The bacterial microbiome of *Rhipicephalus sanguineus* ticks in the Mnisi community, South Africa

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INTRODUCTION:

Rhipicephalus sanguineus, the brown dog tick, is almost exclusively a parasite of domestic dogs and is well adapted to living with its canine host in kennels or human dwellings [7], where it may also bite people in the safety of their own homes [4]. It is known to transmit various tick-borne diseases. In the Mnisi community, an area of high rural poverty in Bushbuckridge, Mpumalanga, South Africa, *R. sanguineus* is one of the most prevalent ticks found on dogs. The community lies at the human/livestock/wildlife interface where humans are at risk of infection with various tick-borne zoonotic diseases.

AIM:

Investigating the prevalence and diversity of tick-borne bacterial pathogens and symbionts in *R. sanguineus* ticks collected from domestic dogs in the Mnisi community that may impact human and animal health.

METHODS:

Ticks were collected monthly from dogs from households in two Mnisi villages (Eglington and Claire B) (**Fig. 1**) and morphologically identified. Household conditions were noted. Household characteristics that could influence the tick population and/or tick microbiome were established during questionnaire interviews, and assessed for association with tick infestation (considered as a binary outcome: low infestation ≤10 ticks and high infestation ≥11 ticks) using univariate and multivariate (Generalised Linear Model) analysis in R Console version 3.2.1 (R Core Team, 2017) at 5% level of significance. We then analyzed the bacterial community of adult male *R. sanguineus* ticks from two Mnisi villages (Eglington and Claire B). For microbiome analysis, we collected 10 ticks/dog/household in each of the villages. Ticks were kept in a humidity and temperature controlled chamber for two days to allow them to digest their blood meal. Ticks were surface sterilized, and dissected to remove their midguts (MG) and salivary glands (SG) and then pooled (either MG or SG pools). Genomic DNA was extracted and PCR amplified using universal 16S rDNA barcoded primers. Sequencing was done at Washington State University, USA using Pacific Bioscience's circular consensus sequencing strategy. Sequence data was analysed using CLC genomics workbench, NCBI BLASTn and the Ribosomal Database Program (RDP).

RESULTS:

Of the 1409 **ticks collected**, *R. sanguineus* (48%) was the most abundant species found on domestic dogs followed by *Amblyomma hebraeum* (16%) and *R. simus* (9%).

Preliminary analysis of the **influence of climate** on the prevalence of *R. sanguineus* ticks indicated that they were more abundant after periods of rainfall (data not shown).

Out of the 12 variables studied in the **univariate analysis**, five (manual removal of ticks, dipping dogs in dip tanks, using dogs for herding, confining dogs , and the age of dogs) were associated with increased odds of high tick infestation amongst dogs (Odds ratio>1), although the associations were not statistically significant (**Table 1**). An increase in the number of chickens in a household was associated with reduced odds of high tick infestation amongst dogs (OR<1: 34.7% for >10 chickens vs 50.8% for ≤10 chickens) (**Table 1**). Six variables that had *p*≤0.5 from univariate analysis were included in the **multivariable** procedure. The most adequate logistic model comprised two variables (rearing pigs, rearing goats), based on Akaike Information Criteria. Households that kept goats or pigs were associated with reduced odds of high tick infestation in dogs (**Table 2**).

Microbiome sequencing results showed that the *R. sanguineus* microbiome is composed of an average of 86-94% *Coxiella* spp. in the MG and SG (**Fig. 2**). The microbiomes of ticks from the two villages were essentially the same, indicating a core microbiome with slight differences between sites. We found multiple *Coxiella* species within the microbiome of these ticks. Notably, 3.5% of sequences detected from the MG and 2.4% from the SG of one pool of ticks (Claire B) were identified as *Anaplasma phagocytophilum* (**Fig. 2**).

DISCUSSION:

Risk factor analysis showed that rearing goats or pigs led to reduced odds of tick infestation amongst dogs, probably due to the distribution of the ticks to other animal species apart from dogs, to maintain the tick populations.

The *R. sanguineus* microbiome was dominated by *Coxiella* spp., including *C. burnetii* (the causative agent of Q-fever) and *Coxiella*-like bacteria (CLB). Q-fever is a zoonotic disease of ruminants and humans and is endemic in South Africa with up to 59% prevalence in vulnerable communities [6]. Interestingly, 4/5 of the common detection methods for *C. burnetii* cross-react with CLBs, thus possibly overestimating *C. burnetii* infection rates [5]. *C. burnetii* was thought to be the only pathogenic organism within the *Coxiella* genus; however, several CLBs have recently been identified infecting humans and animals, causing non-specific clinical symptoms similar to Q fever [1,2,3]. Thus, it is possible that Q fever in SA is due to non-burnettii *Coxiella* infections. New means of detecting and characterizing *C. burnetii* and CLBs are needed.

Notably, *A. phagocytophilum* sequences were also detected in the *R. sanguineus* microbiome. This transstadially tick-transmitted Gram-negative obligate intracellular pathogen is an emerging tick-borne pathogen in humans and animals worldwide. To date, there have been no official diagnoses of *A. phagocytophilum* in humans in South Africa.
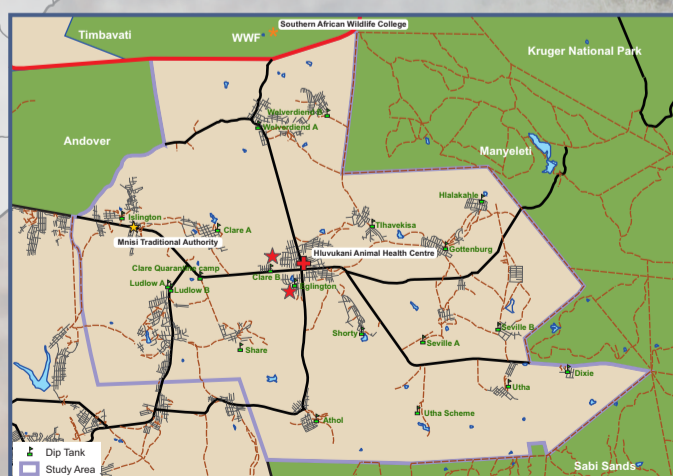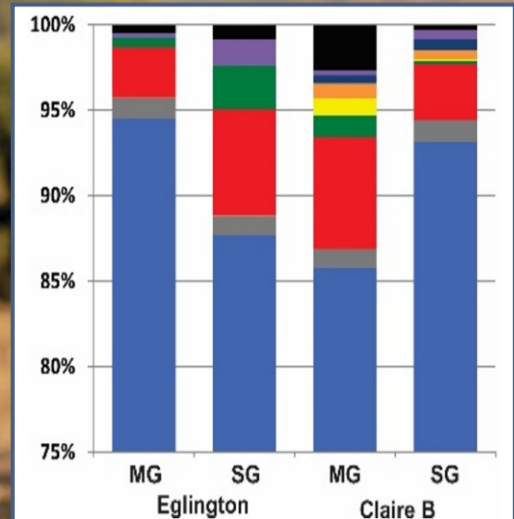
Figure 2: Bacterial microbiome of *R. sanguineus* from Mnisi. *Coxiella*-like Endosymbiont CRt. = blue, *Coxiella* symbiont = gray, *Coxiella* spp. = red, *E. coli/Shigella* = green, *Staphylococcus* spp. = yellow, *Actinobacter* spp. = orange, *A. phagocytophilum* = dark blue, Unclassified group = purple, and rare (<1%) = black. Note: the Y axis scale starts at 75%.

Table 1: Descriptive statistics and univariate associations between potential individual-level exposure factors and tick infestations amongst dogs in Mnisi

Characteristic of household	Category	No. of dogs with high infestation (>11 ticks) /total (%)	Odds ratio (95% CI)	*p*
Number of dogs at household	1 to 2	34/74 (45.9)		
	≥3	14/36 (38.9)	0.75 (0.33,1.68)	0.48
Tick control by manual removal	No	25/66 (37.9)		
	Yes	23/44 (52.3)	1.79 (0.83, 3.89)	0.17
Tick removal by dip tank	No	35/81 (43.2)		
	Yes	13/29 (44.8)	1.07 (0.45, 2.51)	1.0
Veterinary assistance in tick control	No	44/97 (45.4)		
	Yes	4/13 (30.8)	0.54 (0.15, 1,86)	0.33
Using dogs for hunting	No	45/103 (43.7)		
	Yes	3/7 (42.9)	0.97 (0.21, 4.54)	0.97
Using dogs for herding	No	33/80 (41.3)		
	Yes	15/30 (50.0)	1.42 (0.61, 3.31)	0.52
Allow dogs to roam through community	No	22/48 (45.8)		
	Yes	26/62 (41.9)	0.85 (0.39, 1.82)	0.68
Confining dogs to the household	No	40/93 (43.0)		
	Yes	8/17 (47.1)	1.18 (0.42, 3.32)	0.79
Having cattle at the household	No	29/63 (46.0)		
	Yes	19/47 (40.4)	0.79 (0.37, 1.71)	0.56
Having pigs at the household	No	44/92 (47.8)		
	Yes	4/18 (22.2)	0.31 (0.09, 1.02)	0.05
Having goats at the household	No	42/81 (51.9)		
	Yes	6/29 (20.7)	0.24 (0.09, 0.66)	0.005
Number of chickens at the household	≤10	31/61 (50.8)		
	>10	17/49 (34.7)	0.51 (0.24, 1.11)	0.09
Age of dog sampled for ticks	≤1 year	11/26 (42.3)		
	>1 year	34/75 (45.3)	1.13 (0.46, 2.78)	0.82

Table 2: Results of a multivariable logistic regression analysis on the level of tick infestation among dogs from households in Mnisi

Variable (category)	Odds ratio	95% Confidence interval	*p*-value
Reared goats*	0.25	0.09, 0.68	<0.01
Reared pigs*	0.32	0.94, 1.08	0.07

*Reference category is 'not rearing goats or pigs'

Figure 1: Map of the Mnisi community area, Bushbuckridge Municipality, Mpumalanga, South Africa. Collection sites are indicated by red stars.

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