INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH AND KNOWLEDGE
ISSN-2213-1356 www.ijirk.com

Seroprevalence of *Coxiella burnetii* in sheep, goats and impalas at the Amboseli Park corridor, Kenya

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Abstract
Coxiella burnetii is an obligate intracellular bacterium that is the causative agent of Q fever, an important zoonotic. Domestic ruminants, mainly goats and sheep, are the main source of Q fever outbreaks in humans. Very scant information is available on the role and status of Q fever in wildlife in Kenya. This sero-epidemiological survey was conducted to investigate the seroprevalence and associated factors of coxiellosis in sheep, goats and impalas at the Amboseli National Park wildlife-livestock corridor in Kenya. 5 ml whole blood was collected through jugular venepuncture from 300 sheep, 200 goats and 20 impalas. Sera were then collected and tested for antibodies against Coxiella burnetii using ELISA CHEKIT Q fever test kit. The seroprevalence at 95% confidence interval in sheep was 6% (2.7%, 9.3%), 21.7% (17%, 26.4%) in goats and 25% (6%, 44%) in impalas. The study also concluded that species and sex were significant risk factors. A semi-structured questionnaire was also administered in all the households to gather general household data and assess pastoralist knowledge on Q fever, none of the respondents had ever heard of the disease. These findings demonstrate that Q fever could be circulating among the wildlife, livestock and humans in the study area and further investigation is required.

Keywords: *Coxiella burnetii; Q fever; seroprevalence; wildlife-livestock corridor*

Introduction

Amboseli National Park is located in Loitokitok sub-county of Kajiado County, Kenya; it is the second most visited animal park after Maasai Mara National Park. The land outside the park is divided into group ranches (manyattas) occupied predominantly by the pastoral Maasai community. This study was designed to investigate the presence of Q fever at the wildlife-livestock corridor zone, which is rich in wildlife biodiversity that interacts with livestock, their owners and visitors, this interaction may result in zoonotic disease transmission.

Direct risks of Q fever include infection of humans and animals while indirect risks include loss of income from livestock due to reduced production and reproduction. Potasman *et al* (2010) showed that 4 (8%) of 50 safari travelers to Kenya contracted Q fever.

Q fever, first described in 1937 (Maurin and Raoult, 1999; Parker *et al*., 2006; Marrie, 2009; Wardrop *et al.*, 2016), is a worldwide zoonosis that has long been considered an under-reported and under-diagnosed illness because symptoms frequently are nonspecific, making diagnosis challenging (CDC, 2013). *Coxiella burnetii*, the causative agent for Q fever, has been described as one of the most infectious organisms known and is considered as a potential agent for bioterrorism (Jones *et al*., 2006). *Coxiella burnetii* is shed in milk, urine, feces, amniotic fluids and placenta (Jones *et al*., 2006; CDC, 2013). The organisms contaminate dust and are so highly infectious that a single inhaled organism can cause clinical illness in an animal or person (CDC, 2013). Q fever can also be spread by ticks (Barandika *et al*., 2007; Mediannikov *et al*., 2010; Knobel *et al*., 2013). Human coxiellosis is a significant yet under-diagnosed cause of respiratory illness in Kenya (Potasman *et al.*, 2010; Knobel *et al*., 2013; DePuy *et al*., 2014).

Coxiella burnetii infects a broad range of mammalian hosts, including cattle, goats, sheep (Barandika *et al*., 2007; Astobiza *et al*., 2010; Kshash, 2012; Knobel *et al*., 2013; DePuy *et al*., 2014) , wild ruminants (Barandika *et al*., 2007; Hernandez *et al*., 2007; Dorko *et al*., 2009), sea mammals (Kersh *et al*., 2012), camels (Knobel *et al*., 2013; Mohammed *et al*., 2014), fish, amphibians, reptiles (Hernandez *et al*., 2007; Knobel *et al*., 2013) as well as humans (Fennolar *et al*., 2001; McQuiston *et al*., 2002; Marrie, 2009; Potasman *et al.*, 2010; Mediannikov *et al*., 2010; Porter *et al.,* 2011; Wardrop *et al.*, 2016) . In animals, the organism is mainly found in the reproductive system and may primarily cause abortion or infertility (Barandika *et al*., 2007; Astobiza *et al*., 2010; Kshash, 2012; DePuy *et al*., 2014; Mohammed *et al*., 2014). In humans, however, the disease is associated with acute flu-like illness, hepatitis, pneumonia and chronic endocarditis (Fennolar *et al*., 2001; Marrie, 2009; Mediannikov *et al*., 2010; Wardrop *et al.*, 2016). At present, direct detection and quantification by PCR and serological ELISA should be considered as methods of choice for clinical diagnosis (Field *et al*., 1983; Cowley *et al*., 1992;Fournier *et al.,* 1998; Kitterberger *et al*., 2009; CDC, 2013).

There is limited information about Q fever in wildlife, livestock and humans in Kenya, this can results in misdiagnosis hence underreporting. In order to have proper diagnosis, treatment, control and prevention of Q fever there is need for more information about the disease. This study provided a valuable opportunity for generating sheep, goats and impalas coxiellosis seroprevalence data which could be linked to human health outcomes.

Materials and Methods

Twenty manyattas (pastoral group ranches) were selected conveniently based on their proximity to the Amboseli National Park where 10 sheep and 15 goats in each manyatta were sampled randomly. In addition, 20 impalas were captured conveniently through darting and net capture from inside the National Park. From each animal, 5 ml whole blood was collected through jugular venepuncture using gauge18 needles into plain vacutainer tubes after disinfecting the skin with 70% alcohol. The blood was then left to stand for 1 hour in a cool box so as to clot slowly with little or no hemolysis to form clear serum. The sera were then transferred

into well labelled cryo-vials and stored in a refrigerator at -5°C before transport to the Department of Clinical Studies, University of Nairobi laboratory where it was store at -20°C awaiting analysis. A semi-structured questionnaire was administered in the study area to gather data on; general household practices, owner/household head, livestock production and management, Q fever and other zoonotic diseases knowledge.

Serological evaluation of *Coxiella burnetii* antibodies in the sera was done using ELISA CHEKIT Q fever test kit (IDEXX, Westbrook, Maine), according to the protocol recommended by the manufacturer. Results were expressed as a percentage of the optical density reading of the test sample (value), calculated as value = 100 × (S − N)/ (P − N), where S, N, and P are the OD of the test sample, the negative control, and the positive control, respectively. Sera were considered to be ELISA positive if they had a value of 40% or more. All data collected from the questionnaires and from blood analysis were entered into Microsoft office Excel 2007 spreadsheet file which were then exported to the statistical packages, SPSS 16.0 and STATA for statistical analysis. The seroprevalence of Q fever in sheep, goats and impalas was determined based on serological results at 95% confidence interval. Frequency tables showing the ELISA results (positive or negative) versus the risk factors were generated and using mixed logistic regression and linear regression, association between sero-positivity and potential risk factors was determined. Fishers Exact test was used to test for confounding between the various risk factors.

Results

Q fever Seroprevalence in sheep, goats and impalas

The seroprevalence of *Coxiella burnetii* in sheep goats and impalas at the wildlife-livestock interface of Amboseli National Park is displayed in Table 1.

Table 1: Seroprevalence of Q fever in impalas, sheep and goats at the Amboseli Park corridor, Kajiado County, Kenya in 2016

Species	Number positive	Total	Seroprevalence %	95%) Confidence Interval	
				Lower	Upper
Impala	5	20	25	6	44
Sheep	12	200	6	2.7	9.3
Goat	65	300	21.7	17	26.4

Significant risk factors to Q fever sero-positivity in sheep and goats

Based on the results of the mixed linear regression, there was significant association between species (p=0.007), sex (p=0.0005) and sero-positivity to Q fever antibodies. Logistic regression showed association in species (p=0.000) but not sex. There was no indication of confounding or interaction in any of the factors.

Table 2: Sero-positivity of Q fever in sheep and goats by sex at the Amboseli Park corridor, Kajiado County, Kenya in 2016

Species	**Sex**	**ELISA results**		**Total**	**%Seropositivity**
		Positive	Negative		
Sheep	Male	1	36	37	0.5
	Female	11	152	163	5.5
	Total	12	188	200	6
Goat	Male	11	62	73	3.7

	Female	54	173	227	18
	Total	65	235	300	21.7
Sheep & Goats	Male	12	98	110	2.4
	Female	65	325	390	13
	Total	77	423	500	15.4

Questionnaire data gathered in the study area showed that knowledge on Q fever in the pastoral communities was very poor as all the interviewed animal owners had never heard of the disease. However, other diseases such as brucellosis, rabies, poxvirus and tuberculosis were well understood (71%) and the pastoralists took preventive measures such as boiling milk before drinking (100%), avoiding sharing sleeping quarters with animals (100%) and up to date animal vaccinations (86%).

Discussion

The results of this study recorded presence of Q-fever in impalas, sheep and goats at the Amboseli National Park wildlife-livestock corridor. However, the seroprevalence rate of coxiellosis in sheep and goats in this study was lower compared to other reports in Kenya which approximates 32% in goats, and 18.2% in sheep (Knobel *et al*., 2013; DePuy *et al*., 2014). The potential risk factors to Q fever in sheep and goats identified were:

1. Access to the game park for pasture and watering
2. Presence of ticks on animals
3. The type of tick control method(s) employed and acaricide used
4. Species and sex

However, only species and sex had statistically significant association with sero-positivity. These findings show that Q fever is a significant yet under-diagnosed cause of abortion or infertility in sheep and goats (CDC, 2013; Knobel *et al*., 2013; DePuy *et al*., 2014) in the study area, this view is further supported by the fact that all the interviewed pastoralists confirmed that abortions in their livestock was a common occurrence.

Despite a very small sample size of impalas, Q fever was present at a seroprevalence of 25%. It is therefore important to use a larger sample size and understand the role of wildlife in the epidemiology of infectious pathogens including *Coxiella burnetii* (Kruse *et al*., 2004; Dorko *et al*., 2009; Ndeereh, 2016). The infection dynamics and route by which transmission of infection from wild animals to livestock may occur is still unclear (Kruse *et al*., 2004; Barandika *et al*., 2007), and greater understanding of this is necessary to determine the factors involved. Very scant data is available in Kenya about Q fever seroprevalence status of any wildlife species, however, several reports outside Kenya exist on sero-epidemiology of Q fever in different species that include various mammals, birds, reptiles and fish (Kruse *et al*., 2004; Barandika *et al*., 2007; Hernandez *et al*., 2007; Dorko *et al*., 2009; Kersh *et al*., 2012). This study therefore confirms that wildlife species have the potential to contribute significantly to reservoirs of Q-fever infection (Kruse et al., 2004; Marrie, 2009) for both livestock (Kruse *et al*., 2004; Barandika *et al*., 2007; Hernandez *et al*., 2007; Dorko *et al*., 2009; Porter *et al.,* 2011) and humans (Mediannikov *et al*., 2010) hence wildlife surveillance may be a useful tool in monitoring patterns of infection and potential disease risk.

This was the first study conducted to investigate the sero-epidemiology of Q fever in impalas, sheep and goats at the Amboseli National Park corridor in Kenya. Q fever should be of public health concern (Kshash, 2012) in this area which has unique human-livestock-wildlife interfaces that can potentially facilitate transmission of infectious pathogens across different species. However, the disease remains unreported in the entire sub-Saharan Africa (Knobel *et al*., 2013). Generally within Kenya, there seems to be a low level of knowledge towards many zoonotic diseases including Q fever amongst pastoral communities (Knobel *et al*., 2013; DePuy

et al., 2014; Ndeereh, 2016) which is consistent with the findings of this study raising concerns about the potential risks of Q fever amongst local populations. Education for proper diagnosis, treatment and prevention of Q fever could be needed; this will require interdisciplinary and cross-cultural work to understand how this and other disease cycles in the region could be embedded in livestock management practices. The low level of knowledge on Q fever also raises concerns about the potential risks posed by the diseases in local residents, the diseases could be circulating unnoticed in the area and therefore be amongst the 'fevers of unknown origin' recorded in most medical facilities.

The study further identified certain practices which could also predispose the local residents to zoonotic transmission of the diseases. These included;

1. Sharing of habitats and other resources such as water between humans, livestock and wildlife
2. Own treatment of livestock by most pastoralists through buying drugs
3. Lack of livestock abortion investigation
4. Handling of abortus without gloves and feeding aborted material to dogs or leaving the abortus in the field

Close contact of pastoralist to wild animals can also expose them to tick bites (Mediannikov *et al*., 2010). Q fever could be a possible cause of acute lower respiratory illness among the pastoralists (Knobel *et al*., 2013) in the study area; it could also be a common infection to visitors (Potasman *et al*., 2010) who frequent the Amboseli ecosystem. Inhalation of aerosols contaminated by the parturient fluids of infected animals is the main mode of human infection with *Coxiella burnetii* (CDC, 2013). Further investigation on of the role of domestic dogs and cats (Knobel *et al*., 2013) is required as all the households visited keep them as pets.

Conclusions

These findings demonstrate that Q fever could be circulating among the wildlife, livestock and humans in the study area and further investigation is required. Extensive epidemiological surveillance is needed to fully understand the complex ecology of *Coxiella burnetii.* Q fever is listed in the OIE *Terrestrial Animal Health Code* and Member Countries and Territories are obligated to report occurrences of the disease to the OIE.

Acknowledgments

This study was made possible through the facilitation of One Health Central and Eastern Africa (OHCEA) and significantly supported by the Kenya Wildlife Service (KWS). Sincere gratitude to Dr. Dennis Makau, Miss. Jane Kamau P.M, Dr. Mwangi N.J, Dr. Michael Njoroge, Mr. Daniel Kanchori and all Amboseli location pastoralists.

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