



**HAL**  
open science

## Detection in and circulation of Bluetongue virus among domestic ruminants in Madagascar

Soa Fy Andriamandimby, Cyril Viarouge, Jean-Pierre Ravalohery, Jean Marc Reynes, Corinne Sailleau, Luciano Michael Tantely, Nohal Elissa, Eric Cardinale, Amadou Alpha Sall, Stéphan Zientara, et al.

### ► To cite this version:

Soa Fy Andriamandimby, Cyril Viarouge, Jean-Pierre Ravalohery, Jean Marc Reynes, Corinne Sailleau, et al.. Detection in and circulation of Bluetongue virus among domestic ruminants in Madagascar. *Veterinary Microbiology*, 2015, 176 (3-4), pp.268-273. 10.1016/j.vetmic.2015.02.009 . hal-01290712

**HAL Id: hal-01290712**

**<https://hal.science/hal-01290712>**

Submitted on 28 Feb 2018

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Title: Detection in and Circulation of Bluetongue Virus among domestic ruminants in**

2 **Madagascar**

3

4 **Running Title:** Bluetongue Virus in Madagascar

5

6 **Authors**

7 Soa Fy Andriamandimby<sup>1</sup>, Cyril Viarouge<sup>2</sup>, Jean-Pierre Ravalohery<sup>1</sup>, Jean-Marc Reynes<sup>1</sup>,

8 Corinne Sailleau<sup>2</sup>, Michael Luciano Tantely<sup>3</sup>, Nohal Elissa<sup>3</sup>, Eric Cardinale<sup>4</sup>, Amadou Alpha

9 Sall<sup>5</sup>, Stephan Zientara<sup>2</sup>, Jean-Michel Heraud<sup>1</sup>

10

11 **Affiliations**

12 <sup>1</sup> Virology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar

13 <sup>2</sup> Anses-Laboratoire Santé Animal-UMR 1161, Maisons-Alfort, France

14 <sup>3</sup> Medical Entomology Unit, Institut Pasteur de Madagascar, Antananarivo, Madagascar

15 <sup>4</sup> CIRAD, UMR CMAEE, F-97490 Sainte-Clotilde, La Réunion, France; INRA, UMR 1309

16 CMAEE, Montpellier, France

17 <sup>5</sup> Virology Unit, Institut Pasteur de Dakar, Dakar, Sénégal.

18

19 Soa Fy Andriamandimby (soafy@pasteur.mg)

20 Cyril Viarouge (cyril.viarouge@anses.fr)

21 Jean-Marc Reynes (jean-marc.reynes@inserm.fr)

22 Jean-Pierre Ravalohery (jpierre@pasteur.mg)

23 Corinne Sailleau (corinne.sailleau@anses.fr)

24 Amadou Alpha Sall (asall@pasteur.sn)

25 Michael Luciano Tantely (lucinambi@pasteur.mg)

26 Nohal Elissa (nohal\_elissa@yahoo.com)

27 Eric Cardinale (eric.cardinale@cirad.fr)

28 Stephan Zientara (szientara@vet-alfort.fr)

29 Jean-Michel Heraud (jmheraud@pasteur.mg)

30

31 **Corresponding author:** Jean-Michel Heraud, Virology Unit, Institut Pasteur de Madagascar,

32 Antananarivo 101, Madagascar. Tel: +261202241272. Email: jmheraud@pasteur.mg

33

#### 34 **Highlights**

35 • Bluetongue virus serotype 2 (BTV-2) circulates in Madagascar.

36 • BTV is endemic and highly prevalent among cattle

37 • Yearly incidence among cattle suggests low pathogenicity of circulating strains

38

39

40 **Abstract**

41 So far, no published data was available concerning the circulation of Bluetongue virus (BTV)  
42 in Madagascar. During a survey on Rift Valley Fever, we were able to detect a virus  
43 belonging to BTV. Therefore, we conducted a study aiming at characterizing molecularly the  
44 BTV isolated and assess the importance of circulation of BTV in Madagascar. A total of  
45 4,393 sera from ruminants selected randomly by stratification and sampled in 30 districts of  
46 Madagascar were tested for BTV. Moreover, 175 cattle were followed during 11 months.  
47 Phylogenetic analyses were performed from virus isolated from unfed pools of mosquitos.  
48 Overall, the estimated mean seroprevalence of infection at the national level was 95.9% (95%  
49 CI: [95.2-96.5]) in cattle and 83.7% (95% CI: [81.4-85.9]) in small ruminants. Estimation of  
50 incidence rate was 54 per 100 cattle-years assuming that the incidence rate is constant all year  
51 along. Phylogenetic analyses revealed that BTV detected belong to serotype 2.  
52 In conclusion, our results showed that BTV is endemic in Madagascar and highly prevalent  
53 among cattle. In our study we did not work on the vector involved in transmission of BTV in  
54 cattle. Thus, research should be conducted to better describe epidemiology of BTV in  
55 Madagascar including vectors and assess economic impact of the disease associated to BTV  
56 infections.

57

58 **Keywords**

59 Bluetongue virus, *Bovidae*, Madagascar.

60

## 61 **Background**

62 Bluetongue disease is a disease of ruminants caused by Bluetongue Virus (BTV). This  
63 arthropod-borne virus is transmitted primarily by biting midges belonging to the *Culicoides*  
64 genus (reviewed in Coetzee 2012) (Coetzee et al., 2012a; Coetzee et al., 2012b). BTV is  
65 circulating widely and its distribution pattern is related to the distribution of its vectors. BTV  
66 is believed to have expanded in recent years as a possible result of climate warming (Purse et  
67 al., 2005; Tabachnick, 2004). Areas like Northern and Western Europe, previously BTV-free  
68 were recently affected by the disease (Wilson and Mellor, 2009). Direct or indirect economic  
69 impacts were important in some areas of the world (Dungu et al., 2004a; Maclachlan, 2010;  
70 MacLachlan and Osburn, 2006; Tabachnick et al., 1996). BTV can cause severe disease in  
71 certain species of sheep, especially European fine wool and mutton breeds; however in  
72 endemic regions, local ruminants do not express clinical disease (Coetzee et al., 2012b). The  
73 clinical signs of BTV infection can vary between species. Sheep may have mainly fever,  
74 serous bloody nasal discharge, edema erosions and ulcers. Cattle and other ruminants may  
75 have ocular discharge, conjunctivitis, oral mucosal congestion, ulceration of muzzle, and teats  
76 (reviewed in Maclachlan, 2009) (Maclachlan et al., 2009). Previous studies showed that  
77 ruminants of African origin are apparently resistant to the infection (Fernandez-Pacheco et al.,  
78 2008; Mauroy et al., 2008). This resistance to BTV might explain why the disease was never  
79 described in Madagascar. In this study, we describe for the first time the detection of BTV  
80 serotype 2. Moreover, serosurvey amongst cattle revealed high seroprevalence of BTV  
81 infection and large distribution of the virus in Madagascar.

## 82 **Methods**

### 83 **Specimen collection**

84 A total of 12,785 adults mosquitoes were collected on April 2009 in Fianarantsoa and  
85 Ambalavao (Central South of Madagascar) during investigations due to Rift Valley Fever

86 outbreaks. Mosquitoes were pooled (23-30) by species and then stored at -80°C. A total of  
87 390 pools containing monospecies of unfed female mosquitoes were grinded and supernatants  
88 were collected for subsequent viral analysis.

89 To address seroprevalence of BTV infection, we used sera from cattle and small ruminants  
90 collected in Madagascar in August 2008 during Rift valley fever outbreak. Sampling methods  
91 were described in Jeanmaire *et al.*, 2011 (Jeanmaire et al., 2011). Briefly, Madagascar was  
92 divided into 10 sampling areas representing the combination of two stratification factors, the  
93 cattle density and the different ecozones. In these 10 sampling areas, 30 districts were  
94 randomly sampled. In each district 33 to 200 animals were sampled. Only animals that were  
95 born and lived in the same sampling area were considered.

96 For estimation of incidence, we included retrospectively sera collected from a cohort of cattle  
97 from 5 and 3 villages located respectively in the Southwestern (Tulear II district) and  
98 Northwestern (Mampikony district) regions of Madagascar (Figure 1A). These two districts  
99 were chosen because of their agro-system differences. Tulear II is a semi-arid ecosystem and  
100 Mampikony has an environment of a dry and deciduous forest. In these study sites, 20 to 30  
101 cattle were sampled and followed up monthly from May 2010 to April 2011. In collaboration  
102 with veterinary service and the farmers, cattle included in this cohort study were firstly  
103 identified and marked using number hooked around their neck. Sera from these cattle were  
104 collected monthly by a study veterinarian and were stored in -80°C until use.

#### 105 **Viral isolation and detection**

106 Virus isolation was performed on mosquito AP61 cell lines and virus identification was firstly  
107 performed by an indirect immunofluorescence assay (IFI) as previously described (Digoutte et  
108 al., 1992; Ratovonjato et al., 2011). One pool containing supernatant of *Anopheles squamosus*  
109 individuals tested positive for BTV and was subsequently analyzed (see viral detection and  
110 analysis). (Ratovonjato et al., 2011).

## 111 **Nucleic acid sample preparation, RT-PCR reactions and sequencing**

112 Total RNA was extracted from 100  $\mu$ L of infected culture cells using the QIAcube robot  
113 (Qiagen, Courtaboeuf, France) and the QIAamp Viral kit (Qiagen, Courtaboeuf, France)  
114 according to the manufacturer's instructions. Finally, the RNAs were eluted with 50  $\mu$ L of  
115 ultrapure water. Reverse transcription (RT) and amplification (PCR) were performed using a  
116 commercial real-time RT-PCR kit (ADI-352, AES) according to the manufacturer's  
117 instructions. For the segment 2 sequencing, RT-PCR were performed using a single tube RT-  
118 PCR method and following the "One-step RT-PCR Kit" protocol (Qiagen, Courtaboeuf,  
119 France). Group-specific primer-pairs derived from the nucleotide sequence data of genome  
120 segment 2 of BTV available in GenBank were used (not described). Amplified RT-PCR  
121 products were sequenced directly, in both directions, using the primer-pairs (Eurofins MWG  
122 Operon, Ebersberg, Germany). Sequences were assembled by SeqMan (DNASTAR programs,  
123 Lasergene) and compared to the homologous sequences available in GenBank. Sequence  
124 alignments were performed using MegAlign-Clustal V method (DNASTAR software).

## 125 **Serological Analysis**

126 Serological tests were performed using LSIVet™ Ruminant Bluetongue Advanced II - Serum  
127 ELISA Kit (LSI, Lissieu, France). This kit which is based on the principle of a blocking  
128 ELISA, has been shown to detect specific antibodies directed against VP7 of all BTV  
129 serotypes without cross reactions against antibodies induced by Epizootic Hemorrhagic  
130 Disease Virus and has a high level of sensitivity and specificity (Niedbalski, 2011).

## 131 **Data analysis**

132 Statistical analysis was performed using R software version 3.1.2 (R Core Team (2014)). Data  
133 were described nationwide and on stratification by sampling zone. Mean seroprevalence of  
134 antibodies directed against BTV was estimated at the national level and by sampling zone  
135 with accompanying 95% confidence interval (CI). Student's t-test was used to compare mean

136 age of two sub population, Chi-square test was used to compare frequency between two or  
137 more sub population with  $p=0.05$  as degree of signification. Odds ratio (OR) were calculates  
138 to quantify effects of age in seroprevalence. Survival analysis was used to assess incidence  
139 rate, and median time from birth to seroconversion for the cohort population of cattle. Event is  
140 defined as ELISA positive during cohort observation. Time to seroconversion is defined as  
141 time from birth to seroconversion. In this study the time point is defined as the end of the  
142 survey. Animal is right censored if at the time point it did not have IgG against BTV or if  
143 survey of cattle did not reach the time point. Animals were left-censored when time to  
144 seroconversion could not be estimated because they were seropositive for BTV (IgG) at the  
145 beginning of the survey.

## 146 **Results**

### 147 **Virus detection and molecular analysis**

148 Molecular analysis of virus isolated the supernatant of *Anopheles squamosus* individuals from  
149 Ambalavao inoculated in mosquito cells (AP61) revealed that genomic segment 2 (encoding  
150 the VP2 serotype-specific protein) of the isolate belonged to the serotype BTV-2 (Figure 2).  
151 The comparison with homologous sequences available in GenBank showed a nucleotide  
152 identity from 79.5 to 99% (data not shown).

### 153 **Serological results**

154 To assess the seroprevalence of BTV, a total of 4,393 samples (3,395 cattle and 998 small  
155 ruminants: sheep and goats) from 30 of the 114 districts of Madagascar were tested.

156 The cattle population had a range of age from 4 months to 19 years, with a median age at 5  
157 years and a mean at 5.5 (123 missing data). Small ruminant had a range of age population  
158 from 6 months to 7 years, with a median age at 2 years and a mean at 2.4 (244 missing data).  
159 The sex ratio (M/F) was 1.37 (26 missing data) and 0.4 (4 missing data) in cattle and small  
160 ruminants respectively. Fifteen percent of cattle were sampled in slaughterhouses, 46.5% on

161 farms, and 6.2% in markets. Most of the small ruminants (97.7%) were sampled in farms.  
162 Overall, 95.9% (95% IC = [95.2-96.5]) of cattle sampled and 83.8% (95% IC = [81.4-85.9])  
163 of small ruminants sampled showed detectable antibodies levels against BTV indicating past  
164 infections. Twenty-one (0.62%) cattle and 19 (1.9%) little ruminants presented doubtful  
165 results. Due to small amount of sera available, we were not able to re-test samples with  
166 doubtful results. In all sampling areas there were animals with antibodies against BTV with a  
167 seroprevalence in animals ranging from 85.2% (one district of zone 10) to 100% (Figure 1B).  
168 The seroprevalence of infection was significantly higher in males compared to females in  
169 cattle (Pearson's Chi-squared test  $p=0.001$ ) but not in small ruminants (Pearson's Chi-squared  
170 test  $p=0.075$ ). Prevalence of antibodies against BTV increased significantly with the age in  
171 cattle with an OR=1.24 (CI 95% [1.15-1.35], Pearson's Chi-squared test  $p<0.0001$ ) but not in  
172 small ruminants (Pearson's Chi-squared test  $p=0.69$ ). The value of OR showed more  
173 important risk depending on age category (Table 1). There were no significant differences in  
174 mean BTV-seroprevalence between animals sampled in farm, slaughterhouse or market.

175 To assess incidence-rate of BTV infection, a cohort of 182 cattle sampled from 2 regions of  
176 Madagascar was included. Age was missing for 7 animals that were then excluded from our  
177 statistical analysis. This cohort had a range of age from 7 to 12 months, with a median age at  
178 8.7 months. Sex-ratio was 0.9 (2 missing data). From this cohort of 175 cattle, 77.7% (n=136)  
179 had already IgG against BTV; 39 cattle remained seronegative at the beginning of the survey.  
180 Mean age of IgG positive population was significantly higher to mean age of remaining at risk  
181 population (Student's t-Test  $p=0.03$ ). Difference of seroprevalence was significantly more  
182 important in Northwestern region than Southern region (Pearson's Chi-squared test  $p<10^{-3}$ ).  
183 No significant differences were observed between sexes concerning seropositivity of BTV in  
184 this cohort. During this longitudinal survey, 1 animal was lost before its seroconversion; and  
185 19 seroconversions were observed. The median time to seroconversion was 9 months (95% CI:

186 [8-9.5]) (Figure 3). Estimation of incidence rate was 89.5 per 100 cattle-year assuming this  
187 incidence rate is constant.

## 188 **Discussion**

189 Our study confirms the circulation of BTV serotype 2 and the widespread distribution of this  
190 virus amongst domestic ruminant from Madagascar. The molecular analysis of genome  
191 segment 2 of the strain isolated in this study showed a high homology in nucleotides (99%)  
192 with the homologous gene from a strain isolated in 2009 in Reunion Island, 800 km to the  
193 East from Madagascar (Sailleau et al., 2012). These data suggest that the same strain has been  
194 circulating on both islands.

195 No clinical manifestations due to BTV infection were reported in Madagascar during the last  
196 decade except a suspicion of a BTV outbreak among cattle in the southern region of  
197 Madagascar in 1999-2000. Unfortunately, this outbreak was not confirmed by laboratory  
198 testing (Herbas J, personal communication). The high seroprevalence of BTV infection and  
199 the estimated incidence are in favor of a silent circulating of BTV in cattle of Madagascar as  
200 observed in other parts of Africa and in all of BTV enzootic-areas (Gibbs and Greiner, 1994;  
201 MacLachlan and Osburn, 2006). The lack of clinical manifestation may be due to this high  
202 seroprevalence. Indeed, Park *et al.*, have demonstrated that areas with intermediate  
203 seroprevalence have high number of cases reporting whereas low case reporting was  
204 associated with a high seroprevalence (Park et al., 2013). Only one unpublished study has  
205 evaluated seroprevalence of BTV infection in small ruminants of Madagascar (Rasamoelina-  
206 Andriamanivo, Dakar: UCAR, 2005). Indeed, this study showed a seroprevalence of 17.7%  
207 (n=92) amongst 520 sheep sampled in the district of Ambatondrazaka in 2005 (East part of  
208 Madagascar). In the same district, three years later, we found a seroprevalence of antibodies  
209 against BTV of 100% (95% CI, [89.6-100]) amongst cattle using the same assay. Although  
210 we cannot exclude a difference in terms of risk of infection between cattle and sheep, the

211 actual seroprevalence observed amongst both species and the clinically described BTV  
212 epidemic in 1999-2000, could be in favor of a hypothesis of a fairly recent introduction in  
213 Madagascar. Nevertheless, due to lack of historical data at the national level, we could not  
214 confirm our hypothesis.

215 Our results indicate that BTV infection increased significantly with age of ruminants  
216 demonstrating an enzootic circulation. We also demonstrate that annual incidence of the  
217 disease was 54 per 100 cattle-year. This incidence could be in favor of many episodes of  
218 circulation per year or a yearly circulation of BTV. Nevertheless, since BTV infection seems  
219 to be silent we could only address the question of seasonality and/or episodes of circulation  
220 with yearly virological surveillance.

221 Our study has some limitation. Indeed, BTV was detected from a pool of mosquitoes which is  
222 uncommon. No BTV wild strain was present in the laboratory at the time of viral isolation and  
223 detection of the Malagasy strain. Thus, a laboratory contamination at this level can be  
224 excluded. Some fresh blood from cattle was perhaps still present in the female mosquitoes  
225 constitutive of the BTV positive pool. Biting midges may have also contaminated mosquito's  
226 pools. Unfortunately, due to lack of specimen we could not look for DNA material from cattle  
227 or midges in the positive pool. Finally, incidence was estimated considering that rate of  
228 infection was constant all year around which might not be the case. A longer survey period  
229 that includes entomological and virological surveillance will be needed to address the  
230 question of seasonality or period of BTV transmission.

### 231 **Conclusions**

232 Our study reports the first BTV isolation in Madagascar. Madagascar is a BTV endemic-area  
233 and we can conclude that at least serotype 2 of BTV have circulated in Madagascar. Farming  
234 ruminants are largely traditional with local breeds. However importation of ameliorated  
235 breeds in dairy farms raises concern about the susceptibility of these animals to BTV

236 circulating in Madagascar. More studies about the incidence, the circulating serotypes, the  
237 vectors and clinical manifestations of BTV in Madagascar should be conducted at a larger  
238 scale to evaluate the associated risk for cattle. Due to high circulation of BTV in Madagascar,  
239 vaccine implementation can prevent infection amongst introduced breeding ruminants to  
240 avoid economic loss. Nevertheless, this implementation should be discussed due to the risk of  
241 recombination between vaccine and circulating strains (Dungu et al., 2004b).

242

### 243 **Acknowledgements**

244 We would like to thank Dr Harena Rasamoelina-Andriamanivo for providing data on cattle.

### 245 **Funding**

246 This study was funded by an internal grant from IP Madagascar. Sera from cohort of cattle  
247 were obtained from study conducted in the frame of AnimalRisk-OI, a research program on  
248 emerging animal diseases in the Indian Ocean, funded by FEDER POCT (European Union,  
249 Regional Council of Reunion and French government). Sera from transversal study were  
250 obtained during survey of Rift valley fever funded by World Health Organization and Food  
251 and Agriculture Organization through the Central Emergency Response Fund of the United  
252 Nations.

### 253 **Competing interests**

254 The authors declare no conflict of interest. None of the authors have financial or personal  
255 conflicts of interest related to this study. The corresponding author has full access to all data  
256 in the study and final responsibility for the decision to submit this publication.

### 257 **Authors' contributions**

258 JMH and SFA executed and coordinated the study, analyzed data and coordinated all process  
259 of writing of this work. CV, JMR, JPR and CS performed viral diagnosis and analysis. AAS  
260 provided reagents and help writing the paper. MLT and NE conducted vector survey and help

261 design the study. EC helped with serological analysis. SZ helped design the study and  
262 participated to the writing.

263

## 264 **References**

- 265 Coetzee, P., Stokstad, M., Venter, E.H., Myrmel, M., Van Vuuren, M., 2012a.  
266 Bluetongue: a historical and epidemiological perspective with the emphasis on  
267 South Africa. *Virology* 9, 198.
- 268 Coetzee, P., Van Vuuren, M., Stokstad, M., Myrmel, M., Venter, E.H., 2012b.  
269 Bluetongue virus genetic and phenotypic diversity: towards identifying the  
270 molecular determinants that influence virulence and transmission potential.  
271 *Veterinary Microbiology* 161, 1-12.
- 272 Digoutte, J.P., Calvo-Wilson, M.A., Mondo, M., Traore-Lamizana, M., Adam, F.,  
273 1992. Continuous cell lines and immune ascitic fluid pools in arbovirus  
274 detection. *Research in virology* 143, 417-422.
- 275 Dungu, B., Gerdes, T., Smit, T., 2004a. The use of vaccination in the control of  
276 bluetongue in southern Africa. *Veterinary Italy* 40, 616-622.
- 277 Dungu, B., Potgieter, C., Von Teichman, B., Smit, T., 2004b. Vaccination in the  
278 control of bluetongue in endemic regions: the South African experience. *Developmental  
279 Biology (Basel)* 119, 463-472.
- 280 Fernandez-Pacheco, P., Fernandez-Pinero, J., Agüero, M., Jimenez-Clavero, M.A.,  
281 2008. Bluetongue virus serotype 1 in wild mouflons in Spain. *Veterinary Record* 162,  
282 659-660.
- 283 Gibbs, E.P., Greiner, E.C., 1994. The epidemiology of bluetongue. *Comparative Immunology  
284 and Microbiology Infectious Diseases* 17, 207-220.
- 285 Jeanmaire, E.M., Rabenarivahiny, R., Biarmann, M., Rabibisoa, L., Ravaomanana,  
286 F., Randriamparany, T., Andriamandimby, S.F., Diaw, C.S., Fenozyara, P., de  
287 La Rocque, S., Reynes, J.M., 2011. Prevalence of Rift Valley fever infection in  
288 ruminants in Madagascar after the 2008 outbreak. *Vector Borne Zoonotic Diseases*  
289 11, 395-402.
- 290 Maclachlan, N.J., 2010. Global implications of the recent emergence of bluetongue  
291 virus in Europe. *Veterinary Clinician North American Food Animal Practice* 26, 163-171, table of  
292 contents.
- 293 Maclachlan, N.J., Drew, C.P., Darpel, K.E., Worwa, G., 2009. The pathology and  
294 pathogenesis of bluetongue. *Journal of Comparative Pathology* 141, 1-16.
- 295 MacLachlan, N.J., Osburn, B.I., 2006. Impact of bluetongue virus infection on the  
296 international movement and trade of ruminants. *Journal of the American Veterinary Medical Association* 228,  
297 1346-1349.
- 298 Mauroy, A., Guyot, H., De Clercq, K., Cassart, D., Thiry, E., Saegerman, C., 2008.  
299 Bluetongue in captive yaks. *Emerging Infectious Diseases* 14, 675-676.
- 300 Niedbalski, W., 2011. Evaluation of commercial ELISA kits for the detection of  
301 antibodies against bluetongue virus. *Polish Journal of Veterinary Science* 14, 615-619.
- 302 Park, A.W., Magori, K., White, B.A., Stallknecht, D.E., 2013. When more transmission  
303 equals less disease: reconciling the disconnect between disease hotspots and  
304 parasite transmission. *PLoS One* 8, e61501.

- 305 Purse, B.V., Mellor, P.S., Rogers, D.J., Samuel, A.R., Mertens, P.P., Baylis, M.,  
306 2005. Climate change and the recent emergence of bluetongue in Europe. *Nat*  
307 *Rev Microbiol* 3, 171-181.
- 308 Ratovonjato, J., Olive, M.M., Tantely, L.M., Andrianaivolambo, L., Tata, E.,  
309 Razainirina, J., Jeanmaire, E., Reynes, J.M., Elissa, N., 2011. Detection,  
310 isolation, and genetic characterization of Rift Valley fever virus from *Anopheles*  
311 (*Anopheles*) *coustani*, *Anopheles* (*Anopheles*) *squamosus*, and *Culex* (*Culex*)  
312 *antennatus* of the Haute Matsiatra region, Madagascar. *Vector Borne Zoonotic*  
313 *Dis* 11, 753-759.
- 314 Sailleau, C., Zanella, G., Breard, E., Viarouge, C., Desprat, A., Vitour, D., Adam, M.,  
315 Lasne, L., Martrenchar, A., Bakkali-Kassimi, L., Costes, L., Zientara, S., 2012.  
316 Co-circulation of bluetongue and epizootic haemorrhagic disease viruses in  
317 cattle in Reunion Island. *Veterinary microbiology* 155, 191-197.
- 318 Tabachnick, W.J., 2004. *Culicoides* and the global epidemiology of bluetongue virus  
319 infection. *Vet Ital* 40, 144-150.
- 320 Tabachnick, W.J., MacLachlan, N.J., Thompson, L.H., Hunt, G.J., Patton, J.F., 1996.  
321 Susceptibility of *Culicoides variipennis sonorensis* to infection by polymerase  
322 chain reaction-detectable bluetongue virus in cattle blood. *Am J Trop Med Hyg*  
323 54, 481-485.
- 324 Wilson, A.J., Mellor, P.S., 2009. Bluetongue in Europe: past, present and future.  
325 *Philos Trans R Soc Lond B Biol Sci* 364, 2669-2681.  
326  
327

328 **Table 1.** Relation between age group and seroprevalence of Bluetongue virus infection in  
329 cattle population, Madagascar, 2008 survey.

330

331 **Figure 1.** Seroprevalence of Bluetongue virus infection, Madagascar 2008-2009. **A.** Sampled  
332 area. Longitudinal study area: Tulear II in the Southern region (3 sites) and Mampikony in the  
333 Northern Region (2 sites). Sample district area: 30 districts sampled for the survey. **B.**  
334 Seroprevalence of BTV in sampled area. White areas represent districts where no sample was  
335 collected.

336

337 **Figure 2.** Phylogenetic tree based on the full-length segment 2 sequences from BTV-2 strains  
338 selected in GenBank (clustal analysis)

339

340 **Figure 3.** Estimation of survival parameters during longitudinal survey, Madagascar 2010-  
341 2011. Kaplan-Meier curve.

**Table 1.** Relation between age group and prevalence of Bluetongue virus infection in cattle population, Madagascar, 2008 survey.

Age group (years)	OR	p-value*	95% CI**
≤5***	1		
]5-10]	2.5	0.000	[1.6-3.9]
]10-20]	9.5	0.025	[1.3-68.4]

\*p-value: Fisher's exact test (univariate analysis), OR: univariate analysis

\*\*95% CI: Confidence interval of OR

\*\*\*≤5: reference category

Figure1A  
[Click here to download high resolution image](#)

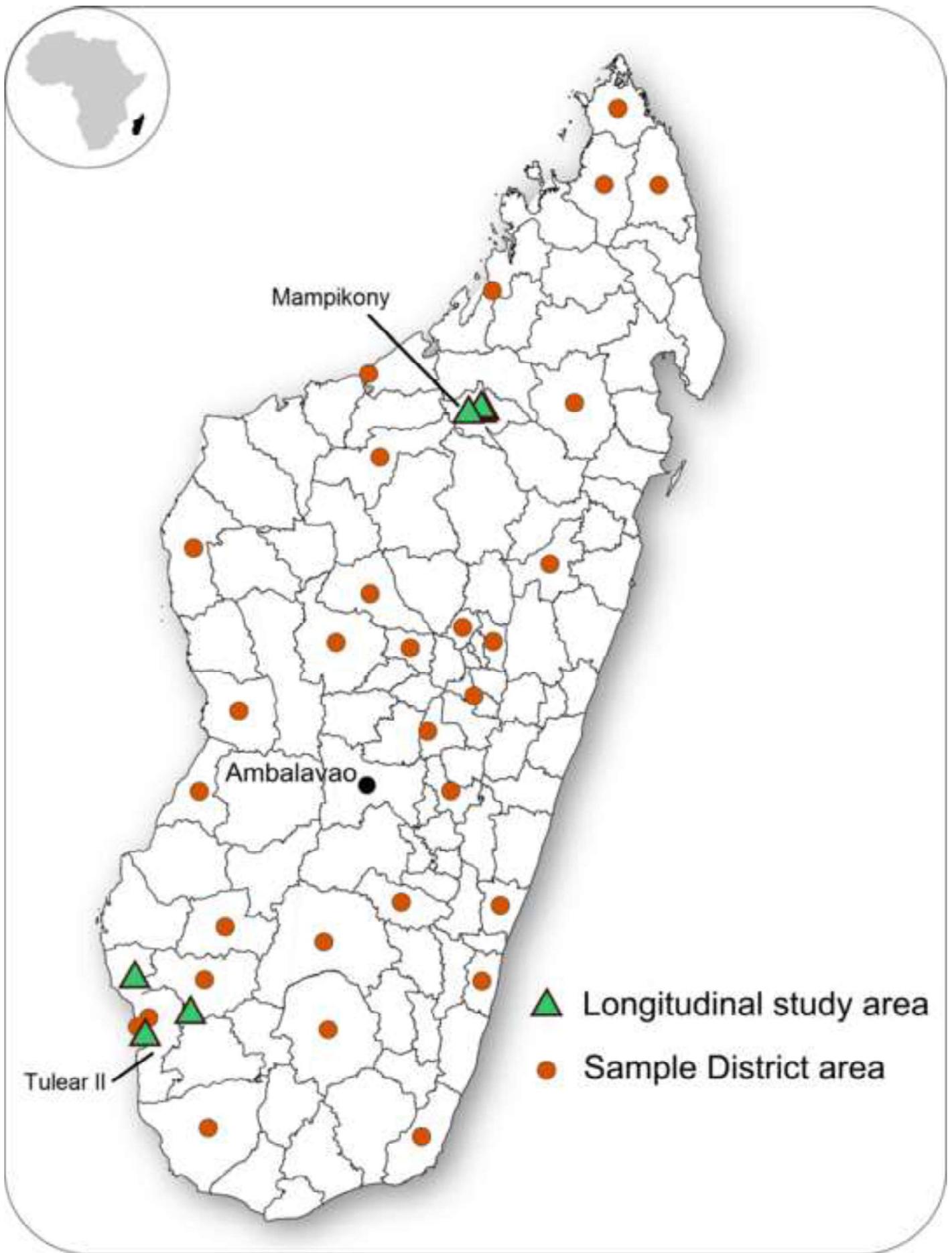


Figure1B  
[Click here to download high resolution image](#)

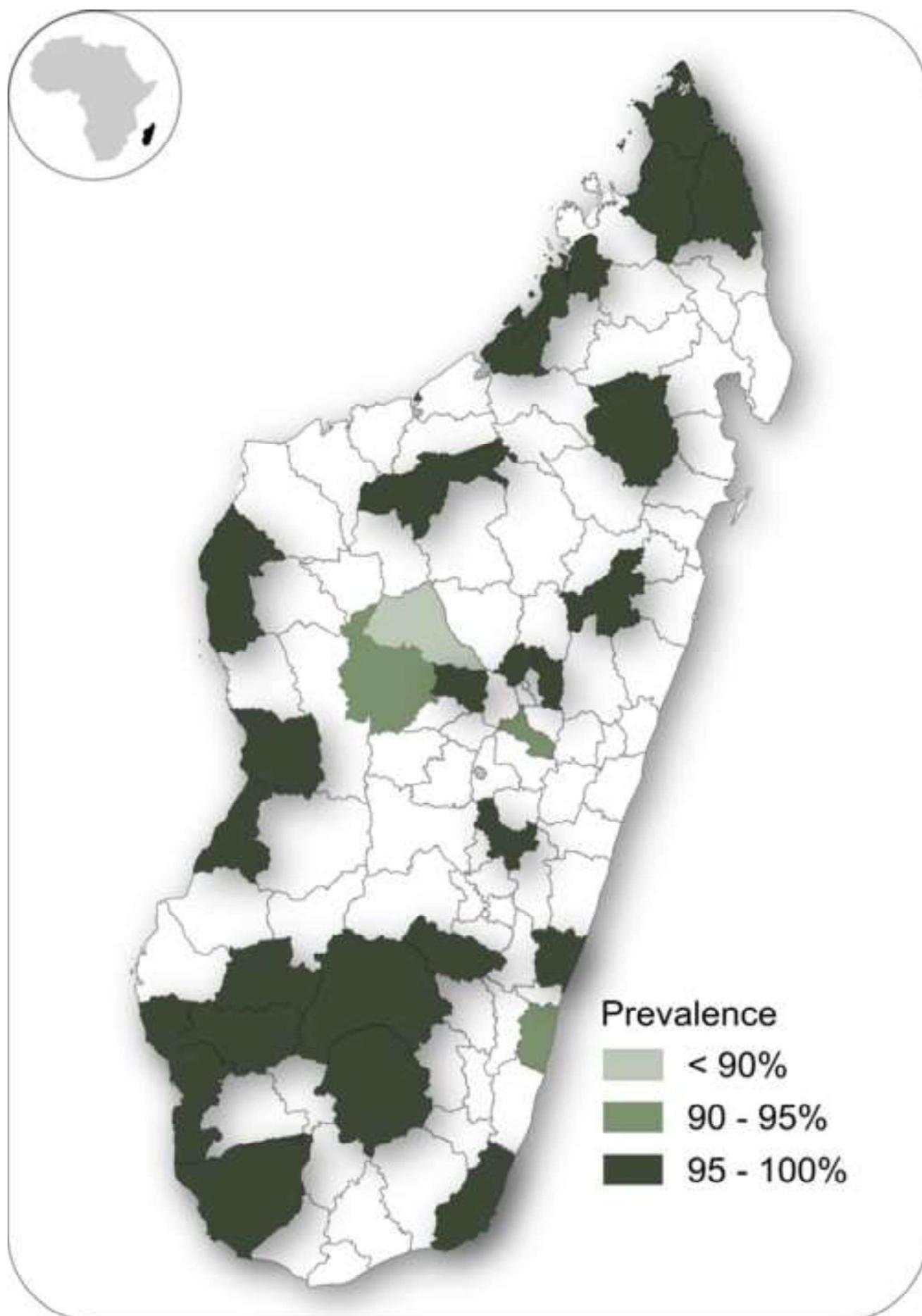


Figure 2

[Click here to download high resolution image](#)

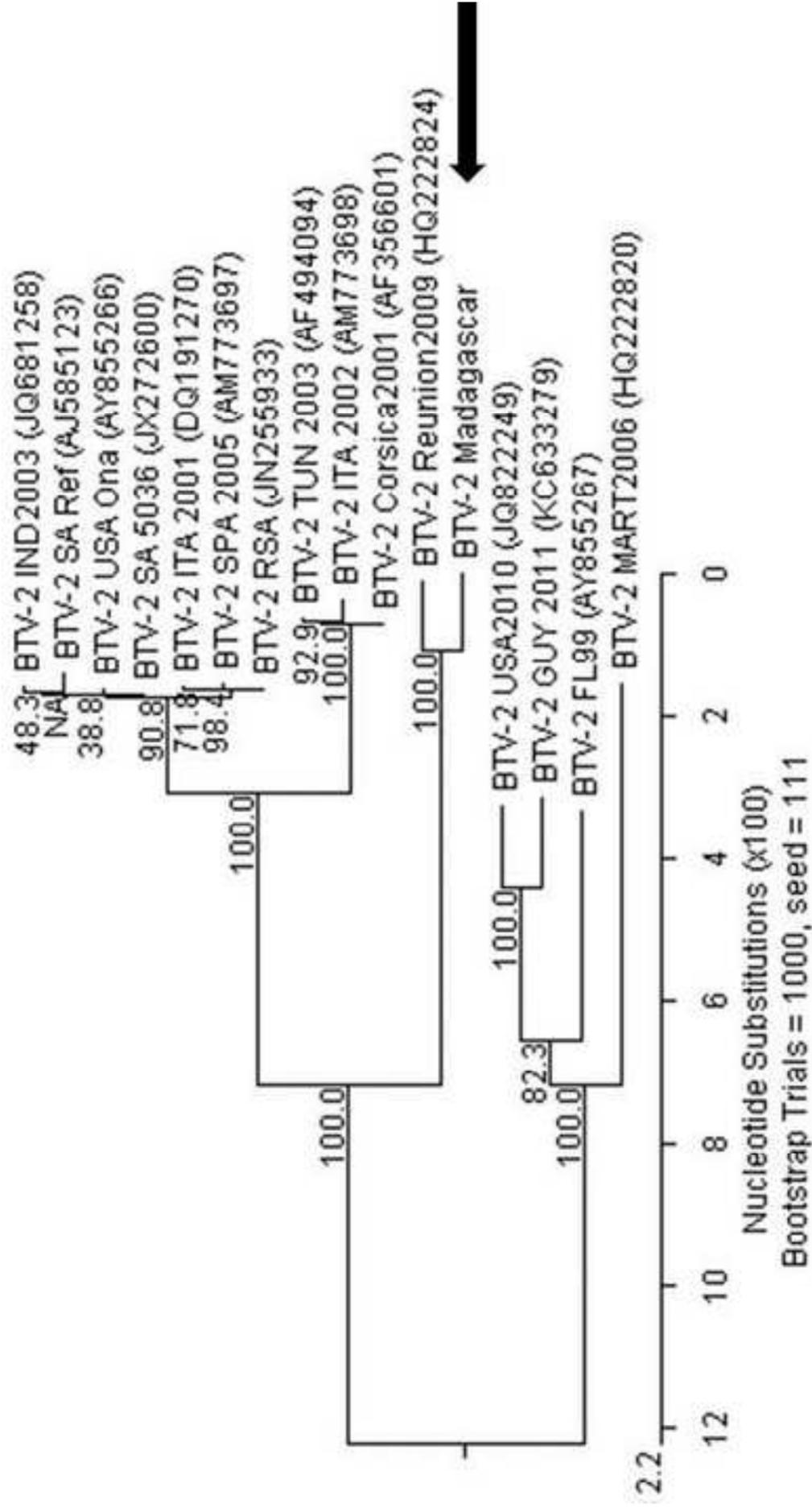


Figure3  
[Click here to download high resolution image](#)

