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Molecular detection of *Bartonella* species and haemoplasmas in wild African buffalo (*Syncerus caffer*) in Mozambique, Africa

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Research Article

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Abstract

The African buffalo (*Syncerus caffer*), a mammal species whose population is declining, can play a role as a reservoir or carrier of a wide number of arthropod-borne pathogens. Translocation procedures have been used as an alternative approach for species conservation. However, the veterinary aspects of this sort of procedures are extremely important to minimize the impact on animal health. In order to detect *Bartonella* and haemoplasmas, two important group of bacterial that have an impact in both human and animal health, EDTA whole-blood samples were screened for the presence of these bacterial pathogens by molecular techniques. As a result, a molecular occurrence of 4.1 and 15.4% for *Bartonella* spp. and haemoplasmas, respectively, was reported among 97 wild buffaloes sampled during a translocation procedure from Marromeu to Gorongosa Reserve, Mozambique. Additionally, phylogenetic analyses of the obtained sequences were conducted. At least, three bovine-associated pathogens, namely *B. bovis*, *M. wenyonii* and '*Candidatus* M. haemobos', as well as a probably new *Bartonella* genotype/species were detected in *S. caffer*. Further studies are needed in order to determine whether these bacterial species may cause impact in buffaloes and other sympatric ruminant species living in the release site.

Introduction

The African buffalo (*Syncerus caffer*) plays a role as a reservoir or carrier of a wide number of arthropod-borne pathogens, such as *Theileria*, *Ehrlichia*, *Babesia* and *Anaplasma* species (Andrew and Norval, 1989; Allsopp *et al.*, 1999; Eygelaar *et al.*, 2015; Machado *et al.*, 2016). Moreover, buffaloes are known to harbour other economically important infectious pathogens, such as *Mycobacterium tuberculosis*, foot-and-mouth disease virus and *Brucella abortus* (Godfroid, 2002; Michel *et al.*, 2006; Van Schalkwyk *et al.*, 2016). Although widely distributed throughout sub-Saharan Africa, the African buffaloes are currently confined to protected areas. The species distribution and numbers have been strongly reduced by habitat loss and hunting (IUCN – Downloaded on 29 July 2016).

Many native and exotic animal species have been selected for translocations procedures around the world (Seddon *et al.*, 2014; Soorae, 2016). The translocation is defined as the human-mediated movement of living organisms from one area to another (Woodford and Rossiter, 1993). Although the translocation of endangered species has become an important conservation approach, the project success depends to a large extent on the care with which wildlife biologists and veterinarians evaluate the suitability of the chosen release site (Woodford and Rossiter, 1993). Adequate managements are extremely important for the translocation procedures since the introduction of pathogens into naive resident wildlife community or the translocation of animals from free-vectors and pathogens sites to enzootic areas can be catastrophic.

In this context, *Bartonella* species and haemotropic mycoplasmas (also known as haemoplasmas) emerge as important arthropod-borne pathogens that have an impact in humans and animals' health (Maggi *et al.*, 2013*a*; Breitschwerdt, 2014).

The *Bartonella* genus comprises a successful group of Gram-negative bacteria parasites (Birtles, 2005), which infects mainly erythrocytes and endothelial cells from a wide range of animal species, including humans (Breitschwerdt *et al.*, 2010; Harms and Dehio, 2012). This success is characterized by the high prevalence of the infection and diversity of host species (Birtles, 2005; Kosoy *et al.*, 2012). Currently, five (*B. bovis*, *B. chomelii*, *B. schoenbuchensis*, *B. capreoli* and *B. melophagi*) out of the 36 named *Bartonella* species/subspecies described have been associated with ruminants (Buffet *et al.*, 2013; Breitschwerdt, 2017).

Contrariwise, haemoplasmas are cell wall-less uncultivated epicellular bacteria that attach to red blood cells surface of a wide range of animals, including humans (Neimark *et al.*, 2001; Maggi *et al.*, 2013*b*). Among the haemoplasmas, *Mycoplasma ovis*, *Mycoplasma wenyonii* and '*Candidatus* Mycoplasma haemobos' have been recognized as pathogens of domestic

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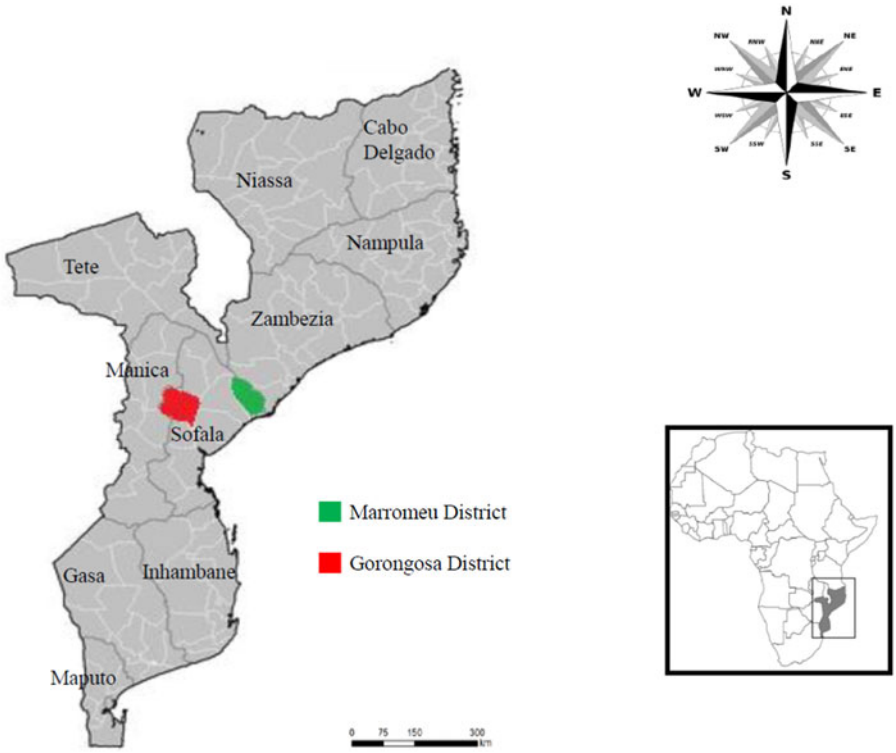

Fig. 1. Map of Mozambique country highlighting the districts where the buffalo were translocated.

ruminants worldwide. In addition, an expanding number of *Candidatus* to new *Bartonella* and *Mycoplasma* species/genotypes have globally been reported in ruminants (Stoffregen *et al.*, 2006; Watanabe *et al.*, 2010; Sato *et al.*, 2012; Maggi *et al.*, 2013*b*; Dahmani *et al.*, 2017).

Although the impact of bartonellae and haemoplasmas in livestock is still unknown, the gaps in the biology of these bacteria warrant further investigation. Indeed, there are limited data on the occurrence, distribution, genetic diversity, pathogenicity and transmission of arthropod-borne agents among wild ruminants.

The elucidation of these bacterial cycles in nature, including the identification of hosts, vectors and the species distribution in a particular ecotope shows great importance (Gutiérrez *et al.*, 2014). Additionally, the African buffalo plays a role as reservoirs for vector-borne pathogens and may represent a serious threat to the livestock industry (Andrew and Norval, 1989; Allsopp *et al.*, 1999; Eygelaar *et al.*, 2015; Van Schalkwyk *et al.*, 2016).

Therefore, the present study aimed to investigate the occurrence of *Bartonella* and haemoplasmas infection in wild buffaloes (*S. caffer*) submitted to translocation in Sofala province, Mozambique, Africa.

Material and methods

Blood collection of African buffalo

The present study was carried out when Carlos L. Pereira was the Director of Conservation Gorongosa National Park, Mozambique. Animal management and welfare during the fieldwork with Cape buffaloes were conducted in accordance with national legislation on the use of animals for research implemented by the National Administration for the Conservation Areas (ANAC) of Mozambique. In 2011, blood samples were collected from 97 wild African buffalo (*S. caffer*) in Marromeu Reserve, Mozambique. This reserve is a special buffalo protection area located in the Marromeu district (Sofala Province) (Fig. 1), with an area of 1.500 km^2 (http://www.jenmansafaris.com). Sampled animals were apparently healthy young male and female individuals. Approximately 10 mL of blood samples were collected of each animal before they were transferred from Marromeu Reserve (the site where there was contact with cattle) to the Gorongosa National Park (the site where there was no contact with cattle), distant around 300 kilometers from each other. The EDTA–blood were mixed (v/v) with ethanol, transported to the laboratory and posterior kept at −20 °C until sent an aliquot (~1 mL) to Brazil (Machado *et al.*, 2016; Rodrigues *et al.*, 2017).

Blood samples and DNA extraction

EDTA–blood samples collected from buffaloes were mixed (v/v) with ethanol for further DNA extraction. In Brazil, the blood samples from these buffaloes were incubated in a lysis buffer (1% SDS, 100 mM EDTA at pH 8.0, 20 mM Tris–HCl at pH 8.0 and 350 mg mL^{-1} of proteinase K) at 37 °C for 18 h and centrifuged at 14.000***g*** for 5 min. The DNA was purified using Wizard Purification Systems (Promega). The concentration of each DNA sample was determined in a NanoDrop 2000c spectrophotometer (Thermo Scientific, San Jose, CA, USA) (Machado *et al.*, 2016).

Molecular diagnosis of Bartonella *and haemoplasmas species in African buffalo blood samples*

A previously described broad range Taqman quantitative real-time PCR (qPCR) protocol based on *nuoG* gene was used aiming to detect *Bartonella* species DNA as previously described (André *et al.*, 2016). The qPCR amplifications were conducted in low-profile multiplate unskirted PCR plates (BioRad, CA, USA) using a CFX96 Thermal Cycler (BioRad, CA, USA). Serial dilutions were performed aiming to construct standard curves with different concentrations of plasmid DNA (pIDTSMART – Integrated DNA Technologies) (2.0×10^7–2.0×10^0 copies μL^{-1}) encoding an 83 bp insert of *nuoG Bartonella henselae* (André *et al.*, 2016). qPCR assays were performed including duplicates of each buffalo DNA sample. All the duplicates showing a difference in Cq values higher than 0.5 were retested. Amplification

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efficiency (E) was calculated from the slope of the standard curve in each run using the following formula ($E = 10^{-1/\text{slope}}$).

Additionally, two specific conventional PCR (cPCR) protocols based on 16S rRNA were used to amplify *M. wenyonii* (~530 bp) and '*Candidatus* M. haemobos' DNA (279 bp) as previously described (Nishizawa *et al.*, 2010; Su *et al.*, 2010). *Bartonella* (KX086714), '*Candidatus* M. haemobos' (KY328834) and *M. wenyonii* (KY328836) DNA samples previously obtained from naturally infected rodents and buffaloes (Gonçalves *et al.*, 2016; Santos *et al.*, 2018), respectively, were used as positive controls. Ultra-pure sterile water and blood samples from calves previously tested negative for both pathogens were used as negative controls in all PCR assays described above. In order to prevent PCR contamination, DNA extraction, reaction setup, PCR amplification and electrophoresis were performed in separated rooms.

Molecular characterization of Bartonella *and haemoplasma species*

Samples from qPCR assays-positive buffalo were submitted to cPCR assays targeting four protein-coding genes with great potential to differentiation among related *Bartonella* species (La Scola *et al.*, 2003; Kosoy *et al.*, 2017), namely *gltA* (350 bp), *rpoB* (825 bp), *ftsZ* (515 bp) and *groEL* (752 bp) genes, as previously described (Norman *et al.*, 1995; Birtles and Raoult, 1996; Renesto *et al.*, 2001; Zeaiter *et al.*, 2002; Paziewska *et al.*, 2011). *Bartonella* DNA previously detected in rodents was used as positive control (Gonçalves *et al.*, 2016). On the other hand, in order to better characterize the initial PCR assay results, haemoplasma-positive buffalo samples were additionally submitted to a cPCR assay targeting a 16S rRNA larger fragment (~800 bp), as previously described (Maggi *et al.*, 2013*b*). *Mycoplasma haemofelis* DNA previously detected in cats in Brazil was used as positive control (Santis *et al.*, 2014).

All cPCR products were purified using Silica Bead DNA Gel Extraction Kit (Fermentas, SP, Brazil). Purified amplified DNA fragments were submitted to sequence confirmation in an automatic sequencer (ABI Prism 310 Genetic Analyser – Applied Byosystem/Perkin Elmer) in both directions using the same primers used for PCR detection. In order to correctly determine the nucleotide composition, the electropherograms were submitted to PhredPhrap analysis (Ewing *et al.*, 1998). The Phred quality score (peaks around each base call) was established higher than 20 (99% in the accuracy of the base call). Subsequently, the sequences were submitted to BLASTn and phylogenetic analyses.

Phylogenetic analyses of gltA, ftsZ *and 16S rDNA sequences*

The sequences obtained from *gltA* and *ftsZ Bartonella* and 16S rRNA haemoplasmas cPCR assays were identified by BLASTn, using the Megablast (highly similar sequences – using default parameters). Subsequently, obtained sequences were aligned with those retrieved from GenBank database using Clustal/W (Thompson *et al.*, 1994), adjusted in Bioedit v. 7.0.5.3 (Hall, 1999), and submitted to phylogenetic analysis. The maximum likelihood (ML) phylogenetic analysis was inferred with RAxML-HPC BlackBox 7.6.3 (Stamatakis *et al.*, 2008). Also, in order to perform a robust phylogenetic analysis among the protein-coding gene sequences, the *Bartonella gltA* and *ftsZ* nucleotide sequences were submitted to other two methods. Phylogenetic analysis based on Bayesian inference (BI) was done using MrBayes on XSEDE (v. 3.2.6) (the *a posteriori* probability values higher than 50% were accessed with 10^6 replicates; the first 25% trees were discarded as burn-in). Finally, these sequences were analysed by neighbour-joining (NJ) using the MEGA5.05 software. The AIC (Akaike information criterion) available on MEGA 5.05 software was applied to identify the most appropriate model of nucleotide substitution.

Results

Occurrence and molecular characterization of Bartonella *and haemoplasma species in African buffalo*

Out of 97 African buffalo blood samples submitted to *Bartonella*-qPCR, four (4.1%) were positive. The mean amplification efficiency was $E = 90.7\%$ [(ranging from 90.5 to 90.9%); slope = −3.568; $r^2 = 0.996$]. All blood samples showed low number of *Bartonella*-DNA copies μL^{-1} (#45 = 0.046×10^2; #50 = 0.041×10^2; #62 = 0.037×10^2; and #44 = 0.002×10^2 copies μL^{-1}). Among the four blood samples positive to *Bartonella* in qPCR assay, only the sample #62 showed to be positive in *gltA* and *ftsZ* cPCR assays. None blood samples showed positive results in cPCR assays targeting *rpoB* and *groEL* genes. The *gltA* nucleotide sequence amplified in the present study shared 99% identity with *B. bovis* (KF199897) detected in cattle from Guatemala (Bai *et al.*, 2013). On the other hand, the obtained *ftsZ* nucleotide sequence shared 98% identity with *Bartonella* sp. (AB703117) previously detected in a Japanese Sika deer (*Cervus nippon centralis*) (Sato *et al.*, 2012).

Additionally, 15.4% (15/97) samples were positive to haemoplasmas. Among them, 14.4% (14/97) and 4.1% (4/97) showed positive results to *M. wenyonii*, and '*Candidatus* M. haemobos', respectively. Four (4.1%) samples were simultaneously positive to *M. wenyonii* and '*Candidatus* M. haemobos'. The two haemoplasmas amplicons sequenced shared 99 and 99% identity with *M. wenyonii* (KX171205) and '*Candidatus* M. haemobos' (EF616468) nucleotide sequences detected in cattle from Mexico and Switzerland, respectively. None sample was simultaneously positive to *Bartonella* and haemoplasmas. The 16S rDNA sequences amplified in the present study showed query coverage ranging from 99 to 100%. All nucleotide sequences submitted to BLASTn and phylogenetic analyses were deposited in GenBank under the following access numbers: *Bartonella* (MF774324–MF774325) and haemoplasmas (MF981847 and MF992084).

Phylogenetic analysis

The phylogenetic analyses performed among the protein-coding gene sequences (*gltA* and *ftsZ*), using different methods (ML, MI and NJ) yielded congruent tree topologies (Figs 2, 3 and S1–S4). Additionally, according to BLASTn analysis, the *Bartonella gltA* and *ftsZ* sequences amplified from the same animal (#62) showed a distinct phylogenetic positioning in ML, BI and NJ analyses (Figs 2, 3 and S1–S4). The *gltA* sequence (MF774324), which shared 99% identity with *B. bovis*, clustered with other *B. bovis* sequences, including *B. bovis* sequences previously amplified in water buffalo from Thailand (Bai *et al.*, 2013), showing high support index (ranging from 97 to 100%) (Fig. 2 and S1, S2). On the other hand, although the *ftsZ* sequence (MF774325) clustered with other ruminant-associated *Bartonella* sequences, it was not closely related to any of these sequences, remaining in a separate branch in all different methods and supported by a high support index (ranging from 80 to 100%) (Fig. 3 and S3, S4).

Also in agreement to BLASTn analysis, the 16S rRNA sequences (MF981847 and MF992084) belonging to *M. wenyonii* and to '*Candidatus* M. haemobos' when submitted to ML analysis were phylogenetically positioned near to other *M. wenyonii* and '*Candidatus* M. haemobos' sequences, respectively, detected around the world and supported by high bootstrap values (87 and 100%, respectively) (Fig. 4).

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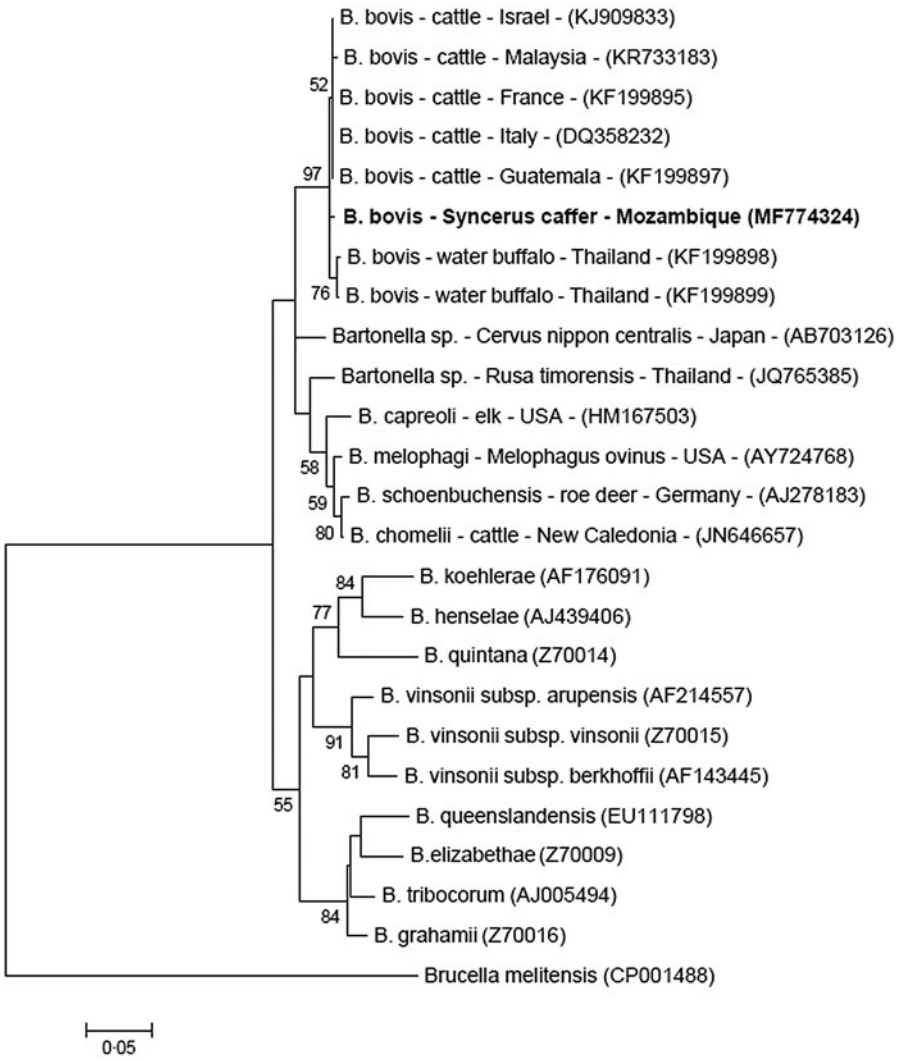

Fig. 2. Phylogenetic relationships within the *Bartonella* genus based on the *gltA* gene. The tree was inferred by using the maximum likelihood (ML) method with the GTR + G + I model. The sequences detected in the present study are highlighted in bold. The numbers at the nodes correspond to bootstrap values higher than 50% accessed with 1000 replicates. *Brucella melitensis* was used as an outgroup.

Discussion

In the present study, the occurrence of *Bartonella* and haemoplasmas species was assessed in African buffaloes translocated from Marromeu to Gorongosa National Park in Mozambique. Although *Bartonella* and/or haemoplasmas species have been previously reported in domestic animals (dogs, cats and cattle) (Gundi *et al.*, 2004; Willi *et al.*, 2006; Dahmani *et al.*, 2017), wild animals (rodents, bats and cheetahs) (Kosoy *et al.*, 2010; Kamani *et al.*, 2013; Krengel *et al.*, 2013) and haematophagous arthropods (soft ticks and bat flies) (Billeter *et al.*, 2012; Mediannikov *et al.*, 2014) from African continent, the present work presented, for the first time, the occurrence of these arthropod–bacteria species in *S. caffer*.

Similarly to the occurrence of *Bartonella* in water buffalo [*Bubalus bubalis* (6.8%; 7/103)] from Thailand (Bai *et al.*, 2013), a low occurrence was observed among the animals analysed in the present study (4.1%; 4/97). Additionally, the prevalence of haemoplasmas reported in the African buffaloes was lower than that previously reported in buffaloes (32%; 8/25) from China (Su *et al.*, 2010). However, it is important to highlight that the animals selected in the latter study were showing different clinical signs, such as emaciation, anorexia and decreased milk yields (Su *et al.*, 2010). In addition to animal health status, the difference in the occurrence of *Bartonella* and haemoplasmas observed in different countries have been attributed to different factors, such as distribution and abundance of arthropod vectors, and environment and landscape features, which could influence the exposure to these agents (Bai *et al.*, 2013).

Syncerus caffer is frequently infested by species of *Hyalomma*, *Rhipicephalus* and *Amblyomma* ticks (Carmichael, 1976; Anderson *et al.*, 2012; Kariuki *et al.*, 2012). These ticks are responsible for transmission of several pathogens frequently reported in African buffalo, such as *Theileria* spp., *Anaplasma* spp., *Ehrlichia* spp. and *Babesia* spp. (Andrew and Norval, 1989; Eygelaar *et al.*, 2015; Machado *et al.*, 2016). Therefore, it suggests that *Bartonella* and haemoplasma species detected in *S. caffer* in the present study may be potentially transmitted by these tick species. In addition to possible role of ticks in the transmission of *Bartonella*, several studies have reported the isolation or molecular detection of *Bartonella* species in other blood-sucking arthropods associated with wild and domestic ruminants (Chung *et al.*, 2004; Dehio *et al.*, 2004; Halos *et al.*, 2004; Duodu *et al.*, 2013; Gutiérrez *et al.*, 2014). Regarding the haemoplasma transmission, few studies have accessed the mechanisms of transmission by arthropod vectors. Prullage *et al.* (1993) demonstrated the mechanical transmission of *Mycoplasma suis* by *Stomoxys calcitrans* and *Aedes aegyptii* among susceptible splenectomized pigs. Woods *et al.* (2005) showed that *Ctenocephalides felis* is a possible vector of *M. haemofelis* and *'Candidatus* M. haemominutum' among cats. Additionally, bovine-associated *Mycoplasma* species were molecularly detected in ticks belonging to *Dermacentor andersoni* (Neimark *et al.*, 2001), *Rhipicephalus (Boophilus)*

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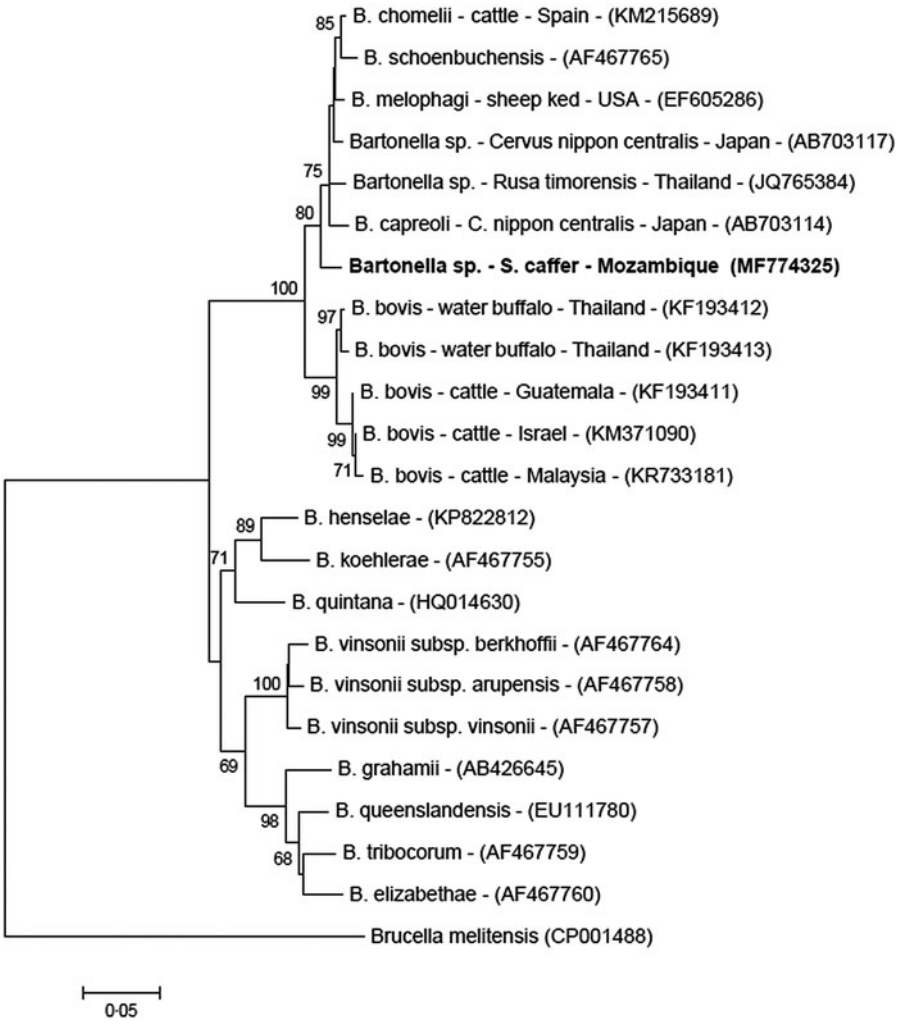

Fig. 3. Phylogenetic relationships within the *Bartonella* genus based on the *ftsZ* gene. The tree was inferred by using the maximum likelihood (ML) method with the GTR + G model. The sequences detected in the present study are highlighted in bold. The numbers at the nodes correspond to bootstrap values higher than 50% accessed with 1000 replicates. *Brucella melitensis* was used as an outgroup.

microplus and *Haemaphysalis bispinosa* species (Mohd Hasan *et al.*, 2017), among horn (*Haematobia irritans*), stable (*S. calcitrans*), horse (*Tabanus* spp.) (Hornok *et al.*, 2011) and house (*Musca domestica*) flies, as well as in lice (*Haematopinus eurysternus*) (Hofmann-Lehmann *et al.*, 2004). These findings suggested that these arthropods may have an active role in the maintenance and transmission of ruminants-associated *Bartonella* and haemoplasma species. However, experimental studies aiming to analyse the vectorial competence of selected arthropod species are much needed.

In contrast to the phylogenetic analysis (ML) performed with haemoplasmas sequences amplified from *S. caffer* blood samples, which clustered with other *M. wenyonii* and '*Candidatus* M. haemobos' sequences detected around the world, the *Bartonella gltA* and *ftsZ* sequences detected in the same animal (#62) were positioned in different branches in all different methods analysed (ML, BI and NJ). This uncertainty about the phylogenetic positioning could be explained by two hypotheses. Firstly, there would have been a coinfection with different *Bartonella* species/genotypes (*B. bovis* identified by *gltA* sequence, and a new *Bartonella* genotype closely related to other ruminant-associated *Bartonella* identified by *ftsZ* sequence). Alternatively, the amplified sequences might have represented an infection with a genotype that went through recombinant events. Indeed, the latter phenomena have been already reported in *Bartonella* species from wild rodents (Harrus *et al.*, 2009; Paziewska *et al.*, 2011), cattle (Gutiérrez *et al.*, 2014) and bats (Bai *et al.*, 2015). These distinct possibilities reinforce the great challenge on *Bartonella* identification based on direct molecular detection in blood, tissue or ectoparasite samples (Gutiérrez *et al.*, 2014; Kosoy *et al.*, 2017). Although the multiple loci sequencing approach was attempted in the present study for a better understanding of sequences phylogenetic positioning and possible recombinant events and/or infection by multiple *Bartonella* species (Kosoy *et al.*, 2017), the unique positive sample (#62) in cPCR assays was negative in PCR targeting additional genes (*rpoB* and *groEL*), thus precluding to solve this issue. Additional culturing of isolates would have benefited the differentiation of these genotypes/species (Kosoy *et al.*, 2017).

Considering that buffaloes usually do not show clinical signs of tick-borne diseases, a surveillance on this animal species is much needed since they act as carriers for arthropod-borne pathogens to the livestock (Andrew and Norval, 1989; Allsopp *et al.*, 1999; Eygelaar *et al.*, 2015; Van Schalkwyk *et al.*, 2016). Furthermore, since these animals can migrate over large distances, they could spread different pathogens for susceptible wildlife. In the present study, at least three bovine-associated pathogens, namely *B. bovis*, *M. wenyonii* and '*Candidatus* M. haemobos' were reported in *S. caffer*. Regarding the pathogenic potential of the above-mentioned agents, while *B. bovis* have been associated with bovine endocarditis (Mailard *et al.*, 2007; Erol *et al.*, 2013), bovine haemoplasmas species have been associated with anaemia, transient fever, decreased milk production, anorexia, weight loss and infertility (Smith *et al.*, 1990; Su *et al.*, 2010; Hoelzle *et al.*, 2011).

In the Marromeu Reserve, the site where animals were caught, buffaloes and cattle used to share the same area. Although little is known about the origin, evolution and dispersion of *Bartonella* and haemoplasmas species, the low prevalence detected in

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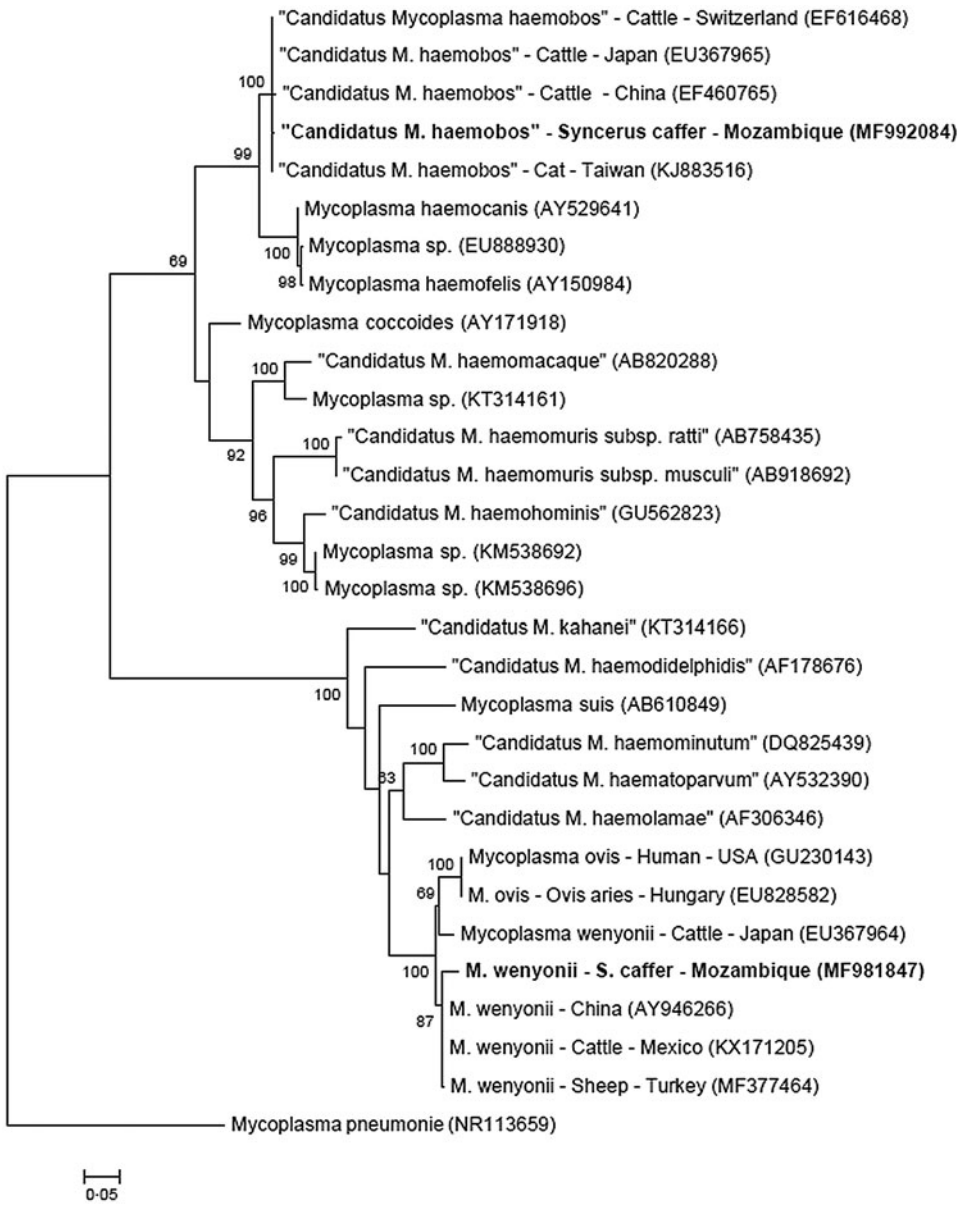

Fig. 4. Phylogenetic relationships within the *Mycoplasma* genus based on a fragment of 800 bp of the 16S rRNA gene. The phylogenetic tree was inferred by using the maximum likelihood method with the GTR + G + I model. The sequences detected in the present study are highlighted in bold. The numbers at the nodes correspond to bootstrap values higher than 60% accessed with 1000 replicates. *Mycoplasma pneumoniae* was used as an outgroup.

African buffaloes, mainly to *Bartonella*, coupled to a wide distribution and the constant detection of these bacterial in cattle around the world, suggest that these pathogens may have been transmitted *via* arthropod-vectors from cattle to buffaloes. On the other hand, there is no contact among cattle and wildlife in Gorongosa National Park, the site where the animals were released. Therefore, the sylvatic animals residing in this area, mainly other ruminant species may be exposed to *B. bovis, M. wenyonii*, '*Candidatus* M. haemobos' and *Anaplasma* spp., as previously reported (Machado *et al.*, 2016). Although it is difficult to assess the impact of these infections in such population, this situation can be catastrophic to immunologically naïve wildlife (Woodford and Rossiter, 1993).

Even though translocation procedures have intended conservation benefits, this attempt may also impose risks to animal health (IUCN/SSC, 2013). During management procedures, it is important to consider that a translocated animal does not represent a single species, but is rather a biological package containing a selection of viruses, bacteria, protozoa, helminths and arthropods. Different reports have showed the complexity of the translocation procedures, either by diseases introduced through the animals or diseases contracted from the resident animals at the release site (reviewed by Woodford and Rossiter, 1993). Therefore, the veterinary aspects, prior, during and after translocation procedures are extremely important in order to minimize the impact on animal health. Indeed, few studies have been conducted regarding arthropod-borne pathogens in the capture (Marromeu Reserve) and the release (Gorongosa National Park) sites. For instance, Rift Valley fever phlebovirus was detected in buffaloes and cattle sampled in both places between the years of 2013 and 2014 (Moiane *et al.*, 2017). Recently, *Trypanosoma vivax* and *T. vivax*-like DNA was amplified in buffaloes and tsetse flies (*Glossina* spp.) captured in Gorongosa between the years of between 2007 and 2014 (Rodrigues *et al.*, 2017). These findings reinforce the need for further studies in order to better assess the impact of the translocation process on resident fauna.

Although in the present study we were unable to solve the issue regarding the possible occurrence of co-infection or recombination events by distinct *Bartonella* species, future studies using cloning of amplicons prior to sequencing and the isolation of bartonellae in blood or chocolate agar may help solving this problem, improving the molecular identification and characterization of this important bacterial group in wild ruminants.

In summary, our study showed the molecular occurrence of *B. bovis*, a possible new *Bartonella* genotypes/species or a genotype

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resulted from recombination events, *M. wenyonii* and '*Candidatus* M. haemobos' in African buffaloes submitted to translocation from Marromeu to Gorongosa National Park. Additionally, African buffaloes and other sympatric ruminants living in the release site (Gorongosa National Park) should be evaluated in the future in order to assess the impact of these pathogens in the ecosystem.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/pao.2018.10.

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Conflicts of interest

None.

Ethical standards

Not applicable.

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