and cattle grazing in an area on a route taken by herds on transhumance (OR 1.40, 95 % CI 1.04–1.89) appeared to be positively associated with *Leptospira* seropositivity. These two factors reflect the increased risk associated with mixing with infected animals. In contrast to what one might expect, animals that have been on transhumance during the past year appear to be protected (OR 0.56, 95 % CI 0.42–0.75). One of the questions in the questionnaire asked 'why did you not take your herd on transhumance this dry season'. One-third of the farmers responded that it was because too many animals die during transhumance. It is possible that farmers with higher disease burdens and hence more deaths do not take their animals on transhumance, and this may act as a confounder.

In terms of seropositivity to *C. burnetii*, the multivariable analysis indicated that the odds increase in female cows (OR 1.55, 95 % CI 1.11–2.15) and if buffaloes are seen during grazing or transhumance (OR 1.89, 95 % CI 1.17–3.05). Furthermore, herdman's ethnic group Mbororo (OR 1.62 95 % CI 1.00–2.63) and any other ethnic group (OR 2.50 95 %CI 1.14–5.52) were positively associated with *C. burnetii* seropositivity when compared to the Fulbe ethnic group. This may be due to differences in husbandry regimes in each ethnic group not captured by the current questionnaire, due to its focus on FMD-related issues. For example, high concentrations of the pathogen can be found in the placenta of carrier animals so different strategies on disposal of birth products may explain the different risks in each ethnic group (Watanabe and Takahashi 2008).

Buffalo seem to be implicated in both *Brucella* and *Coxiella* seropositivity. Antibodies to *Brucella* spp. were found in buffalo (Waghela and Karstad 1986; Chaparro et al. 1990) in Africa, but no recent studies have identified *C. burnetii* in buffalo in Africa, although they were found in buffalo in other areas such as in India (Sodhi et al. 1980). It is therefore possible that seeing buffalo during grazing or transmission reflects the increased risk of a cattle becoming infected when in contact or sharing the same grazing area with infected animals of different species. This finding emphasises the role of wildlife in infectious disease transmission and highlights the need for inclusion of wildlife in future research.

The high compliance rate, 90.7 % (147/162 herds selected), achieved minimises non-response bias (Bronsvoort et al. 2003). Another strength of this study is the stratified, two-stage random sampling design used, which provided optimum spatial coverage. Additionally, the use of a well-designed, pretested questionnaire carefully translated into the local language increases the accuracy of the risk factor data. Lastly, the mixed-effects analysis used empowers this study to deal with pseudoreplication that may arise due to the fact that animals may be correlated in space as they belong to the same herd and therefore not completely independent. Using herd as a random effect has the advantage of explaining the variance due to

the cluster design, while maintaining the power of the study (Paterson and Lello 2003).

One limitation of this study is the sampling frame used. According to a previous publication providing detailed information on the initial study, the rinderpest vaccination records used from the 1998/1999 MINEPIA campaign were incomplete and therefore underestimating the number of herds. Nevertheless, this was the best available option for the location and available resources (Bronsvoort et al. 2003). Additionally, the sample size calculation was calculated based on the initial aim of this study, i.e a risk factor analysis for FMD. Lastly, inherent sources of bias in cross-sectional studies using imperfect diagnostic tests include confounding, recall and misclassification bias. Most of these are minimised by the multivariable mixed-effects analysis used.

Leptospirosis and Q fever can have life-threatening effects in humans and brucellosis and can cause long periods of convalescence with residual disability. Additionally, during the acute stages of these three diseases, their flu-like symptoms can be misdiagnosed as malaria in the developing world setting, which can result in the administration of the wrong treatment, longer periods of disease and increase in antibiotic resistance (Kunda et al. 2007; WHO 2010). In their review on malaria misdiagnosis based on research mostly located in developing countries, Amexo et al. (2004) have found a mean malaria overestimation by clinical diagnosis of 61 %, highlighting the magnitude of the problem. The main risk of transmission to humans comes from animal reservoirs, and this increases the importance in measuring and identifying ways of decreasing the burden of these diseases in animals. This paper has identified potential risk factors, with wildlife, namely, buffaloes, playing a major role. Further research is needed to confirm these findings and improve the knowledge of the epidemiology of these three pathogens in Africa, taking particular consideration of the wildlife involvement in disease transmission.

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