

Prevalence of selected zoonotic diseases and risk factors at a human-wildlife-livestock interface in Mpumalanga Province, South Africa

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Running title: Zoonoses at a human-wildlife-livestock interface (46 characters)

Abstract

A lack of surveillance and diagnostics for zoonotic diseases in rural human clinics limits clinical awareness of these diseases. We assessed the prevalence of nine zoonotic pathogens in a pastoral, low-income, HIV-endemic community bordering wildlife reserves in South Africa. Two groups of participants were included: malaria-negative acute febrile illness (AFI) patients, called febrilers, at three clinics (n=74) and secondly farmers, herders and veterinary staff found at five government cattle dip-tanks, called dip-tanksters (n=64). Blood samples were tested using one PCR (*Bartonella* spp.) and eight antibody-ELISAs, and questionnaires were conducted to assess risk factors.

Seventy-seven percent of febrilers and 98% of dip-tanksters had at least one positive test.

Bartonella spp. (PCR 9.5%), spotted fever group *Rickettsia* spp. (IgM 24.1%), *Coxiella burnetii*. (IgM 2.3%) and *Leptospira* spp. (IgM 6.8%) were present in febrilers and could have been the cause of their fever. Dip-tanksters and febrilers had evidence of past infection to *Rickettsia* spp. (IgG 92.2% and 63.4%, respectively) and *C. burnetii* (IgG 60.9% and 37.8%, respectively). No

Brucella infection nor current *Bartonella* infection was found in the dip-tanksters, although they had higher levels of recent exposure to *Leptospira* spp. (IgM 21.9%) compared to the febrilers. Low levels of West Nile and Sindbis, and no Rift Valley fever virus exposure were found in either groups. The only risk factor found to be significant was attending dip-tanks in febrilers for Q fever ($p=0.007$).

Amoxicillin is the local standard treatment acute febrile illness, but would not be effective for *Bartonella* spp. infections, spotted fever group rickettsiosis, Q fever infections, nor the viral infections. There is a need to revise acute febrile illness treatment algorithms, educate medical and veterinary staff about these pathogens, especially spotted fever group rickettsiosis and Q fever, support disease surveillance systems and inform the population about reducing tick and surface water contact.

Keywords: Zoonosis; South Africa, surveillance, one health, human-wildlife-livestock interface

Introduction

Zoonotic diseases are caused by pathogens naturally transmitted directly or indirectly (e.g. by vectors) between animals (usually vertebrates) and humans. Of all currently known human pathogens, 61% are considered to be zoonotic, whilst 73% of all emerging and re-emerging infections are also zoonotic (Jones et al. 2008). An estimated 2.5 billion human cases related to zoonotic infections occur globally each year, causing 2.7 million deaths (Grace et al. 2012a). Zoonoses are estimated to account for one quarter of the disease burden in low-income

Table I. Selected studies of zoonotic aetiologies of acute febrile illness in sub-Saharan Africa.

Disease	Population	Result	Reference
Brucellosis	Febrile hospital patients with a history of animal contact, north-eastern Kenya (n=1067)	13.7% qPCR-positive for <i>Brucella abortus</i> , 11% ELISA seropositive for <i>Brucella</i> spp. IgM/IgG	(Njeru et al. 2016)
Leptospirosis	Febrile hospital inpatients (n=870), northern Tanzania	9% serologically confirmed acute leptospirosis by MAT* (representing 20 antigen serovars)	(Biggs et al. 2011; Prabhu et al. 2011)
Spotted fever group rickettsiosis		8% serologically confirmed by ≥ 4 -fold increase in IgG to <i>Rickettsia conori</i> by IFA**	
Q fever		5% serologically confirmed acute Q fever by ≥ 4 -fold increase in IgG to <i>Coxiella burnetii</i> by IFA**	
Spotted fever group rickettsiosis	Population based infectious disease study (n=357) and febrile clinic patients (n=699), western Kenya	57% had detectable IgG against SFG antigen and 7% were PCR-positive (<i>gltA</i> assay) for <i>Rickettsia</i> spp. (<i>R. felis</i> DNA was identified)	(Maina et al. 2012)
Bartonellosis	Febrile patients, Democratic Republic of the Congo (n=155)	4.5% serologically positive for <i>Bartonella henselae</i> , <i>B. quintana</i> , or <i>B. clarridgeiae</i> spp. by IFA**.	(Laudisoit et al. 2011)

* Microscopic agglutination test; ** immunofluorescent assay

countries (Grace et al. 2012b). Poverty raises the risk of zoonotic disease in communities living in close contact with their livestock or with wildlife (Grace et al. 2017).

Studies in sub-Saharan Africa investigating acute febrile illness (AFI) patients have found evidence of present or past zoonotic infections (Table I). In populations with a high prevalence of HIV infection, this proportion of zoonotic infections may be even higher due to immunosuppression, HIV also increases the clinical severity of many zoonoses and results in a more protracted, chronic illness (Froberg et al. 2004; Biggs et al. 2011).

A lack of human surveillance for zoonotic diseases (Rodríguez-Prieto et al. 2015) and laboratory capacity causes a lack of clinical awareness, leading to under-diagnosis and consequently mismanagement of cases (John et al. 2008). In this light, the early detection of new and re-emerging human pathogens is also problematic (Zumla et al. 2016). Gaps in data on the burden of zoonotic infections are especially pressing in the study site, a pastoral setting where humans, their domestic animals and wildlife have frequent direct and indirect contact. The zoonoses selected for this study were based on prior information concerning infection prevalence and laboratory capacity. The study aimed to document the prevalence of nine zoonoses in this setting; investigate the proportion of acute febrile illness patients and high-risk cattle farmers, herders and veterinary staff that have evidence of exposure or infection to these zoonoses; and identify risk factors for these zoonoses. Implications for public health surveillance, diagnostic and treatment protocols used in this setting are explored.

Materials and methods

Study site

The study was implemented in three rural government primary health care clinics and five government cattle dip-tanks in Bushbuckridge Municipality, Mpumalanga Province, South Africa (Figure 1). The prevalence of HIV infection in the province is 14.5% and the municipality is situated in the Ehlanzeni District, where levels of HIV are amongst the highest of all districts in the country (between 16% and 22%) (Shisana et al. 2014).

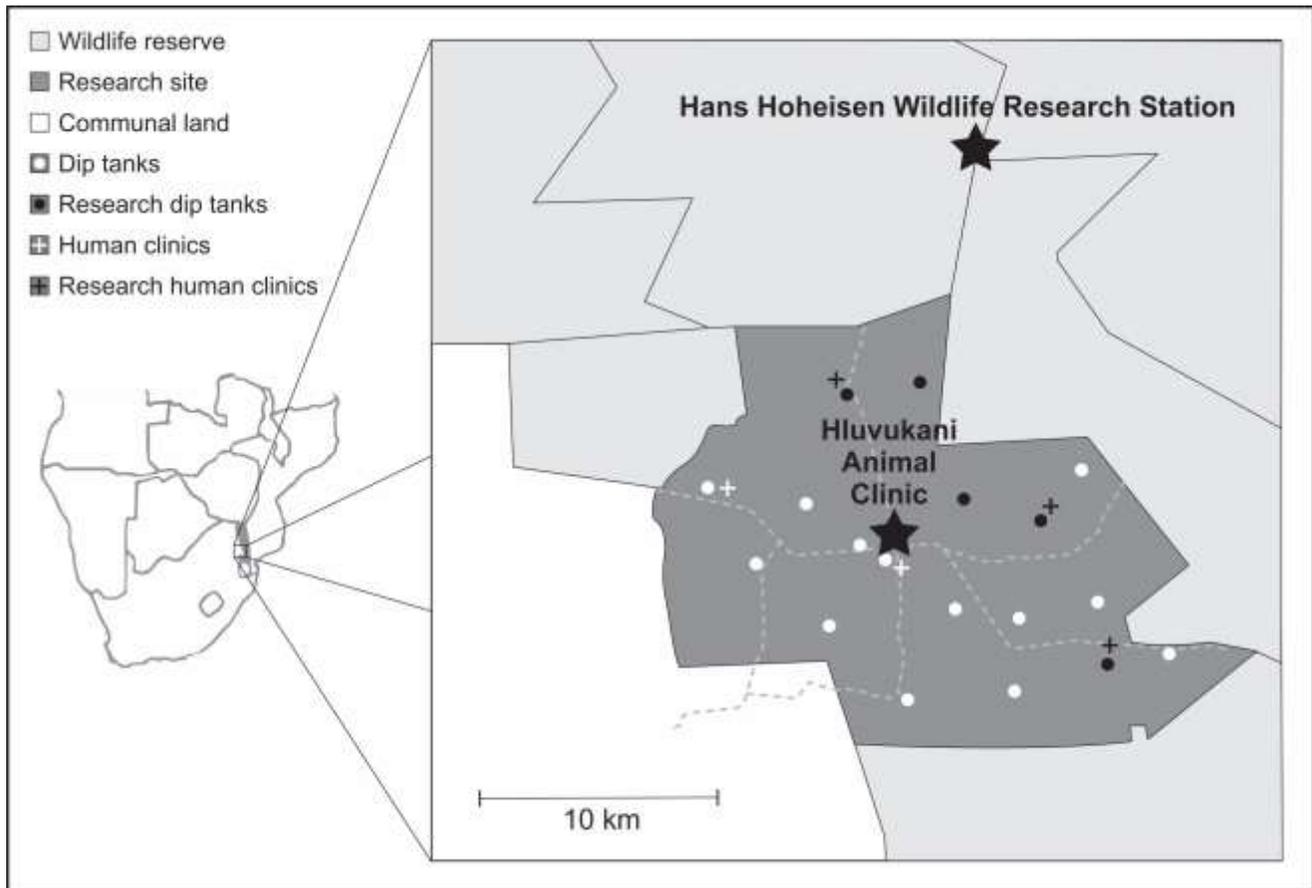


Figure 1. Location of the research site, Bushbuckridge Municipality, Mpumalanga Province, South Africa.

The study area was in the Mnisi Community Programme area, containing the Hans Hoheisen Wildlife Research Station and Hluvukani Animal Clinic, and is a joint initiative by the University of Pretoria with the Mpumalanga Veterinary Services and the Mnisi Traditional Authority, to promote sustainable livelihoods through research into human and animal health, animal production and natural resource utilisation. Approximately 40,000 people live in 8,500 households (Statistics South Africa (SSA) 2012), with 72% of all households owning at least one species of animal and agro-pastoralist farming being the primary subsistence activity (Berrian et al. 2016). Chickens are owned in 55% of households, cattle in 25%, goats in 16%, pigs in 5% and companion animals in 36% (Berrian et al. 2016). The community shares three-quarters of its boundary with wildlife reserves. In the communal area there are hares, small antelope, rodents, small carnivores and in the wildlife reserves, all wildlife typical of “big five” reserves in this region. In these wildlife areas brucellosis has been detected in buffalo, other antelope, zebra, lions, hyenas and other animals, including monkeys (De Vos and Van Niekerk 1969; Gorsich et al. 2015). Malaria is endemic in the area, but the average annual incidence of the infection has decreased over the past 10 years, due to intensive control activities (Maharaj et al. 2013). The contribution of malaria to the syndrome of AFI has thus reduced.

Participant recruitment

The sample size calculation was done using an infinite population, where 59 samples are required to detect a disease with 95% chance when the actual prevalence of the disease is 5% (Thrusfield 1995). In addition, 64 samples give unilateral 95% confidence intervals of proportions of 12% maximum, which was deemed sufficient for the study.

The study involved two groups of participants. The first comprised patients diagnosed with AFI at the clinics, hereafter called febrilers. An AFI case was defined as a patient with documented axillary temperature $\geq 37.5^{\circ}\text{C}$, or a history of fever within the previous 72 hours, whom the clinic staff would routinely test for malaria, in terms of the clinic standard operating procedure.

Patients who tested negative for malaria were referred to the study nurse to be enrolled in this study. All patients ≥ 18 years of age who met this case definition were eligible for enrollment from October 2012 to June 2013. Information was captured onto a standard case information form (CIF). This CIF inquired about a variety of symptoms and questions related to risk factors for zoonotic disease, and diagnosis, treatment and outcome. The selected risk factors were a variety of contacts (such as handling and consumption) with various domestic animals, presence at dip-tanks and tick bites. Blood samples (one coagulated for serum and one EDTA anticoagulated) for laboratory tests were drawn at the time of presentation (acute sample) and the patients were asked to return two to three weeks later to provide follow-up convalescent samples. A study nurse facilitated enrolment of volunteers at all the clinic sites.

The second group comprised cattle farmers, herders and government veterinary staff (animal health technicians and veterinarians), who attended any of five cattle dip-tanks for weekly stock inspections and tick control in the area in one week in June 2013 (Figure 2). This group were called dip-tanksters. They were recruited for interviewing and provided blood samples as described above for the febrilers, but without follow-up samples. A CIF with the risk factors detailed above was also completed, but excluded the clinical symptoms, diagnosis or treatment and outcome information. All information was captured onto a Microsoft Excel database.



Figure 2. Sampling of dip-tanksters at a dip-tank.

Laboratory methods

Laboratory testing for all samples was performed at the National Institute for Communicable Diseases. Tests for a panel of nine known endemic bacterial and viral zoonoses in South Africa were used in this study (Table 2).

Table 2. Panel of tests performed on blood samples from acute febrile patients and healthy subjects. Sensitivity and specificity are included if available.

Disease agent/s	Diagnostic assay	Samples tested	Interpretation of results	Reference
Bartonellosis	PCR*: <i>Bartonella</i> spp. 16S/23S rera internal transcribed spacer (ITS) region (in-house) and sequencing.	Acute whole blood samples from all participants. All positive amplicons were sequenced	Fragment sizes variable depending on species approximately 640 – 788 bp for outer primers and 481 – 573 bp for inner primers	(Trataris et al. 2012)
Brucellosis	Serology (total antibodies): Brucellacapt® assay (Vircell S.L., Spain)	Acute serum samples from all participants	Titres higher than 1:320 were deemed positive	96% sens. & 97.5% spec (Casao et al. 2004)
Chikungunya, Rift Valley fever, Sindbis fever and West Nile fever viruses	HAI:** in-house assay	Serum samples from all participants	Titres higher than 1:20 were deemed positive	(Lennette and Schmidt 1979)
Chikungunya, Rift Valley fever, Sindbis fever and West Nile fever viruses	IgM capture ELISA***: in-house assay	Serum samples that tested positive per arbovirus HAI	Percentage positivity values higher than the calculated run-based or population based cut-off values	RVF: 96.5% sens. & 99.4% spec. (Paweska et al. 2005)
Leptospirosis	IgM ELISA: Panbio® <i>Leptospira</i> (Standard Diagnostics Inc., Republic of Korea).	Convalescent serum samples or acute samples where convalescent samples not available	Index values calculated using run-based cut-off values as per manufacturer's recommendations	90.8% sens. & 55.1% spec. (Desakorn et al. 2012)

Spotted fever group <i>Rickettsiae</i> spp.	IgG IFA:**** <i>Rickettsia conorii</i> kits (Vircell S.L., Spain)	All AFI participants and all dip-tank participants	IgG: titer of 1:40 deemed positive	85% sens. & 100% spec. (Do et al., 2009)
	IgM IFA: <i>Rickettsia conorii</i> kits (Vircell S.L., Spain)	All AFI participants	IgM: titer of 1:192 or greater deemed positive, or fourfold rise in titer as per manufacturer's recommendations	94% sens. & 95% spec. (Do et al., 2009)
Q fever	IgG ELISA: Panbio® <i>Coxiella burnetii</i> (Q fever) (Standard Diagnostics Inc., Republic of Korea)	Convalescent serum samples, or acute samples where convalescent samples not available	Index values calculated using run-based cut-off values as per manufacturer's recommendations	71% sens. & 96% spec. (Field et al. 2002)
	IgM ELISA: Panbio® <i>Coxiella burnetii</i> (Q fever) (Standard Diagnostics Inc., Republic of Korea)	Acute serum samples if convalescent sample not available or if tested positive with IgG ELISA	Index values calculated using run-based cut-off values as per manufacturer's recommendations	99% sens. & 88% spec. (Field et al. 2000) ¹

* PCR: polymerase chain reaction; ** HAI: haemagglutination inhibition assay; ***ELISA: enzyme-linked immunosorbent assay;

****IFA: indirect immunofluorescence assay

The DNA for the bartonella test was extracted according to Trataris et al. 2012, from packed red blood cells (obtained after spinning down EDTA tube) using the UltraClean® BloodSpin® DNA

Isolation Kit (MoBio Laboratories, Czech Republic). The tests for leptospirosis, Q fever, brucellosis and HAI tests were not done in duplicate. The convalescent samples of the acute febrile illness patients were tested and, if found positive, then the corresponding acute samples were tested to detect seroconversion. Positive HAI samples were then tested with IgM ELISA.

Statistical analysis

Data were analysed in multivariable logistic regression models, separately for the clinic and dip-tank data. Test results of each test were used as binary response variables (in different models). Backward selection of explanatory variables was applied using $p = 0.05$ as a cut-off. The tested explanatory variables were age (continuous), dip-tank attendance, eating wild animals, history of tick bites, herding of livestock, slaughtering of animals and eating livestock (binary) for both the clinic and dip-tank data. Confidence intervals (CI) of proportions >0 and <1 were calculated using logistic predictors whereas the exact method (assuming a binomial distribution) was used for proportions = 0 or 1.

Ethical clearance

Ethical approval was obtained from the University of the Witwatersrand Human Ethics Committee (certificate number M120667) and signed informed consent obtained from study participants. Further permissions were secured from the Mpumalanga Provincial Government for the study to be done at the three clinics.

Results

Participant demographics and symptoms

In the febrilers, of 119 eligible participants, 74 (62%) were enrolled (45 were excluded as they declined consent or the study nurse could not access them). There were twenty-three (16/23 were adult) AFI patients malaria test positive during the study period, who were not included in the study. The median age of the 74 febrilers was 34 years (interquartile range [IQR] 25-47 years), 46/74 (62%) were females, of which two were pregnant. About half were febrile on examination (38/74, 51%). The median duration of illness was 3 days (IQR 2-7 days). About 10% (6/74) were referred to hospital, and 62% (46/74) received an antibiotic. A quarter did not return for follow-up blood sampling (20/74; 27%). Only 4 febrilers had with no systemic symptoms other than fever, 36/74 (49%) presented with one other symptom, 20/74 (27%) with two and 4/74 (5%) with three other symptoms. The majority presented with muscle pain (85%), while fewer had respiratory symptoms (14%). A total of 64 dip-tanksters were recruited, of whom, 46 (72%) were male and a median 55 years old (IQR 42-68 years). The median duration of dip-tank attendance was 15 years (IQR 6-30 years).

Table 3. Results of laboratory tests for selected zoonotic pathogens in AFI and diptank participants with 95% confidence interval

	Febrilers			Dip-tanksters		
	Positive	Sample size	% positive (95% CI)	Positive	Sample size	% positive (95% CI)
SFG IgG	45	71	63.4 (51.6-73.7)	59	64	92.2 (82.6-96.7)
SFG IgM*	13	53	24.5 (14.8-37.8)	-	-	-
Q fever IgG	28	73	38.3 (28.0-49.9)	39	64	60.9 (48.6-72.1)
Q fever IgM**	1	43	2.3 (0.3-14.8)	0	39	0 (0-7.4)
<i>Bartonella</i> spp. PCR	7	74	9.5 (4.6-18.5)	0	64	0 (0-4.6)
<i>Leptospira</i> IgM	5	74	6.8 (2.8-15.2)	14	64	21.9 (13.4-33.6)
<i>Brucella</i> Ig	1	74	1.4 (0.2-9.0)	0	64	0 (0-4.6)
Chikungunya Ig	0	73	0 (0-4.0)	3	64	4.7 (1.5-13.6)
West Nile virus Ig	3	73	4.1 (1.3-12.0)	2	64	3.1 (0.8-11.7)
Sindbis Ig	1	73	1.4 (0.2-9.1)	2	64	3.1 (0.8-11.7)
Rift Valley fever Ig	0	73	0 (0-4.0)	0	64	0 (0-4.6)

*SFG rickettsiosis IgM (recent) done on the 45 AFI participants who were positive on SFG rickettsiosis IgG (past) and 19 IgG negative and one IgG untested participants, **IgM for Q fever done on acute specimens for which there was no convalescent specimen and in participants that were positive for IgG.

Clinical and laboratory findings

In summary, 77% (57/74) of febrilers tested positive for at least one of the zoonotic pathogens included in the panel (Table 3). The most prevalent past infection was the spotted fever group (SFG) (*Rickettsia* spp.), with 63.4% (45/71, 95% CI 51.6-73.7%) testing positive for anti-*Rickettsia* IgG, while anti-*Rickettsia* IgM was positive in 24.5% (13/53, 95% CI 14.8-37.8%) of

febrilers, indicating a more recent or current infection. Of the 13 anti-*Rickettsia* IgM positive patients, twelve were anti-*Rickettsia* IgG positive. Antibodies (IgG) to *Coxiella burnetii*, the causative agent of Q fever, were detected in 38.3% (28/73, 95% CI 28.0-49.9%) of patients. Other bacterial pathogens included bartonellosis with 9.5% PCR-positive (7/74, 95% CI 4.6-18.5%), that were identified as *Bartonella vinsonii* subspecies *berkoffii* (n=1) (which has a dog reservoir), *B. henselae* (n=2) (domestic cat reservoir), *B. quintana* (n=1) (human reservoir (Zeaiter et al. 2002)), *B. thailandensis* (n=1) (rodent reservoir (Saisongkorh et al. 2009)) and two *Bartonella* positives are unnamed, but rodent borne. Leptospirosis with 6.8% (5/74, 95% CI 2.8-15.2%) group-specific IgM antibodies and brucellosis with 1.4% (1/74, 95% CI 0.2-9.0%) group-specific total antibodies were also found. West Nile and Sindbis fever antibodies were detected in 4.1% (3/73, 95% CI 1.2-12.0%) and 1.4% (1/73, 95% CI 0.2-9.1%) of febrilers respectively. No chikungunya or Rift Valley fever (RVF) antibodies were found.

In the dip-tanksters, the proportion with anti-*Rickettsia* IgG antibodies was also high at 92.2 % (59/64, 95% CI 82.6-96.7%), and 60.9% (39/64, 95% CI 48.6-72.1%) had anti-*Coxiella* IgG antibodies. No evidence for exposure to *Brucella* spp. was detected, but 21.9% (14/64, 95% CI 13.4-33.6%) tested positive for *Leptospira* IgM antibodies. For viral aetiologies, 3.1% (2/64, 95% CI 0.8-11.7%) of dip-tanksters tested positive for West Nile virus IgM, 4.7% (3/64, 95% CI 1.5-13.6%) for chikungunya and 3.1% (2/64, 95% CI 0.8-11.7%) for Sindbis virus antibodies. No RVF antibodies were detected.

The AFI participant arbovirus HAI positive samples were retested with IgM ELISA and had the following results: the three WNV positive samples had two negative results and one untested and the Sindbis positive was also IgM positive. Amongst dip-tank participants: for chikungunya one of the three HAI-positive samples was also IgM positive, one was negative and the last untested; both WNV HAI-positive samples were IgM negative, and both Sindbis HAI-positive samples were also IgM positive.

The only risk factor that was statistically significantly associated with a disease was attending dip-tanks in febrilers for Q fever IgG ($p=0.007$). Thirteen of the 74 (18%) febrilers were found to have previously attended dip-tanks and of those tested 77% (10/13) were IgG positive compared to 31% (19/62). None of the risk factors for the dip-tanksters were associated with the test results.

Discussion

Our study looked at both acutely febrile participants in clinics and cattle dip-tank participants at high risk for zoonoses. Undifferentiated acute febrile illness is among the most common presenting signs in patients seeking medical care in Africa (Perkins et al. 1997; Crump et al. 2003; Van Hemelrijck et al. 2009). Despite this, the syndrome is frequently misdiagnosed, and the aetiologies still poorly understood. Febrile illness is often misclassified as malaria, especially in adults living in areas with low to moderate malaria transmission (Reyburn 2004; Chandler et al. 2008; Nankabirwa et al. 2009). Misdiagnosis leads to delays in appropriate treatment and higher case-fatality rates (Chandler et al. 2008).

This study showed that 77% of febrilers and 98% of dip-tanksters had at least one positive test. Bartonellosis (PCR 9.5%), spotted fever (IgM 24.1%), and less likely, leptospirosis (IgM 6.8%) and Q fever (IgM 2.3%) could have been the cause of the acute febrile illness in the febrilers, as IgM and PCR suggest recent or current infection. This is a valuable finding, relevant to the management of clinic patients, as amoxicillin is the standard treatment for patients with AFI, but it would not be effective for these diseases. Therefore, the AFI treatment algorithms in this and similar settings need to be reconsidered and health staff educated on these infections.

The febrilers also showed a high past exposure (IgG) to SFG *Rickettsia* spp. (63.4%) and Q fever (37.8%), not previously described in this population. The high proportion of exposure to *Rickettsia* spp. is understandable, given that 70% of tick pools and 100% of flea pools taken from dogs in the area were positive for *Rickettsia* spp. (Kolo et al. 2016). Over 70% (50/71) of febrilers had either an SFG *Rickettsia* spp. or Q fever positive result, while 95% of dip-tanksters had either an SFG *Rickettsia* spp. or Q fever positive result. We expect veterinary staff or livestock herders and farmers presenting at the dip-tanks with their livestock to be at a higher risk for zoonotic diseases (especially for those diseases transmitted by vectors/reservoirs and associated with livestock) in this population. However, the only diseases where dip-tanksters had a confidence interval higher than febrilers was spotted fever (IgG). There are differences between proportions of participants positive in the febrilers and dip-tanksters but this is not strongly statistically supported in this study.

Dip-tanksters are on average 21 years older than febrilers and therefore have had more time for exposure to pathogens. Although, the number of zoonotic illnesses per individual was not correlated with years of dip-tank attendance in the dip-tank group, contact with cattle dip-tanks was positively associated with Q fever exposure in AFI patients. The higher proportion of spotted fever IgG in dip-tank participants could be from cattle owners having higher levels of exposure to ticks, related to their dogs and other domestic animals that they will interact with daily. There was little evidence of other risk factors contributing to positive serology. The lack of positive associations may be due to a relatively small sample size and test-positives. Also, the environment is homogenous such that even if people say they do not own or work with animals, some contact is inevitable, due to the free-ranging nature of the animals and their close proximity to humans; for example, almost a fifth of the febrilers had some contact with the dip-tanks.

The higher bartonellosis test-positive proportion in febrilers could be as the test was detecting organisms and not antibodies, which would be more closely associated with patients [presenting with fever than compared to dip-tanksters. *Bartonella* infection rate in other studies was also found to be high in HIV-infected individuals (Frean et al. 2002; Trataris et al. 2012), and although our participants were not tested for HIV, HIV prevalence may have been higher in the febrilers that had an average age of 34 years compared to the dip-tankster with an average age of 55 years, which correlates with the provincial age and HIV prevalence demographics (Shisana et al. 2014). The *Bartonella* species found in the febrilers were associated with humans (n=1) and domestic dogs and cats (n=3) and rodents (n=2). *Bartonella*

infections have been found to be common in cats (23.5%) and dogs (9%) in a southern African study (Trataris et al. 2012), and in fleas (36.3%) elsewhere in Africa (Leulmi et al. 2014).

A review of leptospirosis in sub-Saharan Africa, noted very few human studies, with most data being from animals studies (De Vries et al. 2014). In our study evidence of leptospirosis was higher in the dip-tanksters, although with confidence intervals overlapping, which again makes sense given that transmission to humans is from the urine of infected animals, commonly rodents, pigs and other domestic animals. Cattle in a neighbouring province had a leptospirosis prevalence of almost 20% (Hesterberg et al. 2009) and a South African study found leptospirosis in 4.7% (25/530) of dogs tested (Roach et al. 2010). What is more difficult to understand is the dip-tanksters having a threefold higher IgM (which should indicate current or recent infection), than AFI patients. The dip-tank participants may be more consistently exposed to *Leptospira* spp., but not present with fever at the clinic. The *Leptospira* ELISA IgM is known to have a low specificity (55%) and diagnostic accuracy, which may influence the reliability of the results (Desakorn et al. 2012).

The brucellosis results are reflective of what has been found in domestic animals in the area. Although brucellosis is present in the region (Hesterberg et al. 2008), unpublished studies suggest that there is no brucellosis in the cattle, goats and dogs in the study area (Dr G Simpson, Pers. comm., 15th June 2017). This indicates that the control mechanisms of heifer vaccination and movement control may be keeping the disease at bay, even though there are high

seroprevalences in buffalo (8.7-47.6%) across the neighbouring reserve fences (Gorsich et al. 2015).

The febrilers had 5.5% (4/73) showing at least one positive arbovirus positive result compared to the dip-tanksters with 10.9% (7/64). Little is known about the epidemiology of chikungunya virus in the region, although baboons (*Papio ursinus*) were previously found to be the primary vertebrate reservoir (Mcintosh et al. 1977). The vectors are tree canopy-associated *Aedes fucifer-taylori* group, which are less likely to be found in a peri-domestic situation, and which rarely feed on humans. The dip-tank participants may be more at risk as they take their cattle and goats into the surrounding bushveld for grazing. The presence of West Nile and Sindbis virus infections, although at low levels, is of interest as patients are not routinely tested for these diseases. There are also veterinary implications associated with their presence.

Vectors can move pathogens between wildlife and domestic animals species (Bengis et al. 2004). This phenomenon is of growing concern with the expanding human populations, habitat destruction, intensification of wildlife and potentially greater contact between humans and wildlife (Bengis et al. 2004). The diseases investigated in this study can be transmitted to humans through vectors, food, contact or contamination of the environment, which has implications for vector, hygiene and environmental control. Malaria control in the area, for example, has had a negative impact on some mosquito populations, which may affect other mosquito-transmitted diseases. Further vector control, disease control in animals, as well as food and other hygiene improvements, would be beneficial in decreasing the burden of these

diseases. There is also a need to educate the population about reducing tick and surface water contact.

A limitation of our study was that sample sizes were small. There was only one nurse covering three clinics for AFI patients, which made it difficult to access all the eligible patients. The dip-tank participants were opportunistically selected in a non-random manner. This could influence the extrapolation of the results to all the dip-tank attendees.

Conclusions

Bartonella spp., *Rickettsia* spp., *Coxiella burnetii* and *Leptospira* spp. are present and could be undetected causes of fever and illness in a community bordering wildlife reserves in the north-east of South Africa. Some acute febrile illness patients (9.5%) were infected with *Bartonella* spp. associated with human, rodent and domestic dog and cat reservoirs. A high proportion of cattle dip-tank attendees and acute febrile illness patients had evidence of past infection or background exposure to *Rickettsia* spp. and *Coxiella burnetii*, which is of relevance to public and veterinary health. Current acute febrile illness treatment algorithms at the clinics prescribe amoxicillin, which would not be effective for these diseases. The low proportion of brucellosis and viral infections is encouraging, but should continue to be monitored. The vector control strategies for livestock and public health should take into account ticks and fleas as they transmit *Bartonella* spp. and *Rickettsia* spp. Animal husbandry should be adapted to include means of infection prevention, such as disinfection, after contact with animal excretions to

decrease Q fever, leptospirosis and potentially brucellosis. This is an opportunity for public and animal health organisations to discuss control programs to tackle common afflictions. Wider zoonotic surveillance should be continued, and veterinary and public health services should be aware of these diseases, their diagnoses and treatments, and educate their patients and owners in prevention methods and care seeking.

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