Title of dissertation				
Molecular epidemiological study of African trypanosomiasis and piroplasmosis at the interface of human-wildlife-livestock populations in Zambia				
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The intimate interaction of human, their livestock and wildlife populations at the human-wildlife-livestock interface poses opportunities for a complex multi-directional inter-specie transmission of pathogens of zoonotic and veterinary importance. The transmission of these pathogens across the interface impacts public health, limits livestock production and potentially decimates wildlife populations raising conservation concern. Trypanosomiasis and piroplasmosis are important parasitic diseases that humper livestock production at the interface. The presence of wildlife reservoirs play an important role in the transmission of these two diseases. Using molecular tools of NGS, this thesis aims to investigate the diversity of trypanosomes and piroplasmas circulating in wildlife reservoir populations at the interface. The description of cryptic parasite communities in wildlife populations at the interface is important to provide data to counter disease transmission and pre-empty specific infectious disease control measures. This approach is also important to identify the precise local strain or genotypes for vaccine development and roll-out especially in the control of piroplasmosis. Apart from the impact that these two diseases have on livestock, the study also reveals the circulation of zoonotic trypanosomes in wildlife population of the Kafue ecosystem.

The first chapter of this thesis describes a human clinical case of HAT caused by a vector-borne parasite of *Tr. b. rhodesiense.* The parasite was diagnosed on microscopy and confirmed with the *Tr. b. rhodesiense*-specific human SRA gene LAMP analysis. This is the first case of HAT in the Kafue national park after 50 years of non-HAT cases in the area despite the presence of the vector and reservoir. This diagnosis reveals that the Kafue national park is an emerging foci for HAT.

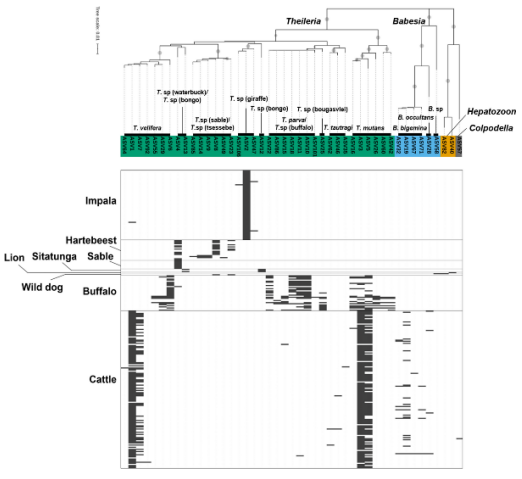
Following the demonstration of human infection of *Tr. b. rhodesiense* in the Kafue national park in chapter one, the second chapter investigated the prevalence and diversity of circulating trypanosomes in wildlife reservoir population in the Kafue ecosystem. Various trypanosomes circulating in different

mammalian wildlife species were detected by applying a high throughput ITS1-PCR/MinION sequencing method in combination with SRA-PCR/Sanger sequencing method. The prevalence of trypanosomes in hartebeest, sable antelope, buffalo, warthog, impala and lechwe were 6%, 37%, 13%, 11%, 2% and 11%, respectively. A total of six trypanosomes species or subspecies were detected in the wildlife examined, including *Tr. b. brucei*, *Tr. godfreyi*, *Tr. congolense*, *Tr. simiae* and *Tr. theileri*. Importantly human infective *Tr. b. rhodesiense* was detected in buffalo, sable antelope, and vervet monkey. The results from this chapter reaffirmed that the Kafue ecosystem is a genuine neglected and re-emerging foci for HAT.

The third chapter looks at the piroplasmas circulating in wildlife and cattle of the Kafue ecosystem and their potential spillover from wildlife to cattle. To investigate piroplasm diversity in wildlife and cattle population, PCR was utilized 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. 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. These results demonstrate possible spillover of these hemoprotozoan parasites from wildlife to cattle population in the wildlife-livestock interface. These results illustrated the diversity of piroplasma and the specificity of their hosts. The deep amplicon sequencing of the 18S rRNA V4 hyper-variable region for wildlife was informative enough to speculate possible ecological cycle including transmission from wildlife to domestic animals in the Kafue ecosystem. The application of this approach to reveal the diversity of parasite community may contribute to the establishment of appropriate and effective disease control strategies.

Photos

Collection of blood samples from wildlife in the Kafue ecosystem, Zambia



Phylogenetic tree showing diversity of piroplasms circulating in livestock and wildlife species in the Kafue ecosystem, Zambia