CASE REPORT: TREATMENT OF TRYPANOSOMES INFESTATION IN A MALAYAN TIGER (*Panthera tigris* ssp. *jacksoni*)

***Zubaidah, K., Siti-Suzana, S., Nur-Mala, A., Mohd-Zamri, R. & Khairul-Anuar, A.L.**

National Wildlife Rescue Centre (NWRC) Sungkai, Department of Wildlife and National Parks (PERHILITAN) Peninsular Malaysia, 35600 Sungkai, Perak.

*Corresponding author's email: zubaidah@wildlife.gov.my

ABSTRACT

A male adult Malayan Tiger (*Panthera tigris* ssp. *jacksoni*) was presented with clinical signs of decreased appetite, inactive and gradual loss of body weight. Diagnosis of trypanosomiasis was made based on thin blood smear examination. The hematology and biochemistry results revealed anaemia and hyperproteinaemia and present of marked enlargement of the liver upon abdominal ultrasound. Early treatment with Immiticide® (melarsomine dihydrochloride) only showed partial recovery of the infection. Follow up treatment with two doses of Berenil® (diminazene aceturate) at 3.5 mg/kg by intramuscular injection resulted in full recovery.

Keywords: Malayan tiger, trypanosomes, melarsomine dihydrochloride, diminazene aceturate

Received (19-May-2020); Accepted (11-January-2021); Available online (25-March-2021).

Citation: Zubaidah, K., Siti-Suzana, S., Nur-Mala, A., Mohd-Zamri, R. & Khairul-Anuar, A.L. (2021). Case report: treatment of trypanosomes infestation in a Malayan tiger (*Panthera tigris* ssp. *jacksoni*). *Journal of Wildlife and Parks,* **36**: 39-44.

INTRODUCTION

Trypanosoma is an extracellular protozoan blood parasite that affects most domestic animals and wildlife. The major veterinary importance species are *Trypanosoma congolense*, *T. vivax*, *T. brucei brucei*, *T. simiae, T. evansi, T.*

equiperdum and *T. theleri* (Rogers & Randolph, 1993; Touratier, 1999; Christine & Grinnage-Pulley, 2015). The 'African sleeping sickness' is a zoonotic disease caused by *T. brucei rhodesiense* and *T. brucei gambiense.* These parasites are transmitted mechanically by biting flies of the genera *Tabanus, Lyperosia, Stomoxys*, and *Atylotus* (Brun *et al*., 1998). The incubation period of the disease is 1- 4 weeks (Khan *et al*., 2015).

The primary clinical signs are intermittent fever, anaemia and weight loss as described by Christine and Grinnage-Pulley (2015). Khan *et al.* (2015) reported that Bengal tiger in Lahore Zoo infected by trypanosomes displayed clinical signs of anorexia, intermittent fever, watery eyes, pale mucus membrane, bilateral enlargement of prescapular lymph node, reluctant to walk, and seizure. Late detection and treatment of the disease may lead to pathology of the brain tissues causing confusion and sleep disorders. Significant losses due to coma and death will be the ultimate consequences (Maya *et al.,* 2007). Thus, early diagnosis of the disease and treatment is vital to ensure the recovery of infected individuals.

In wildlife, trypanosomes infection had been reported in deer, elephant, capybara, jaguars, Australian marsupial, and wild chimpanzees (Choudury & Misra, 1972; Smith *et al*., 2008; Jirků *et al*., 2015). Many cases of trypanosomiasis had been reported in tigers. Parija and Bhattacharya (2001) reported the death of 12 tigers (*Panthera tigris tigris*) in Nandakanan Zoo, India due to *T. evansi* infection. The same organism also caused the death in male Royal Bengal Tiger in Chennai (Manohar *et al*., 2003). Trypanosomiasis contributes to 25% of the tiger's mortality in Maharashtra State captive facilities based on a retrospective study (Kinge *et al*., 2010). This infection has also caused death in five and four tigers in Lahore Zoo, Pakistan and National Zoo in Bangladesh, respectively (Khan *et al*., 2015).

CASE REPORT

A male adult Malayan tiger approximately 15 years old, was reported to have a decrease in appetite, inactive, and loss of body weight. The animal was observed to have unkempt hair coat, indicating the lack of grooming behaviour due to sickness, lethargic, prolapsed of third eyelid and loss of normal aggressive behaviour. The tiger was kept individually and was fed six times per week consists of fresh meat, frozen meat and processed chicken at 6% of body weight.

The tiger was fasted for 24 hours. General anaesthesia was achieved using a combination of ketamine HCI (Ilium Ketamil®, Troy Lab, Australia, 3 mg/kg) and detomidine (Dormosedan®, Zoetis, USA, 0.07 mg/kg). Upon anaesthesia, physical examination showed pale mucous membrane and paw with 5% dehydration. Twenty kilogram of body weight loss was recorded. Abdominal ultrasound revealed the liver image extended up to the middle abdomen and has a blunt edge that indicated hepatomegaly. Unfortunately, no palpation of the prescapular lymph node was done in this case. The blood results revealed a PCV of 19% (normal range; 36 – 45%) indicating anemia, decrease in alanine aminotransferase 5.32 U/L (normal range; 21.20 – 109.00 U/L) and creatinine 6.42 µmol/L (normal range; 141 – 406 µmol/L), slight elevation of total protein 110 g/L (normal range; 37 – 87 g/L) and urea nitrogen 36.90 mmol/L (normal range; 2.30 – 17.20 mmol/L).

The infection diagnosis was based on demonstration of extracellular, unicellular organisms with morphology consistent with *Trypanosoma* in thin blood smear (Veterinary Research Institute, Ipoh). These organisms had a blade-like, triangular to elongated shape that tapered to a posterior flagellum and a pointed anterior. An undulating membrane extended along much of the organisms' length, a dark, basophilic focal kinetoplast was located in the anterior of the organism, and a round, basophilic to amphophilic nucleus was located around the mid-section. The cytoplasm was a pale basophilic colour (Smith *et al*., 2008).

First treatment attempt was made using melarsomine dihydrochloride (Immiticide®, MERIAL, France) at a dosage 0.25 mg/kg, once a day for four days (Tarello, 2005). During the second day of treatment, the tiger started to eat independently and became more responsive, shown by roaming inside the night stall and charging upon stimulation. Unfortunately, after four days of immiticide therapy, the infection relapsed indicated by recurrence of clinical signs.

Treatment of diminazene aceturate (Berenil® RTU, 7%, MSD) was given intramuscularly at 3.5 mg/kg and repeated after 48 hours (Khan *et al*., 2015). A day after, the tiger became more alert and ate 80% of the food given. At the end of the fourth day, the appetite and behaviour return to normal and no relapses occurred. Re-examination of blood at 1-, 3-, 4-, and 15-months post- treatment showed no presences of *Trypanosoma* species.

DISCUSSION

Trypanosomiasis is known to infect a wide range of animal hosts. Transmission of the parasites is via a mechanical vector, accidental contact with infected blood during fighting between two individuals (Smith *et al*., 2006), exposure to infected exotic host (Parija & Bhatttacharya, 2001) and by feeding on infected raw meat (Raina *et al*., 1985; Maraghi *et al*., 1995). However, the source of infection, in this case, is unknown as only one tiger was affected clinically. The tiger was kept in the facility for six years with sixteen other tigers in separated enclosure with no recent history of cage mate fighting or pairing for mating.

In addition, blood examination of five healthy tigers in the facility within six months of trypanosomiasis occurrence was negative for the organism.

The dissemination of parasites into the lymphatic system and lymph node resulting in vigorous immune response, and immune complexes cause inflammation, contributing to all clinical signs of the disease (Christine & Grinnage-Pulley, 2015). Swelling of prescapular lymph nodes is one of significant physical findings in affected tiger (Khan *et al*., 2015) and its examination should be performed during the full examination. *Trypanosoma* sp. can be detected in blood smear microscopically. Polymerase chain reaction (PCR) also can be used to confirm the presence of infection and identification of parasite using blood, tissue samples and faeces (Jirků *et al*., 2015). In this case, the diagnosis was based on the demonstration of parasites in thin blood smear, clinical signs and positive response to treatment (therapeutic diagnosis).

Berenil® is a registered drug used for trypanosomiasis treatment for over 60 years. It is widely used as trypanocide because of its high therapeutic index and low incidence of resistance compared to other compounds. High cost, risk of drug resistance and relapses of infection are few problems related to the use of Berenil® (Kuriakose & Uzonna, 2014). The trypanocide action of Berenil® towards the parasites consists of the development of akinetoplast parasites, complete unfolding and inhibition of DNA replication. A study by Kuriakose *et al.* (2012) shown that Berenil® also modulates the host immune response. Treatment with immiticide at a dosage of 0.25 mg/kg is reported curative for trypanosomiasis in domestic cats (Tarello, 2005), but in this case, relapses occurred. Desquesnes *et al*. (2011) reported that *T. evansi* infection in camel is effectively treated by using the same dosage of immiticide, but a double dose is required for dairy cattle. Thus, a higher dosage may be required for complete recovery from trypanosomiasis in tiger. As prophylaxis measures, vector controls such as the use of insecticide on animals (fipronil spray) and environment, bush clearing, placement of nets over cages and use of prophylactic drugs can be implemented. In conclusion, trypanosomes infestation in tiger was effectively treated using Berenil®. With early recognition of sick animals and proper treatment given, the prognosis is good.

ACKNOWLEDGEMENT

We would like to thank the Director General DWNP, staffs of National Wildlife Rescue Centre (NWRC), Sungkai, Perak, Dr Donny Yawah, Dr Jeffrine Rovie Ryan Japning, Mrs Premaalatha a/p Bathmanaban from Veterinary Research Institute (VRI) Ipoh, Dr Mark Hiew Wen Han and Dr Puteri Azaziah Megat Abdul Rani from University Putra Malaysia (UPM) for their support and involvement in this case.

REFERENCES

Brun, R., Hecker, H. & Lune, Z.R. (1998). *Trypanosoma evansi* and *T. equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review). *Veterinary Parasitology*, **79**: 95-107.

Choudury, A. & Misra, K.K. (1972). Experimental infection of *T. evansi* in the cat. *Trans. R. Soc. Trop.Med. Hyg*, **31**: 231-239.

Christine, P. & Grinnage-Pulley, T.L. (2015). Trypanosomiasis. MSD Veterinary Manual. Merck & Co., Inc., Kenilworth, NJ, USA.

Desquesnes, M., Kamyingkird, K., Vergne, T., Sarathapan, N., Pranee, R. & Jittapalapong, S. (2011). An evaluation of melarsomine hydrochloride efficacy for parasitological cure in experimental infection of dairy cattle with *Trypanosoma evansi* in Thailand. *Parasitology*, **138**(9): 1134-1142.

Jirků, M., Votýpka, J., Petrželková, K.J., Jirků-Pomajbíková, K., Kriegová, E., Vodička, R., Lankester, F., Leendertz, S.A.J., Witting, R.M., Boesch, C., Modrŷ, D., Ayala, F.J., Leendertz, F.H. & Lukeš, J. (2015). Wild chimpanzees are infected by *Trypanosoma brucei*. *International Journal for Parasitology: Parasites and Wildlife*, **4**(3): 277-282. doi: 10.1016/j.ijppaw.2015. 05.001.

Khan, B.N, Ali, Z., Yasmeen, R., Bibi, F., Ziaulah & Mehboob, N. (2015). Trypanosomes infestation in Royal Bengal Tiger (*Panthera tigris tigris*) at Lahore Zoological Gardens and its therapy. *The Journal of Animal & Plant Sciences*, **25**(3 Supp. 2): 477-482.

Kinge, Y.A., Sarode, D.B. & Dakshinkar, N.P. (2010). Mortality pattern in captive wild carnivores in Maharashtra State. *Veterinary World*, **3**(1): 23-25.

Kuriakose, S. & Uzonna, J.E. (2014). Diminazine aceturate (Berenil), a new use for an old compound. *International Immunopharmacology*, **21**: 342-345.

Kuriakose, S., Muleme, H.M., Onyilagha, C., Singh, R., Jia, P. & Uzonna, J.E. (2012). Diminazine aceturate (Berenil) modulates the host cellular and inflammatory responses to *Trypanosoma congolense* infection. *PLoS One*, **7**(11): e48696.

Manohar, B.M., Selvaraj, J., Jayathangaraj, M.G. & Khan, P.N. (2003). Pathology of *Trypanosoma evansi* infection in a tiger. *Indian Veterinary Journal*, **80**: 505-507.

Maraghi, S., Wallbanks, K.R. & Molyneux, D.H. (1995). Oral transmission of trypanosomes of the subgenus Herpetosoma from small mammals. *Parasitology Research*, **81**: 693-695.

Maya, J.D., Cassels, B.K. & Iturriaga-Vàsquez, P. (2007). Mode of action of natural and synthetic drugs against *Trypanosoma cruzi* and their interaction with the mammalian host. *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol*, **146**(4): 601-20.

Parija, S.C. & Bhattacharya, S. (2001). The tragedy of tigers: lesson to learn from Nandankanan episode. *Indian Journal Medical Microbiology*, **19**: 116-118.

Raina, A.K., Kumar, R., Rajora, V.S. & Singh, R.P.S. (1985). Oral transmission of *Trypanosoma evansi* infection in dogs and mice. *Veterinary Parasitology*, **18**: 67-69.

Rogers, D.J. & Randolph, S.E. (1993). Distribution of tsetse and ticks in Africa; past, present and future. *Parasitology Today*, **9**: 266-271.

Smith, A., Clark, P., Averis, S., Lymbery, A.J., Wayne, A.F., Morris, K.D. & Thompson, C.A. (2008). Trypanosomes in a declining species of threatened Australian marsupial, the brush-tailed bettong *Bettongia pennicillata* (Marsupialia: Potoroidae). *Parasitology,* **135**: 1329-1335.

Tarello, W. (2005). *Trypanosoma evansi* infection in three cats. *Revue de Médecine Vétérinaire,* **156**(3): 133-134.

Touratier, L. (1999). The office International des epizooties adhoc group on non-tsetse animal trypanosomes, its origin, scope and perspectives. *Memórias do Instituto Oswaldo Cruz*, **94**: 191- 194.