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# Serological survey of *Neospora caninum* and *Besnoitia besnoiti* in cattle and goats from smallholder farms in Angónia, Tete Province, Mozambique

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**ABSTRACT**

Rearing cattle and goats is an important economic activity for smallholder farmers in Mozambique where animals are raised in traditional production systems in communal pastures. Despite its importance, there is little information on their health conditions, particularly the occurrence of infections by parasites that can cause economic losses, especially *Neospora caninum* and *Besnoitia besnoiti*. A serological study to estimate the prevalence of *Neospora caninum* and *Besnoitia besnoiti* was undertaken in Angónia district of Central Western Mozambique between April and May 2010. Animals were randomly selected at acaricide dip tanks upon the consent of the owners, and all the selected animals were regarded as one flock in each locality. A serological survey for these protozoa was undertaken using the Indirect Fluorescent Antibody Test. A prevalence of anti-*N. caninum* IgG was estimated in 297 cattle serum samples and 8.4% were positive at the dilution of 1:200 to 1:800, while in goats from the 132 samples 3.8% were positive at 1:50 and 1:100 dilutions. Ninety four cattle samples were examined and 39.4% were positive for anti-*B. besnoiti* antibodies, which represents a high prevalence. This is the first evidence of seroprevalence of *N. caninum* in cattle and goats, and of *B. besnoiti* in cattle in Angónia, Mozambique.

Key words: *Besnoitia besnoiti,* Mozambique, *Neospora caninum*, ruminants, sero-prevalence

**RÉSUMÉ**

L'élevage du bétail et des chèvres est une activité économique importante pour les petits agriculteurs du Mozambique où les animaux sont élevés dans les systèmes de production traditionnels dans les pâturages communaux. Malgré son importance, il existe peu d'informations sur leurs conditions de santé, en particulier l'apparition d'infections par des parasites pouvant entraîner des pertes économiques, en particulier *Neospora caninum* et *Besnoitia besnoiti*. Une étude sérologique visant à estimer la prévalence de *Neospora caninum* et *Besnoitia besnoiti* a été entreprise dans le district d'Angónia du centre-ouest du Mozambique entre avril et mai 2010. Les animaux ont été sélectionnés au hasard dans des réservoirs d'immersion d'acaricides sur consentement des propriétaires et tous les animaux sélectionnés ont été considérés en tant qu'un même troupeau dans chaque localité. Une étude sérologique de ces protozoaires a été effectuée en utilisant le test d'anticorps fluorescent indirect. Une prévalence de l'anti-N. L'IgG de caninum a été estimée dans 297 échantillons de sérum bovins et 8,4% étaient positifs à la dilution de 1:200 à 1:800, tandis que chez les chèvres des 132 échantillons, 3,8% étaient positifs à 1:50 et 1:100 dilutions. Quatre-vingt-quatre échantillons de bovins ont été examinés et 39,4% étaient positifs pour anti-B. Besnoiti anticorps, ce qui représente une forte prévalence. C'est la première preuve de séroprévalence de *N. caninum* chez les bovins et les chèvres, et de *B. besnoiti* chez les bovins à Angónia, au Mozambique.

Mots-clés: *Besnoitia besnoiti*, Mozambique, *Neospora caninum*, ruminants, séroprévalence



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## INTRODUCTION

*Neospora caninum* is an intracellular protozoan parasite that is closely related to *Toxoplasma gondii*, reported worldwide as one of the major protozoan pathogens that has canids as definitive hosts (Mcallister *et al.*, 1998; Gondim *et al.,* 2004; King *et al.,* 2010; Dubey *et al.,* 2011). Studies in several countries have identified *N. caninum* infection as responsible for reproductive diseases and as one of the major causes of abortions in cattle (Dubey and Schares, 2011), as well as neonatal mortality in sheep and goats (Pena *et al.,* 2007; Corbellini *et al.,* 2001; Varaschin *et al.,* 2012). This protozoan parasite is maintained in cattle as a chronic infection which can be passed on to the fetus during pregnancy (Anderson *et al.,* 2000) and causes an expressive economic global impact. According to Reichel *et al.* (2013), the global economic impact caused by *N. caninum*-related losses exceeded US $1.298 billion per annum, ranging as high as US $2.380 billion, and annual losses on individual dairy farms were estimated to reach a median of US $1,600.00, while on beef farms these costs amounted to just US $150.00.

*Besnoitia besnoiti* is a cyst-forming apicomplexan parasite and is the causative agent of bovine besnoitiosis (Álvarez *et al.,* 2014), a relatively common disease of cattle in Southern Africa. However, the infection is usually clinically unapparent (Bigalke and Prozesky, 2004). The infection can cause adverse effects both during the acute and chronic phases of the disease that can compromise animal welfare (EFSA, 2010; Álvarez *et al.,* 2014) and is responsible for economic losses due to poor body condition, sterility in bulls, high morbidity and eventual death (Álvarez-García *et al.,* 2013; Álvarez-García *et al.,* 2014). *Besnoitia besnoiti* has been described in several countries worldwide, particularly in some countries of Sub-Saharan Africa such as Angola, Botswana, Cameroon, Congo, Kenya, Namibia, Nigeria, South Africa, Swaziland, Zimbabwe and other East African countries (Bigalke, 1981; Bigalke and Prozesky, 2004) and Mozambique (Ferreira *et al.,* 1982). Outbreaks of the disease have been registered in Portugal (Cortês *et al.,* 2006), Spain (Fernandez-Garcia e*t al.,* 2009), France (Jacquiet *et al.,* 2010), Germany (Mehlhorn *et al.,* 2009; Rostaher *et al.,* 2010), Italy (Gollnick *et al.,* 2010), Switzerland (Lesser *et al.,* 2012) and Hungary (Hornok *et al.,* 2014). Thus, *Bovine besnoitiosis* is recognized as a disease of economic importance in several countries.

*Neospora caninum*, for example, is considered to be one of the major abortive pathogens in cattle, and cases of abortion have been registered in sheep and goats (Varaschin *et al.,* 2012). In Tete province of Mozambique including Angónia district, rearing of cattle and goats are key activities by smallholder farmers. Thus, the production of ruminants plays an important role in income generation for rural livelihoods in those regions.

Despite the importance of these protozoan parasites, data regarding the serological occurrence of *N. caninum* both in bovines and in goats, and of *B. besnoiti* in cattle in Mozambique, specifically in Angónia, are lacking. Therefore, the aim of this study was to determine the serological occurrence of *N. caninum* in cattle and goats, and of *B. besnoiti* in cattle, in the smallholder farms in Angónia district, in Central-Western Mozambique.

## RESEARCH APPROACH

**Location and study animals.** The study was conducted in Angónia district between April and May 2010. Angónia is located in the semi-arid Tete province, in the central-western region of Mozambique, holding one of the largest goat and cattle herds, which are traditionally raised in communal pastures. Angónia is located in a plateau region with an altitude of over 1000 m, characterized by temperate-humid climate, with an annual average of 23ºC temperature and relative humidity of 80%. The soil is deep and flat or gently rolling relief. The monthly average rainfall is 265 mm, with rainfall varying from 400 to 1200 mm, and rainfall concentration in the period from December to February (Figure 1).

For determining the occurrence of anti-*N. caninum* and anti-*B. besnoiti* antibodies, animals were randomly selected at acaricide dip tanks upon the consent of the owners, and all the selected animals were regarded as one flock in each locality since the number of heads per smallholder farmer was low. A total of 297 cattle and 132 goats were selected through a non-probabilistic convenience sampling since no data on bovine and *Caprine neosporosis*, and on *Bovine besnoitiosis* were available in Angónia district.

Thereafter, whole blood samples were collected

by jugular venipuncture using plain vacutainer system from Angone cattle in six flocks from six localities (Ulóngué, Chivomodzi, Ntengoumodzi, Vila Mouzinho, Madzawa, Chizuzu) and from Landim goats in ten flocks from three localities (Ulóngué, Mangàne, Kampessa) in Angónia District. The samples were stored in a cooler with ice and transported to the Parasitology Laboratory at Zootechnical Station of the Agricultural Research Institute of Mozambique (IIAM) in Angónia, where serum was obtained from the blood samples.

**Serum samples.** The study was conducted with a non-probabilistic sample of convenience because of the absence of any data on *Caprine neosporosis*, and on *Bovine besnoitiosis* in Angónia district. Cattle blood samples (n= 297) and goat blood samples (n= 132) were collected by jugular venipuncture using plain vacutainer system from smallholder farms in Angónia. The samples were stored at a cooler with ice and transported to the Parasitology Laboratory at Zootechnical Station of the Agricultural Research Institute of Mozambique (IIAM) in Angónia. After clot retraction, samples were submitted to centrifugation at 3,000 rpm for 10 minutes. The resulting sera were stored at –20 ºC until serological testing for *N. caninum* and *B. besnoiti.* Ninety four of the cattle sera samples were also tested for *B. besnoiti*. Since the positivity was high for *B. besnoiti* in the first batch of samples tested, the number of samples was defined by convenience for testing for *B. besnoiti* antibodies. Therefore IFAT was only performed in 94 bovine sera from three localities.

**Antigen production and Indirect Fluorescent Antibody Test (IFAT).** The Indirect Fluorescent Antibody Tests (IFATs) were performed for determination of anti-*N. caninum* and *B. besnoiti* IgG antibodies at the Parasitic Diseases Laboratory, Veterinary Medicine Hospital, Federal University of Bahia (UFBA), Brazil. For antigen production tachyzoites of *N. caninum* of the strain NC-Bahia (Gondim *et al.,* 2001) and from the Spanish *B. besnoiti* strain Bb Spain-1 (Fernandez-Garcia *et al.,* 2009) were used. Production of slides and IFAT were performed as described by Gondim *et al.* (2001) and Uzêda *et al.* (2014). Antigen slides, containing a concentration of 1500 tachyzoites/µl and previously prepared, were washed in PBS pH7.2 for five minutes, dried at room temperature, and a 1:200 dilution of serum sample in PBS was added to each well as described by Schares *et al.* (2010) with minor modifications. Slides were incubated at 37$^0$C for 30 minutes and washed twice in PBS for five minutes. A 1:400 dilution of fluorescein isothiocyanate (FITC) conjugate (anti-bovine IgG whole molecule, SIGMA, St. Louis, USA) in PBS with 0.05% Evans blue was added and then the slides were incubated at 37$^0$C. Two additional washes were performed before drying and mounting the slides with glycerin 90%. Slides were examined under a microscope with UV emission (Olympus® CX 31). Serum from a naturally-infected bovine was used as positive and a previously negative tested serum was used as negative control (Schares *et al.,* 2010). A bovine calf serum (Hyclone, Utah, USA) was also used as negative control. Samples were considered as positive only if total peripheral fluorescence was detected in more than 50% of tachyzoites in each well. Positive sera were diluted two-fold until the end point. Information on other factors such as age, management, among others, was unavailable.

**Statistical analysis.** A Chi-Square test was done to check for possible differences between the localities surveyed since no registration of animals is done at the traditional production systems in rural communities. The comparison of the positivity for anti-*N. caninun* and anti-*B. besnoiti* antibodies between localities in goats and cattle, respectively, was performed using Pearson's Chi-Square Test with 95% confidence interval (SPSS version 21). Seroprevalence values were calculated for both cattle and goats for the two protozoan pathogens, and then tested by Pearson's Chi-Square test to consider possible differences between the localities surveyed.

## RESULTS

The IFAT applied for *N. caninum* antibodies at individual serum samples indicated an overall serological frequency of 8.4% (25/297) at dilutions 1:100 to 1:800 at a cut-off point of 1:200 (Table 1). It was found that 30% (3/10) and 83.3% (5/6) of goats and cattle flocks showed positive results, respectively. The highest positivity rates were observed in cattle herds of locations in Ntengoumodzi (16.1%) and Chivomodzi (13.7%), and for goats in Ulónguè (5.9%) and Kampessa (4.1%). Regarding *B. besnoiti*, only three cattle herds were evaluated, and positive animals were found in all of them. The Chivomodzi locality had 71% positivity for cattle. Statistical difference was found on positivity between localities for anti-*N. Caninum* IgG antibodies presence in cattle ($p<0.05$), but in goats it was not observed ($p>0.05$), while statistical difference was also observed for anti-*B. besnoiti* IgG antibodies presence in cattle ($p<0.05$).

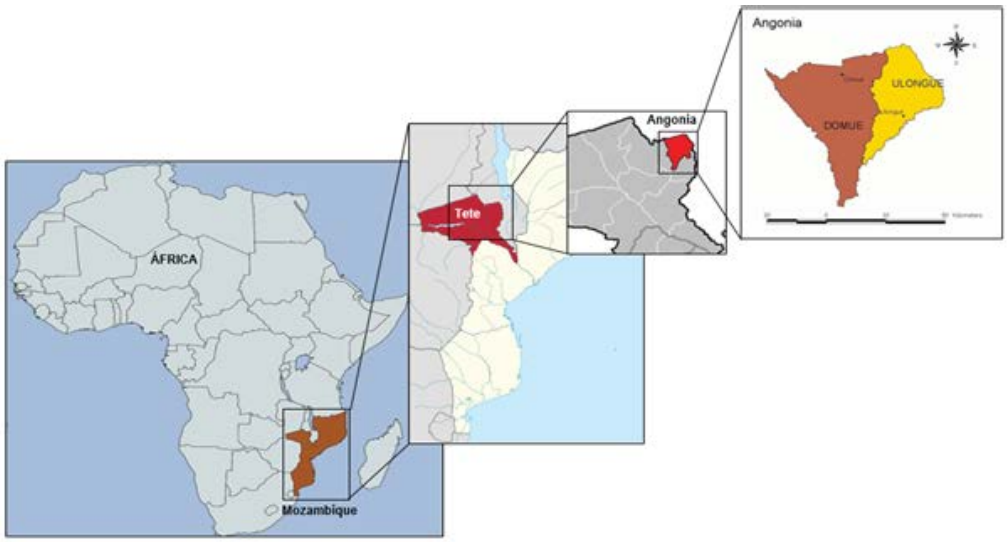


Figure 1. Map of Mozambique indicating Angónia district in the northern region of Tete Province

Table 1. Positivity for anti-*N. caninum* IgG antibodies determined by IFAT in 297 serum samples from Angone cattle, Angónia district, Tete province, Mozambique in 2010

| Localities | Positive samples | N | % Positivity |
|---|---|---|---|
| Ulóngué | 3 | 42 | 7.1 |
| Chivomodzi | 7 | 51 | 13.7 |
| Ntengoumodzi | 9 | 56 | 16.1 |
| Vila Mouzinho | 2 | 51 | 3.9 |
| Madzawa | 4 | 48 | 8.3 |
| Chizuzu | 0 | 49 | 0.0 |
| Total | 25 | 297 | 8.4 |

A sero-prevalence of 3.8% (5/132) was found in goat serum samples tested using IFAT at 1:50 and 1:100 dilutions at a cut-off point of 1:50 (Table 2).

Table 2. Positivity for anti-*N. caninum* IgG antibodies determined by IFAT in 132 serum samples in Landim goats, Angónia district, Tete province, Mozambique in 2010

| Localities | Positive samples | N | % Positivity |
|---|---|---|---|
| Ulóngué | 2 | 34 | 5.9 |
| Mangane | 0 | 25 | 0.0 |
| Kampessa | 3 | 73 | 4.1 |
| Total | 5 | 132 | 3.8 |

Thirty seven (39.4%) of individual sera were identified as positive for antibodies against *B. besnoiti* at the dilutions of 1:100 to 1:800 at the cut-off of 1:200 (Table 3).

Table 3. Positivity for anti-*B. besnoiti* IgG antibodies determined by IFAT in 94 serum samples from Angone cattle, Angónia district, Tete province, Mozambique in 2010.

| Localities | Positive samples | N | % positivity |
|---|---|---|---|
| Chivomodzi | 22 | 31 | 71.0 |
| Ntengoumodzi | 12 | 45 | 26.7 |
| Vila Mouzinho | 3 | 18 | 16.7 |
| Total | 37 | 94 | 39.4 |

**DISCUSSION**

The study established the seroprevalence of *N. caninum* in cattle and goats and of *B. besnoiti* in cattle from Angónia district, Mozambique. This is the first survey to report the serological occurrence of the two protozoan parasites, and it is possible that *neosporosis* and *besnoitiosis* are important diseases but are probably underestimated in Africa, particularly in Mozambique. A prevalence of antibodies against *N. caninum* of 8.4% for cattle was estimated, while for goats the prevalence of antibodies against *N. caninum* was estimated at 3.8%. The prevalence of *N. caninum* infection found in this study in cattle in Angónia region of Mozambique was comparable to that reported in Tanzania. In a sero-epidemiological study using ELISA in Tanzania and Ghana, an average of 8.1% were seropositive to *N. caninum*, with a range from 0% to 15% in Ngorongoro District in Tanzania, a neighboring country to Mozambique, however no Neospora-seropositive cattle were found in Ghana (Latham, 2003). Serologic prevalence of *N. caninum* varies depending on the country, region sampled, type of serological test used, and cut-off level used to determine the exposure, presence of definitive hosts, and seropositivity for up to 87% in some dairies has been reported to occur (Dubey, 2003). Some serological surveys in cattle in Brazil indicate prevalence from low (1%) to high (31.7%) in asymptomatic cattle (Dubey and Schares, 2011).

In goats, the overall prevalence of 3.8% from this study was considered to be low or similar compared to results from other studies using IFAT. Data on sero-epidemiological studies on *N. caninum* in goats are scarce in Africa. Several studies on the prevalence of *N. caninum* have been conducted elsewhere. In a survey conducted in Pernambuco, in the Northeastern region of Brazil, for example, a prevalence of 26.6% in goats was reported (Tembue *et al.,* 2011), while in São Paulo, a Southeastern region of Brazil a prevalence of 6.34 % was reported (Figliuolo *et al.,* 2004a). Another study conducted in Western Maranhão in Brazil reported that 17.39% of serum samples showed anti-*N. caninum* antibodies using IFAT (Moraes *et al.,* 2011). The low prevalence in goats reported from this study is in agreement with observations by other authors. A low prevalence of 1% was registered in Rio Grande do Norte by Lima *et al.* (2008) and a prevalence of 3.3% was registered in Paraíba state by Faria *et al.* (2007), while in Argentina a prevalence of 6.6% was reported by Moore *et al.* (2007). Gazzonis *et al.* (2016) reported a prevalence of 5.7% in goats from Northern Italy, 6% of tested animals were positive to *N. caninum* in the northern-western Spain (Díaz *et al.*, 2016), and a prevalence of 10.7% was observed by Andrade *et al.* (2013) in goats of Minas Gerais, Brazil. According to Figliuolo *et al.* (2004b), Dubey and Schares (2011), and Gazzonis *et al.* (2016), neosporosis is not as common in goats compared to cattle and sheep. The results from the current study also indicate that prevalence may vary according to the area sampled, and that small ruminants are only occasionally infected by *N. caninum* compared to cattle that are commonly affected by *neosporosis*, considered to be of major importance than in goats (Dubey and Schares, 2011; Gazzonis *et al.,* 2016).

There were significant differences in sero-positivity between localities for anti-*N. caninum* IgG antibodies in cattle, varying from 0 to 16.1% in the six localities sampled, but not in goats, where seropositivity varied from 0 to 5.9 only in the three localities. However, the prevalence in Ulóngue where both species were surveyed was 7.1% in cattle and 5.9% in goats which indicates that local factors contributed to the differences between localities. The fact that the locations surveyed for cattle and goats are different contributed to the significant differences observed since prevalence varies highly depending on the sampling area (Dubey and Schares, 2011). Thus, the results showed that both Angone cattle and Landim goats from Angónia district were exposed to *N. caninum*, and that the occurrence of anti-*N. caninum*

antibodies was higher in cattle than in goats and this was in agreement with other past studies (Latham, 2003).

The occurrence of antibodies against *B. besnoiti* in cattle from this study was estimated using IFAT. The occurrence of *B. besnoiti* in Mozambique was reported earlier based on clinical symptoms and histopathological examinations only (Ferreira *et al.,* 1982; Ferreira, 1985, Cortês *et al.,* 2003). Studies carried out in Africa show that *besnoitiosis* is an important disease in the continent (Kumi-Diaka *et al.,* 1981; Penzhorn and Krecek, 1997; Njagi *et al.,* 1998; Jacquiet *et al.,* 2010). Data on the serological prevalence of *B. besnoiti* in cattle in Mozambique, including Angónia district, are scarce. This study provides the first serological evidence of this protozoan parasite in this region. The IFAT for *B. besnoiti* IgG was employed using a 1:200 cut off, which is considered to be specific for antibodies against the parasite (Schares *et al.,* 2010).

The study results reported an overall seropositivity of 39.4% of *B. besnoiti* in cattle from smallholder farmers in Angónia region, varying from 16.7% to 71% in the localities surveyed, which represents a high exposure. This was significantly different across localities ($p<0.05$), indicating that *besnoitiosis* may be a relatively common disease of cattle in the region such as in other Southern Africa countries as reviewed by Bigalke and Prozesky (2004). It has also been reported that *Bovine besnoitiosis* is highly prevalent in tropical and subtropical regions, and in Africa (Penzhorn and Krecek, 1997), and the prevalence also varies according to the sampling area, production systems and on the test used (Talafha *et al.,* 2015). Some studies on sero-prevalence of *B. besnoiti* have been conducted in South Africa and elsewhere using serological techniques such as indirect immunofluorescence tests (IFATs), and reported higher sero-prevalence rates in beef herds, more often raised under extensive conditions, than in dairy herds (EFSA, 2010). As an example, prevalence of 53.5% in dairy and of 62.5% in beef cattle was reported in Mato Grosso do Sul by Benetti *et al.* (2009) and Andreotti *et al*. (2010) respectively, while a prevalence of 73% in beef and 80.9% in dairy were registered in Argentina by Moré *et al.* (2008a) and by Moré *et al*. (2009), respectively. This study on cattle raised under extensive production systems indicated relatively high seropositivity rates which corroborates with studies conducted in South Africa that reported a higher seropositivity rate of 61.7% with immunofluorescence tests in animals without clinical signs (Janitschke *et al.*, 1984).

*Neosporosis* and *Besnoitiosis* are important diseases that are probably underestimated in Africa, particularly in Mozambique, and can represent economic losses to goats and cattle production by smallholder farmers in some localities due to reproductive disorders. The serological results from this study serve as a baseline indicator of the level of exposure of cattle and goats to *Neospora* and *Besnoitia* parasites and the potential risk of these infections in the region, as noted by other authors (Anderson *et al.,* 2000). Therefore, the development and standardization of serological tests may improve the diagnosis of these protozoan infections and contribute to the knowledge of the real situation of occurrence of these diseases in the country and in Africa in general.

## CONCLUSION

The results of this study show that cattle and goats in Angónia are exposed to both *Neospora caninum* and *Besnoitia besnoiti* protozoan parasites. This is the first serological evidence of *N. caninum* in cattle and goats, and of *B. besnoiti* in cattle in this region of Mozambique. These results serve as a baseline indicator of potential protozoan parasitic infections in cattle and goats in Mozambique and in particular in Angónia district and Tete province. The study should prepare the Department of Veterinary Services in Mozambique to prioritize controlling the diseases, and to develop strategies for wider disease surveillance, and thereafter control these infections/diseases in ruminants at provincial level based on evidence. Furthermore, the results indicate a need for further studies to determine the role of both protozoan parasites in causing reproductive disorders in cattle and goats.

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**STATEMENT OF NO CONFLICT OF INTEREST**

We the authors of this paper hereby declare that there are no competing interests in this publication.

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