Short Communication

First Report of Leishmania infantum in a Captive Panther from Pakistan

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ABSTRACT

Visceral leishmaniasis is a neglected zoonotic protozoal disease caused by Leishmania infantum and Leishmania donovani that is transmitted by sandflies (Phlebotamine flies). In November 2020, a case of leishmaniasis was diagnosed in a captive tiger through microscopy and L. infantum was confirmed by PCR and sequencing analysis. DNA sequencing of the amplicon revealed close homology with Leishmania sequences available in GenBank. Alignments and phylogenetic analyses of the Leishmania infantum from a tiger in Pakistan indicated 94-100 % identity with Leishmania from animals and 98.8-100% with Leishmania from humans suggesting the need for screening of animals before transporting, and of humans before taking care of captive animals, in order to prevent transboundary spread of Leishmania.

In November 2020, a blood sample of a male brown tiger (Panthera tigris tigris) from Lahore Zoological Garden (Lahore, Pakistan) was received at the Diagnostic Laboratory of the Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan for the evaluation of hemoparasites. The tiger was imported from United Arab Emirates (UAE) in April, 2019 and was healthy. In October 2020, the tiger received a wound and a blood sample was collected at that time. The approximate age of tiger was 3-4 years. Later on the tiger died possibly due to the previous injury or due to the previous injury or weakness (Alves et al., 2018; Herwaldt, 1999; Murray et al., 2005). In central Asia, the Mediterranean region and the United States, VL is caused by L. infantum (Cavalera et al., 2020; Herwaldt, 1999). Following the bite of an infected sandfly, L. infantum follows hematogenous route and infects phagocytes of spleen, liver, bone marrow, and lymph nodes (Ribeiro et al., 2018). An animal infected with Leishmania generally shows no clinical signs, however, in immuno-compromised animals, above mentioned signs are noted.

Reservoir host for VL (L. infantum) is dog (Asfaram et al., 2019). Previously, wild cats were considered resistant to VL. However, VL in Panthera tigris, Leopardus pardalis, Puma concolor, Panthera onca, and Panthera leo has recently been reported (Tolentino et al., 2019). The principal mode of transmission of Leishmania is via the bite of a biological vector: Sandflies of genus Phlebotomus (Serafim et al., 2020). The present study reports a case of Leishmania infantum in captive tiger.

Materials and methods

In November 2020, a blood sample of a male brown tiger (Panthera tigris tigris) from Lahore Zoological Garden (Lahore, Pakistan) was received at the Diagnostic Laboratory of the Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan for the evaluation of hemoparasites. The tiger was imported from United Arab Emirates (UAE) in April, 2019 and was healthy. In October 2020, the tiger received a wound and a blood sample was collected at that time. The approximate age of tiger was 3-4 years. Later on the tiger died possibly due to the previous injury or Leishmaniasis in November 2020. Blood smears were prepared, staining with Giemsa stain, and examined under oil emersion lens (100X).

The blood sample was subjected to PCR. Briefly DNA was extracted by Phenol-Chloroform-Isomyl alcohol method (Chacon-Cortes and Griffiths, 2014). PCR was performed with L. infantum primer pair targeting a 570 bp internal transcribed spacer region of rRNA (Tolentino et al., 2019). The amplification was performed

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0030-9023/2022/0001-0001 $ 9.00/0

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in a thermocycler (Thermo Fisher Scientific, Gloucester, UK) in a final volume of 20 µl containing 10 µl of dream 
taq master mix (Thermo Fisher Scientific, Waltham, MA, 
USA), 2 µl of each primer, 2 µl DNA template, and 4 
µl diethyl pyrocarbonate (DEPC) water. The DNA was 
amplified using standard amplification settings with initial 
denaturation at 95°C for 5 min followed by 35 cycles 
(denaturation at 95 °C for 30 seconds, annealing at 61.5 
°C for 1 min, and extension at 72 °C for 1 min) followed 
by final extension at 72°C for 10 min. The PCR product 
was subjected to gel electrophoresis using gel apparatus 
(BioBase, Shandong, China). The gel was analyzed under 
Ultraviolet illuminator (Mupid-One, Nippon Genetics, 
Japan). The gel band was excised with a sterile scalpel 
blade and sent to Advance Bioscience International, 
Lahore for sequencing. The obtained sequence was 
aligned using online BLASTN tool (blastn; ncbi.nlm. 
nih.gov) using default parameters. The partial sequences 
(n=7) of 18S subunit ribosomal RNA of Leishmania (from 
animals and humans) were retrieved from public data base 
(GenBank; ncbi.nlm.nih.gov) and used to align with the 
sequence obtained in the current study using laser gene 
(version 11, DNASTAR Inc., Madison, WI). Phylogenet 
tic trees were constructed by maximum likelihood method 
and Tamura-Nei model using the molecular evolutionary 
genetics analysis software: MEGA X (version 10.2.2) with 
1000 bootstrap replications (Kumar et al., 2018; Tamura 
and Nei, 1993). 18S RNA sequence of L. braziliensis and 
L. amazonensis was used as an out-group with sequences 
of L. infantum from animal’s and human’s origins for 
phylogenetic analyses, respectively.

Results

The microscopic examination revealed the presence 
of inclusion bodies consistent with amastigote stage of 
Leishmania (Fig. 1a). As a result of PCR gel analysis, a 
band was visualized at expected size (Fig. 1b) that was 
sequenced. The sequence obtained from the tiger was 
identified as L. infantum. The sequence has been deposited 
in public data base, GenBank with an accession number 
(MW730712). The sequence alignments indicated the highest percent 
identity of 99-100% and identity of 98.7-99.5%. The sequence alignments indicated the highest percent 
identity of 94-100%; Fig. 2a) and humans (98.8-100%; Fig. 2b).

Discussion

L. infantum infects a range of hosts including 
domestic and wild canines and felines (Lima et al., 2019). 
In Pakistan, leishmaniasis has been reported previously 
with a wide variety of hosts including dog (Canis lupis), 
cattle (Bos taurus), goat (Capra aegagrus hircus), sheep 
(Ovis aries), buffalo (Bubalus bubalis), donkey (Equus 
asinus), wild rats (Rattus), and Indian gerbils (Tatera 
indica) (Tiwananhagorn et al., 2012). This is the first 
report of Leishmaniasis in a captive tiger from Pakistan. 
Unfortunately, the tiger died of an injury and an underlying 
Leishmaniasis, in November, 2020 thus closing down the 
case follow-up whereas the efforts to collect an evidence 
for the presence of vector continued. We evaluated the 
tigers’ enclosure and its periphery for the presence of 
sandflies. As no sandflies could be retrieved during several 
attacks of fly-capture using electric traps from the vicinity 
of tiger’s cage, thus it is highly likely that tiger caught 
the infection before being shifted to the facility. Other 
possibilities include the occurrence of this infection at the 
place where the tiger was born and lived (in UAE) before 
being imported, or during the transport. It’s well known 
that this disease appears in the host, much longer after 
the introduction of the parasite by the bite of an infected 
sandfly, thus occurrence of infection during transport 
seems less likely as against its occurrence in UAE before 
being imported to Pakistan. The UAE is located in Middle 
East a region known to be endemic for Leishmaniasis, 
predominantly for the cutaneous form whereas it’s not 
absolutely free from VL. The VL has also been reported 
in feline and canine hosts in the Middle Eastern countries 
(Lima et al., 2019) that are considered endemic for this 
disease owing to vast-population displacements from 
Leishmania-infected regions like from Iraq (Salam et al., 
2014). Challenges in the sandfly population control are also 
contributing to a rise in the case numbers of leishmaniasis 
in the Middle East (Stoops et al., 2013). Interestingly, all 
Leishmania sequences from humans that clustered with L. 
infantum sequence of the current study, were from India 
(Fig. 2b), indicating a potential case of zooanthroponosis. 
Large part of workforce in Middle East is sourced from 
India, another endemic region for this disease. It can be 
hypothesized that a Leishmania-infected care taker from 
India might be the source of infection for this tiger born in 
UAE in a captive facility.

Leishmania infantum is not as common as L. major 
and L. tropica in Pakistan (Durrani et al., 2011). However, 
disease caused by Leishmania infantum in Pakistan is not 
reported suggesting the finding could be coincidental or 
least important as a potential reservoir, if host, parasite, 
and environment factors were appropriate. Unfortunately, 
we could not evaluate the tiger’s caretakers in UAE or 
Pakistan which should constitute a crucial step in 
establishing the source of infection as asymptomatic 
human infection is common (Michel et al., 2011).
Leishmaniasis, a neglected tropical disease, is a public health as well as an animal health challenge. The zoonotic potential Leishmaniasis is particularly problematic in a captive environment located in an endemic region. High sequence identity and clustering of our sequence with the sequences of *L. infantum* from animals and humans from GenBank database highlights that infected captive animals may constitute a risk to other susceptible animals and humans kept in close proximity. Moreover, reverse zoonoses, is suspected in this case, suggesting that human caretakers could be a source of infection for captive wild animals. Vector control based on chemical or electrical insect killers especially during the fly-season, is an important tool to mitigate such a risk.

Fig. 1. (a) Microscopic image captured with an i-Phone-7 from stained blood smear of tiger blood sample examined at 1000x magnification in an Olympus CX-21 microscope. The inset contains images of two monocytes that were edited with photos editor of windows, with a Zeke Filter on it, for a clearer view of the multiple intracellular Leishmanial amastigotes (Thick yellow arrow pointed to one of those) that actually helped in diagnosis. (b) An image of agarose gel indicating a band at the size expected for *L. infantum* detection in a tiger blood. C+ and C- represent positive and negative controls, respectively.

Fig. 2. Phylogenetic analyses of *L. infantum* from a tiger in Pakistan (a) with Leishmania sequences from animals and (b) humans.
As part of an active surveillance as well as a preventive strategy, thorough-screening of animals must be ensured before their movement across the regions.

**Conclusion**

The tigers should be screened for *Leishmania infantum* especially before transboundary transportation as tigers can be reservoirs of visceral leishmaniasis. Moreover, the health status of the caretakers and other human beings working in close proximity of captive animals should be carefully monitored and only *Leishmania* free caretakers should be allowed access to captive animals.

**Acknowledgements**

The authors would like to thank Prof Dr. Mohamed Gharbi (Laboratoire de Parasitologie, Université de la Manouba, École Nationale de Médecine Vétérinaire de Sidi Thabet, 2020 Sidi Thabet, Tunisia) for providing the *L. infantum* control positive DNA.

**Funding**

This research received no specific funding from any Public, Private or Non-profit Organization.

**Ethical statement**

The samples were taken by authorized veterinarians for diagnostic purposes according to the protocol of the zoo.

**Statement of conflict of interest**

The authors have declared no conflict of interest.

**References**


