



Research Article

Identification and treatment of Trypanosomiasis in Lion

Shoab Shafqat^{1*}, Saba Ismail², Yasir Mahmood², Maryam Umar², Danish Shafqat³, Muhammad Kashif Obaid⁴, Xiao-Qing Zan⁵

¹Department of Environmental Health, Faculty of Public Health, Universitas Indonesia, Jakarta, Indonesia

²Department of Zoology, The Islamia university of Bahawalpur, Pakistan

³Faculty of Veterinary Science, University of Agriculture Faisalabad, Pakistan

⁴State Key Laboratory for Animal Disease Control and Prevention, Key Laboratory of Veterinary Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu, China

⁵College of Animal Science and Technology, Ningxia University, Yinchuan 750021, People's Republic of China

*Correspondence: shoab.shafqat@ui.ac.id

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ABSTRACT

Hemoparasitic diseases, like trypanosomiasis, negatively impact the health and working ability of infected animals. In this study, blood samples were collected from lions (5) including 2 lionesses kept at Bahawalpur Zoo in Pakistan. Overall prevalence of Trypanosoma infection was 20% based on microscopic smear examination. The research included a thorough clinical examination with hematological and biochemical tests and treatment of infected lion species. The infected animal exhibited clinical symptoms indicative of a Trypanosoma infection, such as intermittent fever, rapid panting, and signs of discomfort. Blood samples taken without anticoagulant were analyzed for various hematological parameters. Serum samples were examined to measure components such as total proteins and albumin levels, enzymes like alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. Urine sample were collected directly through the urinary bladder for chemical and physical examination. Hematological examinations revealed significant deviations, such as decreased hemoglobin and hematocrit values, indicating anemia along with an increase in the erythrocyte sedimentation rate. Serum analysis showed increased alanine phosphate and decrease albumin. Other biochemical analyses showed hyperglycemia, uric acid elevation, and mild liver involvement, which are common findings in severe cases of trypanosomiasis. The treatment therapy reversed hematology and serum biochemistry and helped infected animals for overall recovery.

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Introduction

Trypanosomiasis, a disease caused by parasites belonging to the Trypanosoma genus, is transmitted by vectors and can impact both animal and human populations. Tsetse flies are vectors for animal trypanosomiasis diseases, such as nagana and surra, affecting livestock and wildlife in Africa (Namangala and Odongo 2013). Trypanosomiasis

in animals can cause signs including anemia, weight loss, and decreased productivity (Osório et al. 2008). The parasitic protozoan genus Trypanosoma multiplies in blood of target animals under tropical and subtropical regions can harm a variety of species, including wild animals (Green et al. 2020). Trypanosomiasis can severely impact lion populations by hindering a lioness's hunting ability.

Since lionesses are vital for providing food for their prides, an infected lioness can cause nutritional stress for the entire pride, affecting the overall health of the lions (Kasozi et al. 2021).

Historically, the range of the lion (*Panthera leo*) once extended across parts of Africa, southern Europe, and southwestern Asia. However, over the centuries, human activities, infectious diseases, habitat destruction, and hunting have significantly reduced their range. Today, lions are primarily found in sub-Saharan Africa, with small, isolated populations and no presence in Europe or southwestern Asia (Mandowara 2023). The population of African lions has dropped by approximately 50% over the past two decades (IUCN 2006). Lions are presently categorized as Vulnerable on the IUCN Red List of Threatened Species and the United States Fish and Wildlife Service is contemplating altering the classification of African lions to Threatened. In 1960, there were approximately 450,000 lions, but by 1994, the lion population had dwindled to just 100,000 (Nowell and Jackson, 1996; Muriuki et al. 2017). The current estimates vary between 23,000 and 32,000 (Bauer and Van Der Merwe 2004; IUCN, 2006; Riggio et al. 2013). In general, lion populations have shrunk to just 25% of their historical habitat range (Riggio et al. 2013). Human encroachment causing habitat loss, a rise in infectious diseases, and the loss of genetic diversity due to fragmented and isolated populations are also considered significant contributors to population decreases.

Lions are mainly infected by *Trypanosoma brucei* subspecies, specifically *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* which are the causative agents of African trypanosomiasis, also known as sleeping sickness in humans. Another species, *Trypanosoma evansi*, can also infect lions, causing a disease known as surra. In lion, the effects of *Trypanosoma* infection can be severe and may include symptoms such as weakness, lethargy, loss of appetite, anemia, and even death in some cases. The protein-based diet helps wild animals fight against trypanosomes by strengthening their immune system. Additionally, medicines like Injection Neurobion, Decadron, Methocobal, and Syrup Vidaylin M, as well as Mosegar, help improve nerve function, reduce inflammation, and enhance nutrition and the immune system. ORS/Distal water is used to prevent dehydration (Murray and Dexter, 1988).

Materials and Methods

Study area

Bahawalpur is a city in the Punjab province of Pakistan. The climate is dry. The average annual temperature is 25.7 °C or 78.3 °F. This research conducted at Bahawalpur Zoo is situated at the coordinate's 29° 24' 9.95" N latitude and 71° 40' 53.77" E longitude, marking its precise location in the city of Bahawalpur, Pakistan. The Bahawalpur

Zoo is home to approximately 870 animals, including a substantial collection of mammals, exceeding 180 in total.

Study animal

The Bahawalpur Zoo boasts a diverse collection of wildlife mammals including Asiatic lion, Bengal tiger, Asian black bear, Black Buck, Chinkara deer, Chital, Hog deer, Himalayan brown bear, Llama, Mouflon, Nilgai (blue bull), Plains zebra, Red deer, Red-necked wallaby, Rhesus macaque, Sambar, and the Small Indian civet (Bahawalpur Zoo, n.d.). The suspected animal displayed intermittent fever (with a temperature between 103.5 and 105°F), rapid panting, a pulse rate of 76 beats per minute, an enlarged pre-scapular lymph node, yellowish urine, a reluctance to move, less food intake, signs of discomfort, and vomiting.

Treatment therapy for infected animal

The protein-based diet helps wild animals fight against trypanosomes by strengthening their immune system. Additionally, medicines like Injection Neurobion, Decadron, Methocobal, and Syrup Vidaylin M, as well as Mosegar, help improve nerve function, reduce inflammation, and enhance nutrition and the immune system. ORS/Distal water is used to prevent dehydration

Blood collection and parasitological examination

Blood samples of 5 lions were collected including 2 lionesses at Bahawalpur Zoo. In addition, urine samples, including serum proteins (both total proteins and albumin levels), also taken.

Blood samples were obtained from femoral veins of right leg of the lions according to instructions and the guidelines of International Animal Ethics and Welfare Committee. The area around the selected vein was sterilized to minimize the risk of infection. A needle and syringe were used to draw blood from the selected vein. The blood is collected into appropriate tubes for analysis.

Urine collection

Urine was collected directly from urinary bladder for physical and chemical examination. These samples were handled carefully to prevent contamination and kept in appropriate containers for processing in the laboratory for parasitological investigation.

Hematological and serum biochemistry

Blood samples were taken without anticoagulant were analyzed for various hematological measurements, including total red blood cell count, hemoglobin levels, total white blood cell count, differential white blood cell counts, hematocrit, and mean corpuscular hemoglobin concentration.

The serum samples were analyzed to assess various components, including serum proteins, various enzymes such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, mineral content, lactate dehydrogenase, and a product of lipid peroxidation.

Results

Out of 5 lions, including 2 lionesses, one lioness was affected by Trypanosoma infection, resulting in an overall prevalence of 20% based on microscopic smear examination. The diagnosis of trypanosomiasis was based on clinical observations and the microscopic identification of the organism, leading to the initiation of clinical treatment.

During the clinical examination, the infected animal displayed intermittent fever (with a temperature between 103.5 and 105°F), rapid panting, a pulse rate of 76 beats per minute, an enlarged pre-scapular lymph node, yellowish urine,

a reluctance to move, signs of discomfort, and vomiting.

Hematological examination

The microscopic examination showed the presence of Trypanosoma parasite in infected lioness blood. Hematology tests showed erythrocyte indices, including red blood cell counts, hemoglobin concentration, and MCHC, decreased significantly, while hematocrit and MCV increased significantly in the infected lioness. Moreover, the increased white blood cell count indicated an inflammatory response to the infection

Table 1: Hematological values of healthy and infected lioness pretreatment, during treatment and post treatment

Parameters	Healthy Lioness	Infected Lioness		
		Pretreatment	During treatment	Post treatment
Erythrocyte counts (10 ⁶ /μL)	5.5-8.5	2.04	16.57	43.6
Hemoglobin quantity (g/dL)	8-15	3.3	7.2	11.6
Hematocrit (%)	30-45	18.7	29.1	38.9
Mean corpuscular volume (fL)	45-75	49.0	46.2	53.1
Mean corpuscular hemoglobin (pg)	16-24	16.2	15.2	15.8
Mean corpuscular hemoglobin concentration (g/dL)	30-35	33.0	32.9	29.8
Leukocyte counts (10 ³ /μL)	5-20	2.5	24.3	43.6
Neutrophil (%)	20-75	63	86.1	106.1
Lymphocyte (%)	0-10	30	9.8	5.0
Eosinophil (%)	2-10	4	3.8	4.3
Monocyte (%)	2-10	3	0.3	2.7
Basophil (%)	rare	0	0	0
Platelet Count	300,000-700,000	19000	480,000,000	16,019,000

Serum analysis:

The serum examination of the infected lioness indicates elevated levels of serum urea (mg/dL), uric acid (mg/dL), and creatinine (mg/dL). Additionally, increased levels of random blood

sugar (mg/dL) and albumin have been observed in the infected animal. Other biochemical analyses showed hyperglycemia, uric acid elevation, and mild liver involvement, which are common findings in severe cases of trypanosomiasis.

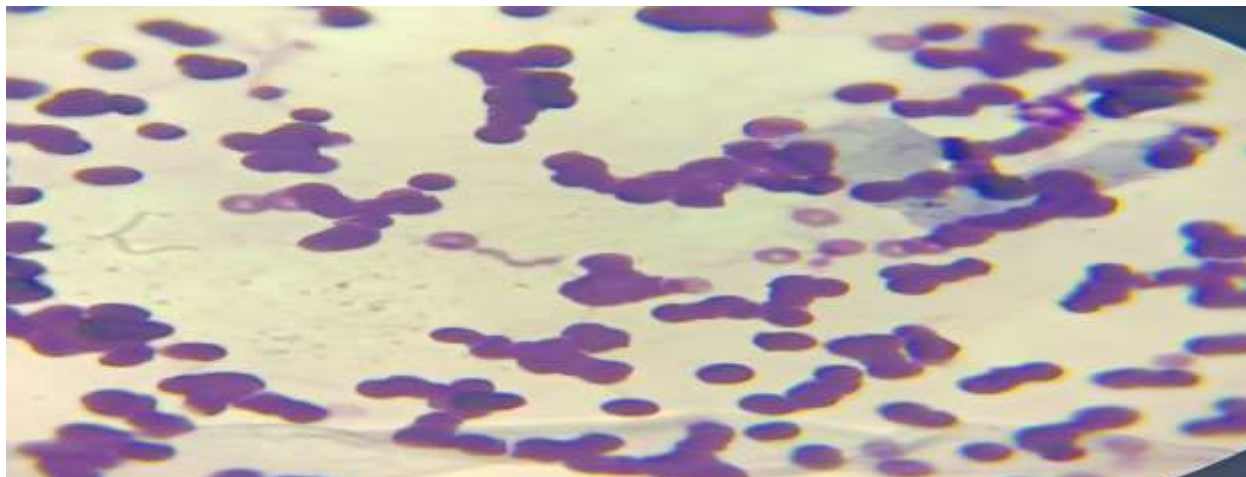


Fig. 1: Microscopic capture presenting the *Trypanosoma* in the blood of lion

Discussion

Pakistan has a tropical and subtropical climate, which provides a conducive environment for many blood protozoan infections, such as *Trypanosoma*, to thrive in wild animals. Infectious diseases pose severe problems for animal health and productivity (Khan et al. 2021). Despite the significance of trypanosome infections, few studies have been conducted on this topic, and no studies have specifically focused on lions in Pakistan expect (Khan et al. 2021). Therefore, in this study, we aim to investigate the presence of trypanosome infections in lions kept under tropical and subtropical climatic conditions in Pakistan.

In present studies, the prevalence of *Trypanosoma* infectious in lions was 20% kept at Bahawalpur Zoo, Pakistan. The symptoms exhibited by the infected lioness were alarming, indicating a severe health issue. Clinical observations revealed intermittent fever, rapid panting, enlarged lymph nodes, yellowish urine, and reluctance to move, confusion, and vomiting. These symptoms are similar, as mentioned in literature (Nelson and Couto 2019) which prompted a comprehensive diagnosis process involving careful blood collection and parasitological investigations. The initial diagnosis was based on clinical observations and the microscopic identification of *Trypanosoma* organisms in the lioness's blood, leading to the initiation of clinical treatment.

The hematological examination provided crucial insights into the lioness's condition. Hemoglobin levels and hematocrit values showed a significant decrease, indicating anemia, a common consequence of trypanosomiasis evident from studies on cats (Da Silva et al. 2011). Additionally, the erythrocyte sedimentation rate was elevated, further confirming the presence of an inflammatory condition. Microscopic examination confirmed the

presence of *Trypanosoma* organisms outside the red blood cells, corroborating the diagnosis. However, intriguingly, the blood biochemical parameters did not display significant deviations. This contrast between hematological and biochemical findings emphasizes the importance of a comprehensive diagnostic approach, combining clinical observations, hematological analysis, and microscopic examination for accurate disease diagnosis.

The lioness's treatment therapy worked very well and helped the lioness with her fully recovery. A protein-based diet, consisting of beef, liver, bone marrow, milk, and chicken, was administered to bolster her immune system and aid in combating the trypanosome infection as described by (Giordani et al. 2016). Additionally, a range of medicines, including Injection Neurobion, Decadron, Syrup Vidaylin M, ORS/Distal water, Syrup Mosegar, and Inj. Methocobal, were administered throughout the treatment duration to alleviate symptoms and promote recovery as mentioned by (Control and Trypanosomiasis 1998). Same medications were used for the treatment of *Trypanosoma* infection in a study conducted in India (Agrawal et al. 2023).

Furthermore, the study provided recommendations for additional medicines, such as Capsule Fefolvit, Tablet Zyloric-300, Cecon, and Syrup Bisleri, to enhance the treatment regimen. These recommendations signify the holistic approach adopted to ensure the lioness's comprehensive care and recovery (Schroeder 2009). The results of this study underscore the importance of early detection, comprehensive diagnosis, and multidimensional treatment strategies in managing trypanosomiasis in wildlife. This case serves as a valuable reference for future research and conservation efforts, emphasizing the need for continued vigilance and proactive healthcare measures in captive animal populations.

The comprehensive approach taken in this study, from ethical considerations and meticulous diagnosis to tailored treatment and dietary strategies, showcases the importance of interdisciplinary collaboration. This collaboration, involving veterinarians, wildlife biologists, and nutritionists, was essential in addressing the complexity of trypanosomiasis in the afflicted lioness (Khan et al. 2023).

The study's detailed hematological analysis provided crucial insights into the impact of trypanosomiasis on the lioness's blood parameters. The significant decrease in hemoglobin and hematocrit levels, coupled with an elevated erythrocyte sedimentation rate, aligns with typical manifestations of the disease, emphasizing the reliability of hematological assessments in diagnosing trypanosomiasis in lions (Kucykowicz 2019). Additionally, the presence of *Trypanosoma* organisms outside the red blood cells, as observed in the microscopic examination, corroborated the hematological findings, reinforcing the accuracy of the diagnosis (Pessoa-e-Silva et al. 2017).

The lioness's response to the prescribed treatment regimen is noteworthy. The combination of protein-rich diet and targeted medications resulted in a positive outcome, underscoring the significance of a balanced nutritional approach in conjunction with medical interventions as mentioned in (Cliff and Anna 2021). This successful recovery emphasizes the importance of understanding the nutritional needs of animals during illness, highlighting avenues for future research in wildlife nutrition and health.

Furthermore, the study's recommendations for additional medicines offer a valuable resource for clinicians and wildlife caretakers facing similar cases (Crissey et al. 2003). The tailored nature of these recommendations, considering the lioness's specific symptoms and requirements, reflects the study's attention to individualized care. This

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personalized approach is vital in captive animal management, where every case may present unique challenges.

Conclusion-

In conclusion, trypanosomiasis poses a significant threat to lionesses and lion populations. Understanding the disease's impact, diagnostic methods and treatment strategies is crucial for their conservation. Moreover, a One Health approach is essential for addressing the broader implications of trypanosomiasis in shared ecosystems. Conservation efforts and research are essential to safeguard these magnificent animals and their habitats.

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Ethical Statement

No Ethical permissions were required for this article.

Availability of Data and Material

The data can be obtained from the corresponding author on a reasonable request.

Consent to Participate

All the authors gave their consent for equal participation.

Consent for Publication

All the authors gave their consent for publication.

Competing Interest

The authors declare that they have no relevant financial or non-financial interests to disclose.

Author Contribution

All the authors contributed equally to this article.

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