Tick-borne diseases in the Union of the Comoros are a hindrance to livestock development: circulation and associated risk factors

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Abstract

Tick-borne diseases (TBD) occur in many temperate countries and are economically important in most tropical and subtropical areas, affecting dairy and beef cattle, as well as small ruminants. Four major

tick-borne diseases have been detected in eastern and southern Africa: East Coast fever (ECF) caused by *Theileria parva,* Theiler 1904, anaplasmosis caused by either *Anaplasma marginale,* Theiler 1910, *Anaplasma centrale,* Theiler 1911, and *Anaplasma ovis,* Bevan 1912, babesiosis caused by *Babesia bovis,* Babes 1988 and *Babesia bigemina,* Smith & Kilborne 1893, and heartwater caused by *Ehrlichia ruminantium* Cowdry 1925. A cross-sectional survey was undertaken to determine the antibody prevalence of these TBDs and to identify the risk factors for TBD infections in the Union of the Comoros. In 2016 and 2017, 903 individual animal serum samples were collected from 429 separate farms, where the farmers answered individual questionnaires. The antibody prevalence of anaplasmosis, babesiosis (*B. bigemina*) and heartwater was determined by enzyme-linked immunosorbent assays (ELISA) and the antibody prevalence of ECF was assessed using an immunofluorescence antibody test (IFAT). The relationship between TBD seropositivity and livestock-related variables was assessed by multivariate analyses with standard logistic regression models. The results showed that these four TBDs were present in the Union of the Comoros with a global antibody prevalence of 15% (95% CI [12.7%; 17.3%]) for anaplasmosis, 9.2% (95% CI [6.5%, 11.9%]) for *B. bigemina* babesiosis, 5.3% (95% CI [3.2%, 7.4%]) for ECF and 4.6% (95% CI [3.2%, 6%]) for heartwater. We compared these findings with the abundance and distribution of several tick species known to be TBD vectors and we found a significant correlation between *Rhipicephalus appendiculatus* and ECF, and between *Amblyomma variegatum* and heartwater. We also found that two major variables were significantly correlated with *B. bigemina* antibody prevalence (“island” and “breeding area”), four variables were significantly correlated with anaplasmosis antibody seroprevalence (“island”, “number of cattle per farmer”, “number of farmers per village” and “breeding area”), two were significantly correlated with ECF antibody prevalence (“number of farmers in village” and “presence of ticks”), and three were significantly correlated with heartwater (“island”, “number of cattle per farmer” and “number of farmers in the village”). Our findings confirmed livestock exposure to the four targeted TBDs of major concern for livestock development. Consequently, raising farmers' awareness and setting up a period of quarantine should be considered a priority.

1. Introduction

Among the tick-borne diseases (TBD) reported in the South-West Indian Ocean, including eastern and southern Africa, East Coast fever (ECF) (caused by *Theileria parva,* Theiler 1904), anaplasmosis (caused by *Anaplasma marginale,* Theiler 1910, *A. centrale,* Theiler 1911 or *A. ovis,* Bevan 1912), bovine babesiosis (caused by *Babesia bovis,* Babes 1988 and *B. bigemina,* Smith & Kilborne 1893) and heartwater (caused by *Ehrlichia ruminantium,* Cowdry 1925) are economically important diseases affecting dairy and beef cattle, as well as goats and sheep, and they are directly linked to tick abundance (Adjou Moumouni et al., 2015; Bram, 1975; Hove et al., 2018; Jongejan and Uilenberg, 2004; Kerario et al., 2017; Ringo et al., 2018; Worthington and Bigalke, 2001). The sovereign state of the Union of the Comoros comprises three islands, Anjouan, Moheli and Grande Comore, located in the South-West Indian Ocean at the northern end of the Mozambique Channel and lying north-west of Madagascar. The country relies mostly on ruminant livestock production, which is the main source of income for the state. In 2004, the livestock population was estimated at 64,000 cattle, 96,000 goats and 16,000 sheep (Saido, 2005). Each year, many live zebus are imported from Tanzania, mainly for the traditional "*Grand Mariage*" celebrations (De Deken et al., 2007). The animals are imported with no thorough quarantine and with limited veterinary controls. Transboundary and vector-borne diseases are known to have a major impact on livestock production. For example, in Tanzania where these four tick-borne diseases occur, economic losses were estimated at 364 million US dollars (Kivaria, 2006). In 1989, Du Plessis et al. reported the isolation of *E. ruminantium* from *Amblyomma variegatum* ticks collected on the islands of the Union of the Comoros. In 2002, there was a huge outbreak of ECF, leading to a 10% loss of livestock. Its origin was legal cattle imports from Tanzania (De Deken et al., 2007; Norval et al., 1992). Although the national epidemiological surveillance network set up by the national veterinary services suspected tick-borne diseases, there was no laboratory diagnosis of heartwater, bovine babesiosis, or anaplasmosis, the only investigation being a molecular biology diagnosis for ECF in 2003 (De Deken et al., 2007). Clinical signs common to these four tick-borne diseases are regularly observed: fever, inappetence and mortality, with a specific pattern of nervous signs for heartwater, hemoglobinuria and anemia for bovine babesiosis, and enlarged lymph nodes for ECF. Three species of ticks have been reported in the Union of the Comoros, namely *A. variegatum*

known to be a biological vector of heartwater, along with *Rhipicephalus microplus* associated with bovine babesiosis and anaplasmosis, and *Rhipicephalus appendiculatus* associated with ECF (Worthington and Bigalke, 2001; Yssouf et al., 2011).

To clarify the TBD epidemiological situation in the Union of the Comoros, a cross-sectional study was conducted on the indigenous domesticated ruminant population, focusing on the acquisition of specific antibodies and on tick distribution, combined with an analysis of risk factors to identify variables that might be linked to TBD infections.

2. Materials and methods

2.1.Livestock cross-sectional study, design and sampling

The study was conducted from April 2016 to July 2017 on the three islands, Grande Comore, Anjouan and Moheli. The sampling size was calculated using an expected prevalence of 20% and a relative precision of 20%. The inflation coefficient and intra-class coefficient were used applying the method developed by Toma and collaborators (2001). The total number of samples was distributed across the three islands, taking into account the number of animals per island based on the 2004 census (Saido, 2005). The study was designed as follows: three animals per farm with an overall objective of 903 samples (n=458 cattle, n=420 goats, n=25 sheep) (Fig. 1). The difference between the expected number of samples and the actual number of samples was due to field constraints (Table 1). Five ml of whole blood was collected from the jugular vein of the animals in Vacutainer tubes (Becton Dickinson, USA). Samples were left to clot at 15°C and the serum was separated from whole blood by centrifugation, then stored at -20°C. The research protocol was implemented with the approval of the Vice-Presidency of Agriculture, Fisheries and Environment of the Union of the Comoros. Farmers in each village gave their verbal consent to being included in the study. No personal data were collected, only information concerning livestock practices was requested.

2.2. Risk factor analysis

A questionnaire was completed during an interview with the farmers (n=429). The data collected concerned farm characteristics, locations, breeding practices, the existence of a water point nearby, purchasing and selling practices, knowledge of the different biological vectors (flies, ticks, mosquitoes) present on their animals in 2016-2017, clinical signs specific to the four TBDs (ECF, heartwater, babesiosis, anaplasmosis), TBD incidence and mortality, the use and frequency of treatments against ectoparasites (frequency, type of molecule and treatment) and insects. The questionnaire was pre-tested on five breeders and distributed in the local language by a team of two people trained for the purpose. The final questionnaire had 46 questions, of which 78% were closed.

2.3. Serological assays for the detection of specific TBD antibodies

Only bovine samples (n= 457) were used to test for antibodies against bovine-specific diseases, *B. bigemina* babesiosis and ECF. All the ruminant samples were tested for anaplasmosis and heartwater (n=902). *Anaplasma* spp-specific antibodies were tested using the commercial *Anaplasma* antibody test kit, cELISA v2 (VMRD, Pullman, Washington, USA) based on the major surface protein 5 (MSP 5) with a sensitivity of 96% and a specificity of 95% (Torioni de Echaide et al., 1998). The percentage of inhibition was calculated for each sample as follows: Value (%) =100×[1-(sample OD/negative control OD)] according to the manufacturer's recommendations. Test samples with < 30% inhibition were considered negative and ≥ 30% were considered positive. Specific anti-*B. bigemina* antibodies were tested in serum samples using the commercial SVANOVIR® *B. bigemina*-Ab ELISA kit (Biosellal, Lyon, France), with a sensitivity of 96% and a specificity 97.5%, (Tebele, 1996). Positivity (percentage) was calculated for each sample as follows: Value (%) = (sample OD/positive control OD) x100. Test samples with < 25% inhibition were considered negative, 26-39% doubtful and ≥ 40% were considered positive. *Babesia bovis* antibodies could not be tested due to the lack of a specific and reliable commercial kit.

Specific anti-*T. parva* antibodies were tested in serum samples using an indirect fluorescent antibody test (IFAT) based on *T. parva* piroplasm prepared by ARC, Onderstepoort Veterinary Institute (OVI),

South Africa, using positive and negative control sera. A titer > 1/80 was considered positive (Burridge and Kimber, 1972). The sensitivity and specificity of the test were 95.24% and 99%, respectively (ARC-OVI, 2018). Specific anti-*E. ruminantium* antibodies were tested in serum samples using an indirect ELISA based on the MAP-1B antigen, with a sensitivity varying between 91.6% to 95.4% and a specificity of 99.4% (Mondry et al., 1998; van Vliet et al., 1995). The amount of serum available was not enough for some of the animals and therefore restricted the number of pathogens tests. *Anaplasma* spp.*, B. bigemina,* and *T. parva* tests were run as a priority, which explains the difference in the total number of samples analyzed for each of the pathogens.

2.4. Tick sampling, identification, distribution

Ticks were collected from the three islands and identified in 2010 (Yssouf et al. (2011)). Figure 1 shows tick sampling sites using QGIS © 2.6 software (Sherman et al., 2017).

2.5. Statistical analysis

Statistical analyses were performed with R studio (R studio team, 2015). A Spearman test was used to estimate the correlation between antibody prevalence and tick abundance. $P < 0.05$ was considered statistically significant. The 95% confidence interval was also calculated.

A risk factor analysis, based on the individual questionnaires, was undertaken in two steps. First, a univariate analysis was carried out between the presence of TBD in livestock (the outcome variable) and the explanatory variables. Variables that were significantly associated with the presence of TBD ($\chi 2$ test; $p < 0.25$) were kept to be tested for inter-correlation; if a strong correlation between variables was observed ($p < 0.05$), only the most explanatory variable related to the outcome variable was kept. The second stage involved a logistic multiple-regression model. The contribution of each factor to the model was tested with a likelihood-ratio χ^2 using a backward stepwise procedure. At the same time, the best parsimonious models were compared to the full model using the Akaike information criterion (Akaike, 1974). The validity and goodness-of-fit of the final model were assessed using Pearson's $\chi 2$

test and measurement of residual deviance (pseudo-R^2). The odds ratio (OR) and the 95% confidence interval (CI) were calculated.

3. Results

3.1. Anaplasma spp., *B. bigemina, T. parva* and *E. ruminantium, antibody prevalence*

In all, 903 sera (458 bovine sera and 445 goat and sheep sera) were tested to determine the overall anaplasmosis antibody prevalence in the Union of the Comoros, which was estimated at 15% (95% CI [12.7%; 17.3%]) all species combined, at 15.5% (95% CI [12.2%; 18.8%]) for cattle, and at 13% (95%CI [9.9%; 16.1%]) for goats and sheep. Specific anti-*B. bigemina* antibody prevalence was estimated at 9.21% (95% CI [6.5%; 11.7%]). Both infections are present on all three islands, although Grande Comore and Anjouan appear to be more infected by *Anaplasma* spp. than Moheli, and Grande Comore and Moheli are more infected by *B. bigemina* than Anjouan (Table 2). Both infections are transmitted by the same tick species, *R. microplus*, but anaplasmosis can affect cattle, sheep and goats while babesiosis, caused by *B. bigemina*, affects cattle only. *R. microplus*was collected from 16 of the 17 study sites (Fig. 2).

A generally low heartwater antibody prevalence of 4.6% (95% CI [3.2%; 6%]) was detected in the Union of the Comoros, with the highest antibody prevalence of 7.4% (95% CI [4.9%; 9.9%] in goats and sheep versus 1.9% (95% CI [0.6%; 3.2%]) for cattle (Table 2). The tick species *A. variegatum* was broadly distributed in 15 of the 17 sites sampled, except on Anjouan, for which the lower level of *A. variegatum* abundance was correlated with the lowest antibody level, 1.35% (Table 2, Fig. 2).

ECF antibody prevalence, at 5.3% (95% CI [3.2%; 7.4%]), was only detected on the island of Grande Comore (Table 2). The tick species *R. appendiculatus* was very abundant and was found at seven of the nine sites on Grande Comore (Fig. 2).

The abundance of *R. appendiculatus* and *A. variegatum* was positively correlated with the prevalence of ECF (p=0.01) and heartwater (p=0.04) antibodies. The abundance of *R. microplus* was not

significantly correlated with either the prevalence of antibodies to Anaplasma spp. (p=0.35) or antibodies to *B. bigemina* (p=0.64).

3.2.Analysis of risk factors

In all, five of the 17 variables tested in the screening analysis were significantly correlated with TBD infections. Table 3 summarizes the three variables identified in association with the occurrence of *B. bigemina* antibodies in the Union of the Comoros, two of which were significantly associated. The logistic multiple-regression model indicated that the risk of *B. bigemina* babesiosis decreased when the farm was located on the island of Anjouan and when animals grazed near the forest. Table 4 summarizes the five variables identified in association with the risk of anaplasmosis in the Union of the Comoros, four of which were significantly associated. The logistic multiple-regression model indicated that the risk increased when there were a large number of cattle per farmer and a large number of farmers per village. The risk decreased when farms were located on Moheli and Anjouan and when animals grazed near the forest. Table 5 summarizes the three variables identified in association with ECF in the Union of the Comoros, two of which were significantly associated. The risk of ECF infection was lower when there were a large number of farmers per village, whereas the risk of ECF infection increased with an increase in the presence of ticks, and when animals were not imported. Table 6 summarizes the three variables identified and significantly associated with heartwater in the Union of the Comoros. The risk of heartwater infection decreased when the farmers were located on Anjouan and when there were a large number of cattle per farmer. The risk increased when there were a large number of farmers per village.

4. Discussion

This was the first study to investigate the prevalence of TBD antibodies and the risk factors associated with TBD infection in the Union of the Comoros. Our findings confirmed livestock exposure to the four targeted TBDs of major concern for livestock development, namely anaplasmosis, *B. bigemina* babesiosis, heartwater and ECF, by assessing specific antibody prevalence. Apart from anaplasmosis, babesiosis and heartwater were regularly suspected by the veterinary services, although no laboratory

206 confirmation has earlier been made. ECF had already been reported in 2002 on the island of Grande
207 Comore (De Deken et al., 2007). The four species of ticks known to be biological vectors of these
208 TBDs were reported in 2010, with *R. microplus* and *A. variegatum* present on all three islands, and *R.*
209 *appendiculatus* on the island of Grande Comore (Yssouf et al. 2011). The fact that *R. appendiculatus*
210 was only found on Grande Comore could be explained by the movements of animals between the
211 Union of the Comoros and neighboring African countries. Until 2000, the Union of the Comoros
212 imported ruminants from Madagascar to all three islands. The presence of *A. variegatum* and *R.*
213 *microplus* had been reported in Madagascar, suggesting that this import route was the most likely
214 source of introduction for the two vectors in the Union of the Comoros (Stachurski et al., 2013;
215 Uilenberg et al., 1979; Yssouf et al. 2011). A free trade bill was signed in 2000, after which the Union
216 of the Comoros stopped imports from Madagascar and started importing legally from Tanzania to
217 Grande Comore, the only island where *R. appendiculatus* is present. This last tick vector species was
218 consequently introduced into the Union of the Comoros in 2002. Indeed, the main movement of
219 animals is from the islands of Moheli and Anjouan to Grande Comore for traditional ceremonies. (De
220 Deken et al., 2007; Lynen et al., 2007; Stachurski et al., 2013).

221 A correlation was found for two diseases, heartwater and ECF, where antibody prevalence could be
222 compared to the distribution of tick species (Yssouf et al., 2011). However, given the time lapse
223 between the two studies an update is needed to confirm this assertion for the other diseases.

224 Highly specific ELISA kits showed that the level of specific antibodies in livestock mounted against
225 *Anaplasma* spp. (15 %) and *B. bigemina* (9.2%) was higher than against *E. ruminatium* (4.6%) and *T.*
226 *parva* (5.3%). Specific *B. bovis* antibodies could not be tested due to a lack of reliable commercial
227 kits. Given the high sensitivity and specificity of the *B. bigemina* ELISA used in this study, cross-
228 reactivity in the detection of *B. bovis* versus *B. bigemina* was not likely to occur. These results appear
229 consistent, since *R. microplus* is the tick species most often found on local animals in the Union of the
230 Comoros, i.e. 1311 ticks, accounting for 77% of the 2010 collection, versus 253 *A. variegatum* and
231 126 *R. appendiculatus* (Yssouf et al., 2011). *R. microplus* is known to be an invasive tick species

232 easily able to replace indigenous ticks, as reported in South Africa for another tick species,
233 *Rhipicephalus decoloratus* (Chevillon et al., 2007; Madder et al., 2011; Nyangiwe et al., 2013).

234 Although anaplasmosis and *B. bigemina* babesiosis are the most prevalent diseases in the Union of the
235 Comoros, and particularly on the island of Grande Comore, farmers consider ECF to be the disease
236 that most affects the development of their livestock (Boucher et al., 2018). Farmers have better
237 knowledge of ECF due to the considerable losses that occurred in 2004 following its introduction into
238 the country (De Deken et al., 2007). ECF antibodies were only detected in five regions of Grande
239 Comore. When these findings were compared with those of a previous TBD study taking a
240 participatory epidemiology approach, there was concordance in the five regions that tested positive for
241 specific ECF antibodies. Indeed, ECF incidence was estimated at more than 10% in the participatory
242 study. However, in other regions, some farmers reported ECF-specific clinical signs, but *T. parva*
243 seroprevalence was nil. Based on the serology findings, those regions were considered ECF-free
244 (Boucher et al., 2018). Overall, the results of the participatory TBD epidemiology studies tallied with
245 the serological data.

246 The levels of specific antibodies for the four TBDs were much lower than those observed in
247 neighboring East African countries, including Tanzania, from where most, if not all, animals were
248 imported. *A. marginale* and *B. bigemina* antibody prevalence in cattle ranged from 20% to 63% in
249 Tanzania, Kenya and Mozambique (Alfredo et al., 2005; Swai et al., 2005; Swai et al., 2007a;
250 Wesonga et al., 2017). ECF antibody prevalence ranged from 40% to 48% in Tanzania and Kenya
251 (Swai et al., 2007b; Wesonga et al., 2015), whereas heartwater seroprevalence was 50% for cattle and
252 66% for small ruminants in Tanzania (Swai et al., 2008, 2009). The diversity and the performance
253 (specificity, sensitivity) of the ELISA kits used in these studies, as well as the sampling design
254 (national versus regional in some cases), may be some of the factors explaining these differences.

255 The levels of antibodies specific to *Anaplasma* spp, *B. bigemina* and *E. ruminantium* were found to be
256 influenced by farm location. Antibody prevalence was found to be lowest on Anjouan, as was the
257 number of ticks recorded in 2010 (Yssouf et al., 2011), which may have been related to lower
258 humidity on that island. However, Grande Comore is the island most exposed to the occurrence of

several imports of zebus carrying their vectors from Tanzania on a yearly basis (De Deken et al., 2007; DGE, 1993). Exposure to *Anaplasma* was found to be greater when farmers owned many cattle. The probability of being exposed to ticks and of being infected was found to increase with an increase in the number of cattle owned by a farmer. The opposite was found for heartwater, where a large number of cattle appeared to result in less exposure to the pathogen *E. ruminantium*. This result might be explained by the type of serological test that was used, the indirect MAP1-B-ELISA recommended by OIE. Indeed, the sensitivity observed in cattle was lower than the one observed in sheep and goats, as cattle could become seronegative after a 6-month period (Mahan et al., 1998; Semu et al., 2001). A large number of farmers per village increased the risk of exposure to heartwater and anaplasmosis and reduced the risk of exposure to ECF. Heartwater and anaplasmosis are diseases that are much less familiar to farmers (Boucher et al., 2018). The risk of being infected by anaplasmosis and *B. bigemina* babesiosis has been found to be lower for animals grazing near forests than for animals in agricultural zones. *R. microplus* tick species are mostly present in forest areas (Estrada-Peña et al., 2006). Moreover, one study showed that there is no difference in the population dynamics of ticks between forests and grasslands, but a higher density of cattle in grassland areas can increase the tick-host encounter rate (Nava et al., 2013). Importing cattle increased the risk of livestock being infected with *T. parva* due to (i) imports from Tanzania, where ECF is still present and, (ii) imports from the islands of Moheli and Anjouan, where there are naive cattle that rapidly develop the disease (De Deken et al., 2007; Swai et al., 2007b; Yssouf et al., 2011). None of the risk factors is linked to control and drug treatment practices, but to the fact that most Comorian farmers do not apply preventive or curative treatments (Boucher et al., 2018).

To conclude, our results demonstrated that all three islands of the Union of the Comoros were affected by tick-borne infections (anaplasmosis, *B. bigemina.* babesiosis, heartwater and ECF) but Anjouan and Moheli had not yet been exposed to *T. parva*, which was only detected on Grande Comore. The prevalence of ECF antibodies observed on the island of Grande Comore was consistent with the incidence estimated by participatory epidemiology in 2015, and with the tick species *R. appendiculatus* only being found on Grande Comore. The type of breeding area, the island on which

the livestock was raised, the number of cattle per farm, the presence of ticks and the density of farmers per village, appeared to have an impact on TBD occurrence. These risk factors, especially the breeding area, could be used to raise livestock farmers' awareness of appropriate control measures against these diseases and, lastly, our results stressed the need for setting up quarantine facilities for surveillance of imported animals.

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Conflict of Interest Statement

The authors declare that they have no conflict of interests.

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Figure legends

Fig. 1: Spatial distribution of ruminant samples collected during the livestock cross-sectional study in 2016-2017, and spatial distribution of ticks in 2010.

Fig. 2: Spatial distribution of antibodies (prevalence)to *Anaplasma* spp, *B. bigemina*, *T. Parva* and *E. ruminantium*, Union of the Comoros in 2016/2017, and distribution of their biological vectors in 2010 (A: *Anaplasma* spp, B: *B. bigemina* , C: *T. parva*, D: *E. ruminantium).*

Table 1 : Distribution of the samples. Planned sampling (actual sampling).

	Number of regions	Number of villages	Number of farms	Number of samples	Cattle /goats and sheep
Grande Comore	12 (12)	24 (37)	120 (178)	360 (367)	180/180 (187/180)
Anjouan	5 (5)	30 (33)	150 (172)	450 (446)	225/225 (226/220)
Moheli	3 (3)	6 (5)	30 (33)	90 (90)	45/45 (45/45)

Table 2: Observed *Anaplasma* spp., *E. ruminantium*, *B. bigemina* and *T. parva* seroprevalence. Confidence intervals (CI) were calculated using a normal approximation binomial distribution. All bovine samples (n= 457) and goat and sheep samples (n=445) were tested for *Anaplasma* spp and *E. ruminantium*. Only bovine samples were tested for the bovine specific pathogens *B. bigemina* and *T. parva* . The amount of serum available was not enough for some of the animals and therefore restricted the number of pathogens tests. *Anaplasma* spp., *B. bigemina* and *T. parva* tests were run as a priority.

	***Anaplasma* spp**						***E. ruminantium***						***B. bigemina***		***T. parva***	
	Total		Cattle		Goats and sheep		Total		Cattle	Goats and sheep			Cattle		Cattle	
	Number positive/ Total number	Seroprevalence % [95%CI]	Number positive/ Total number	Seroprevalence % [95%CI]	Number positive/ Total number	Seroprevalence % [95%CI]	Number positive/ Total number	Seroprevalence % [95%CI]	Number positive/ Total number	Seroprevalence % [95%CI]	Number positive/ Total number	Seroprevalence % [95%CI]	Number positive/ Total number	Seroprevalence % [95%CI]	Number positive/ Total number	Seroprevalence % [95%CI]
Grande Comore	83/367	22.6% [18.3%; 26.9%]	31/186	19.9% [14.2%; 25.6%]	46/180	25.6% [19.2%; 32%]	28/328	8.5% [5.5%; 11.5%]	4/159	2.5% [0.1%; 4.9%]	24/169	14.2% [8.9%; 19.5%]	26/185	14.1% [9.1%; 19.1%]	24/185	13% [8.2%; 17.8%]
Anjouan	49/446	11% [8.1%; 14%]	39/226	17.3% [12.4%; 22.2%]	10/220	4.6% [1.8%; 7.4%]	6/446	1.4% [0.3%; 1.5%]	1/226	0.4% [0%; 1.2%]	5/220	2.3% [0.3%; 4.3%]	10/226	4.4% [1.7%; 7.1%]	0/226	0%
Moheli	3/90	3% [0%; 7%]	1/45	2% [0%; 6%]	2/45	4% [0%; 10%]	6/90	7% [2%; 12.3%]	3/45	7% [0%; 15%]	3/45	7% [0%; 15%]	6/45	13% [3%; 23%]	0/45	0%
Total	135/902	15% [12.7%; 17.3%]	71/457	15.5% [12.2%; 18.8%]	58/445	13% [9.9%; 16.1%]	40/804	4.6% [3.2%; 6%]	8/430	1.9% [0.6%; 3.2%]	32/434	7.4% [4.9%; 9.9%]	42/456	9.2% [6.5%; 11.9%]	24/456	5.3% [3.2%; 7.4%]

Table 3: Final multivariate logistic regression model for risk factors associated with *Babesia bigemina* babesiosis (n= 247 cattle herds), Union of the Comoros, 2016-2017.

Variables		**Number of positive herds/Total number of herds (herd antibody prevalence %)**	**AOR (95% CI)**	**p-value**
Island	Grande Comore	20/95 (21)	Ref	0.008
	Moheli	6/22 (27)	1.3 (0.4,4.24)	0.661
	Anjouan	9/119 (8)	0.29 (0.11,0.75)	0.01*
Breeding area	Agricultural	15/94 (16)	Ref	0.01
	Village	19/112 (17)	1.35 (0.59,3.06)	0.479
	Forest	1/30 (3)	0.12 (0.01,0.96)	0.046*
Number of cattle per farmer	Small (below the median)	8/97 (8)	Ref	0.119
	Large (above the median)	27/139 (19.4)	2.04 (0.81,5.13)	0.129

AOR=Adjusted odds ratio, CI=Confidence interval, Ref.= reference category cell

Intercept=-1.7524; Model deviance=175.99; AIC=187.99, Model Df=7($P < 0.001$). * $p< 0.05$.

Table 4: Final multivariate logistic regression model for risk factors associated with anaplasmosis (n=406 cattle, goats, sheep herds, Union of the Comoros, 2016-2017.

Variables		Number of positive herds/Total number of herds (herd antibody prevalence %)	AOR (95% CI)	p-value
Island	Grande Comore	59/186 (31.7)	Ref	<0.001
	Moheli	2/25 (8)	0.13 (0.03,0.58)	0.008*
	Anjouan	25/136 (18.4)	0.53 (0.31,0.9)	0.012*
Number of cattle per farmer	Small (below the median)	53/226 (23.5)	Ref	<0.001
	Large (above the median)	25/121 (20.7)	2.74 (1.59,4.7)	<0.001*
Number of farmers per village	Small (below the median)	33/176 (18.8)	Ref	0.021
	Large (above the median)	53/171 (31)	1.85 (1.09,3.12)	0.022*
Livestock raising method	Fixed wooden stake and enclosure	24/82 (29)	Ref	0.058
	Moveable wooden stake and free movement	62/265 (23.4)	0.55 (0.3,1.02)	0.056
Breeding area	Agricultural	47/142 (33.1)	Ref	0.043
	Village	30/162 (18.5)	0.59 (0.33,1.05)	0.075
	Forest	9/43 (21)	0.39 (0.16,0.93)	0.034*

AOR=Adjusted odds ratio, CI=Confidence interval, Ref.= reference category cell

Intercept=-0.6782; Model deviance= 347.22; AIC=363.22, Model Df=11 ($P < 0.001$). * $p< 0.05$.

Table 5: Final multivariate logistic regression model for risk factors associated with East Coast fever (n=105 cattle herds), island of Grande Comore, Union of the Comoros, 2016-2017.

Risk factor		Number of positive herds/Total number of herds (herd antibody prevalence %)	AOR (95% CI)	P-value
Provenance	Local	9/81 (11)	Ref	0.081
	Imported	8/23 (35)	3.43 (0.84,13.96)	0.085
Number of farmers in the village	Small (below the median)	12/39 (31)	Ref	0.001
	Large (above the median)	5/65 (8)	0.12 (0.03,0.48)	0.003*
Presence of ticks	No	2/62 (3)	Ref	<0.001
	Yes	15/42 (36)	16.49 (3.15,86.39)	<0.001*

AOR=Adjusted odds ratio, CI=Confidence interval, Ref.= reference category cell

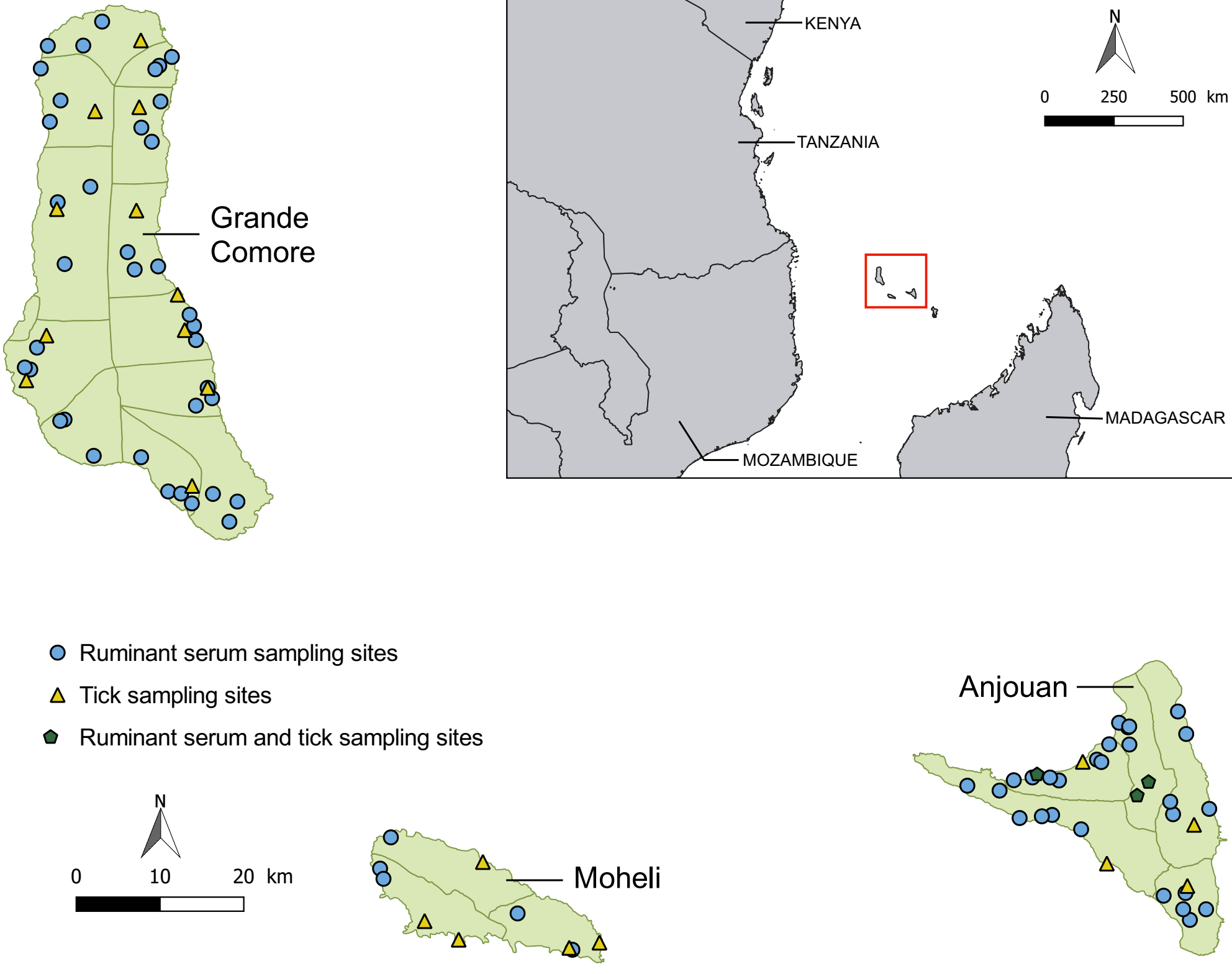
Intercept=-2.7320; Model deviance=59.474; AIC=67.47, Model Df=5(P < 0.001). * p< 0.05

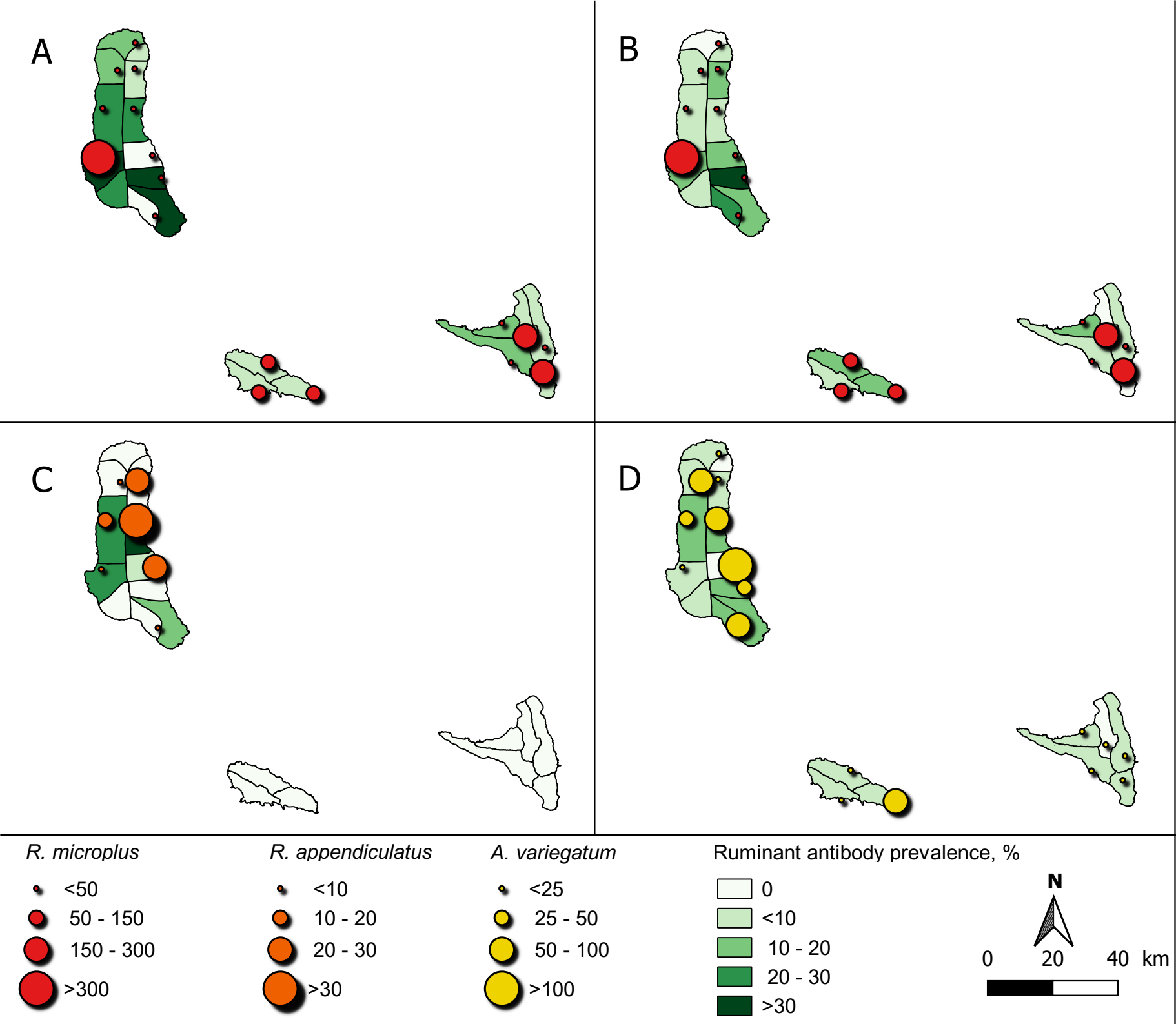
Table 6: Final multivariate logistic regression model for risk factors associated with heartwater, n=398 cattle, goats and sheep herds, Union of the Comoros, 2016-2017.

Risk factor		Number of positive herds/Total number of herds (herd antibody prevalence %)	AOR (95% CI)	p-value
Island	Grande Comore	22/196 (11.2)	Ref	0.01
	Moheli	5/25 (20)	1.99 (0.65,6.07)	0.228
	Anjouan	6/136 (4.4)	0.33 (0.13,0.84)	0.021*
Number of cattle per farmer	Small (below the median)	26/220 (11.8)	Ref	0.006
	Large (above the median)	7/137 (5.1)	0.32 (0.13,0.77)	0.011*
Number of farmers in village	Small (below the median)	10/181 (5.5)	Ref	0.02
	Large (above the median)	23/176 (13.1)	2.47 (1.12,5.44)	0.025*

AOR=Adjusted odds ratio, CI=Confidence interval, Ref.= reference category cell

Intercept=-2.2199; Model deviance=199.38; AIC=209.38, Model Df=6 ($P < 0.001$). * $p< 0.05$


Grande Comore
KENYA
TANZANIA
MOZAMBIQUE
MADAGASCAR
N
0 250 500 km
Ruminant serum sampling sites
Tick sampling sites
Ruminant serum and tick sampling sites
N
0 10 20 km
Moheli
Anjouan


A
B
C
D
R. microplus
<50
50 - 150
150 - 300
>300
R. appendiculatus
<10
10 - 20
20 - 30
>30
A. variegatum
<25
25 - 50
50 - 100
>100
Ruminant antibody prevalence, %
0
<10
10 - 20
20 - 30
>30
N
0
20
40 km