

# Investigating *Rickettsia africae* infection in *Amblyomma hebraeum* ticks in Mnisi, Bushbuckridge Municipality, South Africa

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## Introduction

*Rickettsia africae* is an intracellular gram-negative bacteria which belongs to the Spotted Fever Group (SFG). It is transmitted by *Amblyomma hebraeum* ticks in Southern Africa and *Amblyomma variegatum* in West, Central and Eastern Africa (Kelly *et al.*, 1992). It causes African Tick Bite Fever (ATBF) in humans and has mostly been reported in tourists who visit Southern Africa (Raoult *et al.*, 2001). Transovarial and transstadial transmission of *R. africae* in *A. hebraeum* ticks has been proved (Kelly & Mason, 1991).

## Aim

The aim of the study was to investigate *R. africae* infection in *A. hebraeum* ticks in Mnisi.

## Objectives

To determine *R. africae* infection rates at different developmental stages (larvae, nymphs, and adults) of the ticks.

To determine the efficiency of transovarial transmission of *R. africae* in *A. hebraeum* ticks.

## Materials and Methods

### Study area

This cross-sectional study was performed in the Mnisi Community, Mpumalanga province, South Africa. Two dip tanks, Utah and Welverdiend, were selected for the collection of samples.

### Sample collection and processing

The calculated sample size (n) was 106 for all stages. Adult ticks and engorged female ticks were collected from cattle. Larvae were collected by dragging at the selected dip tanks. Engorged females were incubated under laboratory conditions, until they oviposited and egg masses were collected.

DNA was extracted from all the samples and were screened by real-time PCR (qPCR) targeting the *gltA* gene which is *Rickettsia* genus specific. *OmpA* gene, belonging to the SFG was amplified from the *gltA* gene positive samples using conventional PCR (cPCR). The amplified products were sent for sequencing to Inqaba Biotech.

## Results

### PCR results from DNA of adult ticks:

From Utah, out of the 96.23% (n=106) *gltA* gene positive samples, 14.71% (n=102) were positive for the *ompA* gene. From Welverdiend, out of the 95.28% (n=106) samples that tested positive for the *gltA* gene, 14.85% (n=101) were positive for the *ompA* gene. Resulting sequences were 99.98% identical to *R. africae*.

Table 1: PCR results from larvae:

Dip tanks	Utah	Utah	Wolverdiend	Wolverdiend
Reaction	qPCR	cPCR	qPCR	cPCR
Positive	73	18	49	10
Negative	33	55	57	39
Minimum Infection Rate % (MIR)	68.87	16.96	46.23	9.43

### Transovarial transmission efficiency

The transmission efficiency of *R. africae* in *A. hebraeum* engorged ticks collected from Utah was 100% and 71% in the engorged females collected from the cattle in Welverdiend.

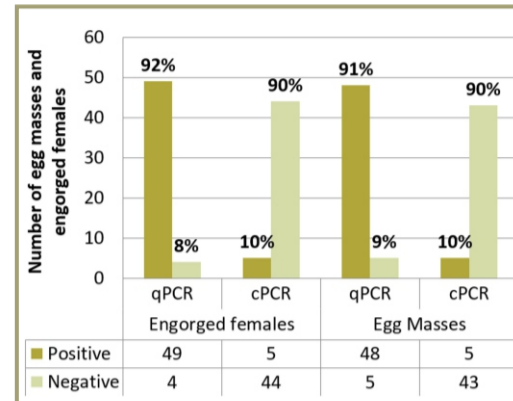


Figure 1: PCR results of the engorged females and egg masses - Utah

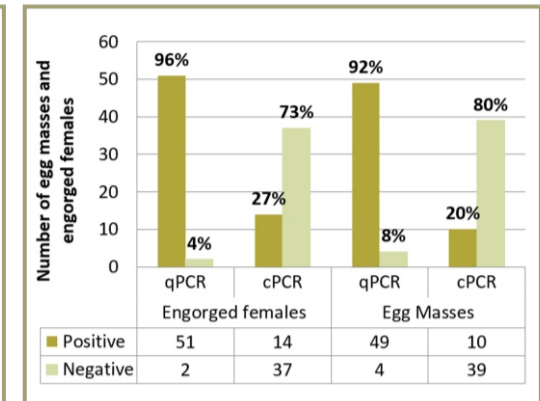


Figure 2: PCR results of the engorged females and egg masses - Welverdiend

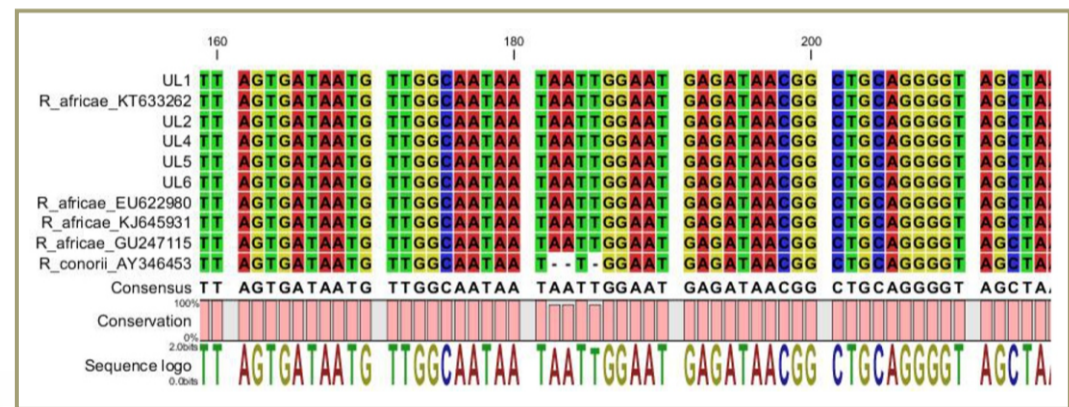


Figure 3: Alignment of part of the *ompA* gene sequences from the study (UL1-UL6) against *R. africae* and *R. conorii* Genbank reference strains.

All the cPCR positive amplicons for adult ticks, larvae and the engorged females were 99.98% identical to *R. africae*.

## Discussion and Conclusion

The results indicate a substantial percentage of *A. hebraeum* ticks from the study area are infected by *R. africae*. There are no notable differences in the infection rates in ticks even though the selected dip tanks have different vegetation and they are in different geographical locations. The dip tank in Welverdiend is in the proximity of human dwellings and the dip tank in Utah is close to game parks. This shows that the *Rickettsia* is evenly distributed in ticks from these areas. Transovarial transmission is high and could mean that the tick vector also acts as a reservoir for this pathogen. The presence of the pathogen in the tick vectors from this area poses a risk to the human population.

## References

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Photo: Nan Smith