Risk factors for bacterial zoonotic pathogens in acutely febrile patients in Mpumalanga Province, South Africa

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Abstract

Endemic zoonoses, such as Q fever and spotted fever group (SFG) rickettsiosis, are prevalent in South Africa, yet often undiagnosed. In this study, we reviewed the demographics and animal exposure history of patients presenting with acute febrile illness to community health clinics in Mpumalanga Province to identify trends and risk factors associated with exposure to *Coxiella burnetii* , the causative agent of Q fever, and infection by SFG *Rickettsia* spp. Clinical and serological data and questionnaires elucidating exposure to animals and their products were obtained from 141 acutely febrile patients between 2012 and 2016. Exposure or infection status to *C. burnetii* and SFG *Rickettsia* spp. was determined by presence of IgG or IgM antibodies. Logistic regression models were built for risk factor analysis. Clinical presentation of patients infected by SFG rickettsiosis was described. There were 37/139 (27%) patients with a positive *C. burnetii* serology, indicative of Q fever exposure. Patients who had reported attending cattle inspection facilities ("dip tanks") were 9.39 times more likely to be exposed to Q fever (95% CI: 2.9–30.4). Exposure risk also increased with age (OR: 1.03, 95% CI: 1.002–1.06). Twenty-one per cent of febrile patients (24/118) had evidence of acute infection by SFG *Rickettsia* spp. Similarly, attending cattle inspection facilities was the most significant risk factor (OR: 8.48, 95% CI: 1.58–45.60). Seropositivity of females showed a significant OR of 8.0 when compared to males (95% CI: 1.49–43.0), and consumption of livestock was associated with a decreased risk (OR: 0.02, 95% CI: 0.001–0.54). A trend between domestic cat contact and SFG rickettsiosis was also noted, albeit borderline non-significant. In this

endemic region of South Africa, an understanding of risk factors for zoonotic pathogens, including exposure to domestic animals, can help clinic staff with diagnosis and appropriate therapeutic management of acutely febrile patients as well as identify target areas for education and prevention strategies.

Impacts

- Undifferentiated acute febrile illness is a common syndrome among patients presenting to healthcare facilities in resource-limited areas. We studied 141 adult patients who presented to community clinics with acute fever in rural South Africa to determine the prevalence and risk factors of two endemic zoonoses, Q fever and spotted fever group (SFG) rickettsiosis.
- Based on questionnaires and serology, exposure to the pathogen causing Q fever (*Coxiella burnetii*) was evident in 27% of patients and was greater for individuals attending cattle inspection facilities ("dip tanks"). Acute SFG rickettsiosis was evident in 21% of patients; the odds of seropositivity were higher for females and those attending dip tanks.
- Q fever and SFG rickettsiosis may be associated with acute febrile illness in adult patients, particularly those having more intense contact with cattle.

1 INTRODUCTION

Zoonoses are a growing global threat to public health, and their incidence is expected to increase with ongoing globalization, landscape disruption, and climate change (Aenishaenslin et al., 2013). Zoonoses, however, are often overshadowed by infections such as malaria and HIV/AIDS, which dominate the global health agenda in terms of research and resources (Maudlin, Eisler, & Welburn, 2009; WHO, 2006). In Africa, zoonotic infections are not only directly responsible for human morbidity and mortality, but also have a profound impact on human well-being as a result of reduced livestock productivity and food security (Molyneux et al., 2011; Perry & Grace, 2009). Despite their importance, the literature is richer on studies describing broader socio-cultural considerations for emerging, rather than endemic zoonoses (Schelling & Hattendorf, 2015); and anthropological studies on zoonoses are limited (Bardosh & Thys, 2012).

In parts of sub-Saharan Africa, endemic bacterial zoonoses are common yet underappreciated causes of febrile illness among patients requiring hospitalization (Crump et al., 2013). While infections can be diagnosed using a variety of laboratory and molecular methods, including serology and DNA amplification by polymerase chain reaction (PCR), these diagnostics can be prohibitively expensive and generally do not provide results at the point of treatment (Molyneux et al., 2011; Petti, Polage, Quinn, Ronald, & Sande, 2006). Additionally, endemic zoonoses frequently present with unspecific symptoms that are difficult to differentiate clinically, and clinicians often have limited knowledge of zoonotic causes of human disease (Frean, Blumberg, & Ogunbanjo, 2008; Halliday et al., 2015; Molyneux et al., 2011). For diseases such as Q fever and rickettsioses, endemic zoonoses in South

Africa, symptoms can vary from mild to severe and even fatal disease (Frean & Blumberg, 2007; Frean et al., 2008). Zoonotic transmission typically results from direct or indirect contact with livestock species (Q fever) or the bite of an infected tick associated with cattle or dogs (SFG rickettsiosis; Maurin & Raoult, 1999; Frean et al., 2008). An enhanced understanding of locally relevant risk factors for transmission and subsequent infection could be useful for patient screening, particularly in low-resource settings, and ultimately, improve patient outcomes.

This epidemiologic study, which was part of a broader zoonotic disease prevalence study (Simpson et al., 2018), aimed to identify risk factors for exposure to *Coxiella burnetii* (Q fever) and acute infection by SFG *Rickettsia* spp. (tick bite fever and/or other rickettsioses). This study focused on acutely febrile patients in the Mnisi Community Programme area in Mpumalanga Province, South Africa, in which crop growing and communal livestock ranching is the primary subsistence activity. The clinical presentation for patients with evidence of acute rickettsiosis was also described. Results of this study are intended to guide diagnosis and therapeutic management of febrile illness in this resource-limited area.

2 METHODS

2.1 Study area

The study was conducted in the northeastern part of South Africa in the Bushbuckridge Local Municipality, Mpumalanga Province. The municipality is one of the Presidential Nodal Points of Development in South Africa, designated as such because of its poverty and lack of basic services (Bushbuckridge Local Municipality, 2010). The study area, situated in the northeastern corner of the municipality, is comprised of approximately 50,000 people in 8,500 households, many of which own domestic animals (primarily cattle, goats, and chickens; Berrian et al., 2016) and forms part of the Mnisi Community Programme within the Hans Hoheisen Research Platform of the University of Pretoria. The study area is part of the Kruger to Canyons Biosphere Region, which incorporates grassland, Afro-montane forest, and lowveld savannah, and shares 75% of its boundary with private and provincial wildlife conservation areas (Berrian et al., 2016).

2.2 Study population

Five rural government primary health-care clinics serve the study area, four of which (Gottenburg, Utha, Welverdiend, and Hluvukani) were sites for the study. Data from questionnaires and serological testing were used from an ongoing zoonotic disease prevalence study conducted by the National Institute for Communicable Diseases (NICD) in collaboration with the University of Pretoria (UP) between October 2012 to June 2013 (Phase 1) and September 2014 to December 2016 (Phase 2; Simpson et al., 2014; Simpson et al., 2018). The NICD-UP study enrolled adult patients (≥18 years of age) with a

documented axillary temperature ≥99.5°*F* (≥37.5°C) or a history of fever within the last 48 hr before admission. As part of clinic standard operating procedure, all enrolled patients were screened for malaria using a rapid diagnostic test. For Phase 1, patients were those presenting to Gottenburg, Utha, or Welverdiend clinics and from Hluvukani clinic for Phase 2. Clinic selection was based on source and availability of funding, staff allocation, and staff availability.

2.3 Data collection

After providing informed consent, patients completed a brief questionnaire which captured information pertaining to patient demographics, exposure history to animals and animal products, history of tick and flea bites, and consumption/preparation of animal-source foods. Additionally, patients provided blood for acute serological testing of selected zoonotic pathogens (Visit 1). A convalescent blood sample was collected from patients two to three weeks later (Visit 2). Diagnostic tests and interpretations by pathogen tested are described in Table 1. For this study, patients for whom laboratory diagnostics were available from Visit 1 and/or Visit 2 were included. Patients for whom serological testing was unavailable for both *C. burnetii* and *Rickettsia* spp. were excluded from further analysis.

TABLE 1 Serologic techniques and interpretations for *Coxiella burnetii* and *Rickettsia conorii*

Pathogen	Serologic test	Sample(s) tested	Interpretation
Coxiella burnetii	IgG ELISA	Convalescent serum, or acute serum if convalescent not available	Index values calculated using run-based cut-off values per manufacturer's recommendations
	IgM ELISA	Acute serum if convalescent serum not available or if tested positive with IgG ELISA	Index values calculated using run-based cut-off values per manufacturer's recommendations
Rickettsia conorii	IgM IFA	Acute or convalescent serum[a]	IgM ≥ 1:192 deemed positive for acute infection

Note. ELISA: enzyme-linked immunosorbent assay (Panbio®, Standard Diagnostics Inc., Republic of Korea); IFA: indirect immunofluorescence assay (Vircell S.L., Spain).
[a]Depending on availability.

2.4 Diagnostics

Positive serological evidence for exposure to *C. burnetii* was defined as an elevated (≥1:100 dilution) single Q fever anti-phase II immunoglobulin (Ig) G/M titer (Sivabalan, Saboo, Yew, & Norton, 2017; Waag, Chulay, Marrie, England, & Williams, 1995). A commercial indirect enzyme-linked immunosorbent assay (ELISA) kit (Panbio®, Standard Diagnostics Inc., Republic of Korea) was used and interpreted according to manufacturer's instructions (Table 1).

The indirect immunofluorescence assay (IFA) was used to detect IgM antibodies against *R. conorii* . Samples with IgM titers ≥1:192 were classified as positive for acute SFG rickettsiosis (Table 1). According to the manufacturer (Vircell, S.L., Spain), antibody reactivity to the *R. conorii* antigen should be considered SFG reactive which, in this region,

should include boutonneuse fever-like tick bite fever (caused by *R. conorii*) and African tick bite fever (caused by *R. africae* ; Frean et al., 2008).

2.5 Outcome variables (classification of pathogen exposure or infection)

For Q fever, patients with a positive IgG antibody titer on Visit 2 were classified as exposed. In the event, they did not return for Visit 2, patients with either a positive IgG or IgM antibody titer on Visit 1 were classified as exposed. For SFG rickettsiosis, patients with a positive IgM antibody titer during Visit 1 and/or Visit 2 were considered cases (acute infection by SFG *Rickettsia* spp.) (Table 1).

2.6 Explanatory variables

Risk factors were assessed in the form of closed-ended questions including the following data: (a) presence of domestic animals in household (self or neighbour), by species; (b) activities practiced involving animals or their products; and (c) consumption of animal-source foods. Questions were developed as binary response variables (yes/no) and did not specify time or frequency. A total of 23 explanatory variables were evaluated and are listed in Tables 2 and 3.

2.7 Clinical presentation

The clinical presentation of patients was assessed by a clinic nurse and included body temperature, duration of illness, and the presence or absence of gastrointestinal signs (e.g., diarrhoea, vomiting, abdominal pain), respiratory signs (e.g., cough, tachypnea, abnormal lung auscultation), bleeding, muscle or joint pain or rash. Patient records, including physical examination findings, provisional diagnosis, outcome (e.g., referred, recovered) and treatment, were reviewed and recorded by a study nurse.

2.8 Data management and statistical analysis

Questionnaire results, physical examination findings, and serological data were entered and maintained in a Microsoft Excel spreadsheet and exported into SAS v9.4 (Cary, NC, USA) for descriptive analysis. Univariate and bivariate analyses were performed, including descriptive statistics (e.g., median, inter-quartile range—IQR) and correlation analysis. Univariable and multivariable logistic regression were performed to identify significant predictors of pathogen exposure or acute infection.

Our general strategy to develop a predictive model for exposure to *C. burnetii* or infection by SFG *Rickettsia* spp. consisted of the following steps. Initially, a univariable analysis was performed using a logistic regression model at the patient level. The dependent variable was dichotomous, where $Y = 1$ if an individual i had a test-positive result for *C. burnetii* (or SFG rickettsial infection) as previously defined and $Y = 0$ otherwise; each patient-level risk factor

was tested independently as an explanatory variable. X_i and $X_1,\ldots, X_p$ were the candidate risk factors (X_i $i = 1,\ldots, p$ are indicator variables reflecting dichotomous or categorical risk factors). Variables with a $p < 0.25$ in the univariable analysis were further evaluated in a multivariable logistic regression analysis. A manual forward selection approach was applied to fit the final model. For this approach, the best univariable model was selected based on the Akaike information criterion (AIC) values (the lower the better). The remaining variables were then added one at a time to form a multivariable model. The best multivariable model was selected as that with the lowest AIC value. This procedure was repeated until the addition of one or more variables failed to improve the model fit. The model with the lowest AIC was considered to be the most appropriate model for the data.

Correlation among the quantitative independent variables was assessed using the Pearson correlation coefficient. Values > 0.7 were indicative of high correlation; in that case, only the variable most significantly associated with the response remained in the model to avoid multicollinearity problems in the final model. All two-way interaction terms of the variables remaining in the final model were assessed for significance based on the likelihood ratio test.

2.9 Ethical considerations

Ethics approval for the original study and data collection was obtained through the University of Witwatersrand Human Ethics Committee (certificate no.: M120667). Approval from the same committee was also obtained for this sub-study (certificate no.: M1704131).

3 RESULTS

A total of 141 acutely febrile patients were available for analysis, of which 88 (62%) were females and 53 (38%) were males. The median age for females was 36.7 years (IQR = 26.9–49.9) and 30.1 years (IQR = 24.7–41.3) for males (Kruskal–Wallis test, $p = 0.0688$). In Phase 1, 73 patients were enrolled from the three participating clinics (2012:6, 2013:67); in Phase 3, 68 patients were enrolled from the Hluvukani clinic (2014:4, 2015:50, 2016:14). Nearly all patients (140/141) reported domestic animals within the household (self or neighbour), including chickens (96%), dogs (93%), cattle (70%), goats (64%), cats (58%), and pigs (21%). Seventy-nine per cent (111/141) of patients reported domiciliary (self or neighbour) rodents, and 83% (116/140) reported a history of tick bites. Twenty-seven per cent (38/141) reported a history of consumption of unpasteurized milk from village cows and/or goats.

On presentation (Visit 1), body temperature of the patients ranged from 34.6–39.9°C (median = 38, IQR = 37.5–38.5); and 33 patients (23%) were considered historically febrile (<37.5°C on presentation). Median duration of illness was two days (IQR: 2–4). A blood sample was collected from each patient; due to processing errors, three patients did not

complete acute serologic testing. One patient, a 22 year-old male, was identified as malaria positive. At presentation, he was febrile (38°C) and subsequently tested negative for all zoonotic pathogens evaluated by the study. Ninety patients (64%) returned for convalescent sampling (Visit 2). Median time from acute to convalescent samples was 20 days (IQR: 15–25).

3.1 Seroprevalence and risk factors of Q fever

A total of 139 acutely febrile patients were available for analysis (Figure 1), among whom the prevalence of Q fever exposure was 27% (37/139) (IgG: 36, IgM: 1). The most significant risk factor for Q fever exposure was attendance at cattle inspection facilities ("dip tanks") (OR: 9.39, 95% CI: 2.9–30.4). Exposure risk also increased with age (OR: 1.03, 95% CI: 1.002–1.063). When compared to year 2016, patients sampled in 2015 had a decreased odds of exposure to *C. burnetii* (OR: 0.12, 95% CI: 0.02–0.64). Other livestock husbandry procedures were reported in a greater proportion in exposed patients, including milking (27% vs. 12%, $p = 0.0293$), herding (46% vs. 16%, $p = 0.0002$), and handling livestock faeces (51% vs. 34%, $p = 0.0685$); however, these variables did not retain their significance in the multivariable model (Table 2). Clinical presentation was not considered as the available test results allowed for the evaluation of Q fever exposure, not acute infection.

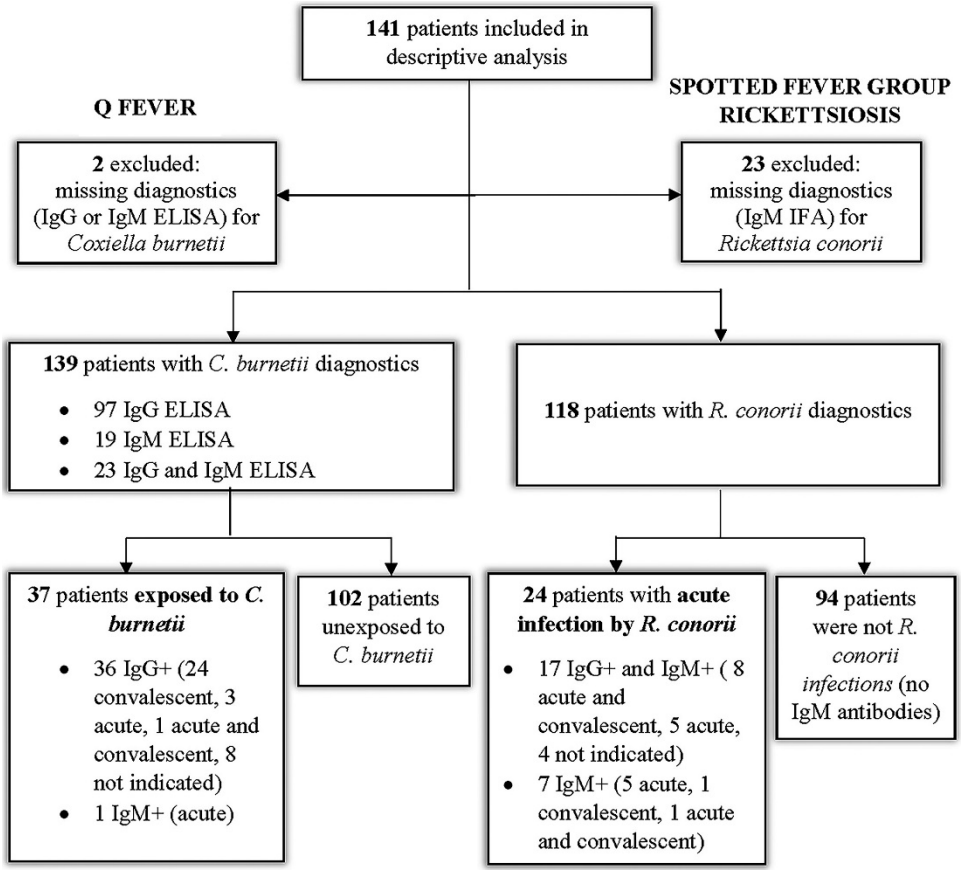

Figure 1. Flowchart of acutely febrile patients in Mpumalanga Province, South Africa who met study inclusion and exclusion criteria to further evaluate risk factors for Q fever exposure and acute spotted fever group (SFG) rickettsiosis infection

TABLE 2 Frequency (%) of risk factors for exposure to *Coxiella burnetii* in acutely febrile patients in Mpumalanga Province, South Africa. Odds ratios (OR) and 95 per cent confidence intervals (CI) of the univariable and multivariable logistic regression models

Risk factor	Patients, *n* (%)		Univariable OR	95% CI	Multivariable OR	95% CI
	Total (% with *C. burnetii* exposure) *n* = 37	Total (% without *C. burnetii* exposure) *n* = 102				
Age, years (median), IQR	43.6 (34.1–55.5)	31.4 (24.2–42.4)	1.040	1.015–1.066	1.032	1.002–1.063
Gender						
Male	16 (43)	36 (35)	1.397	0.649–3.007		
Female (reference)	21 (57)	66 (65)	1.000			
Clinic						
Utha (reference)	6 (16)	14 (14)	1.000			
Gottenburg	10 (27)	11 (11)	2.121	0.588–7.658		
Welverdiend	8 (22)	24 (24)	0.778	0.224–2.706		
Hluvukani	13 (35)	53 (52)	0.572	0.184–1.776		
Year						
2012	5 (14)	1 (1)	11.247	0.972–130.159	2.888	0.183–45.484
2013	19 (51)	48 (47)	0.891	0.245–3.242	0.458	0.116–1.806
2014	2 (5)	2 (2)	2.250	0.229–22.144	1.280	0.093–17.653
2015	7 (19)	42 (41)	0.375	0.090–1.557	0.124	0.024–0.637
2016 (reference)	4 (11)	9 (9)	1.000	0.008–1.029	1.000	0.022–5.453
Dog[a]						
Yes	34 (92)	95 (93)	0.835	0.204–3.414		
No (reference)	3 (8)	7 (7)	1.000			
Cat[a]						
Yes	24 (65)	57 (56)	1.457	0.668–3.179		
No (reference)	13 (35)	45 (44)	1.000			
Cattle[a]						
Yes	29 (78)	68 (67)	1.812	0.749–4.389		
No (reference)	8 (22)	34 (33)	1.000			
Goat[a]						
Yes	22 (59)	68 (67)	0.733	0.338–1.591		
No (reference)	15 (41)	34 (33)	1.000			
Pig[a]						
Yes	9 (24)	21 (21)	1.240	0.508–3.023		
No (reference)	28 (76)	81 (79)	1.000			
Chicken[a]						
Yes	36 (97)	98 (96)	1.469	0.159–13.581		
No (reference)	1 (3)	4 (4)	1.000			
Rodent[a]						
Yes	32 (86)	78 (76)	1.969	0.691–5.614		
No (reference)	5 (14)	24 (24)	1.000			
Tick bites						
Yes	31 (84)	83 (82)	1.120	0.407–3.082		
No (reference)	6 (16)	18 (18)	1.000			
Dip tank						
Yes	15 (41)	10 (10)	6.273	2.486–15.827	9.389	2.900–30.401

Risk factor	Patients, n (%)		Univariable OR	95% CI	Multivariable OR	95% CI
	Total (% with *C. burnetii* exposure) n = 37	Total (% without *C. burnetii* exposure) n = 102				
No (reference)	22 (59)	92 (90)	1.000		1.000	
Feed livestock						
Yes	14 (38)	52 (51)	0.585	0.271–1.264		
No (reference)	23 (62)	50 (49)	1.000			
Milk livestock						
Yes	10 (27)	12 (12)	2.778	1.082–7.132		
No (reference)	27 (73)	90 (88)	1.000			
Herd livestock						
Yes	17 (46)	16 (16)	4.569	1.976–10.565		
No (reference)	20 (54)	86 (84)	1.000			
Handle livestock faeces						
Yes	19 (51)	35 (34)	2.021	0.942–4.335		
No (reference)	18 (49)	67 (66)	1.000			
Dehorn livestock						
Yes	4 (11)	7 (7)	1.645	0.453–5.982		
No (reference)	33 (89)	95 (93)	1.000			
Slaughter livestock						
Yes	33 (89)	86 (84)	1.534	0.478–4.928		
No (reference)	4 (11)	16 (16)	1.000			
Consume livestock						
Yes	37 (100)	98 (96)	–	–		
No (reference)	0	4 (4)				
Consume cow milk						
Yes	23 (62)	49 (48)	1.777	0.823–3.836		
No (reference)	14 (38)	53 (52)	1.000			
Consume unboiled cow milk						
Yes	12 (32)	29 (28)	1.208	0.537–2.721		
No (reference)	25 (68)	73 (72)	1.000			
Consume goat milk						
Yes	0	5 (5)	-	–		
No (reference)	37 (100)	97 (95)				
Consume unboiled goat milk						
Yes	0	2 (2)	-	–		
No (reference)	37 (100)	100 (98)				
Consume wild animals						
Yes	35 (95)	86 (84)	3.255	0.711–14.904		
No (reference)	2 (5)	16 (16)	1.000			
Fit statistics for the multivariable model AUC (ROC) = 0.800						
AIC = 138.337						

Note. AIC: Akaike information criterion; AUC: area under curve; ROC: receiver operating characteristic.
*Phrased as "*Have you had any of these animals in your, or your neighbours' household?*".

TABLE 3 Frequency (%) of risk factors for probable infection by spotted fever group *Rickettsia* spp. in acutely febrile patients in Mpumalanga Province, South Africa ($n = 118$). Odds ratios (OR) and 95 per cent confidence intervals (CI) of the univariable and multivariable logistic regression models

	Patients, n (%)					
Risk factor	Total (% with acute *Rickettsia* spp. infection) $n = 24$	Total (% without acute *Rickettsia* spp. infection) $n = 94$	Univariable OR	95% CI	Multivariable OR	95% CI
Age, years (median) (IQR)	32.3 (24.5–47.1)	34.6 (26–49.2)	0.992	0.964–1.021		
Gender						
Male	6 (25)	41 (44)	0.431	0.157–1.183	0.125	0.023–0.671
Female (reference)	18 (75)	53 (56)	1.000			
Clinic						
Utha (reference)	2 (8)	12 (13)	1.000			
Gottenburg	4 (17)	11 (12)	2.182	0.332–14.36		
Welverdiend	7 (29)	15 (16)	2.800	0.489–16.036		
Hluvukani	11 (46)	56 (60)	1.179	0.231–6.019		
Year						
2012	0	6 (6)	-	-		
2013	13 (54)	32 (34)				
2014	1 (4)	3 (3)				
2015	10 (42)	40 (43)				
2016 (reference)	0	13 (14)				
Dog[a]						
Yes	22 (92)	88 (94)	0.750	0.142–3.972		
No (reference)	2 (8)	6 (6)	1.000			
Cat[a]						
Yes	19 (79)	52 (55)	3.069	1.057–8.910	2.73	0.88–8.464
No (reference)	5 (21)	42 (45)	1.000		1.000	
Cattle[a]						
Yes	17 (71)	64 (68)	1.138			
No (reference)	7 (29)	30 (32)	1.000	0.427–3.037		
Goat[a]						
Yes	15 (63)	60 (64)	0.944	0.374–2.387		
No (reference)	9 (37)	34 (36)	1.000			
Pig[a]						
Yes	5 (21)	21 (22)	0.915	0.305–2.743		
No (reference)	19 (79)	73 (78)	1.000			
Chicken[a]						
Yes	24 (100)	89 (95)	-	-		
No (reference)	0	5 (5)				
Rodent[a]						
Yes	20 (83)	74 (79)	1.351	0.415–4.405		
No (reference)	4 (17)	20 (21)	1.000			
Tick bites						
Yes	20 (83)	77 (83)	1.104	0.334–3.647		
No (reference)	4 (17)	17 (18)	1.000			
Dip tank						

Risk factor	Patients, *n* (%)		Univariable		Multivariable	
	Total (% with acute *Rickettsia* spp. infection) n = 24	Total (% without acute *Rickettsia* spp. infection) n = 94	OR	95% CI	OR	95% CI
Yes	7 (29)	17 (18)	1.865	0.669–5.198	8.478	1.576–45.597
No (reference)	17 (71)	77 (83)	1.000		1.000	
Feed livestock						
Yes	13 (54)	45 (48)	1.287	0.524–3.163		
No (reference)	11 (46)	49 (52)	1.000			
Milk livestock						
Yes	5 (21)	15 (16)	1.386	0.448–4.288		
No (reference)	19 (79)	79 (84)	1.000			
Herd livestock						
Yes	9 (38)	23 (24)	1.852	0.716–4.793		
No (reference)	15 (62)	71 (76)	1.000			
Handle livestock faeces						
Yes	11 (46)	34 (36)	1.493	0.603–3.697		
No (reference)	13 (54)	60 (64)	1.000			
Dehorn livestock						
Yes	1 (4)	8 (9)	0.467	0.056–3.930		
No (reference)	23 (96)	86 (91)	1.000			
Slaughter livestock						
Yes	22 (92)	80 (85)	1.925	0.407–9.114		
No (reference)	2 (8)	14 (15)	1.000			
Consume livestock						
Yes	22 (92)	93 (99)	0.118	0.010–1.364	0.024	0.001–0.539
No (reference)	2 (8)	1 (1)	1.000			
Consume cow milk						
Yes	15 (63)	50 (54)	1.405	0.560–3.528		
No (reference)	9 (37)	44 (46)	1.000			
Consume unpasteurized cow milk						
Yes	7 (29)	29 (31)	0.923	0.345–2.466		
No (reference)	17 (71)	65 (69)	1.000			
Consume goat milk						
Yes	0	5 (5)	-	-		
No (reference)	24 (100)	89 (95)				
Consume unpasteurized goat milk						
Yes	0	2 (2)	-	-		
No (reference)	24 (100)	92 (98)				
Consume wild animals						
Yes	20 (83)	82 (87)	0.732	0.213–2.510		
No (reference)	4 (17)	12 (13)	1.000			
Fit statistics for multivariable model:						
AUC (ROC) = 0.752						
AIC = 110.874						

Note. AIC: Akaike information criterion; AUC: area under curve; ROC: receiver operating characteristic.
[a]Phrased as "Have you had any of these animals in your, or your neighbours' household?"

3.2 Seroprevalence and risk factors of SFG rickettsiosis

A total of 118 acutely febrile patients were available for analysis (Figure 1), of whom 24 (20%) were acutely infected by SFG *Rickettsia* spp. One-third (8/24) of cases were also positive for Q fever exposure. The most significant risk factor for SFG rickettsiosis was

attendance at cattle inspection facilities (OR: 8.48, 95% CI: 1.58–45.6). Seropositivity of females showed a significant OR of 8.0 when compared to males (95% CI: 1.49–43.0), and consumption of livestock was associated with a decreased odds (OR: 0.02, 95% CI: 0.001–0.54). Presence of a cat in the household (self or neighbour) was positively (albeit borderline non-significant) associated with disease outcome (OR: 2.73, 95% CI: 0.88–8.46) (Table 3). There was no evidence that clinical signs varied at presentation between patients with acute SFG rickettsiosis and patients without (Table 4). Among positive cases, muscle pain and respiratory symptoms were the most common clinical presentations (83% and 46%, respectively). Therapeutic management of cases included antibiotics in 67% (16/24) of patients. Although only two of these patients, in retrospect, received doxycycline, all recovered.

TABLE 4 Summary of clinical signs in acutely febrile patients with acute spotted fever group rickettsiosis

Clinical sign	Patients with acute rickettsiosis (%), $n = 24$	Patients without acute rickettsiosis (%), $n = 94$	p
Body temperature, °C (median) (IQR)	37.5 (37.2–38.3)	38 (37.6–38.5)	0.1427[b]
Duration of illness, days (median) (IQR)	2 (1–5)	2 (2–3)	0.8949[b]
Gastrointestinal	4 (17)	17 (18)	1.000[c]
Respiratory	11 (46)	30 (32)	0.2012[d]
Muscle pain	20 (83)	65 (69)	0.2084[c]
Rash	2 (8)	5 (5)	0.6294[d]

Note. IQR: inter-quartile range.
[a]Defined as presence of IgM antibodies against *R. conorii* on acute and/or convalescent sera. [b]Kruskal–Wallis test, [c]Fisher's exact test, [d]Chi-square test.

4 DISCUSSION

Q fever infection in humans occurs most frequently by inhalation of infectious aerosols generated by animals or animal products (e.g., parturient fluids; Maurin & Raoult, 1999). Zoonotic transmission can also occur by consumption of contaminated unpasteurized dairy products, direct contact with contaminated milk, urine, faeces, saliva, and tick bites (Maurin & Raoult, 1999; Porter, Czaplicki, Mainil, Guatteo, & Saegerman, 2011). Thus, Q fever is typically considered an occupational hazard among persons working with animals or animal products, and our results support that conclusion. Patients with a contact history with livestock that involved more intensive husbandry (e.g., milking, herding, handling faeces) were more likely to be exposed to *C. burnetii* . The most significant predictor of exposure was attendance at cattle inspection facilities. The study site falls within the foot and mouth disease (FMD) control zone, in which the South African government (Mpumalanga Veterinary Services) mandates weekly inspection of cattle at registered facilities for FMD surveillance. At this inspection, cattle are typically exposed to an acaricide by plunge dipping or pour-on treatment (Berrian et al., 2016). Farmers who attend these facilities with their animals are likely to be the primary livestock caretakers and/or dependent on livestock for a

living, suggesting the most frequent and direct contact with cattle. Inspection facilities may also have a higher concentration of infectious fomites or tick vectors.

Clinical presentation of Q fever is non-specific and can vary from mild, or even asymptomatic, to severe disease, particularly in elderly patients or those who are otherwise debilitated. When appropriate treatment is delayed, complications can be life-threatening (Frean & Blumberg, 2007). Maurin and Raoult (1999) reported death as an outcome of symptomatic acute Q fever in 1%–2% of cases. This clinical polymorphism contributes to the disease being underdiagnosed and underreported (Porter et al., 2011). Although this study evaluated exposure and not acute infection, it is important to note that, in the study area, the standard of care for patients with non-malarial acute febrile illness (AFI) is amoxicillin, which would not effectively treat Q fever.

In South Africa, tick bite fever (TBF) is the most commonly diagnosed rickettsial disease. Additionally, in a study by Kolo et al. (2016), 70% of tick pools taken from dogs in the study area were positive for *Rickettsia* spp., 30% of which were positive for *R. africae. Rickettsia felis* was also reported in 100% of flea pools from dogs by PCR, while sequencing confirmed this was *Rickettsia asemboensis* (a *R. felis* -like organism; Kolo et al., 2016). *Rickettsia asemboensis* was first isolated in western Kenya from cat fleas (*Ctenocephalides felis)* , a known vector of human pathogens (Jiang et al., 2013; Maina et al., 2016). In South Africa, boutonneuse fever-like TBF is usually transmitted by dog ticks (e.g., *Rhipicephalus sanguineus*), with dogs, rodents, and ticks themselves serving as the reservoirs. In contrast, African TBF is typically transmitted to humans by specific cattle and game ticks (e.g., *Amblyomma hebraeum* ; Frean et al., 2008). Thus, contact with cattle at inspection facilities is a logical risk factor for African TBF.

A trend between exposure to cats and SFG rickettsiosis was also noted in this study, albeit borderline non-significant. In a study by Matthewman et al. (1997), the prevalence of cats with antibodies to *R. conorii* was 19% in South Africa. Cats are also known to be parasitized by *Rhipicephalus* spp. which are vectors of *R. conorii.* Thus, domestic cats may play a role in the epidemiology of human rickettsiosis in the study area, possibly amplifying the infection rate of ticks or bringing infected ticks into closer contact with humans. This finding is particularly interesting given the relatively low proportion of households in the area (9%) reporting ownership of cats in a prior study (Berrian et al., 2016). In the current study, 58% of patients reported contact (either in their or their neighbours' household) with cats, possibly suggesting a broad geographic range of free-roaming cats. These findings may justify an examination of the population ecology of domestic cats in the study area. Additionally, given the increased risk of SFG rickettsiosis in females, we recommend taking an ethnographic

approach to explore gender-specific factors that may influence tick exposure (e.g., domestic responsibilities, use of personal protective measures, clothing).

While our regression analysis can determine associations between exposure variables and outcomes of interest, the lack of temporal sequence prohibited determination of causality. As more data are accumulated, models can be updated and additional hypotheses can be tested. Designs that do not depend on patient recollection of exposure should also be explored to limit recall bias. Future studies may also wish to explore acute Q fever infection, in which case diagnostics should involve measurement of both IgM and IgG antibodies in paired serum (Wegdam-Blans et al., 2012).

The use of IgM detection by IFA as a diagnostic indicator for *R. conorii* has been questioned. Immunologic reactions resulting in false positive IgM findings may occur, influencing clinical diagnostic interpretation, and IgM antibodies may not appear in reinfections. However, given the fact that high titers of IgG can exist during the patient's lifetime, and IgM is generally only measurable in sera during two to three months post-infection, IgM is a suitable, albeit not specific, marker of new infection.

Patients suffering from *R. conorii* infection have been shown to develop IgM antibodies that cross-react to a variety of bacterial antigens, including *Legionella* and *C. burnetii* (McQuiston et al., 2014; Raoult & Dash, 1995). Thus, the eight patients were positive for both Q fever exposure and acute SFG rickettsiosis may be indicative of cross-reactivity rather than co-infection. To improve predictive value of a positive serological test, future studies may consider the addition of clinical signs more specific to SFG rickettsiosis, such as the presence of eschars. Additionally, comparing the rise in IgG antibody titers from acute to convalescent sera could be used to confirm acute infection (Simpson et al., 2018); however, only returning patients (completed Visits 1 and 2) would be considered in the analysis, necessitating adjustments to sample size and study costs.

In the present study, clinical presentation of rickettsiosis did not vary significantly from non-cases; thus, relying on clinical signs for diagnosis would not be recommended. Given the significance of cattle contact in disease outcome, integrating structured patient questionnaires to determine animal contact history in the clinical setting is recommended. This linking of behavioural risk factors and human health may improve detection and therapeutic management of these neglected zoonoses.

Only two patients (8.3%) with rickettsiosis in this study received doxycycline, the most effective chemotherapeutic for this disease (Frean & Blumberg, 2007). Given the high prevalence of SFG rickettsiosis and exposure to Q fever in the study population and the

significance of cattle contact at inspection facilities, algorithms that suggest doxycycline for empiric treatment may be considered for these higher-risk patients. Improving diagnostics and re-evaluating treatment algorithms in the clinical setting may improve patient care, reduce expenditures, and contribute to judicious use of antimicrobials.

Q fever and rickettsiosis may be important contributors to acute febrile illness in this area. The identification of cattle inspection facilities as a significant risk factor could be used to justify a cooperative effort by public health and veterinary services, such as integrated human-animal health education and preventive service campaigns, which have been particularly successful in rural, low-income settings (Schelling, Wyss, Béchir, Moto, & Zinsstag, 2005). In the Mnisi study area, cattle inspection facilities could provide a key entry point for the delivery of both human and animal health services and education. Investing in the detection and control of endemic zoonoses would provide benefits across sectors, not only improving human and animal health, but also livelihoods in this resource-limited community (Molyneux et al., 2011).

5 CONCLUSION

Q fever and SFG rickettsioses are highly prevalent zoonoses in this region of South Africa that are difficult to recognize clinically. By conducting health centre-based surveillance, we determined that close contact with cattle, particularly at inspection facilities, was a significant risk factor for both Q fever exposure and acute SFG rickettsiosis. This is an important finding as these facilities could be strategically targeted locations to increase awareness, education, and prevention strategies with relatively few additional efforts. We suggest using these results to implement community outreach that aims to prevent zoonotic disease transmission and to guide clinical algorithms to make more timely and accurate diagnoses. A One Health approach, which acknowledges linkages between human, animal, and environmental health and encourages cross-sectoral collaboration, should be adopted in the clinical setting to improve patient management as well as guide future community-based research.

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CONFLICT OF INTEREST

The authors declare no personal or financial conflict of interest.

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