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Short communication

Canine *Trypanosoma evansi* infection in Afghanistan

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ABSTRACT

In 2012, a dog in Afghanistan was diagnosed with trypanosomosis. We here describe the clinical picture of the animal and how the parasite was identified as *Trypanosoma evansi* by DNA extraction from a blood smear and molecular analysis with taxon-specific PCR assays.

1. Introduction

Trypanosoma (*T.*) *evansi* causes surra in livestock and wildlife and is mechanically transmitted by biting flies (Luckins and Dwinger, 2004). *T. evansi* belongs to the subgenus *Trypanozoon*, together with *T. brucei* and *T. equiperdum*. Camels and horses are particularly susceptible to surra (OIE, 2010). *T. evansi* infection can lead to severe immunosuppression and increased susceptibility to opportunistic infections. The disease is endemic in large parts of Asia, Africa, Latin America and the Canary Islands of Spain. According to the most recent report on animal health status from the World Organisation for Animal Health (OIE, 2011), Afghanistan did not report any case of surra so far, although the disease is known to be endemic in most parts of central Asia (Luckins, 1988) and has been reported in Iran (OIE, 2011; Pourjafar et al., 2013) and Pakistan (OIE, 2011; Ul Hasan et al., 2006; Shahzad et al., 2010). On the 6th of March 2012, a 7-years old male Dobermann was presented to the Nowzad Dogs Charity Clinic in Kabul, Afghanistan with a confirmed *T. evansi* infection. Here we report the clinical characteristics of the dog and how we identified the *T. evansi* parasites in the blood of the dog.

2. Materials and methods

2.1. Microscopic analysis

Three unstained fixed blood slides were transferred to the OIE Reference Center for Surra at the Institute of Tropical Medicine (ITM) Antwerp (Belgium). The blood slides were Giemsa stained and analysed using an Olympus BX41 microscope (Olympus, Aartselaar, Belgium).

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2.2. DNA extraction

To identify the trypanosome based on its genomic profile, total DNA from a non-stained blood smear of the dog was extracted based on the method described by Meredith et al. (1993) The blood smear was overlaid with 267 μL lysis buffer containg 50 mM NaCl, 10 mM EDTA (pH 8.0) and 50 mM Tris-HCl (pH 7.4). The cellular material was carefully scraped off with a pipette tip and collected in a 1.5 mL micocentrifuge tube. Sixty μg proteinase K and sodium dodecyl sulphate (1% final concentration) were added and the solution was incubated overnight at 60 °C. The lysed sample was subjected to the classic phenol–chloroform extraction and ethanol/sodium acetate precipitation as described by van Eys et al. (1989) The DNA pellet was dried using the DNA SpeedVac (Savant Instruments, Farmingdale, NY) for 5 min and dissolved in 50 μL of molecular biology graded water (Lonza, USA). The DNA sample was kept at 4 °C until use. Lysis mixture with no blood added was subjected to the complete extraction procedure to exclude possible sample contamination during extraction.

2.3. Taxon-specific PCRs

Since the blood smears were archived at ambient temperature for several months, we first verified if the DNA extraction was successful by amplifying a part of the dog's cytochrome b gene using the universal PCR primers described by Kocher et al. (1989). In a next step, we systematically applied several PCR assays to determine if the trypanosome belonged to the *Trypanosomatidae* family, the *Trypanozoon* subgenus and the *T. evansi* species. An overview of the PCR assays used in the study is presented in Table 1. Each PCR assay was done in 25 μL reaction volumes containing 1x Qiagen PCR buffer (Qiagen, Germany), 2.5 mM $MgCl_2$ (Qiagen), 200 μM of each deoxynucleotide triphosphate (Roche, Germany), 0.8 μM of each primer (Sigma, Belgium), 0.1 mg/ml acetylated bovine serum albumin (Promega, USA), 0.5 units of HotStar Taq polymerase (Qiagen, Germany) and 2.5 μL sample DNA. Cycling conditions were 94 °C for 15 min to activate the HotStar Taq polymerase, followed by 40 cycles of 94 °C for 30 s, a PCR-specific annealing temperature for 30 s, 72 °C for 30 s, and a final extension of 72 °C for 5 min. Amplification was conducted in 200-μL thin-wall PCR tubes (ABgene, UK) in a T3 thermocycler 48 (Biometra, Germany). Amplified products were analysed by electrophoresis in a 2% agarose gel (Eurogentec, Belgium) and UV illumination (Syngene, UK) after ethidium bromide staining of the DNA (Sigma, Belgium).

3. Results

3.1. Clinical presentation

On admission to the clinic, the dog showed general malaise, lethargy, loss of appetite, and severe uveitis. Body temperature was 38.8 °C and antibiotics (Betamox and Polyfax eye cream) were prescribed. Six days later the dog re-presented to the clinic with diarrhoea, heart murmurs, complete loss of vision and severe hyphema. The dog's complete blood count showed normal packed cell volume (PCV, 37%) and total white blood cell count (13.1×10^9/L). Canine diabetes was excluded as the glucose, cholesterol and liver enzyme levels in the blood were normal. However, blood slides showed long slender trypomastigotes at very high concentration. At that time the dog also presented canine distemper and his physical condition was getting worse. We decided that the dog was too weak for anti-trypanosomal treatment with Berenil and first had to recover from the canine distemper. We performed regular home visits and the dog's physical condition was getting worse every day. On the 28th of March the dog was euthanized at the clinic.

3.2. Taxon-specific PCRs

The positive cytochrome b PCR indicated that DNA extraction of the archived blood smears was successful (Fig. 1A). The *Trypanosomatidae* (Fig. 1B), *Trypanozoon* (Fig. 1C) and *T. evansi* (Fig. 1D) specific PCR assays were all positive indicating that the trypanosomes observed in the dog's blood belonged to the *T. evansi* species.

4. Discussion

T. evansi causes surra in wild and domestic animals, mainly camels, cattle, buffaloes, equines, goats and sheep. In contrast to *T. brucei*, *T. evansi* does not require cyclic transmission in a tsetse fly but is mechanically transmitted by biting flies. Consequently, surra has spread outside the tsetse regions of sub-Saharan Africa and is endemic in northern Africa, the Sahel region, Latin America, and central and eastern Asia. According to the most recent report on animal health status from the World Organisation for Animal Health (OIE, 2011), Afghanistan did not report any case of surra so far. However, the disease is known to be endemic in many countries of central Asia (Luckins, 1988) and surra has been reported in Iran (OIE, 2011; Pourjafar et al., 2013) and Pakistan (OIE, 2011; Ul Hasan et al., 2006; Shahzad et al., 2010). This is the first case of surra reported in Afghanistan and to the OIE data bank. This canine infection suggests that *T. evansi* is endemic in the country and warrants further investigation into the epidemiological situation of surra in Afghanistan. We do not know how the dog in our study actually got infected with the parasite. The dog was born in Germany and arrived in Kabul in the summer of 2010. The animal never left Kabul city but has been fed with cooked and raw goat and sheep meat that could have caused the infection by oral transmission. Oral transmission of *T. evansi* has been reported earlier. Raina et al. successfully infected dogs by feeding them with *T. evansi* infected meat and blood (Raina et al., 1985). Also, in 1971 there was an outbreak of trypanosomosis in the tiger and jaguar populations of the zoological garden of Calcutta (India) due to feeding with *T. evansi* infected meat (Sinha et al., 1971). However, transmission via biting flies cannot be excluded since *T. evansi* infected horses and camels as well as biting flies may be present in Kabul city. Recently, two cases of canine *T. evansi* infections have been reported. In 2007, Hosseininejad observed *T. evansi* in three dogs referred to the Small Animal Hospital of Tehran

Table 1
Technical specifications of the PCR assays used in the study.

Target group	Target gene	Primers	Primer sequences	Ta	Amplicon length	Reference
Vertebrates	Cytochrome b	L14841 H15149	5′-CCATCCAACATCTCAGCATGATGAAA-3′ 5′-GCCCCTCAGAATGATATTTGTCCTCA-3′	52 °C	400 bp	Adapted from Kocher et al. (1989)
Trypanosomatidae	18S rDNA	18S-F	5′-CGCCAAGCTAATACATGAACCAA-3′	60 °C	110 bp	Adapted from Deborggraeve et al. (2006)
		18S-R	5′-TAATTTCATTCATTCGCTGGACG-3′			
Trypanozoon	18S rDNA	M18S-II-F-Tb M18S-II-R-Tb	5′-CGTAGTTGAACTGTGGGCCACGT-3′ 5′-ATGCATGACATGCGTGAAAGTGAG-3′	60 °C	150 bp	Deborggraeve et al. (2011)
T. evansi	RoTat 1.2.	RoTat1.2-F RoTat1.2-R	5′-GCGGGGTGTTTAAAGCAATA-3′ 5′-ATTAGTGCTGCGTGTGTTCG-3′	59 °C	205 bp	Adapted from Claes et al. (2004)

Ta, annealing temperature; bp, base pairs.

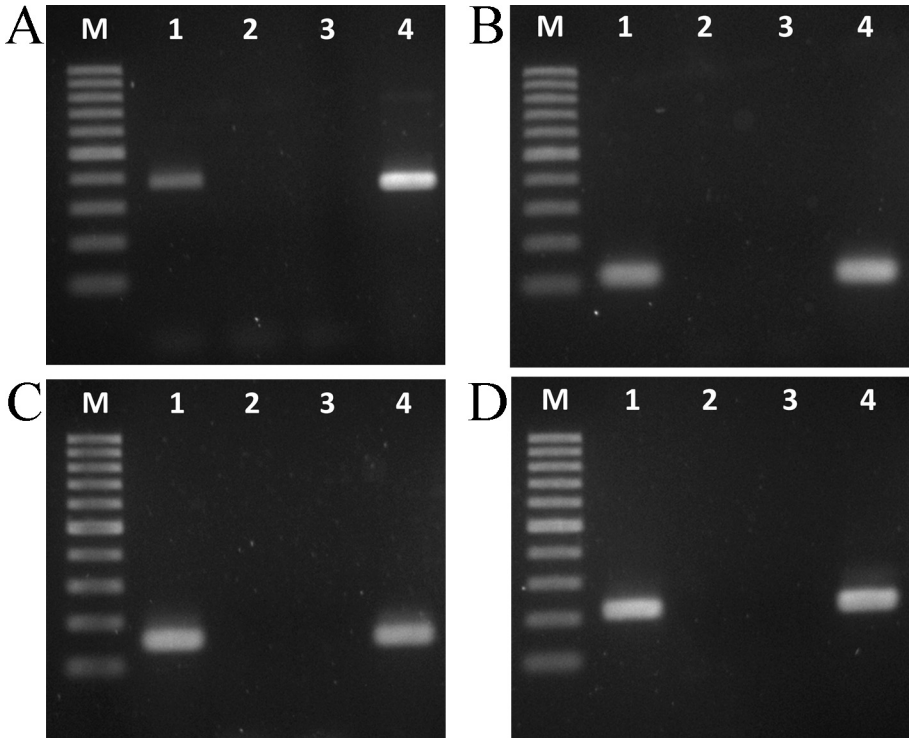

Fig. 1. Molecular analysis of the dog's archived blood smear with PCR assays specific for DNA from vertebrates (A), *Trypanosomatidae* (B), *Trypanozoon* (C) and *T. evansi* (D). M: 100 bp DNA marker (Fermentas), 1: dog smear, 2: negative control DNA extraction, 3: negative control PCR, 4: positive control PCR.

University, Iran (Hosseininejad et al., 2007). However, the authors did not use *T. evansi* specific molecular or serology tests but diagnosed the canine *T. evansi* cases based on the morphology of the trypanosomes in the blood slides and by biochemical analysis of the serum. Recently, Defontis and co-workers reported a *T. evansi* infection in a dog from Germany with a history of travel to Thailand. In that study, the dog was serologically positive in *T. evansi* CATT and confirmed parasitologically by RoTat1.2 PCR on fresh blood (Defontis et al., 2012).

5. Conclusion

In conclusion, we here report the first case of canine *T. evansi* infection in Afghanistan and we provide the detailed protocol how DNA was extracted from blood smears and how *T. evansi* was identified by taxon-specific PCRs.

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