Investigation of the piroplasm diversity circulating in wildlife and cattle of the greater Kafue ecosystem, Zambia

David Squarre
Research Center for Zoonosis Control, Hokkaido University
Yukiko Nakamura
Research Center for Zoonosis Control, Hokkaido University
Kyoko Hayashida
Research Center for Zoonosis Control, Hokkaido University
Naoko Kawai
Research Center for Zoonosis Control, Hokkaido University
Herman Chambaro
Research Center for Zoonosis Control, Hokkaido University
Boniface Namangala
School of veterinary Medicine, University of Zambia
Chihiro Sugimoto
Research Center for Zoonosis Control, Hokkaido University
Junya Yamagishi (junya@czc.hokudai.ac.jp)
Hokkaido Daigaku

Research

Keywords: Piroplasms, meta-barcoding, Kafue ecosystem, Zambia

Posted Date: October 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-53560/v3

Version of Record: A version of this preprint was published on November 30th, 2020. See the published version at https://doi.org/10.1186/s13071-020-04475-7.

Abstract

Background: Piroplasms are vector-borne intracellular hemoprotozoan parasites that infect wildlife and livestock. Wildlife species are reservoir hosts to a diversity of piroplasms and play an important role in the circulation, maintenance and evolution of these parasites. The potential for likely spillover of both pathogenic and non-pathogenic piroplasm parasites from wildlife to livestock is underlined when common ecological niche is shared in the presence of a competent vector.

Method: To investigate piroplasm diversity in wildlife and cattle population of the greater Kafue ecosystem, we utilized PCR to amplify the 18S rRNA V4 hyper-variable region and meta-barcoding strategy using illumina MiSeq sequencing platform and amplicon sequence variant (ASV) based bioinformatics pipeline to generate high resolution data which discriminate sequences down to a single nucleotide difference.

Results: A parasite community of 45 ASVs corresponding to 23 species consisting of 4 genera of *Babesia*, *Theileria*, *Hepatozoon* and *Colpodella,* were identified in wildlife and cattle population from the study area. *Theileria* species were detected in buffalo, impala, hartebeest, sable antelope, sitatunga, wild dog and cattle. In contrast, *Babesia* species were only observed in cattle and wild dog. Our results demonstrate possible spillover of these hemoprotozoan parasites from wildlife, especially buffalo, to cattle population in the wildlife-livestock interface.

Conclusion: We demonstrated that the deep amplicon sequencing of the 18S rRNA V4 hyper-variable region for wildlife was informative. Our results illustrated the diversity of piroplasma and the specificity of their hosts. They led us to speculate possible ecological cycle including transmission from wildlife to domestic animals in the greater Kafue ecosystem. Thus, this approach may contribute to the establishment of appropriate disease control strategies in wildlife-livestock interface areas.

Background

Piroplasmida is an order of intracellular heamoprotozoan parasites that belong to the phylum Apicomplexa. The species of genera *Theileria* and *Babesia* cause clinical disease in vertebrate hosts including domestic and wild animals (1,2). The parasites are transmitted by vectors of ixodid ticks and cause considerable socio-economic impact on livestock production in sub-Saharan Africa, threatening livelihoods and food security (3). The *Theileria* and *Babesia* genera consist of a wide diversity of species and genotypes (4,5).

Wildlife plays an important role in the circulation, maintenance and evolution of these parasites. African buffalos (*Syncerus caffer*), for example, are reservoirs of buffalo-derived *Theileria parva* that causes theileriosis or, corridor disease, in cattle (6,7). This disease is transmitted from buffalo to cattle and not between cattle, because cattle acutely die before piroplasms emerge or infect new ticks (8,9). Conversely, in East Coast fever (ECF) caused by *T. parva* circulating among cattle population, some infected cattle

survive due to immune response and occasional chemotherapy, and then become asymptomatic carriers and lead to the continuous spread of the parasite among cattle (10).

Although *Theileria* is by far the most important piroplasma causing considerable effect on livestock production, *Babesia* also cause a wide range of infectious diseases in domestic animals. Redwater in cattle, canine babesiosis, and equine piroplasmosis are caused by *Babesia bigemina/B. bovis, B. canis,* and *B. caballi/B. equi*, respectively. Several wildlife species are natural hosts of a wide diversity of piroplasma that are either pathogenic or non-pathogenic to domestic animals.

The greater Kafue ecosystem, measuring 68,000 km^2 in size, is a large conservation area in central Zambia. It is composed of the Kafue national park (22,400 km^2) and nine adjacent game management area (GMAs) that act as a buffer to the national park. The national park is host to numerous wildlife species and particularly is devoid of human settlements and livestock. The GMAs that immediately surround the park are notably characteristic of wildlife cohabiting with communities and their livestock, thus forming a wildlife-livestock interface area (11,12). The potential for likely spillover of arthropod-borne pathogens such as piroplasmas from wildlife to livestock occurs when a common ecological niche is shared in the presence of a competent vector (13). In addition to the interface in conservation areas, the growing game ranching industry in Zambia has integrated wildlife and livestock farming, creating widespread patches of ex-situ wildlife-livestock interface areas across the country. The primary source of wildlife for stocking game ranches is conservation areas such as the greater Kafue ecosystem. This is likely to spread parasites and create a vortex of piroplasm parasites across the country.

Methods

Sample collection and DNA extraction

The greater Kafue ecosystem (Figure 1.) is a conservation area located in central Zambia (14°03"S / 16°43"S and 25°13"E / 26°46"E) and measuring about 67,806 km^2 in size. Whole blood samples were collected from wild animals captured during a restocking program conducted between May and August of 2017 and 2018. Approximately 5 mL of blood were collected through venipuncture into EDTA vacutainers and immediately placed on ice. The samples were collected from 253 wild animals consisting of 12 wildlife species (Table 1) during chemical immobilization and physical restraint as previously described (22,23). Additional 232 blood samples were collected from cattle in the interface between the GMA and open area in Zambia's Itezhi-Tezhi district between April and May, 2019 (Table 1).

From each blood sample collected, genomic DNA was extracted using the DNA Isolation Kit for Mammalian Blood (Roche Applied Science, Indianapolis, USA) for wild animal samples and QuickGene DNA whole blood kit S (Kurabo, Osaka, Japan) for cattle samples as per manufacturer's protocol. A final volume of 200 µL of DNA was eluted in tubes and stored at −80 °C until analysis.

RLB-PCR amplification and library preparation

Amplification of the V4 hypervariable region of the 18S rRNA gene was obtained by piroplasma specific RLB-PCR using primers RLB-F and RLB-R (Table 2) (24). A reaction mix of 10 µL containing 5.0 µL Ampdirect plus buffer (Shimadzu, Kyoto, Japan), 3.95 µL PCR grade water, 0.05 µL Bio Taq HS (Bioline, London, UK), 0.5 µL DNA template and 0.25 µL each of the RLB primers. The thermocycler conditions were 94 °C for 10 min denaturation and 40 cycles of 94 °C for 1 minute, annealing at 50 °C for 1 minute, extension at 72 °C for 1.5 minutes and final extension at 72 °C for 10 minutes.

The second PCR adding illumina tail was conducted using 100 times diluted 1st PCR amplicons as template. The reaction volume of 10 µL comprised the same volumes of reagents as the first RLB-PCR but instead replaced the primer with 10 µM illumina tail-tagged RLB primers (Table 2). The thermocycler conditions were the same as the first PCR except amplification was set at 12 cycles.

The illumina tail-tagged amplicons from the second PCR were then diluted 50 times and 1 µL was added to a 20 µL reaction mixture for index PCR. The other reagents included 4 µL of 5 x buffer, 1.4 µL $MgCl_2$ (25mM), 0.5 µL 10mM dNTP mix, 1 µL mixed illumina-index primer (Table 2), 11.975 µL nuclease-free water and 0.125 µL KAPATaq Extra. The indexing PCR was run with thermocycler condition of 95 °C initial denaturation for 5 minutes followed by 15 cycles of 92 °C for 30 seconds, 45 °C for 30 seconds and 72 °C for 30 seconds and final extension at 72 °C for 15 minutes. The obtained amplicons which have unique 8-bp index in both side for each sample were quantified by agarose gel electrophoresis. Then, equal amount of each samples were pooled into one library and gel-purified using Wizard SV Gel and PCR Clean-Up system (Promega, Madison, WI, USA).

Amplicon sequencing and Bioinformatic analysis

The RLB-PCR amplicon library was sequenced with the MiSeq (17,21) using a 300 bp paired-end sequencing protocol and the MiSeq sequencing reagent kits v3 (Illumina, Hayward, CA, USA) with 25% PhiX DNA spike-in control according to the manufacturer's instructions. Quality control and filtering were conducted with Trimmomatic (25) using the following parameter; TRAILING:20, SLIDINGWINDOW:4:15 and MINLEN:36. Concatenation between forward and reverse reads and primer trimming were conducted with AMPtk (26), allowing minimum merged length of 400 bp. Primer sequences to be trimmed were GAGGTAGTGACAAGAAATAACAATA and TCTTCGATCCCCTAACTTTC for forward and reverse reads, respectively.

A set of amplicon sequence variants (ASVs) was generated by DADA2 and LULU in the AMPtk package using the default parameters. The obtained sequences were annotated based on sequence homology with the Basic Local Alignment Tool (BLAST), and non-redundant nucleotide database by NCBI using -max_target_seqs 1, -perc_identity 70, -qcov_hsp_perc 70 and -evalue 1e-20 as a set of parameters (27). Operational taxonomic units (OTUs) was further generated by clustering the ASVs using usearch (28) with 99% identity as clustering threshold. Observed ASVs in each sample were filtered out if number of the assigned reads were less than 1% of the total number of assigned reads.

Phylogenetic analyses

Phylogenetic relationship among ASVs were analyzed using Neighbor-Joining method (29) implemented in MEGA X (30). The evolutionary distances were computed using the maximum composite likelihood method (31) and default parameters with 10,000 bootstraps. Visualization and annotation were conducted using iTOL v5.5 (32). Each clade was annotated based on sequence identity obtained by the BLAST analysis.

Results

Detection of piroplasm parasite by PCR and taxonomical annotation

Out of 253 sampled wild animals, 61.3% (65/106) of impalas, 40% (19/47) of hartebeests, 62.3% (33/53) of buffalos, 74% (3/4) of sitatungas, 25% (1/4) of lions and all the sable antelope (8/8) and wild dogs (2/2) were positive for piroplasmosis by RLB-PCR. In case of cattle, 63.4% (147/232) were RLB-PCR positive. Of the 12 wildlife species sampled and screened, 7 species were infected by piroplasm parasites (Table 1, Additional file 1: Table S1). All the positive amplicons were subjected to sequence analysis to identify their taxonomic classification. In total, 2.80 M raw reads had been obtained from 278 PCR positive samples, then merged into 2.46 M contigs (Additional file 1: Table S1).

A total of 45 ASVs of the V4 hyper-variable region of the 18S rRNA gene were obtained from both wildlife species and cattle sampled from the greater Kafue ecosystem (Table 3). The taxonomic assignment of ASV using BLASTn resulted into the identification of four genera, *Theileria*, *Babesia*, *Hepatozoon* and *Colpodella*, which consisted of 11, 3, 2 and one known species and 36, 6, 2 and one ASVs, respectively (Additional file 1: Table S1).

In the phylogenetic analysis, we observed both *Theileria* and *Babesia* clade (Figure 2). The *Theileria* clade consisted of subclade for *T. velifera*, *T. mutans*, *T. parva* and *T. taurotragi*.

The *T. velifera* subclade consisted of seven ASVs and sequence identity to *T. velifera* was 98.7% to 100% (Table3), suggesting all of these ASV belong to *T. velifera.* The subclade was further divided into two groups based on sequence identity. One was ASV6, 29 and 55 which were detected only in buffalo, ASV7, 64 and 92 were detected only in cattle while ASV1 was detected in cattle and impala (Figure 2, Additional file 1: Table S1).

A similar correlation among sequence identity and hosts was observed in the *T. mutans* clade. ASV26, 42 and 60 were buffalo specific and ASV3, 5, 16 were detected in both buffalo and cattle. All of them had more than 99.5% identity to the reference sequences of *T. mutans* (Figure 2, Additional file 1: Table S1, Table 3).

Interestingly, most of the observed ASVs in the *T. parva* clade were detected only in buffalo except ASV15 (*T. parva*) and ASV11 (*T.* sp buffalo) which were detected in both buffalo and cattle (Na032 and Na142, respectively) (Additional file 1: Table S1). ASV25 showed 100% identity to *Theileria* sp*. bougasvlei* but was adjacent to the *T. parva* clade (Table 3, Figure 2).

T. taurotragi was detected in four cattle. ASV46 and 62 had more than 99.8% identity to a *T. taurotragi* reference sequence but ASV35 had 97.8% identity implying this can be categorized in a different genotype (Table 3).

There were two additional clades in *Theileria* (Figure 2). One consisted of ASV8, 9, 14, 23, 49 and 85. It was almost exclusively detected in hartebeest even though ASV8 and 23 were also detected in a wild dog (Da082). They showed high identity to unspecified *Theileria* spp. The other consisted of ASV2, 47, and 106, which were detected in impala. ASV4 was detected in both hartebeest and sable antelope but also found in a buffalo (Da109). ASV12 and 13 were detected in sitatunga.

The *Babesia* clade consisted of subclade for *B. bigemina* and *B. occultans*. ASVs in the *B. bigemina* subclade showed more than 99.8% identity to *B. bigemina* reference sequences. Both *B. bigemina* and *B. occultans* were detected only in cattle (Figure 2, Table 3, Additional file 1: Table S1).

A *Hepatozoon canis* sequence was detected in a lion and another *Hepatozoon* sp. was detected in a wild dog. Interestingly, a *Colpodellidae* sequence, ASV57, was also detected in cattle (Figure2, Additional file 1: Table S1).

Discussion

We applied the deep amplicon sequencing method to investigate piroplasmas (17,21) in wildlife and cattle population of the Kafue national park and surrounding wildlife-livestock interface area of the greater Kafue ecosystem. Blood samples from 253 wildlife consisting of 12 mammalian species and 232 cattle were collected in 2018 and 2019 respectively. Despite the wildlife and cattle being sampled in different but consecutive years, this may not have affected the comparison and interpretation of the results obtained, because fundamental change of biodiversity in functional and resilient ecosystem like Kafue takes a long period.

Our data show that 45 ASVs and 23 species consisting of 4 genera (*Babesia*, *Theileria*, *Hepatozoon* and *Colpodella*) were detected. Among the 45 ASVs, 14 were identical to previously published sequences. However, 28 ASVs demonstrated percentage identity of 95.7-99.8%, suggesting that novel genotypes may also exist. ASV2, 47, and 106 presented 95.7-95.9% identity to *Theileria* sp. suggesting possible novel *Theileria* spp. or undeposited sequences of known *Theileria* spp. (Astarisk in Table 3).

Within *Theileria* species, 36 ASVs were detected (Table 4). As an important natural reservoir host, buffalo had a diversity of 18 *Theileria* ASVs, which was highest compared to other wildlife species. This finding is consistent with a previous report from a serological study involving buffalos (13). Importantly, three ASVs of *T. parva* (OTU23 comprising ASV15, 86, and 101) were obtained from buffalo, providing important epidemiological insight for cattle in the area in terms of corridor disease transmission. Indeed, *T. parva* ASV15 was detected in cattle (Na032), suggesting possible spillover of *T. parva* from buffalo to domestic cattle. The presence of *T. sp. (buffalo)* (49.1%; 26 of 53) and *T. sp. (bougasvlei)* (18.9%; 10 of 53) in buffalo (Table 4) is of diagnostic importance as it affects the accurate detection of *T. parva* in mixed

infections when performing hybridization PCR assay (33). In addition to buffalo, *T. sp* (buffalo) was also detected in cattle population (0.4%; 1 of 232). This results support the observations and findings from studies conducted in Kenya which also identified *T. sp* (buffalo) from cattle, suggesting that *T. sp* (buffalo) infection in cattle occurs in the field where buffalo and cattle share pasture (20,34). Nevertheless, more knowledge on the infection epidemiology and pathogenicity to cattle will be required. The presence of *T. taurotragi* circulating in cattle population is consistent with findings in other similar studies (35) .The characterization of parasite community and molecular ecology raises awareness on the consequences and limitations of specific diagnostic tests and requires further cautions for the interpretation of the results used for diagnostics or surveillance in a specified area.

Babesia was predominantly observed in cattle but also detected in wild dogs. *Babesia bigemina* (10.3%; 24 of 232) and *B. occultans* (1.7%; 4 of 232) were the only species detected in cattle (Table 4), of which *B. bigemina* is a pathogenic parasite to cattle causing a clinical disorder of babesiosis, also known as redwater. These findings are similar to other comparable studies in southern Africa where the presence of *Babesia* in cattle and wild animals, particularly buffalo, was assessed (36). To the best of our knowledge, this is the first report of the non-pathogenic *B. ocultans* in Zambia. However, its specific vectors, impact on cattle, diagnostic consequence in *Babesia* mixed infection or implication of infection to wildlife are not evaluated.

Despite not being classified in the order of piroplasmida but Eucoccidiorida, Apicomplexan species of *Hepatozoon canis* and *H. sp.* were detected in African lion and wild dog samples, respectively. Divergent to other arthropod-borne parasites transmitted through the vector's salivary glands at the time of feeding, *Hepatozoon* are transmitted to the carnivore host exclusively by ingestion of infected vectors (ticks) during grooming (37,38). They cause subclinical infection in wild carnivores and clinical infection in domestic dogs (39). Previous studies on free ranging wild carnivores in Zambia have indicated the widespread presence of *Hepatozoon sp*. in lions (40). This highlights the considered epidemiologic role of wild carnivores as sylvatic reservoir of *Hepatozoon* and presents the risks of likely spillover of *Hepatozoon* infections to domestic carnivores in the interface area.

Genera of *Colpodella* are part of Alveolata organisms that are originally known to be free-living. However, recent studies have revealed the parasitic nature of *Colpodella* sp. as a Human Erythrocyte Parasite (HEP) that has lately been reported from China to cause relapsing fevers and neurological symptoms in humans (41,42). Furthermore, the detection of *Colpodella* sp. in ticks suggests that this parasite may potentially be transmitted by tick vector(s) (41). We detected a *Colpodella* sequence from one of the cattle samples, with the sequence identity of 79.6% with the reported human cases (GQ411073; *Colpodella* sp. HEP). The sequence detected from our cattle sample showed perfect match (100% identity) to GenBank MN103986 (Colpodellidae clone PL31), reported in raccoon dog in Poland (43). Thus, the detection of *Colpodella* sp. from cattle sample implies to support those findings that some of the *Colpodella* species are associated with vertebrates, and possibly cause disease. It is largely undetermined what vector is involved, how the parasite is maintained, and the risk that the cattle may pose for human

infection. It would be important to determine the zoonotic scale of *Colpodella* infection to rule out incidental infections.

Identification of multiple infection is the another advantage of the deep amplicon sequencing (21). It is known that the African buffalo is simultaneously infected with multiple species of *Theileria* (44). According to our study, the African buffalo is the most infected animal with multiple species of *Theileria* (see Fig 2 and additional data; Table S1). Besides, most of the cattle were also co-infected with *Theileria velifera* and *Theileria mutans*. It is reported that co-infection of multiple *Theileria* spp. in cattle results in dramatically different pathological disorders compare to single-species infections (45). Further studies with expanded sample size might demonstrate similar interactions in wildlife as well. This is particularly important since Zambia's cattle population stronghold is in Itezhi-Tezhi district which is adjacent to the KNP. This is cardinal as accurate diagnosis and effective control (vaccinations) of piroplasm parasites need to take the parasite community data into account. Hartebeest also tended to be co-infected with *Theileria* spp*.* In contrast, the Impalas were mainly infected with *Theileria* spp. isolated from giraffe but hardly co-infected with other piroplasmas.

The identification of tick-borne pathogens in wildlife and cattle population in the study area supports the apparent presence of the known tick vectors implicated in their transmission. Particular *Theileria* species are known to be transmitted by specific ixodid tick species of *Rhipicephalus appendiculatus, R. zambeziensis and Amblyomma variegatum,* while *Babesia* species are transmitted by *R. microplus, R. decororatus and R. evertsi* (46,47)*.* The tick species associated with transmission of *Hepatozoon* species is the *Rhipicephalus sanguineus sensu lato (s.l.)* (48)*.* In order to identify the unknown vectors of some of the parasites described in this study, there is need to conduct tick piroplasm metagenomic analysis. This would further illustrate the piroplasm parasite eco-cycle more precisely.

Conclusion

Molecular epidemiology based on the strength of knowledge of cryptic parasite community and diversity is essential in the control of piroplasmosis. Mapping of the piroplasm parasites in all major livestock landscapes beyond the Kafue ecosystem using metagenomic approach may benefit the piroplasmosis control in Zambia through high resolution data to precisely guide diagnosis, vaccination and movement controls.

Declarations

Acknowledgement

We would like to thank The Department of National Parks and Wildlife (DNPW) and the Itezhi-Tezhi farmers in Zambia for the permission to conduct this research in the greater Kafue ecosystem. The authors also acknowledge the Wildlife Veterinary Unit (WVU) for their role and assistance in the wildlife sample collection.

Ethics approval and consent to participate

The permission to collect blood samples from the free ranging wildlife of the greater Kafue ecosystem and authority to use the samples for this study was granted by the Department of National Parks and Wildlife (DNPW) in Zambia (TJNPW/8/27/1). The blood samples from cattle were collected under the permission from ethics approval Ref. No.2019-Feb-081 (ERES Converge IRB, Lusaka, Zambia).

Consent of publication

Not applicable.

Availability of data and materials

Data supporting the conclusions of this article are included within the article and its additional file. Representative ASV sequences were deposited in the GenBank database under the accession numbers MT814722- MT814766

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by the Japan Society for the Promotion of Science Ronpaku Program (JSPS) Japan, and "Japan Program for Infectious Diseases Research and Infrastructure" from Japan Agency for Medical Research and Development (AMED) under grant number JP20w m0125008.

Authors' contributions

DS, YN collected samples and wrote the Manuscript. DS, YN, NK, KH and JY conducted laboratory experiments and analyzed the data. HC collected samples. JY, KH, BN and CS designed the study, supervised the work and critically revised the manuscript. All authors read and approved the final Manuscript

References

1. Schreeg ME, Marr HS, Tarigo JL, Cohn LA, Bird DM, Scholl EH, et al. Mitochondrial genome sequences and structures aid in the resolution of piroplasmida phylogeny. PLoS One. 2016;11: e0165702.
2. Allsopp MTEP, Cavalier-Smith T, Waal DTDE, Allsopp BA. Phylogeny and evolution of the piroplasms. Parasitology. 1994;108:147–52.
3. Bishop R, Musoke A, Morzaria S, Gardner M, Nene V. *Theileria*: Intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks. Parasitology. 2004;129:S271–83.

4. Criado-fornelio A, Martinez-marcos A, Buling-Saraña A, Barba-Carretero JC. Molecular studies on *Babesia, Theileria* and *Hepatozoon* in southern Europe Part II. Phylogenetic analysis and evolutionary history. Vet Parasitol. 2003;114:173–94.
5. Criado-Fornelio A, Gónzalez-Del-Río MA, Buling-Saraña A, Barba-Carretero JC. The 'expanding universe' of piroplasms. Vet Parasitol. 2004;119:337–45.
6. Kock R, Kock M, Garine-wichatitsky M De, Chardonnet P, Caron A. Livestock and buffalo (*Syncerus caffer*) interfaces in Africa: ecology of disease transmission and implications for conservation and development. In: Melletti M, Burton J, editors. Ecology, evolution and behaviour of wild cattle implications for conservation. University of Cambridge; 2014. p. 431–45.
7. Morrison WI, Hemmink JD, Toye PG. *Theileria parva*: a parasite of African buffalo, which has adapted to infect and undergo transmission in cattle. Int J Parasitol. 2020;50:403–12.
8. Yusufmia SBAS, Collins NE, Nkuna R, Troskie M, Van Den Bossche P, Penzhorn BL. Occurrence of *Theileria parva* and other haemoprotozoa in cattle at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal, South Africa. J S Afr Vet Assoc. 2010;81:45–9.
9. Uilenberg G. Immunization against diseases caused by *Theileria parva*: A review. Trop Med Int Heal. 1999;4:A12–20.
10. Nene V, Musoke A, Gobright E, Morzaria S. Conservation of the sporozoite p67 vaccine antigen in cattle-derived *Theileria parva* stocks with different cross-immunity profiles. Infect Immun. 1996;64:2056–61.
11. Bandyopadhyay S, Tembo G. Household consumption and natural resource management around national parks in Zambia. J Nat Resour Policy Res. 2010;2:39–55.
12. Kock RA. What is this Infamous "wildlife/livestock disease interface?" a review of current knowledge for the African continent. In: Osofsky SA, editor. Conservation and development interventions at the wildlife/livestock interface: implications for wildlife, livestock and human health. IUCN, Gland, Switzerland and Cambridge, UK; 2005. p. 1–13.
13. Munang'andu HM, Siamudaala V, Matandiko W, Mulumba M, Nambota A, Munyeme M, et al. Detection of *Theileria parva* antibodies in the African buffalo (*Syncerus caffer*) in the livestock-wildlife interface areas of Zambia. Vet Parasitol. 2009;166:163–6.
14. Hemmink JD, Sitt T, Pelle R, Klerk-lorist L De, Shiels B, Toye PG, et al. Ancient diversity and geographical sub-structuring in African buffalo *Theileria parva* populations revealed through metagenetic analysis of antigen-encoding loci. Int J Parasitol. 2018;48:287–96.
15. Pienaar R, Latif AA, Thekisoe OMM, Mans BJ. Geographic distribution of *Theileria* sp. (buffalo) and *Theileria* sp. (bougasvlei) in Cape buffalo (*Syncerus caffer*) in southern Africa: implications for speciation. Parasitology. 2014;141:411–24.
16. Eygelaar D, Jori F, Mokopasetso M, Sibeko KP, Collins NE, Vorster I, et al. Tick-borne haemoparasites in African buffalo (*Syncerus caffer*) from two wildlife areas in northern Botswana. Parasites and Vectors. 2015;8:1–11.

17. Glidden CK, Koehler A V., Hall RS, Saeed MA, Coppo M, Beechler BR, et al. Elucidating cryptic dynamics of *Theileria* communities in African buffalo using a high-throughput sequencing informatics approach. Ecol Evol. 202019;10:70–80.
18. Muleya W, Namangala B, Simuunza M, Nakao R, Inoue N, Kimura T, et al. Population genetic analysis and sub-structuring of *Theileria parva* in the northern and eastern parts of Zambia. Parasites and Vectors. 2012;5:255.
19. Geysen D, Bishop R, Skilton R, Dolan TT, Morzaria S. Molecular epidemiology of *Theileria parva* in the field. Trop Med Int Heal. 1999;4:A21–7.
20. Bishop RP, Hemmink JD, Morrison WI, Weir W, Toye PG, Sitt T, et al. The African buffalo parasite *Theileria* sp.(buffalo) can infect and immortalize cattle leukocytes and encodes divergent orthologues of *Theileria parva*antigen genes. Int J Parasitol Parasites Wildl. 2015;4:333–42.
21. Chaudhry U, Ali Q, Rashid I, Shabbir MZ, Ijaz M, Abbas M, et al. Development of a deep amplicon sequencing method to determine the species composition of piroplasm haemoprotozoa. Ticks Tick Borne Dis. 2019;10:101276.
22. Kock MD, Burroughs REJ. Chemical and physical restraint of wild animals: a training and field manual for African species. Greyton: IWVS; 2012.
23. La Grange M. The capture, care, and management of wildlife. Van Schaik; 2006.
24. Gubbels JM, De Vos AP, Van Der Weide M, Viseras J, Schouls LM, De Vries E, et al. Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridization. J Clin Microbiol. 1999;37:1782–9.
25. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20.
26. Palmer JM, Jusino MA, Banik MT, Lindner DL. Non-biological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. PeerJ. 2018; 6:e4925.
27. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215:403–10.
28. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010;26:2460–1.
29. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4:406–25.
30. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35:1547–9.
31. Tamura K, Tao Q, Kumar S. Theoretical foundation of the reltime method for estimating divergence times from variable evolutionary rates. Mol Biol Evol. 2018;35:1770–82.
32. Letunic I, Bork P. Interactive Tree of Life (iTOL) v4: Recent updates and new developments. Nucleic Acids Res. 2019;47:W256–9.

33. Pienaar R, Potgieter FT, Latif AA, Thekisoe OMM, Mans BENJ. Mixed *Theileria* infections in free-ranging buffalo herds: implications for diagnosing *Theileria parva* infections in Cape buffalo (*Syncerus caffer*). Parasitology. 2011;138:884–95.
34. Githaka N, Konnai S, Bishop R, Odongo D, Lekolool I, Kariuki E, et al. Identification and sequence characterization of novel *Theileria* genotypes from the waterbuck (*Kobus defassa*) in a *Theileria parva*-endemic area in Kenya. Vet Parasitol. 2014;202:180–93.
35. Mans BJ, Pienaar R, Latif AA, Potgieter FT. Diversity in the 18S SSU rRNA V4 hyper-variable region of *Theileria* spp. in Cape buffalo (*Syncerus caffer*) and cattle from southern Africa. Parasitology. 2011;138:766–79.
36. Mans BJ, Pienaar R, Ratabane J, Pule B, Latif AA. Investigating the diversity of the 18S SSU rRNA hyper-variable region of *Theileria* in cattle and Cape buffalo (*Syncerus caffer*) from southern Africa using a next generation sequencing approach. Ticks Tick Borne Dis. 2016;7:869–79.
37. Smith TG. The Genus *Hepatozoon* (Apicomplexa: Adeleina). J Parasitol. 1996;82:565–85.
38. Greene CE. Infectious Diseases of The Dog and Cat. 4th ed. Greene CE, editor. Missouri, USA: Elsevier Saunders; 2012. p. 750–757.
39. Cunningham MW, Yabsley MJ. Primer on Tick-Borne Diseases in Exotic Carnivores. In: Fowler's Zoo and Wild Animal Medicine Current Therapy, Volume 7. Elsevier Inc.; 2012. p. 458–64.
40. Williams BM, Berentsen A, Shock BC, Teixiera M, Dunbar MR, Matthew BS, et al. Prevalence and diversity of *Babesia, Hepatozoon, Ehrlichia,* and *Bartonella* in wild and domestic carnivores from Zambia, Africa. Parasitol Res. 2014;113:911–8.
41. Jiang J, Jiang R, Chang Q, Zheng Y, Fricken E Von, Cao W. Potential novel tick-borne *Colpodella* species parasite infection in patient with neurological symptoms Case report. PLoS Negl Trop Dis. 2018;12: e0006546.
42. Yuan CL, Keeling PJ, Krause PJ, Horak A, Bent S, Rollend L, et al. *Colpodella* spp.– like Parasite Infection in woman, China. Emerg Infect Dis. 2012;18:125-7.
43. Solarz W, Najberek K, Wilk-Woźniak E, Biedrzycka A. Raccoons foster the spread of freshwater and terrestrial microorganisms—Mammals as a source of microbial eDNA. Divers Distrib. 2020;26:453–9.
44. Mans BJ, Pienaar R, Latif AA. A review of *Theileria* diagnostics and epidemiology. Int J Parasitol Parasites Wildl. 2015;4:104–18.
45. Woolhouse MEJ, Thumbi SM, Jennings A, Chase-Topping M, Callaby R, Kiara H, et al. Co-infections determine patterns of mortality in a population exposed to parasite infection. Sci Adv. 2015;1: e1400026.
46. Makala LH, Mangani P, Fujisaki K, Nagasawa H. The current status of major tick borne diseases in Zambia. Vet Res. 2003;34:27–45.
47. Simuunza M, Weir W, Courcier E, Tait A, Shiels B. Epidemiological analysis of tick-borne diseases in Zambia. Vet Parasitol. 2011;175:331–42.

48. Qiu Y, Kaneko C, Kajihara M, Ngonda S, Simulundu E, Muleya W, et al. Tick-borne haemoparasites and Anaplasmataceae in domestic dogs in Zambia. Ticks Tick Borne Dis. 2018;9:988–95.

Tables

Table 1. Detection of heamoparasites in wildlife species and cattle from the Kafue ecosystem using RLB-PCR

	Species sampled	Number	RLB-PCR positive	Positive rate (%)
1	Impala *(Aepyceros melampus)*	106	65	61.3
2	Hartebeest *(Alcelaphus buselaphus)*	47	19	40.0
3	Sable antelope *(Hippotragus niger)*	8	8	100
4	Lion *(Panthera leo)*	4	1	25.0
5	Wild dog *(Lycaon pictus)*	2	2	100
6	Sitatunga *(Tragelaphus spekii)*	4	3	74.0
7	Buffalo *(Syncerus caffer)*	53	33	62.3
8	Lechwe *(Kobus leche leche)*	9	0	0.0
9	Cheetah *(Acinonyx jubatus)*	1	0	0.0
10	Vevert monkey *(Chlorocebus pygerythrus)*	1	0	0.0
11	Baboon *(Papio ursinus)*	1	0	0.0
12	Warthog *(Phacochoerus africanus)*	17	0	0.0
13	Cattle (Bos taurus)	232	147	63.4
	Total	**483**	**278**	**57.6**

Table 2. Primers used for piroplasm parasite detection

r's	Primer name	Primer sequence (5`-3`)	Reference
V4 le	Reverse Line Blot - F(RLB-F)	GAGGTAGTGACAAGAAATAACAATA	(24)
	Reverse Line Blot - R (RLB-R)	TCTTCGATCCCCTAACTTTC	
ne l	Illumina RLB-F	ACACTCTTTCCCTACACGACGCTCTTCCGATCT[RLB-F]	
	Illumina RLB-R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT[RLB-R]	
na-	illumina-i5 primers	AATGATACGGCGACCACCGAGATCTACAC[index*]ACACTCTTTCCCTACACGACGCTCTTCCGATCT	
	illumina-i7 primers	CAAGCAGAAGACGGCATACGAGAT[index*]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT	

***index: 8-bp nucleotide to provide unique index to each samples.**

Table 3. Diversity of piroplasmas detected in wildlife and cattle samples collected from the Kafue ecosystem. ASV numbers shown in bold and with asterisk, represents the identity against the reference sequence of 100% and 95.7-95.9%, respectively. OTU[a] = Operational Taxonomic Unit, ASV[b] = Amplicon Sequence Variant

Genus	Species	Genotypes	BLAST Top Hit	ID	OTU[a]	ASV[b]	%identity
Theileria	*T. velifera*	*T. velifera*	*T. velifera* KSA_D_Th6	LC431550	1	**1**	100.00
						7	99.78
						64	99.78
						92	99.78
		T. velifera A	*Theileria cf. velifera A*	GU733375	2	6	98.69
		T. velifera B	*Theileria cf. velifera (Syncerus caffer*) clone H4a	JN572701	1	**29**	100.00
						55	99.78
	T. mutans	*T. mutans*	*Theileria mutans* isolate MT15	KU206320	11	**3**	100.00
						16	99.78
		T. mutans MSD	*Theileria sp.* B15a	JN572700	12	**5**	100.00
		T. mutans-like 1	*Theileria cf. mutans 1 (Syncerus caffer)* clone C21b	JN572699	13	26	99.56
		T. mutans-like 2	*Theileria cf. mutans 2 (Syncerus caffer)* clone Q15d	JN572696	14	42	98.68
						60	98.46
	T. parva		*Theileria parva* isolate F45P16	MH929322	23	**15**	100.00
			Theileria parva	AF013418	23	86	99.78
						101	99.56
	T. sp (buffalo)		*Theileria sp. ex Syncerus caffer* MCO-2011 clone V8b	HQ895982	23	**10**	100.00
						11	99.78
						18	99.56
						22	99.56
	T. sp (bougasvlei)		*Theileria sp.* KS-2015 isolate CAT79	KP410267	22	**25**	100.00
	T. taurotragi		*Theileria taurotragi*	L19082	20	35	97.80
					21	46	99.78
						62	100.00
	*T. sp (*sable)		*Theileria sp.* BM-2010/sable	GU733378	16	8	99.78
						14	99.57
						85	99.35
	T. sp (waterbuck)		*Theileria sp.* NG-2013c isolate waterbuck 39 clone 6	KF597072	19	4	99.35
	T. sp. (tsessebe)		*Theileria sp.* ex Damaliscus lunatus clone TS22_11	HQ179766	16	9	99.57
						23	99.78
						49	100.00
	*T. sp (*giraffe)		*Theileria sp.* NG-2012b isolate 44 clone 2	JQ928925	15	2*	95.90
						106*	95.66
						47*	95.71
	T. sp (bongo)		*Uncultured Theileria sp.* isolate BNG13	MH569462	17	12	97.82
			Uncultured Theileria sp. isolate BNG10	MH569463	18	13	99.57
Babesia	*B. bigemina*		*Babesia bigemina* clone PR28CL7	MH050387	7	32	100.00
						71	99.77
			Babesia bigemina isolate B_bi11	EF458200	7	67	100.00
			Babesia bigemina clone PR38CL1BBIG	MH047819	7	19	100.00
	B. sp		*Babesia sp.* 9 1093 cl1	KX218437	10	58	98.95
	B. occultans		*Babesia occultans* isolate Trender1	KP745626	6	28	100.00
Hepatozoon	*H. canis*		*Hepatozoon canis* isolate 70	MK645969	8	40	99.60
	H. sp		*Hepatozoon sp.* 2 BCS-2013 isolate L4	KF270665	9	82	99.00
Colpodellidae			*Uncultured Colpodellidae* clone PL31	MN103986	3	57	100.00

Table 4. Prevalence of species detected in the sampled wildlife species and cattle from the Kafue ecosystem. ASVs = Amplicon Sequence Variants

Parasitic species	Impala	Hartebeest	Sable	Sitatunga	Lion	Wild dog	Buffalo	Cattle	Corresponding ASV
T. velifera	1 (0.9%)						26 (49.1%)	136 (58.6%)	1, 6, 7, 29, 55, 64, 92
T. mutans							20 (37.7%)	140 (60.3%)	3, 5, 16, 26, 42, 60
T. parva							8 (15.1%)	1 (0.4%)	15, 86, 101
T. taurotragi								4 (1.7%)	35, 46, 62
T. sp (buffalo)							26 (49.1%)	1 (0.4%)	10,11,18,22
*T. sp (*bougasvlei)							10 (18.9%)		25
T. sp (sable)		15 (31.9%)				1 (50.0%)			8,14,85
T. sp (waterbuck)		12 (25.5%)	8 (100.0%)				1 (1.9%)		4
T. sp. (tsessebe)		8 (17.0%)				1 (50.0%)			9,23,49
T. sp (giraffe)	65 (61.3%)								2,47,106
T. bongo				3 (75.0%)					12, 13
B. bigemina								24 (10.3%)	19, 32, 67, 71
B. sp						1 (50.0%)			58
B. occultans								4 (1.7%)	28
H. canis					1 (25.0%)				40
H. sp						1 (50.0%)			82
Colpodellodae								1 (0.4%)	57
Total head sampled	106	47	8	4	4	2	53	232	

Figures

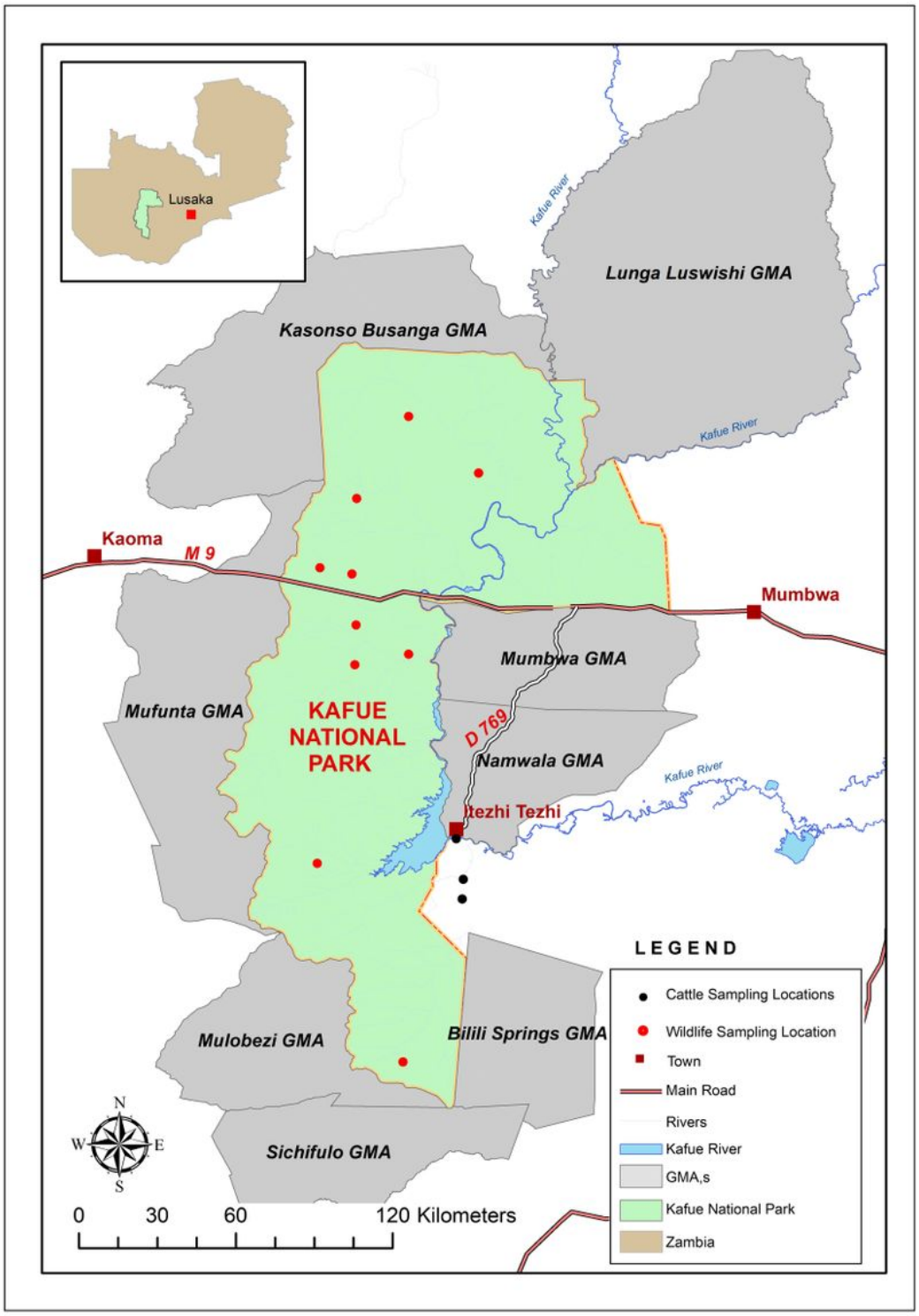

Figure 1

Map of the Kafue ecosystem consisting of the Kafue national Park and the game management areas (GMAs) showing sampling sites of wildlife and cattle

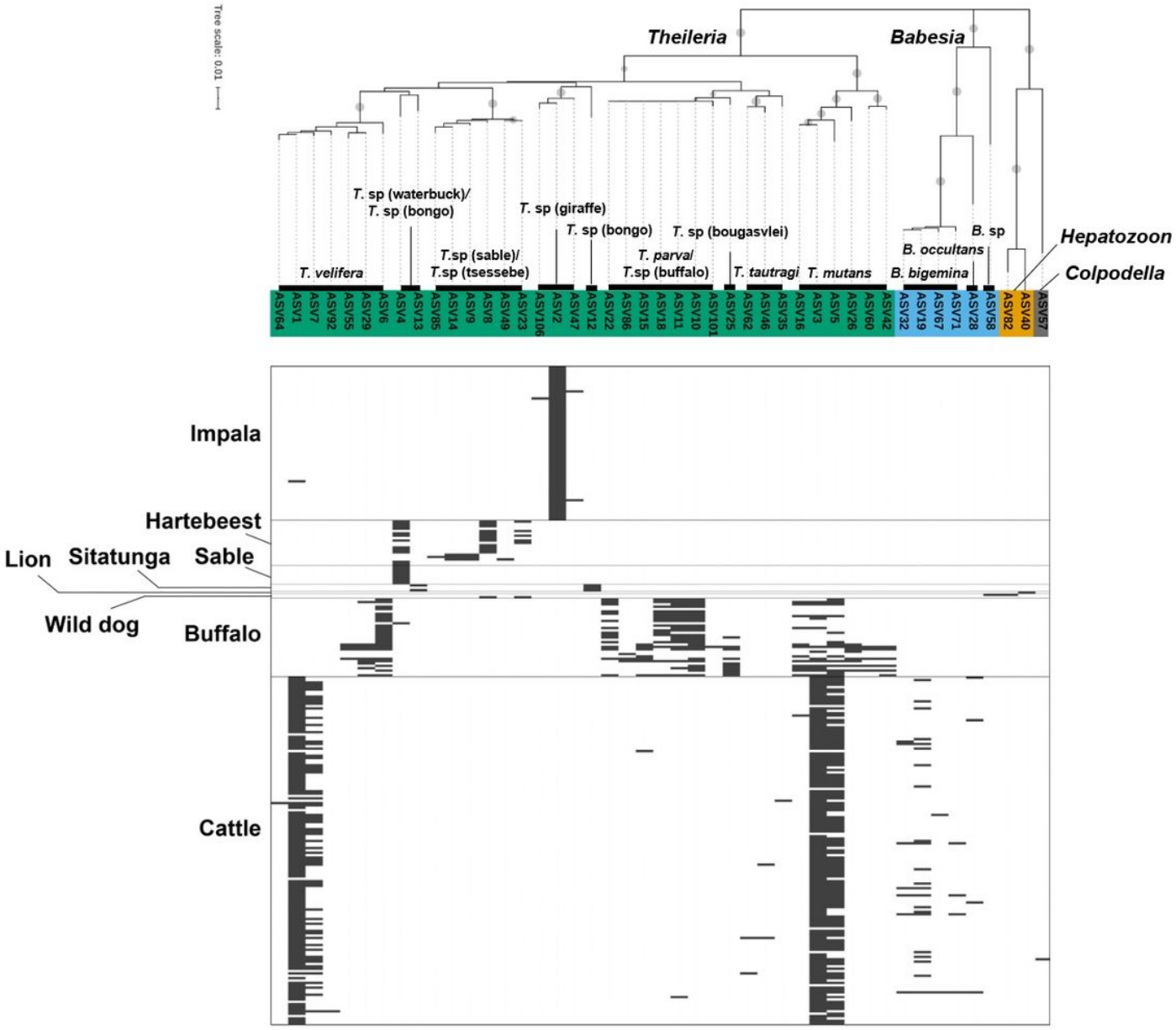

Figure 2

Phylogenetic tree of ASVs and the map of positive ASVs per animal species. On the top is the Neighbor-Joining tree of 45 ASV sequences, using a total of 411 positions in the final dataset. Bootstrap values larger than 70 are shown as proportionate size circles for each node. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Below is a table showing the positive wildlife and cattle samples for each ASVs

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.pptx

- AdditionalfileTableS1.xlsx