Seroprevalence And Risk Factors Of *Coxiella Burnetii* Infecting Cattle Raised In Pastoral Areas Of Narok, Kenya.

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Abstract

Background: There are many prevalence and risk factor studies of infectious diseases such Q fever done in Sub-Saharan Africa but in Kenya, there are limited studies on Coxiellosis. The disease affects animal productivity and the ability to support livelihoods. The under-diagnosis of the disease has proven a challenge for effective control and eradication, since the epidemiology of the disease is still not clearly understood both in the animal and human health systems due to limited availability of technical and financial capacity to allow for significant epidemiological studies. The aim of this study was to determine the sero-prevalence and risk factors of *C. burnetii* in cattle in the Mara region, Narok County of Kenya. This study will provide relevant data for veterinarians and healthcare professionals that can be used for designing strategies for managing Q fever. Ultimately, this will aid in demonstrating that Q fever should not be neglected and should be included while controlling other zoonotic infections using the One Health approach.

Results: A total of 589 sera samples were collected from 390 herds in 5 different villages from 3 zones selected based on the livestock-wildlife interactions and land use patterns in Mara Ecosystem in 2019. The seroprevalence *C. burnetii* was 1.9%. Significant risk factor on univariate analysis at animal level was bull ownership {OR=4.8 (CI 0.4-16.2) P-value 0.016}, while at the herd level the significant factors were: animals grazing in shared areas between villages {OR 2.3 CI 1.0-5.0 P-value 0.046}, mixing of cattle in different herds {OR 0.4 CI 0.2-0.9 P-value 0.029}, bought livestock previous year {OR 10.1 CI 1.3-75.8 P-value 0.025}, bull ownership P-value 0.003, history of abortion in the herd {OR 0.3 CI 0.1-10.9 P-value 0.018} and communal grazing had a borderline significance of (OR=2.2 P-Value). At the multivariable analysis bull ownership was the only significant variable {OR 5.2 CI 1.5-18.4) P-value 0.010}.

Conclusion: The study confirmed the previous exposure of the cattle in Mara ecosystem to the *Coxiella burnetii* indicating the potential risk to human transmission and qualifying the need to have better veterinary and public health interventions.

Background

In Sub-Saharan Africa, livestock keeping is the backbone of livelihoods for most rural inhabitants and plays a key role in rural poverty alleviation strategies [1]. In countries like Kenya, livestock keeping has a bigger socio-economic role; people's lives are intertwined with those of their livestock with a bigger proportion of people directly dependent on livestock products and indirectly through the income generated from livestock sales and their products or employment in livestock-related sectors [1,2,3]. The livestock industry however suffers various challenges that affect its productivity and ability to support livelihoods. Among the challenges, are infectious diseases such as Query fever (Q fever) that not only occurs in animals, but also spreads to man. It is caused by *Coxiella burnetii*, a recently classified bacterium that is prevalent in a variety of biological hosts. Internationally categorized as one among the top thirteen global significant zoonoses, Q fever has been labelled amongst the most contagious infectious diseases [4,5,6,7]. The disease is mainly characterized by abortion in livestock and, acute to chronic disease in humans [5,8]. Cattle, sheep and goats act as the key reservoirs of the disease while the infection is transmitted to human through inhalation of contaminated aerosols [9]. *Coxiella burnetii* can also cause disease in other species including domestic dogs and cats, reptiles, arthropods and birds [7].

Q fever outbreaks in humans and domestic animals have been documented in many developed countries [10,11,12,13,14,15]. In 2007-2009 the largest-ever recorded outbreak of Q fever involving over 3,500 human cases occurred in the Netherlands highlighting the public health impact of the disease [13]. The disease is most likely underdiagnosed in Africa since it is not routinely tested in affected species [16,17,5,18]. In Kenya, only two human outbreaks have been detected though were initially reported as cases of fevers and pneumonias [19,20,21,5]. Although livestock and human populations in Kenya are highly at risk, the disease continues to be given less attention and thus poorly understood. Hence this study was designed to determine the seroprevalence and risk factors of *C. burnetii* in livestock in the Mara region, Narok County, Kenya.

Results

Out of the 589 sera samples only 11 tested positive for *C. burnetii* IgG antibodies resulting to an apparent prevalence of 1.9%. Bull ownership was the only animal factor significantly associated ($P < 0.05$) with the *C. burnetii* seropositivity with herds that were serviced by their own bull having a lower *C. burnetii* seropositivity level than those herds that used a borrowed bull for breeding. Herd level factors at the multivariable model that were significantly associated ($P < 0.05$) with *C. burnetii* were communal grazing, animals sharing grazing areas between villages, inter-herd mixing when grazing, bull ownership, herds with previously purchased livestock and abortions history in the herd. The intra-cluster correlation (ICC) at the herd level was 0.00. The details of the seropositivity are shown in Table 1 below.

Factors associated with *C. burnetii* seroprevalence in the univariable models

Animal-level factors

At the animal level, bull ownership was associated with *C. burnetii* seropositivity ($P < 0.05$) based on univariable analysis (Table 1). Herds with borrowed bulls from other farms were more likely to be seropositive compared to those herds that used their own farm bull (OR 4.8 CI (0.4-16.2); $P = 0.016$).

Descriptive statistics and univariable association

Herd level factors

At the herd level univariable analysis, six factors were associated with the outcome variable (Table 2). Cattle that had common grazing areas were more likely to be *C. burnetii* seropositive with an odds ratio (OR) 2.2 (95% CI 1.0-4.8); P -value 0.05. Cattle that shared grazing areas between villages were more likely to be seropositive OR 2.3 (95% CI 1.0-5.0); P -value 0.04. Animals in different herds that mixed during grazing were more likely to be positively associated with *C. burnetii* OR 0.4 (95% CI 0.2-0.9) -value 0.029. Herds with newly purchased livestock from the previous year were more likely to be positively associated with the outcome variable OR 10.1 (95% CI 1.3-75.8); P -value 0.025. Farms that were serviced with bulls from different farms were more likely to be associated with the outcome variable OR 4.5 (95% CI 2.0-10.3); P -value 0.0003. Herds that had already experienced abortions were more likely to be associated with the outcome variable OR 0.3 (95% CI 0.1-10.9); P -value 0.018 (Table 2).

Multivariable Analyses

In this study, bull ownership was only animal level- risk factor significantly associated *C. burnetii* seropositivity. The details are shown in table 3 below.

In this study, bull ownership and history of abortion in the farm were significantly associated *C. burnetii* seropositivity at the herd level. The details are shown in table 4 below.

Discussion

Representative seroprevalence studies are important in providing accurate information on the occurrence and risk factors of infectious diseases. The results of this study confirmed *C. burnetii* exposure through sero-prevalence antibodies in cattle in the Maasai Mara ecosystem in Narok County, Kenya. The seroprevalence of *C. burnetii* in this study was 1.9% with the rates being higher in males than females. This rate was much lower than those previously reported in cattle in various regions in Kenya in Kajiado County [27] and in western Kenya [29] where the prevalence rates were reported as 89.7% and 28.3% respectively. The low prevalence reported in this study could have been due to differences in livestock production systems. The semi-extensive local production system practiced in this area maybe an attributable probable cause for the low prevalence rates because of the increased land surface area per animal [28]. The overall seroprevalence rates of *C.*

burnetii in cattle in Africa have been found to be $\leq 13\%$ consistent with the current findings [16]. The seropositivity rates in this study were higher in bulls than cows. This is likely because adult females under the influence of estrogens mount stronger innate and adaptive immune responses than males which results in faster clearance of pathogens [30].

The likely explanation for high prevalence rates of the disease in cattle herds grazed together is the lack of awareness about the disease, close contact between animals during herding and when housed together and the sharing of watering points. This is consistent with the findings of this study where communal grazing and inter-herd and inter-village grazing were seen to be greatly associated with *C. burnetii* positivity at the herd level. Increased animal density per land area could result in higher likelihoods of being directly exposed to Coxiellosis [29,31,16]. In addition, both the current study and that of [27] report that cases of abortions in the herd are associated with increased seropositivity to *C. burnetii*. Gravid ruminants are more susceptible to *C. burnetii* than non-gravid ruminants and this explains the reproductive failures associated with the bacteria [32]. High rates of abortion are a public health challenge as the risk of exposure of the disease to humans is increased since it is a zoonotic disease [33]. In a previous epidemiological study [34] among Fulani nomadic pastoralists, seropositive cattle had odds of 6.7 times more likely to have had a reproductive failure the previous year. The results of this study therefore demonstrate the need for animal confinement during parturition in addition to increased awareness on the methods of Q fever transmission to aid in controlling its spread.

The risk variable, newly bought livestock and the correlation with high seropositivity observed in this study agrees with the study done on cattle in western Kenya [35], where farms with recently purchased cattle registered higher *C. burnetii* seropositive cases. This can be best explained by the fact that these new animals were from different herds that may have had pre-exposure to the pathogen and interherd mixing happens in market set ups. The other possible explanation is that the markets have become a pathogen hub where animals that go through this route are more likely to be exposed to a myriad of pathogens and *C. burnetii* being a pathogen that can survive in dust can be transmitted easily in cattle during market days. Animals sent for market may travel from long distances including *C. burnetii* endemic areas or areas with predominantly higher seroprevalence and this increases the likelihood of coxiellosis in these animals. There is also the probability of farmers selling animals with reduced fertility and productivity and if such animals are infected with *C. burnetii*, then farmers are more likely to introduce sick animals to their healthy animals once they purchase them. Based on these plausible transmission pathways [36] may have suggested for the use of vaccination and field side testing in the cattle markets as this will reduce the degree of shedding from the infected animals, the significant reduction in the pathogen load and the number of abortions experienced due to coxiellosis [35].

Bull ownership also showed a spike of significance as those herds that used a borrowed bull were more likely to have an increased seroprevalence than those herds that used their own bulls. Semen has been found to be a plausible medium for *C. burnetii* and it is in line with fact that bulls that have not been tested and cleared to breed are putative causes for coxiellosis in heifers and cows [37]. Cattle owners can only be sure of their own bulls and not be confident in borrowed bulls that is why there was an increased seropositivity seen when borrowed bulls are used for breeding as opposed to own farm bull.

Immunofluorescent antibody test (IFAT) test is the gold standard test set in text for the diagnosis of *C. burnetii* with sensitivity 100% and specificity of 99%, however the disadvantages of using this method are many including the lack of homogenous laboratory standards and the inability to use on large set of samples due to the intense laborious laboratory work. Complement fixation test (CFT; sensitivity 89% and specificity 82%) can also be used but it is not so much a priority while undertaking serological studies due nonspecific output when anti complementary substances are present in the sera and in the same breath some ruminant antibodies have been seen to fix complement [38]. The use of ELISA was used in this study because of the availability and specific output with a sensitivity of 76% and specificity 96.7% observed during cattle sera testing [34].

Conclusion

The evidence from risk factor analysis on *C. burnetii* seroprevalence at the animal and herd level agree with most studies that have been done in Kenya and Africa. Animal husbandry and management important variables in seropositive herds reared in pastoral zones where the sharing of grazing grounds, watering points and bull borrowing is more prevalent in such set ups as opposed to intensified husbandry systems. The farm animal-wildlife interface zones proved to less significant, but more studies should focus on the disease drivers in such interface and remove confounders for a more conclusive grip on the risk factor.

Methodology

This study used stored samples from ILRI'S Repository laboratory. These serum samples were from a previous study (Zoonotic Pathogen Seroprevalence in Cattle in a Wildlife-Livestock Interface, Kenya) done in the Mara region [23].

Study area

The study samples were collected from the Maasai Mara ecosystem in Narok County, Kenya (Fig.1)

Study design and size calculation

A cross-sectional study was done between 2016 and July 2017 [22].

Sample size was calculated using the formula: $n = (1.96)^2 p (1 - p) / d^2$, with a margin error (d) of 0.05.

Prevalence of *Coxiella burnetii* infections in pastoral systems is 26% [23].

Based on formula [24,25] and N was 296.

To adjust for intra-herd correlation, an intra-cluster correlation of 0.5

$K =$ average number of animals per herd = 3; ICC = 0.5 gives a design effect of 2

$296 \times 2 = 592$

Laboratory Analysis

A commercial enzyme-linked immunosorbent assay (ELISA) kit (IDEXX) was used for the detection of phase II IgG antibodies against *C. burnetii* according to the manufacturers' instructions. The assays were performed at the International Livestock Research Institute (ILRI) in Nairobi. Briefly, 100µl of 1:400 dilutions of sera were added to the plate with pre-coated *Coxiella burnetii* antigen and incubated. Plates were washed 3 times and 100µl of the conjugate added and incubated. Both incubation periods lasted an hour each. The plates were washed 3 times and 100µl of tetramethylbenzidine (TMB) substrate added to each well and left at room temperature for 15 minutes. The reaction was culminated using the stop solution provided in the kit and the plates read at 450nm. Samples were run in duplicates as recommended by the manufacturer, animals were considered to be positive if they had an optical density percentage (%OD) 40, negative if OD%30 and ambiguous if between 30 and 40%.

Data collection and handling

Data on herd and animal level factors were collected using a questionnaire developed [23] which was administered during the collection of the animal samples. The questionnaires were administered to the household head or someone familiar with the animals and their management. The variables collected included sex of the animals, herd size, herd management practice i.e. (use of bull or artificial insemination), utilization of communal grazing areas, grazing practices around the

wildlife reserves, mixture of cattle herds during grazing and during watering, introduction of newly bought cattle and the clinical signs observed both in animals and the people handling the livestock.

Data from the household questionnaires together with that of the serological tests were entered in Microsoft Office Excel 2013 Windows 10. The data were then screened for entry errors, cleaned and exported into R studio.

Statistical Analysis

Descriptive statistics were first performed, where proportions, means and medians were calculated. Associations between and across herd and animal level factors were analyzed using multilevel modelling with a positive antibody titre as the outcome of interest. Univariable logistic regression analysis were first done for all the herd and animal level factors using the GLMR function in the LME4 model package (R.3.84 software). All the factors that had a p-value of ≤ 0.2 in the univariable models were then included in multivariable logistic regression model [27]. The factors were considered significant at $p < 0.05$ in the final multivariable model in a forward- backward elimination procedure. The associations were adjustment for intra-cluster correlation derived from the variant components.

Abbreviations

C. burnetii: *Coxiella burnetii*; ELISA: Enzyme-Linked Immunosorbent Assays; OR: Odds ratio; CI: Confidence interval; ILRI: International Livestock Research Institute; GLMR: Generalized linear models in R; LME: Linear mixed effects; OD: Optical densities; TMB: tetramethylbenzidine; IgG: Immunoglobulin G; ICC: Intraclass correlation; IFAT: Immunofluorescent antibody test.

Declarations

Availability of data and materials

The data that support the findings of this study are available from the corresponding author but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of corresponding author.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

GW conducted laboratory work. In addition, she participated in the study design, did the data analysis together with DN and she was involved in preparation of this manuscript. BB, GA, and GG were involved in the conception of the study, study design and the writing of this manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Approval for this study was given by the Faculty of Veterinary Medicine Postgraduate committee of the University of Nairobi and International Livestock Research Institute. Since the information collected for this study was not of a sensitive nature as banked sera samples were used and the ethical approval and guidelines received from International Livestock Research Institute ethical committee was deemed sufficient to conduct the study. Guidelines on handling and processing of biological samples were followed in this study.

Informed consent was sought and received from all the household heads who responded on the administered questionnaire. Data from this questionnaire was stored confidentially and shared with the key stakeholders. Data from the questionnaire was analyzed in accordance with relevant statistical guidelines and regulations.

Laboratory protocols

All laboratory methods were carried out in accordance with relevant laboratory guidelines and regulations.

All experimental protocols were approved by International Livestock Research Institute ethical committee.

Consent for publication

Not applicable.

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Tables

Tables 1-4 are available in the Supplemental Files section.

Figures

Figure 1

Map of Maasai Mara ecosystem in Narok Kenya

Supplementary Files

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