Veterinary Research 3 (1): 1-3, 2010
ISSN: 1993-5412

The Seroprevalence and Endemic Stability of Anaplasmosis in Cattle Around Mafikeng in the North West Province, South Africa

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Abstract: The seroprevalence of *Anaplasma* sp. antibodies in non-vaccinated cattle under a relaxed, non-intensive tick control regime was determined on selected localities around Mafikeng in the North West province of South Africa. The main objective was to assess endemic stability to bovine anaplasmosis in cattle maintained under the above stated conditions. Blood samples were collected from 157 adult cattle randomly selected from 5 different localities employing the above regime. The samples were analyzed using the cELISA method. Seroprevalence ranged from 96.4-100% (μ = 98.2%), indicating a state of endemic stability. The endemic stability could be attributed to the relaxed, non-intensive tick control strategies. The seroprevalence rate on the commercial farm was higher than that of most of the communal localities despite differences in tick control frequency.

Key words: Bovine anaplasmosis, endemic stability, non intensive tick control, seroprevalence, South Africa

INTRODUCTION

Bovine anaplasmosis is caused by the intra-erythrocytic rickettsia called *Anaplasma*, has a world-wide distribution and is endemic in tropical and sub-tropical regions of the world (Bowles *et al*., 2000; De la Fuente *et al*., 2004, 2005). *Anaplasma* is an obligate intracellular organism that is transmitted biologically by ticks, or mechanically by insects and others (De la Fluente *et al*., 2004, 2005; Molad *et al*., 2006). In South Africa, it is transmitted mainly by five Ixodid ticks namely: *Boophilus decoloratus*, *B. microplus*, *Hyalomma marginatum rufipes*, *Rhipicephalus evertsi evertsi* and *R. simus* (Dreyer *et al*., 1998).

Bovine anaplasmosis is associated with significant economic losses related to impaired production, mortalities and control measures (Bowles *et al*., 2000; Regassa *et al*., 2003). Various control measures that include tick control, chemoprophylaxis and vaccination have been blindly applied in South Africa regardless of whether or not endemic stability had been achieved owing to lack of specific serologic data that forms the basis of the application of the principles of endemic stability on disease control (Regassa *et al*., 2003).

South Africa is generally considered an anaplasmosis endemic country, based mainly on tick vector distribution. Serologic evidence of endemicity exists for the Free State and Limpopo provinces (Dreyer *et al*., 1998; Rikhotso *et al*., 2005). However, no such data exists for the areas around the North West Province despite the massive size of South Africa at 1,221,037 km^2 and the resultant geographical and environmental differences among the provinces. This also, despite the importance of serologic data on the choice of control measures applicable to different regions. The current study therefore, sought to determine the endemic status of anaplasmosis in some areas of the North West Province based on serologic studies.

MATERIALS AND METHODS

The study site comprised four communal localities and one commercial farm, all at most 20 km apart. The sites are all in the Mafikeng (25°52'0S and 25°38'60E) district of the North West province of South Africa. Mafikeng is at an altitude of 1278 m above sea level, is semi arid environment with savanna type vegetation, with summer annual rainfall of 539 mm year^{-1}.

Research animals: Between March and August 2007, unvaccinated, adult animals of Tswana, Bosmara and Brahman breeds were randomly selected from several communal owners and one commercial farm owned by correctional services (Prisons services). The Tswana are an indigenous breed, while the Bonsmara are a beef breed containing 5/8 Afrikander (local South African), 3/16

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Hereford and 3/16 Shorthorn genes and are selected for adaptability to a subtropical semi-arid climate, as well as resistance to ticks and tick-borne diseases. During the day, the animals grazed as mixed herds on communal pastures that are known to harbour ticks and were kraaled in the evening. The commercial farm kept their animals in paddocks, which are also known to harbour ticks.

Blood collection: Animals were restrained in a handling facility consisting of a race and crush. Blood was aseptically obtained from the jugular vein into plain vacuum tubes. The samples were transported to the University laboratory and left overnight at room temperature to allow clotting. After centrifugation at 2500 rpm for 10 min, serum samples were preserved at -18°C pending analysis.

The serological test and results processing: The cELISA test kit (VMRD, Inc, Product Code 5002.20) was used for sample analysis following the manufacturer's instructions. This test uses a monoclonal antibody against the *Anaplasma antigen*. We used an inhibition of ≥30% as positive and <30% as negative. In accordance to the test instructions, for the validity of each test, optical densities of the negative controls were allowed to range from 0.4-2.10 and each positive control was required to produce an inhibition of 30% or higher. The controls and serum samples were loaded into three 96 well plates. Immediately after the test procedure, the plates were read with an ELISA microplate reader with 620, 630 and 650 nm filters:

$$\text{Inhibition}(\%) = 100 - \frac{\text{Sample optical density}}{\text{Mean negative control optical density}} \times 100$$

Background information: Information on tick control, acaricides used and vaccination status of the animals was collected through oral interviews with stock owners.

RESULTS

A total of 5 locations (4 communal localities and one commercial farm), involving 157 (μ = 31) adult, unvaccinated animals were sampled. All sampled localities recorded sero-positivity ranging from 96.4-100% (μ = 98.2%) (Table 1). The lowest seroprevalence was recorded at Lekaleng, a communal locality, while the highest was recorded at Rooigrond and Dihatshwane, which are commercial and communal areas respectively.

Table 1: Prevalence of antibodies against *Anaplasma* sp. in cattle (n = 157) at the 4 communal localities and 1 commercial farm

Area	Sample size	Positive (%)
Rooigrond[a,b]	33	100.0
Dihatshwane[c,d]	15	100.0
Top village[c,d]	56	99.6
Lokaleng[c,d]	35	96.4
Mogosane[c,d]	18	98.6
Mean	31	98.2

[a]Commercial farm; [b]Monthly dipping; [c]Communal locality; [d]Uncoordinated dipping

Tick control measures in the communal areas did not follow a particular pattern. Some farmers only dipped their animals after seeing ticks, while others did not dip at all. This, we termed uncoordinated dipping. The acaricides used included Ivomec^R (injectable), Deadline^R and pourons. Most of the farmers used only one type of acaricide, Deadline^R. None of the sampled areas practiced vaccination as a control measure against anaplasmosis, or other tick-borne diseases. On the commercial farm, tick control was carried out once every monthly, alternating the acaricides.

DISCUSSION

Sero-prevalence in this study ranged from 96.4-100% (μ = 98.2%). Although, detailed immunological studies were not intended, seropositivity in unvaccinated animals is clear evidence of prior exposure to natural infection and subsequent immunity to it. The sero-prevalence rates in this study were quite high when compared to the 56.6-82.7% for Limpopo (Rikhotso *et al*., 2005), 44-98.6% for the Free State Province of South Africa (Dreyer *et al*., 1998; Mtshali *et al*., 2007) and 34.4-87.3% in the Tete Province of Mozambique (Alfredo *et al*., 2005). This could be indicative of a higher state of endemicity that has been achieved and maintained over a longer period of time owing the existence of adequate tick loads.

It is generally accepted that endemic stability to tick-borne diseases exists when the number of sero-positive animals in a herd goes above 81% (Dreyer *et al*., 1998). Therefore, based on current results, the sampled areas can be regarded as having achieved endemic stability with regards to anaplasmosis. Endemic stability refers to a situation where, infectious agents do not cause clinical disease in newly infected hosts under normal circumstances of transmission and infection (low dose to an immunocompetent host). Disease is most often the result of the disruption of this relationship (e.g., high infectious dose, stressed host, failure of passive transfer). Disease associated with such agents is much less often due to the introduction or emergence of

a more infectious or more virulent strain (Norval *et al*., 1983). The existence of serologic evidence of endemic stability greatly assists in choosing the ideal method of control by farmers. In the absence of such data, farmers end up blindly choosing inappropriate control methods, such as vaccinating even in the face of endemic stability, which scenario leads to unnecessary losses (Regassa *et al*., 2003).

The tick control strategies used by farmers can be described as relaxed and non-intensive, as they were less frequent than the fortnightly rate defined by Rikhotso *et al*. (2005) and related more to the relaxed, non-intensive strategy of monthly and bi-monthly dipping intervals during summer and winter, respectively (Regassa *et al*., 2003). The non-intensive tick-control strategies could probably have been the main factor for the establishment and maintenance of the endemic stability. An endemically stable state could be achieved through the mere adoption of tick control methods that allow a reasonable number of ticks on cattle rather than relying entirely on intensive tick control and vaccination (Regassa *et al*., 2003).

Endemic stability of anaplasmosis based on serologic evidence has been recorded for the Free State and Limpopo Provinces (Dreyer *et al*., 1998; Rikhotso *et al*., 2005). The current results are therefore, part of increasing serologic evidence for South Africa's endemicity to anaplasmosis.

CONCLUSION

Results in this study show similar seroprevalence rates between communal and commercial farms despite the differences in frequency and pattern of tick control. This appears to be in agreement with the finding that no strong and consistent relationship exists between the intensity of tick control and the degrees of endemic stability and seropositivity to anaplasmosis (Norval *et al*., 1983; Alfredo *et al*., 2005).

ACKNOWLEDGEMENTS

We thank all farmers that participated, NRF and North West University (Mafikeng) for funding and Mr. L.K. Mogapi for assistance rendered.

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