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Molecular Detection of Trypanosoma evansi in Royal Bengal Tiger (Panthera tigristigris)

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1. Abstract

1.1. Background

Trypanosomiasis, widely known as sleeping sickness in humans and Nagana in animals, is a vector-borne disease caused by Trypanosoma species transmitted by tabanid flies. It significantly impacts a wide range of species including large elephants, buffaloes, giraffes, tigers, lions and leopards across various regions.

1.2. Case presentation

This is a presentation of case report of a 7-year-old male Royal Bengal tiger (*Panthera tigristigris*) housed in a facility at Kittur Rani Chennamma Nisargadhama, Belagavi ADDL&Ic, Karnataka state in India. The affected tiger displayed a sudden onset of clinical signs, including intermittent fever, lethargy, decreased appetite and significant weight loss over a two-week period of time. Caretakers reported that the animal was increasingly displayed signs of weakness. The diagnosis was confirmed by microscopic examination of Giemsa-stained blood smears followed by PCR.

1.3. Conclusion

In this case report, trypanosomiasis was diagnosed in a Royal Bengal tiger. After prompt detection, proper treatment procedure was adopted and the animal was cured. Vector control in the susceptible wildlife population as well as surrounding domestic livestock is also critical for the control of the disease.

2. Keywords

PCR; Tiger; Trypanosomiasis

3. Introduction

International Union for Conservation of Nature (IUCN) reports around 3900 tigers are left in the wildlife. In India, 2967 tigers are there accounting about 80% of the total global tiger population [1]. Trypanosomiasis/Surra has been responsible for mortality in number of animals of rare species including large felines in India [2]. Trypanosomes are blood and occasionally tissue parasites belonging to the order Kinetoplastida in the family *Trypanosomatidae*, genus *Trypanosoma*, chiefly transmitted by hematophagous vectors such as Tabanus and Stomoxys flies [3]. *Trypanosoma evansi* is an important hemoparasite posing a threat to the domestic as well as wild animals managed under captivity. The accurate diagnosis of the parasite relies on clinical symptoms, parasitological testing and nucleic acid-based molecular assays. Polymerase Chain Reaction (PCR) diagnosis aids in the early and efficient detection guiding treatment of infections in animals and adopting control strategy for blood sucking vectors present in the area. This research enhanced our knowledge on trypanosomiasis with its accurate identification using smear examination and PCR. Due to the inherent limitations of microscopy and serology-based assays, parasitologists presently rely on molecular techniques targeting gene amplification. In recent years, several types of PCR assays have been employed for solid detection of trypanosomes across the world.

4. Case Presentation

A 7-year-old male Royal Bengal tiger (*Panthera tigristigris*) housed in a facility at Kittur Rani Chennamma Nisargadhama, Belagavi, Karnataka in India displayed a sudden onset of clinical signs including intermittent fever, lethargy, decreased appetite and significant weight loss over a two-week period of time. Caretakers reported that the animal was increasingly displayed signs of weakness.Clinical examination revealed pale mucous membranes indicative of anemia and a notable decrease in overall body condition. Blood sample was drawn and sent to ADDL&IC lab and the tiger was isolated to prevent potential transmission of any infectious agents to other animals. The animal had been maintained in a controlled environment with regular check-ups. A comprehensive clinical examination, including evaluation of vital signs (temperature, heart rate, and respiratory rate), body condition scoring, and assessment of hydration status was performed.

Blood sample (approximately 2 mL) was collected. A Complete Blood Count (CBC) was conducted using an automated hematologyanalyzer to evaluate leukocyte and platelet counts, hemoglobin concentration, and hematocrit levels. Blood smears were prepared, air dried, followed by methanol fixation and Giemsa staining and examined under an oil-immersion lens of a light microscope to detect the presence of Trypanosomes. Hematological examination showed total leucocyte count within normal range, severe left shift with 50% of band cells and possibility of Disseminated Intravascular Coagulation (DIC) due to severe thrombocytopenia. On the basis of clinical finding and microscopic detection of organism, the condition was diagnosed as trypanosomiasis and clinical treatment was undertaken. The haematology values include Hb 12.1g%, WBCs 9250/dl, RBCs 6.19 millions/dl, platelets were low 73000/dl. Serum biochemical values reveled the high SGPT 180u/l, elevated serum amylase 4950u/l. Both haematology and biochemical parameters supported the haemoprotozoan infection. The values are mentioned in table 1.

Genomic DNA was extracted from blood sample using the QIAamp® DNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. A total of 200 µL of whole blood was used for DNA extraction, with a final elution volume of 100 µL and was stored at −20˚C until further use. Hi-PCR® Trypanosoma evansi Semi-Q PCR Kit (HiGenoMB, Himedia, MBPCR212) was used. For extraction and purification of DNA for high yield, the nucleic acid purification was done using HiMedia'sHiPurA® Multi-Sample DNA Purification Kit (MB554) as instructed in the protocol. PCR reactions were performed using 2X PCR TaqMixture 12.5 µL, Primer Mix for T. evansi 2µL, template (extracted DNA) 2.5 µL, molecular biology grade water up to 25µL. For a positive control reaction tube, 5µL of positive control DNA (DS0894) was added along with above mentioned components instead of the extracted DNA template. The tube was centrifuged briefly at 6000 rpm for about 10 seconds to settle down the contents and was placed in the thermocycler with recommended PCR program as: initial denaturation at 95˚C for 10 minutes, 30 cycles of denaturation at 95˚C for 45 seconds, annealing

Table 1: Hematology and Serum Biochemical Values.

PARAMETERS		VALUES
HEMATOLOGY	HEMOGLOBIN	12.1 g%
	TOTAL WBC COUNT	9250 cells/cmm
	DIFFERENTIAL COUNT	
	NEUTROPHILS	81%
	LYMPHOCYTES	12%
	EOSINOPHILS	1%
	MONOCYTES	6%
	MCH	19.6 pg
	MCV	63.0 fl
	MCHC	31.1 gm/dl
	RBC COUNT	6.19 million cells/cmm
	PLATELET COUNT	73,000 cells/cmm
	PCV	39.00%
	RDW	49.0 fl
SERUM BIOCHEMISTRY	RANDOM BLOOD SUGAR	24 mgs%
	SERUM CREATININE	2.7 mgs%
	SERUM TOTAL BILRUBIN	0.6 mgs%
	SERUM DIRECT BILIRUBIN	0.1 mgs%
	SERUM SGOT	62 U/L
	SERUM SGPT	180 U/L
	SERUM ALKALINE PHOSPHTASE	31 U/L
	SERUM AMYLASE	4950 U/L
	SERUM LIPASE	2521 U/L
	SERUM TOTAL PROTEIN	7.2 gm%
	SERUM ALBUMINE	3.1 gm%
	A:G RATIO	0.75
	BUN	27 mgs%
	SERUM URIC ACID	1.2 mgs%
SERUM ELECTROLYTES	SERUM CALCIUM	10.6 mgs%
	SERUM SODIUM Na+	149 mEq/L
	SERUM POTASSIUM K+	3.7 mEq/L
	SERUM CHLORIDE Cl-	117 mEq/L
	SERUM BICARBONATE	22 mEq/L
	SERUM PHOSPHORUS	4.2 mgs%

at 55°C for 30seconds, extension at 72°C for 30 seconds followed by final extension at 72°C for 05 minutes. For analysis, 10 µL of amplicon was loaded on a 2% agarose gel stained with ethidium bromide along with 1µL of 6X gel loading buffer.

Thin blood smear examination revealed the presence of Trypanosoma species (Figure 1). Hematological analysis indicated significant leukopenia and thrombocytopenia, which are consistent with parasitic infections. PCR showed the amplification of trypanosome specific DNA (Figure 2) indicating the confirmation. Following the confirmation of trypanosomiasis, the tiger was treated with Triquin (Quinapyramine Sulphate and Quinapyramine Chloride 3:2 ratio). The dosage was calculated based on the animal's weight (2.5 ml of Triquin injection per 100 kg BW) and administered deep intramuscularly along with Meloxicam @ 0.2mg/kg body weight I/M twice a day for 3 days. Belamyl (B-complex with liver extract) @ 2ml I/M twice a day for 3 days. Following treatment, a follow-up examination showed improvement in clinical signs, and subsequent blood tests revealed a significant reduction in parasitemia, with no detectable Trypanosoma in blood smears after two weeks of treatment. The rapid clinical improvement accompanied by the disappearance of trypomastigotes in blood smears performed after the end of therapy seems to confirm the diagnosis indicating that Triquin (Quinapyramine Sulphate &Quinapyramine Chloride 3:2 ratio) is effective against trypanosomes.

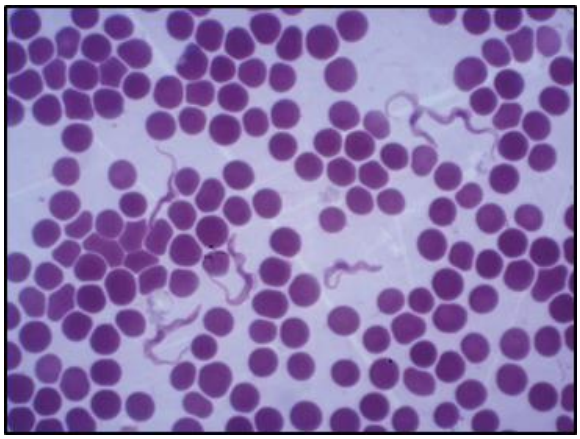

Figure 1: Blood smear showing the extracellular hemoprotozoan trypanosome parasite

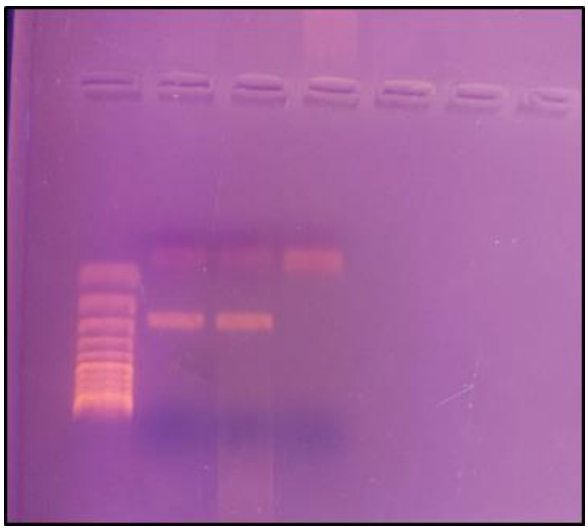

Figure 2: PCR showing the amplification of trypanosome-specific DNA at 830bp
Lane 1: 100 bp DNA ladder, Lane 2: Tiger DNA sample, Lane 3: Positive control and Lane 4: Negative control

5. Discussion

India is hosting the highest number of tigers all over the world. Hence, utmost care should be taken for maintaining a healthy protocol for the tigers so that their criticality for a balanced ecosystem is proven. The present case of trypanosomiasis in tiger highlights the vulnerability of these species to diseases typically associated with domestic animals. *Trypanosoma evansi* is having the widest range of host range among salivariantryapansomes. Trypanosomiasis poses a significant health risk for tigers, lion, leopard, jungle cat, jaguar, wolf, deer, elephant, wild pig etc [4], particularly in environments where they come into contact with infected hosts, such as cattle or wildlife species as mentioned before. Following any abrasion in oral mucosa, the wild carnivores may acquire infection through ingestion of infected meat. Among wild animals, tiger and elephant are highly susceptible to trypanosomiasis [5]. Anemia is the most common cause of death of infected animals [6], which might be due to increase erythrophagocytosis and increased destruction of RBCs by the trypanosome organism [7]. Sporadic reports of trypanosomiasis in tiger due to *T. evansi* have been reported by many authors from different parts of the country.

Pathology of *Trypanosoma evansi* infection in a tiger was reported in India by researchers [8]. There is published report of *T. evansi* detected in big cats like leopards (*Panthera pardus*), jaguars (*Panthera onca*) and tigers (*Panthera tigris*) in India [3]. The disease has been reported in different species including dogs in India and south-east Asia [9], jaguars from Brazilian pantanal wetland [10], horse from Punjab, Pakistan [11], captive tigers and lions in Punjab, Pakistan [12], Iranian one-humped camels [13], dromedaries from Greater Cairo, Egypt [14]. The clinical signs observed in this case, including fever and anemia, are well-documented in other species affected by Trypanosoma. The hematological alterations were leukopenia and thrombocytopenia can result from the immune response to the parasite and indicate the severity of the infection [15,16].

PCR assay targeting ITS1 region has been useful in detecting *T. evansi* infection in goat from Cebu, Philippines [17]. Researchers [18] have used smear microscopy and real time PCR assay for detection of *T. evansi* infection in wild capybaras from Argentina. Workers [11] used formol gel test and PCR assay to detect *T. brucei evansi* in horse from Punjab, Pakistan. Authors [12] used both microscopy and PCR (targeting surface glycoprotein gene) for *T. evansi* detection in captive tigers and lions in Punjab, Pakistan highlighting higher sensitivity of PCR over microscopy. Researchers [13] undertook molecular and hematological investigation of *T. evansi* infection in Iranian one-humped camels (*Camelus dromedarius*) targeting ITS1, 5.8S, and ITS2 ribosomal regions. Diagnosis of the natural infection of captive and free-living procyonids with *T. evansi*was reported in the states of Amapá and Pará, Brazil targeting 205bp fragment of the RoTat 1.2 VSG gene [19]. Parasitological and molecular investigation of Trypanosoma evansi using blood smear examination and PCR-sequencing assays based on the Variant Surface Glycoprotein (VSG) was reported in dromedaries from Greater Cairo, Egypt [14].

In captive wildlife, management practices must consider the risk of trypanosomiasis. Factors such as the proximity to livestock and habitat conditions favoring interaction between wildlife and livestock can facilitate transmission. Monitoring and controlling potential vectors, along with regular health assessments, are crucial for preventing outbreaks in captive populations [20]. In the present case, trypanosome infection in domestic buffalos in the surrounding villages of the biological park have been noticed. This exposure raised concerns about potential vector control and researchers have advised to employ vector traps to manage and lower the risk of illness [21]. Furthermore, public awareness and education about the risks of wildlife-livestock interactions are essential for conservation efforts. The treatment and recovery of the tiger demonstrate the potential for successful intervention with timely diagnosis and appropriate therapeutic strategies. Efforts to manage and mitigate the impact of trypanosomiasis on wildlife involve a combination of strategies including vector control using insecticide-treated traps, baits, or the release of sterile males; surveillance and monitoring of wildlife

health and parasite prevalence; habitat management to reduce fly populations or minimize the risk of transmission between wildlife and livestock etc.

6. Conclusion

In this case report, trypanosomiasis was diagnosed in a Royal Bengal tiger based on microscopic and molecular detection using PCR. After prompt detection, proper treatment procedure was adopted and the animal was cured of the disease. Positive response to treatment also fingered towards an accurate diagnosis. Vector control in the susceptible wildlife population as well as surrounding domestic livestock is critical for the control of the disease.

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