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

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

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23

## 24 **Abstract**

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

27 tick-borne diseases have been detected in eastern and southern Africa: East Coast fever (ECF) caused  
28 by *Theileria parva*, Theiler 1904, anaplasmosis caused by either *Anaplasma marginale*, Theiler 1910,  
29 *Anaplasma centrale*, Theiler 1911, and *Anaplasma ovis*, Bevan 1912, babesiosis caused by *Babesia*  
30 *bovis*, Babes 1988 and *Babesia bigemina*, Smith & Kilborne 1893, and heartwater caused by *Ehrlichia*  
31 *ruminantium* Cowdry 1925. A cross-sectional survey was undertaken to determine the antibody  
32 prevalence of these TBDs and to identify the risk factors for TBD infections in the Union of the  
33 Comoros. In 2016 and 2017, 903 individual animal serum samples were collected from 429 separate  
34 farms, where the farmers answered individual questionnaires. The antibody prevalence of  
35 anaplasmosis, babesiosis (*B. bigemina*) and heartwater was determined by enzyme-linked  
36 immunosorbent assays (ELISA) and the antibody prevalence of ECF was assessed using an  
37 immunofluorescence antibody test (IFAT). The relationship between TBD seropositivity and  
38 livestock-related variables was assessed by multivariate analyses with standard logistic regression  
39 models. The results showed that these four TBDs were present in the Union of the Comoros with a  
40 global antibody prevalence of 15% (95% CI [12.7%; 17.3%]) for anaplasmosis, 9.2% (95% CI [6.5%,  
41 11.9%]) for *B. bigemina* babesiosis, 5.3% (95% CI [3.2%, 7.4%]) for ECF and 4.6% (95% CI [3.2%,  
42 6%]) for heartwater. We compared these findings with the abundance and distribution of several tick  
43 species known to be TBD vectors and we found a significant correlation between *Rhipicephalus*  
44 *appendiculatus* and ECF, and between *Amblyomma variegatum* and heartwater. We also found that  
45 two major variables were significantly correlated with *B. bigemina* antibody prevalence (“island” and  
46 “breeding area”), four variables were significantly correlated with anaplasmosis antibody  
47 seroprevalence (“island”, “number of cattle per farmer”, “number of farmers per village” and  
48 “breeding area”), two were significantly correlated with ECF antibody prevalence (“number of farmers  
49 in village” and “presence of ticks”), and three were significantly correlated with heartwater (“island”,  
50 “number of cattle per farmer” and “number of farmers in the village”). Our findings confirmed  
51 livestock exposure to the four targeted TBDs of major concern for livestock development.  
52 Consequently, raising farmers' awareness and setting up a period of quarantine should be considered a  
53 priority.

## 55           **1. Introduction**

56   Among the tick-borne diseases (TBD) reported in the South-West Indian Ocean, including eastern and  
57   southern Africa, East Coast fever (ECF) (caused by *Theileria parva*, Theiler 1904), anaplasmosis  
58   (caused by *Anaplasma marginale*, Theiler 1910, *A. centrale*, Theiler 1911 or *A. ovis*, Bevan 1912),  
59   bovine babesiosis (caused by *Babesia bovis*, Babes 1988 and *B. bigemina*, Smith & Kilborne 1893)  
60   and heartwater (caused by *Ehrlichia ruminantium*, Cowdry 1925) are economically important diseases  
61   affecting dairy and beef cattle, as well as goats and sheep, and they are directly linked to tick  
62   abundance (Adjou Moumouni et al., 2015; Bram, 1975; Hove et al., 2018; Jongejan and Uilenberg,  
63   2004; Kerario et al., 2017; Ringo et al., 2018; Worthington and Bigalke, 2001). The sovereign state of  
64   the Union of the Comoros comprises three islands, Anjouan, Moheli and Grande Comore, located in  
65   the South-West Indian Ocean at the northern end of the Mozambique Channel and lying north-west of  
66   Madagascar. The country relies mostly on ruminant livestock production, which is the main source of  
67   income for the state. In 2004, the livestock population was estimated at 64,000 cattle, 96,000 goats and  
68   16,000 sheep (Saido, 2005). Each year, many live zebus are imported from Tanzania, mainly for the  
69   traditional “*Grand Mariage*” celebrations (De Deken et al., 2007). The animals are imported with no  
70   thorough quarantine and with limited veterinary controls. Transboundary and vector-borne diseases  
71   are known to have a major impact on livestock production. For example, in Tanzania where these four  
72   tick-borne diseases occur, economic losses were estimated at 364 million US dollars (Kivaria, 2006).  
73   In 1989, Du Plessis et al. reported the isolation of *E. ruminantium* from *Amblyomma variegatum* ticks  
74   collected on the islands of the Union of the Comoros. In 2002, there was a huge outbreak of ECF,  
75   leading to a 10% loss of livestock. Its origin was legal cattle imports from Tanzania (De Deken et al.,  
76   2007; Norval et al., 1992). Although the national epidemiological surveillance network set up by the  
77   national veterinary services suspected tick-borne diseases, there was no laboratory diagnosis of  
78   heartwater, bovine babesiosis, or anaplasmosis, the only investigation being a molecular biology  
79   diagnosis for ECF in 2003 (De Deken et al., 2007). Clinical signs common to these four tick-borne  
80   diseases are regularly observed: fever, inappetence and mortality, with a specific pattern of nervous  
81   signs for heartwater, hemoglobinuria and anemia for bovine babesiosis, and enlarged lymph nodes for  
82   ECF. Three species of ticks have been reported in the Union of the Comoros, namely *A. variegatum*

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

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

## 90 **2. Materials and methods**

### 91 *2.1. Livestock cross-sectional study, design and sampling*

92 The study was conducted from April 2016 to July 2017 on the three islands, Grande Comore, Anjouan  
93 and Moheli. The sampling size was calculated using an expected prevalence of 20% and a relative  
94 precision of 20%. The inflation coefficient and intra-class coefficient were used applying the method  
95 developed by Toma and collaborators (2001). The total number of samples was distributed across the  
96 three islands, taking into account the number of animals per island based on the 2004 census (Saïdo,  
97 2005). The study was designed as follows: three animals per farm with an overall objective of 903  
98 samples (n=458 cattle, n=420 goats, n=25 sheep) (Fig. 1). The difference between the expected  
99 number of samples and the actual number of samples was due to field constraints (Table 1). Five ml of  
100 whole blood was collected from the jugular vein of the animals in Vacutainer tubes (Becton  
101 Dickinson, USA). Samples were left to clot at 15°C and the serum was separated from whole blood by  
102 centrifugation, then stored at -20°C. The research protocol was implemented with the approval of the  
103 Vice-Presidency of Agriculture, Fisheries and Environment of the Union of the Comoros. Farmers in  
104 each village gave their verbal consent to being included in the study. No personal data were collected,  
105 only information concerning livestock practices was requested.

106           2.2. Risk factor analysis

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

115           2.3. Serological assays for the detection of specific TBD antibodies

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

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

132 South Africa, using positive and negative control sera. A titer > 1/80 was considered positive  
133 (Burrige and Kimber, 1972). The sensitivity and specificity of the test were 95.24% and 99%,  
134 respectively (ARC-OVI, 2018). Specific anti-*E. ruminantium* antibodies were tested in serum samples  
135 using an indirect ELISA based on the MAP-1B antigen, with a sensitivity varying between 91.6% to  
136 95.4% and a specificity of 99.4% (Mondry et al., 1998; van Vliet et al., 1995). The amount of serum  
137 available was not enough for some of the animals and therefore restricted the number of pathogens  
138 tests. *Anaplasma* spp., *B. bigemina*, and *T. parva* tests were run as a priority, which explains the  
139 difference in the total number of samples analyzed for each of the pathogens.

#### 140 *2.4. Tick sampling, identification, distribution*

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

#### 143 *2.5. Statistical analysis*

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

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

156 test and measurement of residual deviance (pseudo-R<sup>2</sup>). The odds ratio (OR) and the 95% confidence  
157 interval (CI) were calculated.

### 158 3. Results

#### 159 3.1. *Anaplasma* spp., *B. bigemina*, *T. parva* and *E. ruminantium*, antibody prevalence

160 In all, 903 sera (458 bovine sera and 445 goat and sheep sera) were tested to determine the overall  
161 anaplasmosis antibody prevalence in the Union of the Comoros, which was estimated at 15% (95% CI  
162 [12.7%; 17.3%]) all species combined, at 15.5% (95% CI [12.2%; 18.8%]) for cattle, and at 13%  
163 (95%CI [9.9%; 16.1%]) for goats and sheep. Specific anti-*B. bigemina* antibody prevalence was  
164 estimated at 9.21% (95% CI [6.5%; 11.7%]). Both infections are present on all three islands, although  
165 Grande Comore and Anjouan appear to be more infected by *Anaplasma* spp. than Moheli, and Grande  
166 Comore and Moheli are more infected by *B. bigemina* than Anjouan (Table 2). Both infections are  
167 transmitted by the same tick species, *R. microplus*, but anaplasmosis can affect cattle, sheep and goats  
168 while babesiosis, caused by *B. bigemina*, affects cattle only. *R. microplus* was collected from 16 of the  
169 17 study sites (Fig. 2).

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

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

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



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

### 182 3.2. Analysis of risk factors

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

## 200 4. Discussion

201 This was the first study to investigate the prevalence of TBD antibodies and the risk factors associated  
202 with TBD infection in the Union of the Comoros. Our findings confirmed livestock exposure to the  
203 four targeted TBDs of major concern for livestock development, namely anaplasmosis, *B. bigemina*  
204 babesiosis, heartwater and ECF, by assessing specific antibody prevalence. Apart from anaplasmosis,  
205 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. ruminantium* (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

259 several imports of zebus carrying their vectors from Tanzania on a yearly basis (De Deken et al., 2007;  
260 DGE, 1993). Exposure to *Anaplasma* was found to be greater when farmers owned many cattle. The  
261 probability of being exposed to ticks and of being infected was found to increase with an increase in  
262 the number of cattle owned by a farmer. The opposite was found for heartwater, where a large number  
263 of cattle appeared to result in less exposure to the pathogen *E. ruminantium*. This result might be  
264 explained by the type of serological test that was used, the indirect MAP1-B-ELISA recommended by  
265 OIE. Indeed, the sensitivity observed in cattle was lower than the one observed in sheep and goats, as  
266 cattle could become seronegative after a 6-month period (Mahan et al., 1998; Semu et al., 2001). A  
267 large number of farmers per village increased the risk of exposure to heartwater and anaplasmosis and  
268 reduced the risk of exposure to ECF. Heartwater and anaplasmosis are diseases that are much less  
269 familiar to farmers (Boucher et al., 2018). The risk of being infected by anaplasmosis and *B. bigemina*  
270 babesiosis has been found to be lower for animals grazing near forests than for animals in agricultural  
271 zones. *R. microplus* tick species are mostly present in forest areas (Estrada-Peña et al., 2006).  
272 Moreover, one study showed that there is no difference in the population dynamics of ticks between  
273 forests and grasslands, but a higher density of cattle in grassland areas can increase the tick-host  
274 encounter rate (Nava et al., 2013). Importing cattle increased the risk of livestock being infected with  
275 *T. parva* due to (i) imports from Tanzania, where ECF is still present and, (ii) imports from the islands  
276 of Moheli and Anjouan, where there are naive cattle that rapidly develop the disease (De Deken et al.,  
277 2007; Swai et al., 2007b; Yssouf et al., 2011). None of the risk factors is linked to control and drug  
278 treatment practices, but to the fact that most Comorian farmers do not apply preventive or curative  
279 treatments (Boucher et al., 2018).

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

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

291

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297

## 298 **Conflict of Interest Statement**

299 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.

	<i>Anaplasma</i> spp						<i>E. ruminantium</i>						<i>B. bigemina</i>		<i>T. parva</i>	
	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]
<b>Grande Comore</b>	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%]
<b>Anjouan</b>	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%
<b>Moheli</b>	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%
<b>Total</b>	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

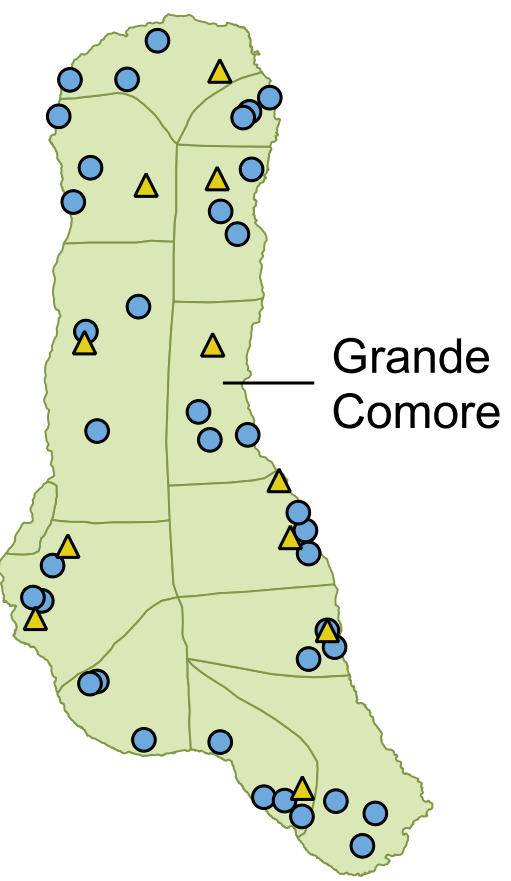
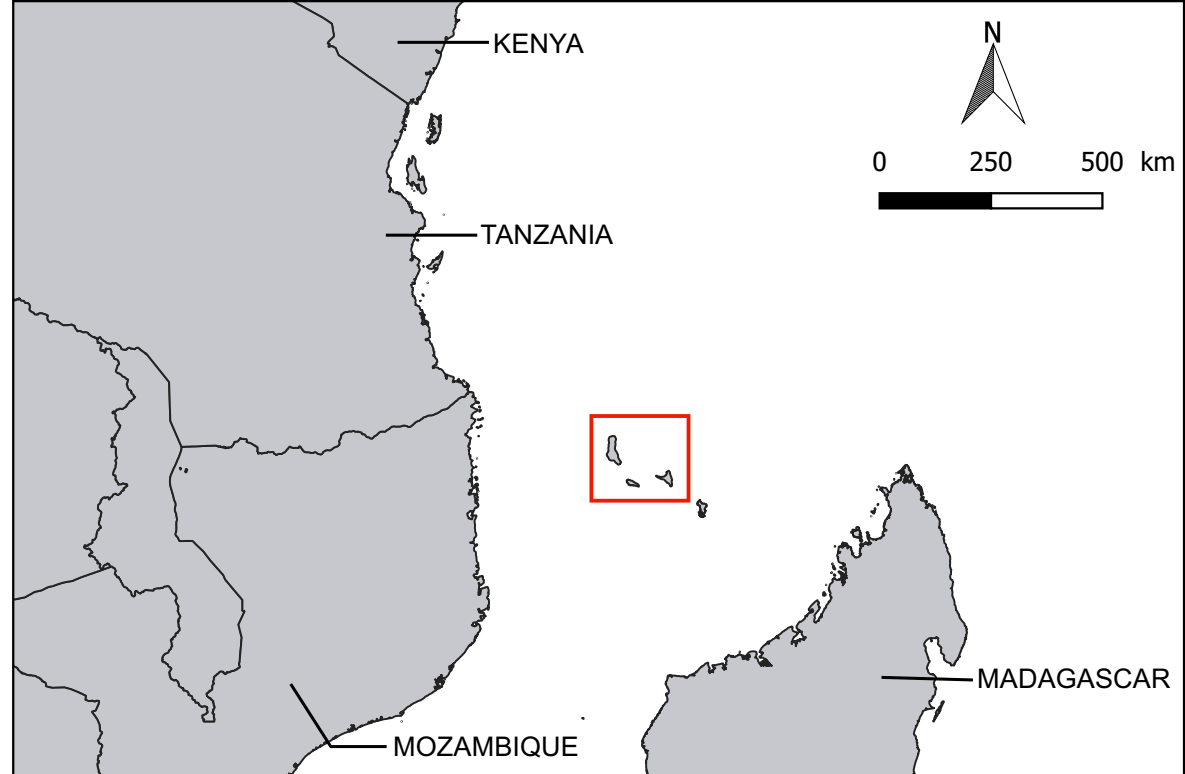
Table 6: Final multivariate logistic regression model for risk factors associated with heartwater, n=398 cattle, goats and sheep herds, Union of the Comoros, 2016-2017.

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*

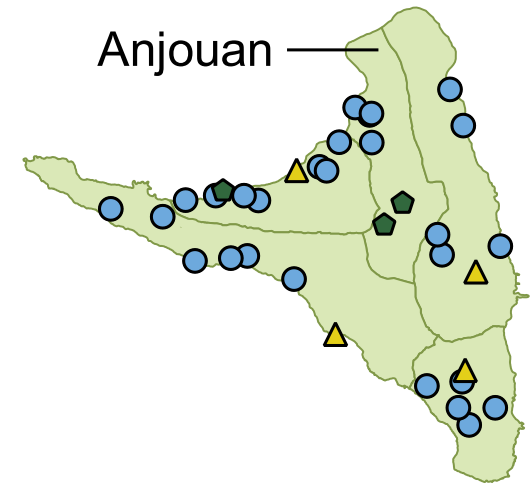
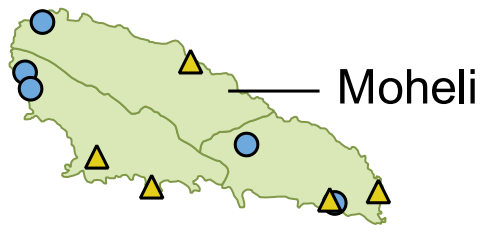
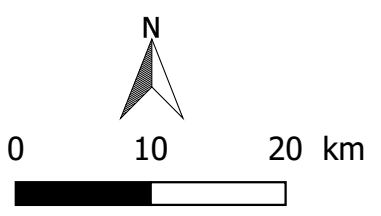
AOR=Adjusted odds ratio, CI=Confidence interval, Ref.= reference category cell

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

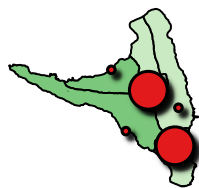
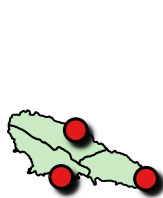
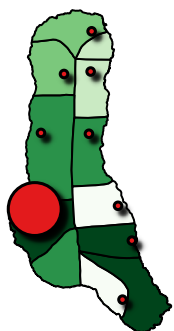




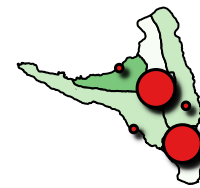
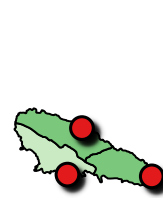
- Ruminant serum sampling sites
- ▲ Tick sampling sites
- ◆ Ruminant serum and tick sampling sites



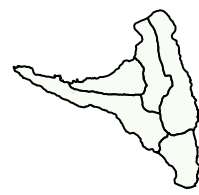
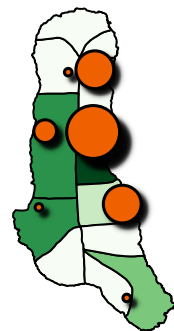
A



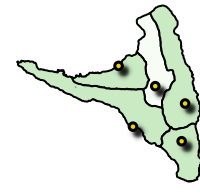
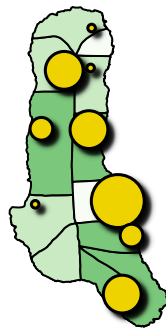
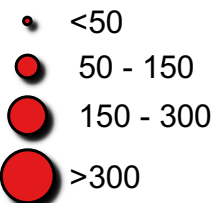
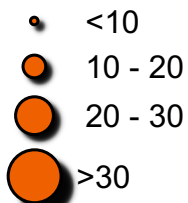
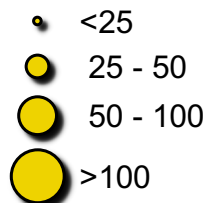
B



C



D

*R. microplus**R. appendiculatus**A. variegatum*

Ruminant antibody prevalence, %

