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## ***Trypanosoma evansi* in camels, donkeys and dogs in India: comparison of PCR and light microscopy for detection - short communication**

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### **ABSTRACT**

The objective of the present study was to compare two methods: PCR and blood smear examination for sensitive and specific detection of *Trypanosoma evansi* in camels, donkeys and dogs. Out of 131 blood samples tested, (61 camels, 44 donkeys and 26 dogs), 26 samples (21 camels, 3 donkeys and 2 dogs) were detected positive by PCR. Blood smear examination revealed the *T. evansi* organisms in only two camels.

**Key words:** *Trypanosoma evansi*, camel, donkeys, dogs, PCR

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### **Introduction**

*Trypanosoma evansi*, a kinetoplastid haemoprotozoan with considerable economic importance, affects a wide range of hosts. The course of the disease and clinical picture varies from host to host thereby making the putative diagnosis more difficult. The cameline surra occurs in an acute or chronic debilitating form causing high morbidity and mortality (RUTTER, 1967; HIGGINS, 1983; PATHAK and KHANNA, 1995). The disease in dogs usually follows an acute course. Although a number of standard trypanosome detection

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methods are in vogue, the sensitivity and specificity of these methods in the detection of infection in subclinical and carrier status is not foolproof. Because of the limitations of immunological techniques (PATHAK et al., 1997) and the difficulty of demonstrating the parasite in blood smears due to the intermittent nature of parasitaemia (NANTULYA, 1990), there is an urgent need for sensitive and unequivocal detection of acute disease conditions and chronic carrier states.

Polymerase chain reaction assay amplifying genetically defined regions of genome of parasites including trypanosomes are currently of great interest. WUYTS et al. (1994) described a PCR targeted to repetitive nuclear sequence of *T. evansi* in mice and bovine calves from Thailand. The present investigation attempts to detect the trypanosome DNA from clotted blood of camels, dogs and donkeys using this PCR and its comparison with blood smear examination after giemsa staining.

### **Materials and methods**

*Animals and samples.* A total of 131 blood samples were collected from 61 camels from Bikaner (Rajasthan state), 44 donkeys from Sabarimala (Kerala state) and 26 dogs from the National Training Centre for Dogs of the Border Security Force, Tekanpur, Gwalior (Madhya Pradesh state), India, in sterile disposable syringes. All the animals were apparently healthy at the time of collection. The donkeys are brought to Sabarimala from Tamil Nadu during every pilgrim season and are used for transport of materials to the hill top temple. The blood samples were allowed to clot and send to Division of Parasitology, Indian Veterinary Research Institute, Izatnagar at room temperature. A pin head sample of the blood clot (approximately 10  $\mu$ L volume) was transferred to a PCR tube, boiled and stored at -20  $^{\circ}$ C till further use. This was used as template DNA for the PCR.

*PCR reactions.* The primers for PCR synthesized by Bangalore Genei, India from a repetitive sequence probe pMUTec6.258 (CHOKESAJJAWATTEE, 1993) were as follows: 5'TGCAGACGACCTGACGCTACT-3' and 5'TCCTAGAAGCTTCGGTGTCCT- 3'. The reaction (25  $\mu$ L) was performed as per the method of BASAGOUDANVAR et al. (1998). A hot start was performed for 7 min at 90  $^{\circ}$ C to completely denature DNA followed by 30 cycles each of 30 sec at 90  $^{\circ}$ C ( to denature), 30 sec at 60  $^{\circ}$ C (to anneal) and 30 sec at 72  $^{\circ}$ C (to extend), then with one extensive polymerization at 72  $^{\circ}$ C for 7 min. At the end of the cycling reaction, approximately 15  $\mu$ L of the product was electrophoresed on a 2 per cent agarose gel for visualizing the product.

Control reactions were performed with 0.5 ng each of parasite template DNA and leucocyte DNA from respective species of animals.

*Microscopy.* Thin blood smears were also prepared at the time of collection of blood. They were methanol fixed and sent to the laboratory. They were examined under an oil immersion objective of a microscope after staining with giemsa stain.

## Results

Giemsa stained blood smears on microscopical examination detected only 2 camels as positive for the presence of *T. evansi*. Smears from donkeys and dogs did not reveal any protozoan organisms.

Out of 131 blood samples from different animals tested, samples from 21 camels (including two blood smear positive camels), 3 donkeys and 2 dogs showed specific PCR signals as a distinct 227 bp band on agarose gel.

The percentage of *T. evansi* positive animals in each of the three species of animals based on two methods is shown in Table 1.

Table1. Percentage of *Trypanosoma evansi* positive animals by PCR and blood smear examination

Species	N° of samples	PCR positive	Prevalence%	Blood smear examination positive	Prevalence%
Camel	61	21	34.4	2	3.3
Donkey	44	3	6.8	0	0
Dogs	26	2	7.7	0	0
Total	131	26	19.84	2	1.5

## Discussion

Although different techniques have been developed, demonstration of organisms by light microscopy or subinoculation into susceptible rodents are routinely used for specific detection of infected animals. Blood smear examination proved to be of limited value in diagnosis of subacute or chronic cases. In the present study only two animals were identified positive by microscopy out of 131 samples tested. According to HERBERT and LUMSDEN (1976), when less than 2,500,000 parasites per mL are present in blood samples, microscopic detection is not feasible.

Antibodies to *T. evansi* infection persist after drug treatment, complicating the differentiation of patent infection from non-patent infections using serological methods. Polymerase chain reaction, free of these hurdles, specifically amplifies genetically defined regions of the genome of the infectious agent. Although the detection of a single DNA molecule is possible, detection levels to a minimum of 5 trypanosomes by PCR assay are

well documented (DIALL et al., 1992; ARTAMA et al., 1992). Amplification of repetitive *T. evansi* specific DNA sequence was possible even with DNA of a single trypanosome (PANYIM et al., 1992; VISESHAKUL and PANYIM, 1990). During the acute phase of experimental *T. evansi* infection in mice, the parasites were detected by PCR three days earlier than microscopy; also the infected mice were consistently positive during the chronic phase when parasites could not be demonstrated using microscopy (IJAZ et al., 1998). But on the contrary, HOLLAND et al. (2004) observed that owing to the inherent detection limits of PCR and fluctuating levels of parasitaemia, the number of ongoing infections will be underestimated.

In the present study, boiling of blood was used to lyse the parasite and to free DNA from blood clot. This caused the denaturation of PCR inhibitors like haemoglobin and cellular endonucleases. Boiling obviates the use of anticoagulants, which are known to act as potent inhibitors in PCR amplification. BASAGOUDANAVAR et al. (1998) reported the stability of unprocessed blood samples in PCR when stored at 28 °C for a period of 15 days after collection. The present study also supports this finding.

Trypanosomosis in camels is of a chronic nature, with animals becoming progressively weaker and emaciated, while in equines and dogs it is of the acute fatal type (GILL, 1991). In the present study, it was found that 27.8 percent of camels from Bikaner, Rajasthan were found to be carriers of the parasite. BASAGOUDANAVAR et al., 1998 reported 15 per cent of prevalence using PCR with a limited sample size of 20 camels from the same geographical area. SURYANARAYANA et al. (1985) examined 126 donkeys at Hissar and detected *T. evansi* in the blood of 13 donkeys. ANWAR and MOHAMMED (1986) reported an outbreak of surra in donkeys in Pakistan. Even though donkeys are exposed to similar vector challenges as horses, they are significantly less infected with trypanosomes, possibly because of the greater feeding preference of vectors for horses or the better ability of donkeys to deter the flies from feeding by skin rippling, head movements and other behavioral avoidance mechanisms (FAYE et al., 2001). Outbreaks of canine trypanosomosis were also well documented from India (ARORA and PATHAK, 1995; BALAKRISHNAN et al., 1994; VARSHNEY et al., 2003).

The fact that the primers used for the assay amplified 227 bp fragment from *T. brucei* and *T. equiperdum* also in addition to *T. evansi* (WUYTS et al., 1994), is hardly problematic for the present investigation since tsetse flies transmitted *T. brucei* and sexually transmitted *T. equiperdum* are nonexistent in the Indian subcontinent. The present investigation emphasizes the need for routine screening of blood samples, especially from valuable dogs, and immediate veterinary care.

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**SAŽETAK**

Svrha ovoga istraživanja bila je usporediti osjetljivost i specifičnost lančane reakcije polimerazom i pretrage krvnih razmazaka svjetlosnim mikroskopom za dokazivanje vrste *Trypanosoma evansi* u deva, magaraca i pasa. Od 131 pretraženoga krvnoga uzorka (61 od deva, 44 od magaraca i 26 od pasa), 26 uzoraka (21 od deva, tri od magaraca i dva od pasa) bilo je pozivno na osnovi pretrage lančanom reakcijom polimerazom. Pretragom krvnih razmazaka svjetlosnim mikroskopom *T. evansi* dokazana je samo u dvije deve.

**Ključne riječi:** *Trypanosoma evansi*, deve, magarci, psi, lančana reakcija polimerazom

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