Coxiella burnetii (Q fever) prevalence in associated populations of humans and small ruminants in The Gambia

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Abstract

OBJECTIVES   To simultaneously estimate the prevalence of antibodies against *Coxiella burnetii* (Q fever) among adults and small ruminants, and *C. burnetii* shedding prevalence among small ruminants in households in the Kiang West district of The Gambia, and to assess associated risk factors.

METHODS   Sera of 599 adults and 615 small ruminants from 125 compounds within 12 villages were tested for antibodies against *C. burnetii* using ELISA. Vaginal swabs and milk samples of 155 small ruminants were tested using PCR to investigate shedding of *C. burnetii*.

RESULTS   A total of 3.8–9.7% of adults, depending on ELISA test cut-off, and 24.9% of small ruminants in Kiang West were seropositive. Having at least one seropositive animal in one's compound was a risk factor for human seropositivity (OR: 3.35, 95% CI: 1.09–14.44). A grazing area within a village was a risk factor for seropositivity in small ruminants (OR: 2.07, 95% CI: 1.26–3.50); others were having lambed (OR: 2.75, 95% CI: 1.37–5.76) and older age of the animals (OR: 2.75, 95% CI: 1.37–5.76 for 1–3 years and OR 5.84, 95% CI: 3.10–11.64 for >3 years); 57.4% of sampled small ruminants were shedding *C. burnetii*.

CONCLUSION   *Coxiella burnetii* infection is endemic among both humans and small ruminants in this area of The Gambia. Human and animal exposure to *C. burnetii* were related at compound level. Further research into the clinical relevance of *C. burnetii* infection in West Africa is needed.

keywords   Q fever, Gambia, seroepidemiologic studies, humans, goats, sheep

Introduction

Q fever is a highly infectious zoonotic disease caused by the obligate intracellular bacterium *Coxiella burnetii* [1]. A variety of wild and domestic host species have been identified, but domestic ruminants such as goats and sheep are the most important source of human infection. The inhalation of contaminated dust particles is the primary route of infection for both humans and animals. *C. burnetii* infection in humans can pass subclinically or present as acute febrile illness, pneumonia or hepatitis. Seropositivity for antibodies against phase II antigens of *C. burnetii* is suggestive of past or acute infection. In approximately 2% of human cases, acute Q fever can develop into chronic Q fever, which mainly manifests as

endocarditis or vascular infection and can be lethal [2]. In non-pregnant goats and sheep, infection usually does not result in symptoms. In pregnant animals, however, the infection is associated with abortions, which may also have economic consequences for the owner [1].

Although the main cause of febrile illness in sub-Saharan Africa has long been considered to be malaria, it is becoming increasingly clear that many other aetiologies of fever may also play a role. In The Gambia, a study in the Farafenni region in 2010 showed that only 11% of febrile episodes in a cohort of children were malaria related [3]. A previous Gambian study had already observed an overestimation of malaria cases of up to 61% if based on clinical diagnosis. Such misdiagnosis may lead to the prescription of incorrect treatments and

reduced antimicrobial efficacy of drugs due to the development of resistance [4]. This calls for further investigation into alternative aetiological agents of fever and their epidemiology.

The relative contribution of bacterial zoonoses in acute febrile cases in sub-Saharan Africa is largely unknown, but some studies suggest that bacterial diseases such as Q fever may account for part of these cases. A fever aetiology study in Northern Tanzania in 2011 showed that acute Q fever infection was identified in 5.0% of a cohort of 870 severe febrile hospital admissions [5]. Also in West Africa, *C. burnetii* infection has been reported in febrile individuals; 40.4% of 168 febrile patients tested positive for antibodies in a study in Mali in 2005, with 9.5% of the seropositive patients showing serological patterns that indicated a recent infection [6].

Exposure to *C. burnetii* has been detected in both humans and small ruminants in various countries across Africa, but the pathogen's epidemiology and the relation between exposure in humans and animals are still poorly understood [7]. In The Gambia, a retrospective serological survey performed on samples collected in 2008 in the Farafenni region showed a Q fever seroprevalence of 8.3% in children [8]. Along with an observed prevalence of 21.6% in sheep and goats in the same region in 2012, these findings suggested that there is considerable exposure to *C. burnetii* [9]. Given that these studies were conducted at different time points, and that populations of small ruminants in the area have a rapid turnover, it is difficult to interpret the relation between the results. To improve understanding of the relation between human and animal *C. burnetii* infections and identify associated risk factors for Q fever infection in The Gambia, we simultaneously estimated the Q fever seroprevalence in human adults and small ruminants.

Materials and methods

This study was carried out in The Gambia from September to December 2014 in 125 compounds divided over 12 villages in the rural Kiang West district (Central River Region), simultaneously sampling humans and small ruminants. Additional small ruminants were sampled at livestock markets and adjoining abattoir sites in two coastal urban areas Brikama and Abuko. This study is part of a larger project on zoonotic diseases in The Gambia.

Sampling

Kiang West is populated by an estimated 15 000 people, 8000 goats and 1600 sheep [10]. In this district, the selection of the villages was based on geographical variation and on a minimum number of inhabitants and small ruminants per village. For each village, compounds with at least five adults and at least five small ruminants were randomly listed. The final selection was made during field visits, choosing the first 10 compounds where a sufficient number of adults and small ruminants were observed. For the human selection, up to 15 individuals who met the inclusion criteria of being healthy and at least 18 years old were randomly selected per compound. The exclusion criteria were as follows: refusal to participate, no longer living in compound and repeatedly not being present at the time of field visits. In the same compounds, a minimum of five small ruminants that met the inclusion criteria of being healthy and more than 6 months old were selected on a random basis, although sheep were occasionally preferred over goats because of a generally lower number of sheep. Animals were included only with permission from the owners. In addition to the small ruminants sampled in Kiang West, 500 animals were selected from two urban sites. These urban sites were livestock markets in Abuko and Brikama and the neighbouring slaughterhouse in Abuko, situated close to the capital, Banjul. Here, the same inclusion criteria for small ruminants were used, but sampling occurred on a convenience basis until the required number was reached.

A structured questionnaire was used to collect information from the participants in Kiang West regarding sex, age, ethnic group, possession of household assets (radio, bicycle, refrigerator, motorbike, car, television, generator, solar panel), occupation and raw milk consumption. Information about the species, breed, sex and age of small ruminants was recorded through observation and inquiry. In Kiang West, additional information on the animals' reproductive history was obtained by consulting flock owners.

Human blood samples were taken using 21G × 1.5 Precision Glide Multi-Sample Needles and collected in serum vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). For the animal blood samples, 20G × 1.5 Precision Glide Multi-Sample Needles and the same serum tubes were used. Within 4 h of collection, human and animal sera were obtained after spinning the blood samples (15 min at a speed of 1500 g), they were divided in aliquots and stored at −20 °C until further analysis [11]. Exclusively in Kiang West, vaginal swab and milk samples were taken from lactating animals. After cleaning the vulva with alcohol-soaked cotton balls, a vaginal swab was taken using a sterile cotton-tipped swab (Copan Flock Technologies Srl., Brescia, Italy). Milk samples were collected in 10-ml plastic tubes after cleaning the teat with alcohol-soaked cotton balls. The first streams of milk were discarded.

ELISA and PCR analyses

Human sera were tested for the presence of IgG and IgM antibodies against *C. burnetii* phase II antigens using commercial ELISA kits (Serion ELISA classic *Coxiella burnetii*, Virion\Serion, Würzburg, Germany) according to the manufacturer's instructions, at the serology laboratory of the Medical Research Council Unit, The Gambia (MRCG). For the IgG analyses, results were expressed quantitatively, in International Units/ml (IU/ml). Samples with IgG values of <20 IU/ml were considered negative, those with 20–30 IU/ml borderline and those with >30 IU/ml positive. Additionally, an alternative interpretation of these results was used after a recent study that suggested lowering the cut-off value for this particular ELISA test to increase its sensitivity for use in seroprevalence studies [12]. In this case, IgG samples with values of <10 IU/ml were considered negative and those with ≥10 IU/ml positive. As for IgM, results were expressed qualitatively with samples with an optical density (OD) >10% below the cut-off control provided by the manufacturer considered negative, OD >10% above the cut-off positive and in-between values borderline. In the data analyses, borderline results of both the IgG and IgM tests were considered positive, and 'seroprevalence' was defined as the percentage of participants who were positive for either IgG or IgM, following an earlier Q fever seroprevalence study in The Gambia [8].

Sera of the small ruminants were exclusively tested for the presence of IgG antibodies against *C. burnetii* using the LSIVet Ruminant Q Fever Serum/Milk ELISA kit (LSI, Lissieu, France), at the Central Veterinary Institute in Lelystad, the Netherlands. Samples with a sample/positive (S/P) ratio of ≤40 were considered negative and those with an S/P ratio of >40 as positive. Seroprevalence was defined as the percentage of small ruminants that were positive for IgG antibodies. DNA extraction from the small ruminants' vaginal swabs and milk samples and subsequent PCR amplification was performed as described by Roest *et al.* [13]. Both milk and swab samples with a cycle threshold (Ct) of ≤36 were considered positive, those ≥40 negative and samples between >36 and <40 borderline. In the statistical analyses, borderline Ct values were included in the category 'positive'.

Statistical analyses

Univariate logistic regression with calculation of odds ratios (OR) was used to identify possible risk factors for *C. burnetii* antibody seropositivity of humans and small ruminants, and for *C. burnetii* shedding in small ruminants. For humans, information on the possession of

household assets acquired from the questionnaires was used to construct socio-economic status (SES) indices. One point was assigned for the possession of each of eight household assets (radio, bicycle, fridge, motorbike, car, tv, generator, solar panel). Following the resulting scores, participants were either classified as having a SES of 'median or lower' or 'higher than median'.

Associated factors with $P < 0.10$ were fitted in mixed-effects logistic regression models to adjust for age and possible clustering on a village level. All analyses were performed using R software version 3.2.1 [14]. The packages *glm* and *lme4* were used for logistic regression analysis.

Ethical approval

The Joint Gambia Government–MRCG Ethics Committee approved the human part of this study. Written informed consent was obtained prior to participation, and all information was anonymised. The veterinary part of the study was conducted in compliance with the Dutch Veterinary Practice Act and Gambian legislation on practicing veterinary medicine.

Results

Study population

Serum samples were available for 599 human adults originating from 12 villages in the Kiang West area, comprising 125 compounds (9–13 per village); 67.3% of the human participants were female, and ages ranged from 18 to 88 years. The largest ethnic group was Mandinka, making up 89.6% of the study population. Smaller ethnic groups were Fula (9.2%) and Jola (0.3%); 80.8% of the respondents reported to have drunk raw cow's milk in the last 14 days. Recent consumption of raw goat or sheep milk was rare, 0.8% and 0.2%, respectively, although larger proportions indicated to have drunk raw goat or sheep milk at some point in their lives (43.7% and 10.2%); 494 and 121 sera were obtained from goats and sheep, respectively, from 121 compounds, of which 119 compounds were also used for the human sampling. An additional 250 goats and 250 sheep sera were collected at the urban sites Abuko and Brikama (Figure 1).

Seroprevalence in humans and small ruminants

Seroprevalence results are presented in Table 1. Fourteen persons (2.3%) tested positive by IgG ELISA and nine (1.5%) by IgM ELISA, with none of them being positive to both tests, adding up to a seroprevalence of 3.8%

Table 1 Serological results of humans and small ruminants in The Gambia

Location	Humans						Small ruminants		
	n	IgG pos.	IgM pos.	Total seroprevalence % (95% CI)	IgG pos.*	Total seroprevalence* % (95% CI)	n	IgG pos.	Seroprevalence % (95% CI)
All villages in KW	599	14	9	3.8 (2.6–5.7)	49	9.7 (7.6–12.3)	615	153	24.9 (21.6–28.4)
1 Dumbuto	50	3	0	6.0 (2.1–16.2)	8	16.0 (8.3–28.5)	50	6	12.0 (5.6–23.8)
2 Jali	50	1	3	8.0 (3.2–18.8)	5	16.0 (8.3–28.5)	50	16	32.0 (20.8–45.8)
3 Janneh Kunda	50	1	0	2.0 (0.1–10.5)	5	10.0 (4.3–21.4)	56	11	19.6 (11.3–31.8)
4 Jiffarong	50	0	1	2.0 (0.1–10.5)	0	2.0 (0.1–10.5)	50	15	30.0 (19.1–43.8)
5 Kantong Kunda	50	1	0	2.0 (0.1–10.5)	8	16.0 (8.3–28.5)	59	28	47.5 (35.3–60.0)
6 Kemoto	47	0	1	2.1 (0.1–11.1)	0	2.1 (0.1–11.1)	29	3	10.3 (3.6–26.4)
7 Keneba	50	0	0	0.0 (0.0–7.1)	0	0.0 (0.0–7.1)	57	19	33.3 (22.5–46.3)
8 Kuli Kunda	52	0	1	1.9 (0.1–10.1)	4	9.6 (4.2–20.6)	50	2	4.0 (1.1–13.5)
9 Manduar	50	4	1	10.0 (4.3–21.4)	8	18.0 (9.8–30.8)	58	19	32.8 (22.1–45.6)
10 Nyorro Jattaba	50	0	1	2.0 (0.1–10.5)	3	8.0 (3.2–18.8)	51	12	23.5 (14.0–36.8)
11 Sankandi	49	1	1	4.1 (1.1–13.7)	2	6.1 (2.1–16.5)	53	8	15.1 (7.9–27.1)
12 Tankular	51	3	0	5.9 (2.0–15.9)	6	11.8 (5.5–23.4)	52	14	26.9 (16.8–40.3)
Both urban sites	–	–	–	–	–	–	500	73	14.6 (11.8–18.0)
A Abuko	–	–	–	–	–	–	415	65	15.7 (12.5–19.5)
B Brikama	–	–	–	–	–	–	85	8	9.4 (4.8–17.5)

KW, Kiang West.
*Based on lower cut-off value, total seroprevalence also includes IgM positives.

(95% CI: 2.6–5.7). Applying a lower cut-off value for the IgG ELISA resulted in 49 (8.2%) IgG positives, which together with the IgM positives amounted to a seroprevalence of 9.7% (95% CI: 7.6–12.3).

A seroprevalence of 24.9% (95% CI: 21.6–28.4) was found in small ruminants in the Kiang West region. Significant differences in seroprevalence figures among the villages were observed. A significantly lower seroprevalence of 14.6% (95% CI: 11.8–18.0) was found in the small ruminants at the urban sites. No significant difference in seroprevalence was found between goats and sheep.

Shedding in small ruminants

A total of 57.4% (95% CI: 49.5–64.9) of the 155 examined small ruminants ($n = 89$) were found to be shedding *C. burnetii*; 41 of 151 milk samples and 68 of 144 vaginal swabs tested positive. Twenty animals tested positive for both the vaginal swab and the milk sample. Detection of *C. burnetii* DNA was significantly more frequent in vaginal swabs with 47.2% (95% CI: 39.2–55.3) than in milk samples with 27.2% (95% CI: 20.7–34.7). For one of the goat vaginal swabs, a CT value of 4.96 was found, indicating a very high concentration of *C. burnetii* DNA. Other borderline and positive CT values ranged from 24.37 to 39.00.

Of the 89 shedding small ruminants, 37 were seropositive (41.6%); 20 of 68 animals with PCR-positive vaginal swabs were seropositive (29.4%) and 28 of 41 animals with PCR-positive milk samples (68.3%).

Risk factors for seropositivity in humans

In Table 2, the characteristics of the human participants and associated risk factors for their *C. burnetii* seropositivity, adjusted for age, are presented. Being an animal farmer and having a seropositive animal in one's compound were associated with presence of antibodies.

The variables 'occupation' and 'seropositive animals in compound' were fitted in a final mixed-effects logistic regression model, correcting for age and village as a random effect. This resulted in a similar association between human seropositivity and having a seropositive animal in one's compound of OR 3.35 (95% CI: 1.09–14.44). The OR for seropositive participants to be animal farmer compared to having another occupation was however slightly lower and no longer statistically significant with OR 2.28 (95% CI: 0.82–7.57).

The combined results of the IgG ELISA based on a lower cut-off value and the IgM ELISA were used in a regression model containing the same explanatory variables. This also resulted in an association between human seropositivity and the presence of a seropositive animal in one's compound with an OR of 2.32 (95% CI: 1.13–5.14). With OR 1.24 (95% CI: 0.66- 2.38), being an animal farmer was not significantly associated with being seropositive.
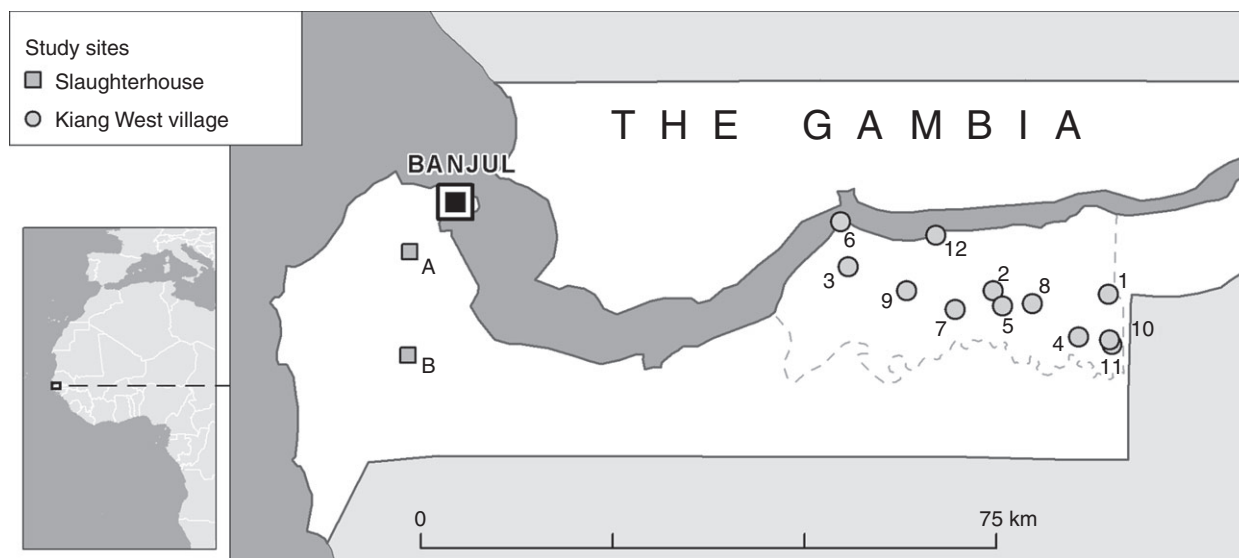
Figure 1 Geographical location of The Gambia with the capital city Banjul and the study sites: the slaughterhouses at the urban sites (A and B) and the villages in Kiang West (1-12).

Table 2 Characteristics of participants and associated risk factors for presence of antibodies against *Coxiella burnetii*

Factor	Category	Population n	Prevalence % (95% CI)	OR† (95% CI)	OR for lower cut-off† (95% CI)
Age	18–29 years	163	2.5 (1.0–6.1)	1 (ref.)	1 (ref.)
	30–49 years	242	4.9 (2.8–8.4)	2.07 (0.71–7.52)	2.15 (1.02–4.96)
	>50 years	194	3.6 (1.8–7.3)	1.49 (0.44–5.77)	2.19 (1.01–5.14)
Gender	Male	196	3.6 (1.7–7.2)	1 (ref.)	1 (ref.)
	Female	403	4.0 (2.5–6.4)	1.05 (0.43–2.82)	0.71 (0.40–1.26)
Ethnicity	Mandinka	537	3.5 (2.3–5.5)	1 (ref.)	1 (ref.)
	Non-Mandinka‡	62	6.5 (2.5–15.4)	1.99 (0.56–5.66)	1.37 (0.54–3.06)
Socio-economic status	Low (<3)	220	3.2 (1.5–6.4)	1 (ref.)	1 (ref.)
	Middle (3–5)	308	4.9 (3.0–7.9)	1.52 (0.63–4.04)	1.13 (0.63–2.04)
	High (>5)	71	1.4 (0.1–7.6)	0.43 (0.02–2.51)	0.60 (0.17–1.66)
Occupation	Other§	262	1.9 (0.8–4.4)	1 (ref.)	1 (ref.)
	Animal farmer	337	4.8 (3.1–7.5)	2.83 (1.03–9.28)	1.37 (0.75–2.58)
Seropositive animals in compound	No	195	1.5 (0.5–4.4)	1 (ref.)	1 (ref.)
	Yes	381	5.0 (3.2–7.7)	3.42 (1.14–14.71)*	2.30 (1.20–4.79)*
Shedding animals in compound	No	156	3.2 (1.4–7.3)	1 (ref.)	1 (ref.)
	Yes	276	5.1 (3.0–8.3)	1.56 (0.58–4.92)	0.96 (0.50–1.92)
Seroprevalence of animals in village	Linear	–	–	1.01 (0.97–1.04)	1.01 (0.99–1.04)
Ratio small ruminant/human in compound	Linear	–	–	1.30 (0.26–5.78)	1.97 (0.72–5.28)
Ratio small ruminant/human in village	Linear	–	–	0.88 (0.13–5.19)	1.44 (0.44–4.53)

*Also statistically significant in final mixed-effects logistic regression model.
†Adjusted for age.
‡Fula, Jola and others.
§Other occupations include student, (plant) farmer and housewife.

Risk factors for seropositivity in small ruminants

Table 3 shows the characteristics of the small ruminants and associated risk factors for presence of antibodies against *C. burnetii*. The presented ORs are corrected for age. Only female animals were included for the factors 'having lambed' and 'history of abortion'.

Table 3 Characteristics of small ruminants and associated risk factors for presence of antibodies against *Coxiella burnetii*

Factor	Category	*n*	Prevalence % (95% CI)	OR† (95% CI)
Age	<1 years	176	9.7 (6.1–14.9)	1 (ref.)
	1–3 years	169	22.5 (16.8–29.4)	2.71 (1.49–5.14)*
	>3 years	268	36.2 (30.7–42.1)	5.31 (3.11–9.56)*
Sex	Male	113	14.2 (8.9–21.8)	1 (ref.)
	Female	501	27.1 (23.4–31.2)	0.90 (0.47–1.78)
Species	Goat	494	25.5 (21.9–29.5)	1 (ref.)
	Sheep	121	22.3 (15.8–30.5)	0.79 (0.48–1.27)
Having lambed‡	No	161	11.2 (7.2–17.0)	1 (ref.)
	Yes	333	34.8 (29.9–40.1)	2.68 (1.38–5.47)*
History of abortion‡	No	427	27.6 (23.6–32.1)	1 (ref.)
	Yes	70	21.4 (13.4–32.4)	0.64 (0.33–1.19)
Grazing area available§	No	393	20.1 (16.4–24.3)	1 (ref.)
	Yes	222	33.3 (27.5–39.8)	1.93 (1.31–2.85)*
Type of grazing in rainy season¶	Tethering	271	27.3 (22.3–32.9)	1 (ref.)
	Herding	344	23.0 (18.8–27.7)	0.72 (0.48–1.08)
No. of small ruminants in village**	Linear (per 100 animals)	–	–	1.07 (1.02–1.12)
No. of small ruminants in compound††	Linear (per 100 animals)	–	–	10.02 (1.43–68.85)

*Also statistically significant in final mixed-effects logistic regression model.
†Adjusted for age.
‡Only females included.
§Village-level factor.
¶Flock-level factor.
**Ranging from 167 to 1603 (median = 338).
††Ranging from 2 to 49 (median = 12).

The presence of a grazing area for small ruminants in a village was associated with seropositivity. Both the number of small ruminants present in a village and in a compound were positively associated with seropositivity, with OR 1.07 (95% CI: 1.02–1.12) and OR 1.02 (95% CI: 10.02–68.85) per 100 animals, respectively. In the case of the female animals, a relation was found between having lambed and seropositivity, with OR 2.68 (95% CI: 1.38–5.47). No significant association was found between seropositivity and a history of abortion.

A mixed-effects logistic regression model was constructed by including all variables with statistically significant ORs except for the exclusively female variable 'having lambed', and performing backward elimination. The final model included the factor 'grazing area available' with OR 2.07 (95% CI: 1.26–3.50) and the factor 'age', with OR 2.97 (95% CI: 1.55- 5.96) for the category 1–3 years and OR 6.18 (95% CI: 3.41–11.99) for the category >3 years. In the same way, a separate mixed-effects logistic regression model was constructed for female small ruminants to fit the exclusively female factor 'having lambed', which remained a statistically significant risk factor with OR 2.58 (95% CI: 1.25–5.58).

Performing the same analysis using the shedding data instead of the seroprevalence data resulted in similar associations. The availability of a grazing area was associated with shedding with OR 2.90 (95% CI: 1.44–6.03). Age was however not significantly associated with shedding, with OR 0.89 (95% CI: 0.38–2.06) for the category >3 years and 1–3 years as the reference category. All animals that were tested for shedding had lambed, so the factor 'having lambed' was excluded from analysis.

Discussion

The *C. burnetii* seroprevalence rates of 3.8% or 9.7% in humans, depending on cut-off, and 24.9% in small ruminants suggest that *C. burnetii* infection is endemic in the Kiang West district of The Gambia. We took samples of humans and small ruminants at the same time and place and the results demonstrate that an individual who lived in the same compound with a *C. burnetii*-seropositive small ruminant was at risk for seropositivity. However, at village level, no significant association was found between human seropositivity and the seroprevalence in the local small ruminant population, possibly explained by a lack of power due to a limited number of villages. This is consistent with findings of a study in 2003 in Chad, in which no significant correlation between human seropositivity and the seroprevalence in animals of the

same nomadic camp was observed [15]. The dynamic composition of nomadic camp members was proposed to explain the absence of this correlation, a situation which however does not apply to the villages of Kiang West.

The seroprevalence of 3.8% among adult humans in this study was lower than the 8.3% found in children in an earlier study in the Farafenni region of The Gambia [8]. A possible explanation for a higher prevalence among children may be found in the consumption of raw goat milk, a practice that is mostly limited to children and the non-active elderly that remain in the compound throughout the day, thus providing additional protein to these groups. Low social value of goats is the main reason that makes farmers reluctant to this [16]. Although drinking contaminated raw milk is not considered to be an efficient route of transmission, it can induce seroconversion [17]. Waning of antibodies could result in observation of a lower seroprevalence in adults. Also, close contact with small ruminants may be more common among children who are not yet of school age and the elderly, as they often take care of the animals in a household or herd the flocks. The intensity of human–animal contact might also explain the observation that livestock farmers were at risk for *C. burnetii* antibody seropositivity, although this association was no longer statistically significant in the final regression model, nor was it in the analysis based on a lower cut-off value. Q fever is generally considered to be an occupational hazard for livestock farmers, but studies in sub-Saharan Africa have so far been inconclusive [18]. For instance, no association between animal contact and seropositivity was found in a study in Mali in 2005 [6]. In a study in Togo in 2013, however, seroprevalence varied by ethnicity, which could be explained by differences in frequency of animal contact [19].

Due to the absence of a gold standard for defining *C. burnetii* seropositivity, assessing Q fever seroprevalence remains complicated. Particularly problematic is the choice of an appropriate cut-off value for ELISA tests, which are usually designed for clinical diagnostic use. For the test that we used to detect human IgG antibodies, a recent German study recommended applying a lower cut-off value to increase sensitivity in seroprevalence studies [12]. Whether this also applies to The Gambia, where *C. burnetii* exposure patterns may be different than in Germany, is unknown. In our risk factor analysis, the association between human seropositivity and having a seropositive animal in one's compound increased in power when applying a lower cut-off value, which supports this recommendation. However, being an animal farmer was no longer identified as a risk factor. We could speculate that due to more frequent interaction with

animals, seropositive livestock farmers may generally have higher antibody titres than seropositive non-livestock farmers, which would explain that an association between seropositivity and this profession is exclusively found when only relatively high titres are considered positive. It is likely that the percentage of adult humans with serological evidence of a previous *C. burnetii* infection ranges from 3.8% to 9.7%, but more research to determine an optimal cut-off value in an endemic, sub-Saharan African setting is needed.

Shedding of *C. burnetii* was very common in small ruminants. No direct relationship between seroprevalence and shedding rates was observed, in accordance with earlier findings [20]. Seroprevalence rates showed considerable variation between villages, ranging from 4.0% to 47.5%. The presence of a separate grazing area in a village, which was found to be a risk factor for seropositivity in small ruminants, may contribute to this geographical variation.

A survey performed in 2012 in another rural area showed a lower overall seroprevalence in small ruminants of 15.1%, compared to the 24.9% we found in Kiang West [9]. In contrast, a seroprevalence of 29.1% was found in goats and sheep sampled from the Abuko abattoir in 2012, which was considerably higher than our observed seroprevalence of 15.7%. At the Abuko abattoir, animals from all over The Gambia and also from abroad are slaughtered [21]. At the time of sampling of the current study, small ruminants from populations with lower Q fever seroprevalence than the animal populations sampled in a different area in 2012 may have been present. A history of abortion was reported for 16.3% of the female goats and 3.4% of the female sheep of the Kiang West populations, but was not significantly associated with Q fever seropositivity, in contrast with findings in other African countries [7]. Given the large proportion of shedders without antibodies, shedding animals without antibodies may have been misclassified as negative, and this may have reduced the power to find associated risk factors. The separate analysis of the shedders does not overcome this problem because their number is much lower. Another explanation could be that due to endemic circulation of *C. burnetii* in the study area, the infection may occur before mating with a lower probability of abortion. Infected animals can have a normal delivery of live lambs and still shed large amounts of bacteria via the placenta and birth fluids [22].

Endemic circulation in small ruminants may also explain the relatively low seroprevalence in adults in the villages in Kiang West, living in close proximity to shedding animals, in comparison with for instance the

rural village Herpen in the Netherlands. In Herpen, a seroprevalence of 33.8% was observed in the village population 7 years after an outbreak [23]. Between 2007 and 2009, the Netherlands experienced the largest Q fever outbreak ever described, where waves of abortions occurred in commercial goat herds, and people became infected through inhalation of contaminated particles, sometimes at a distance of several kilometres from infected farms [24]. Possibly, endemic circulation in animals with human exposure through ingestion of raw milk or direct contact early in life may result in a different immune response and seroconversion rate than exposure through inhalation in an outbreak setting. Both the adaptive and innate immune response play a role in recognition of the infection and the clinical development of Q fever [25].

The present study confirms the importance of *C. burnetii* infection among associated populations of humans and animals in The Gambia. The findings call for further investigation into the clinical relevance of Q fever in this country. Knowledge about the role of *C. burnetii* in acute fever in sub-Saharan Africa is limited, but the few fever aetiological studies that have been performed showed that this pathogen can be responsible for up to 11% of hospitalised febrile cases [7]. The incidence of acute Q fever progressing to chronic Q fever represents an even bigger knowledge gap in this region. Correct diagnosis of the underlying cause of a febrile illness is vital to avoid under- and overprescription of antimalarials and antimicrobials. In the case of Q fever, this is particularly relevant, as *C. burnetii* is not susceptible to the antimicrobial therapy generally used in the empirical management of febrile patients in resource-poor settings [26] and in the absence of any or reliable laboratory results. As a next step, aetiological studies into acute fever could be set up in local clinics in The Gambia to assess the role of Q fever, covering also other neglected bacterial zoonoses such as leptospirosis and rickettsiosis.

References

1. Angelakis E, Raoult D. Q fever. *Vet Microbiol* 2010: **140**: 297–309.
2. ECDC. *Risk Assessment on Q Fever*. European Centre for Disease Prevention and Control: Stockholm, 2010.
3. Ceesay SJ, Casals-Pascual C, Nwakanma NC *et al*. Continued decline of malaria in The Gambia with implications for elimination. *PLoS One* 2010: **5**: e12242.
4. Amexo M, Tolhurst R, Barnish G, Bates I. Malaria misdiagnosis: effects on the poor and vulnerable. *The Lancet* 2004: **364**: 1896–1898.
5. Prabhu M, Nicholson WL, Roche AJ *et al*. Q fever, spotted fever group, and typhus group rickettsioses among hospitalized febrile patients in northern Tanzania. *Clin Infect Dis* 2011: **53**: e8–e15.
6. Steinmann P, Bonfoh B, Peter O *et al*. Seroprevalence of Q-fever in febrile individuals in Mali. *Tropical Med Int Health* 2005: **10**: 612–617.
7. Vanderburg S, Rubach MP, Halliday JE *et al*. Epidemiology of *Coxiella burnetii* infection in Africa: a OneHealth systematic review. *PLoS Negl Trop Dis* 2014: **8**: e2787.
8. Hoek W, Sarge-Njie R, Herremans T *et al*. Short communication: prevalence of antibodies against Coxiella burnetii (Q fever) in children in The Gambia, West Africa. *Tropical Med Int Health* 2013: **18**: 850–853.
9. Klaasen M, Roest HJ, van der Hoek W *et al*. Coxiella burnetii seroprevalence in small ruminants in The Gambia. *PLoS One* 2014: **9**: e85424.
10. The Gambia Bureau of Statistics. The Gambia 2013 Population and Housing Census Preliminary Results, 2013. (Available from: http://www.gbos.gov.gm/uploads/census/The Gambia Population and Housing Census 2013 Provisional Report.pdf) [Accessed 1 October 2014].
11. World Health Organization. Use of anticoagulants in diagnostic laboratory investigations, 2002.
12. Frosinski J, Hermann B, Maier K, Boden K. Enzyme-linked immunosorbent assays in seroprevalence studies of Q fever: the need for cut-off adaptation and the consequences for prevalence data. *Epidemiol Infect* 2015: **00**: 1–5.
13. Roest HI, Ruuls RC, Tilburg JJ *et al*. Molecular epidemiology of Coxiella burnetii from ruminants in Q fever outbreak, the Netherlands. *Emerg Infect Dis* 2011: **17**: 668–675.
14. R Development Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing: Vienna, Austria, 2008. URL: http://www.R-project.org.
15. Schelling E, Diguimbaye C, Daoud S *et al*. Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. *Prev Vet Med* 2003: **61**: 279–293.
16. Osaer S, Goossens B. Trypanotolerance in Djallonke sheep and West African Dwarf goats: Importance of trypanosomosis, nutrition, helminth infections and management factors. PhD Thesis, Utrecht University, The Netherlands, 1999. (Available from: http://dspace.library.uu.nl/handle/1874/33606) [Accessed 12 October 2015].

17. Benson WW, Brock DW, Mather J. Serologic analysis of a penitentiary group using raw milk from a Q fever infected herd. *Public Health Rep* 1963: **78**: 707.

18. Van den Brom R, van Engelen E, Roest HIJ *et al.* Coxiella burnetii infections in sheep or goats: an opinionated review. *Vet Microbiol* 2015: **181**: 119–129.

19. Dean AS, Bonfoh B, Kulo AE *et al.* Epidemiology of brucellosis and Q fever in linked human and animal populations in northern togo. *PLoS One* 2013: **8**: e71501.

20. Arricau-Bouvery N, Rodolakis A. Is Q fever an emerging or re-emerging zoonosis? *Vet Res* 2005: **36**: 327–349.

21. Goossens B, Osaer S, Kora S *et al.* Abattoir survey of sheep and goats in The Gambia. *Vet Rec* 1998: **142**: 277–281.

22. Roest HIJ, van Gelderen B, Dinkla A *et al.* Q Fever in pregnant goats: pathogenesis and excretion of *Coxiella burnetii*. *PLoS One* 2012: **7**: e48949.

23. Karagiannis I, Schimmer B, Van Lier A *et al.* Investigation of a Q fever outbreak in a rural area of The Netherlands. *Epidemiol Infect* 2009: **137**: 1283–1294.

24. Schimmer B, ter Schegget R, Wegdam M *et al.* The use of a geographic information system to identify a dairy goat farm as the most likely source of an urban Q-fever outbreak. *BMC Infect Dis* 2010: **10**: 69.

25. Ammerdorffer A. Innate immune responses against *Coxiella burnetii* human versus goat. PhD Thesis, Radboud University, The Netherlands, 2016. ISBN: 978-94-6233-362-8.

26. Crump JA, Gove S, Parry CM. Management of adolescents and adults with febrile illness in resource limited areas. *BMJ-British Medical Journal* 2011: **343**: d4847.

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