

Detection and characterization of zoonotic haemo-pathogens of non-human primates from Zambia

Jesca Nakayima, Kyoko Hayashida, Ryo Nakao, Akihiro Ishii, Hirohito Ogawa, Ichiro Nakamura, Ladslav Moonga, Bernard M. Hang'ombe, Aaron S. Mweene, Yuka Thomas, Yasuko Orba, Hirofumi Sawa*, Chihiro Sugimoto*

¹Division of Collaboration and Education, Research Center for Zoonosis Control, Hokkaido University, Japan

²Division of Molecular Pathobiology, Research Center for Zoonosis Control, Hokkaido University, N20, W10, Kita-ku, Sapporo 001-0020, Japan

³National Livestock Resources Research Institute (NaLIRRI), Tororo, Uganda

Background

Wildlife may harbor infectious pathogens that are of zoonotic concern acting as a reservoir of diseases transmissible to humans and domestic animals. This is due to human-wildlife conflicts that have become more frequent and severe over recent decades, competition for the available natural habitats and resources leading to increased human encroachment on previously wild and uninhabited areas.

Objectives

1. To establish the status and prevalence of haemo-pathogens in Non-human primates in Zambia
2. To characterize and assess the risk of Human/wildlife conflict regarding disease transmission

Key words: Non-human primates, Reservoir, Haemo-pathogens, Zoonosis, Zambia

Wildlife as Source of Zoonotic Infections

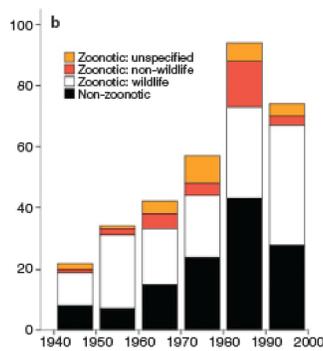
Routes of Transmission

Direct

- Droplet or Aerosol
- Oral
- Contact

Indirect

- Foodborne
- Water-borne
- Fomite
- Vector-borne
- Environmental



EID events: 1940 - 2004

Risk factors

- Companion Animal
- Occupational
- Food-borne
- Recreational Activities
- Farm Settings
- Travel

Common (of 1,407 human pathogens)
58% are zoonotic
70% of emerging diseases are zoonotic
Occur in numerous animal species
Very diverse
Severity
Transmission dynamics
Difficult to predict changes in incidence

Zoonoses: Etiologic Classification

- Viral
- Bacterial
- Parasitic
- Mycotic
- Prion

Ecosystem Health

Animal Health

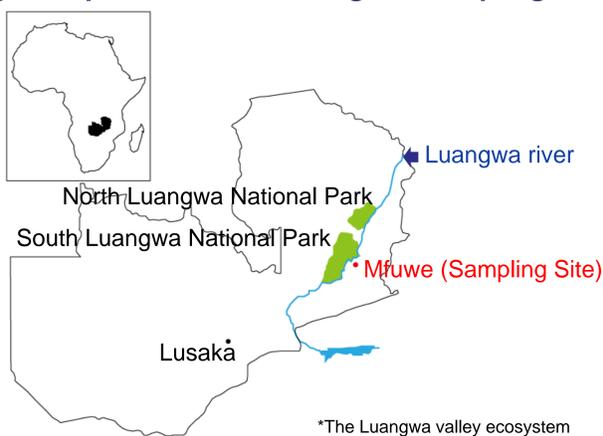
Human Health

Methods

Fig. 1 Map of Zambia showing the sampling site

- The sampling was conducted at Mfuwe in South Luangwa National Park, Zambia (13° 14'42.00" S, 31° 38'54.07" E), 2008.

- A total of 88 spleen DNA samples from baboons and vervet monkeys from Zambia were tested for zoonotic haemo-pathogens using genus or species-specific PCR.



Sampling/ culling was done under the permission from Zambia Wildlife Authority (ZAWA).

Table 1 Primers of PCR for Haemo-pathogen detection

Organism	Target gene	Primer name
Bacteria		
<i>Anaplasma</i> spp.	16S rDNA	EHR16SD/EHR16SR
<i>Borrelia</i> spp.	Fla gene	BflaPAD/BflaPDU
<i>Rickettsia</i> spp.	<i>gltA</i>	RpCS.780p/877p/1273r
<i>Coxiella burnetii</i>	IS1111	Trans1/Trans2
Protozoa		
<i>Babesia microti</i>	18S rDNA	Bab1/Bab4
<i>Leishmania</i> spp.	kDNA minicircle	L.MC-1S/L.MC-1R
<i>Plasmodium</i> spp.	Cytb	Cytb1&Cytb2
<i>Trypanosoma</i> spp.	ITS1	ITS1-CF/ITS1-BR

No. of tested samples was 88.

Work flow for Haemo-pathogen detection

- Culling of primates Baboons & Vervet monkeys

- Spleen DNA extraction

- PCR

- Sequencing & Phylogeny

Results

Table 2: The prevalence of zoonotic haemo-pathogens in non-human primates in Zambia

Parasite species	Primate species Baboon (n=44)	Vervet monkey (n=44)
<i>Anaplasma</i> spp.	5 (11.4%)	7 (15.9%)
<i>Babesia microti</i>	2 (4.6%)	0
<i>Borrelia</i> spp.	0	0
<i>Coxiella burnetii</i>	0	0
<i>Leishmania</i> spp.	0	0
<i>Plasmodium</i> spp.	0*	0*
<i>Rickettsia</i> spp.	16 (36.4%)	19 (43.2%)
<i>Trypanosoma</i> spp.	0	0

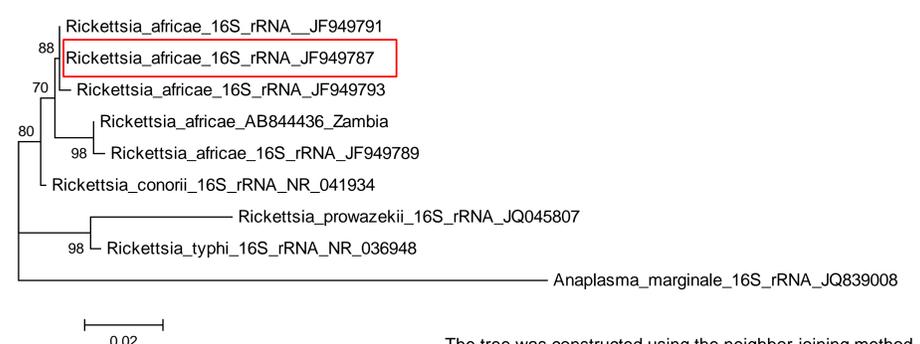
No. of tested samples was 88.

* *Hepatozoon* spp. was detected at 17% prevalence. The *cyto-b* sequence of 23 clones from 17 *Plasmodium cyto-b* PCR positive samples fell within *Hepatozoon* sp., known to infect Old World monkey parasites. This is known to be relatively benign parasite, and ubiquitous in African monkeys and apes.

Table 3. Sequence and BLAST result of PCR-positive samples

Sample ID	Host	Top hit Accession no. (blastn)	Homology	Size (bp)
15	Baboon	JF949789	99% <i>Rickettsia africae</i>	426
16	Vervet	JF949789	99% <i>Rickettsia africae</i>	426
32	Baboon	AY056017	90% <i>Babesia microti</i>	238
56	Baboon	AY056017	87% <i>Babesia microti</i>	238
43	Vervet	CP000235	100% <i>Anaplasma phagocytophilum</i>	345
100	Baboon	CP000235	99% <i>Anaplasma phagocytophilum</i>	345

Fig.2 Phylogenetic positions of the *Rickettsiae* detected in primates from Zambia based on 16S rRNA sequences (426 bp)



- The tree was constructed using the neighbor-joining method. Accession numbers are indicated.

Summary

• *Anaplasma phagocytophilum*, *Babesia microti* and *Rickettsia africae* were detected from the non-human primates. → **Zoonotic pathogens**

• Trypanosomiasis was not detected in the non-human primates despite the fact that the Luangwa valley ecosystem is an endemic focus to trypanosomiasis including Human African trypanosomiasis.

All of these pathogens are transmitted by ticks. However, other vectors and reservoirs like rodents, antelopes and other wildlife and mammalian hosts are present for other pathogens.

• This work was supported by Grant-in-Aid for JSPS fellows and for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), the program of the Funding Research Centre for Emerging and Re-emerging Infectious Disease, MEXT. Special thanks to Zambia Wildlife Authority (ZAWA).