Canine *Trypanosoma evansi* Infection (Surra) in Lahore, Pakistan-Short Communication

Muhammad Asif[1, 2], Kamran Ashraf[1], Tariq Jamil[3], Nisar Ahmad[1], Sami Ullah[2], Shakeel Hussain[1], Mudassar Iqbal[4], Muhammad Nasrullah[5], Abdul Hameed Khan[6] and Muhammad Usman Ghani[6]

[1]Department of Parasitology, University of Veterinary and Animal Sciences, Pakistan
[2]Livestock and Dairy Development Department, Government of Punjab, Pakistan
[3]Department of Microbiology, University of Veterinary and Animal Sciences, Pakistan
[4]University College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan
[5]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Pakistan
[6]Department of Pathology, University of Veterinary and Animal Sciences, Pakistan

Keywords: *Trypanosoma evansi; surra; canine; Punjab; Pakistan*

Abstract. *Surra caused by Trypanosoma evansi is a well-known infection in camel and equines and to a lesser extent in other domestic and wild animals in Pakistan. The purpose of this study was to evaluate the burden and the precise cause of surra in dogs. A total of 160 blood samples were collected, and 3.75% (6/160) were found positive by microscopic examination and 2.5% (4/160) by PCR. One female (25%) and 3 male (75%) dogs were found positive by PCR. Among them, the 2 positive dogs were from Pakistani bully breed and the other 2 from local/non-descriptive breeds. All 4 dogs were between 2–4 years of age. A more precise study is needed for determination of the associated risk factors with this infection. To the best of our knowledge, this is the first-ever report of specific Trypanosoma evansi detection in dogs in Pakistan.*

Introduction

Trypanosoma evansi is a blood borne parasite belonging to the family *Trypanosomatidae*. It causes the surra disease in domestic and wild animals and is widely present in south Asia, Africa and South America (Aregawi et al., 2019; Luckins, 1988). It rarely causes disease in humans (Joshi et al., 2005). The disease is mechanically transmitted by hematophagous flies from genus *Stomoxys*, *Tabanus* and *Glossina* spp. (Desquesnes et al., 2013). Dogs are highly susceptible to surra depicting sever clinical signs that may lead to death. The diagnostic techniques include direct microscopy of a peripheral blood smear coupled with a serological, e.g., immunofluorescence test (IFAT), an enzyme linked immunosorbent assay (ELISA) and a card agglutination test (CATT) and/or molecular biological based detection and differentiation, e.g., polymerase chain reaction (PCR) (OIE, 2019; Tehseen et al., 2015).

Surra was first discovered in 1880 by Griffith Evans at Dera Ismail Khan district of Khyber Pakhtunkhwa (KPK), Pakistan (Luckins, 1988). It is a well-known disease in camels and equines in Pakistan (Bhutto et al., 2010; Hasan et al., 2006; Hussain et al., 2016; Shah et al., 2004; Shahzad et al., 2010; Tehseen et al., 2017; Tehseen et al., 2015). Recent reports in wildlife have also emerged (Muhammad et al., 2007; Shahid et al., 2013). Canine trypanosomiasis has also been previously reported in the country but identification of the aetiology at a species level have been ignored (Gadahi et al., 2008; Rashid et al., 2008). Lahore is the second largest, cultural and provincial capital in the country and a significant population owns dogs as pet animals (Jafri and Rabbani, 1999). Keeping in view the importance and the increasing trend of dog owning, the current study was designed to investigate infection load, precise aetiology, and update the existing knowledge of trypanosomiasis in dogs in Lahore, Pakistan.

Correspondence to Muhammad Asif and Tariq Jamil, Livestock and Dairy Development Department, Government of Punjab, Lahore 54000, Pakistan and Department of Microbiology, University of Veterinary and Animal Sciences, Lahore 54100, Pakistan
Email: asifvet460@gmail.com and drtariqjamil01@gmail.com

Materials and Methods

Study duration and collection of blood samples

A total of 160 blood samples were collected from clinically suspected dogs (⩾ 4 months of age) for trypanosomiasis presented at Pet Center outdoor and Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan from March 01 till April 30, 2015. The infection was suspected based on a history of intermittent fever, anaemia, fascial and pharyngeal oedema, hind limb paralysis and corneal opacity. Blood (3 mL) was drawn for each sample by a sterile syringe through cephalic or saphenous vein puncture and immediately transferred into sterile EDTA (Ethylenediaminetetraacetic acid) coated blood collection tubes to avoid blood clotting.

Microscopic examination

The blood samples were processed by Giemsa staining followed by a microscopic examination at 1000x for *Trypanosoma* spp. examination as per standard procedures of Department of Parasitology, University of Veterinary and Animal Sciences, Lahore, Pakistan.

DNA extraction and amplification

DNA was isolated as described by Britto et al. (1993). Specific primers targeting transferrin protein genes 6 and 7 (ESGA 6/7) were used to amplify a 237 bp fragment. The set composed of a 21-mer forward primer 5′-ACA TTC CAG CAG GAG TTG GAG-3' and a 21-mer reverse primer 5'-CAC GTG AAT CCT CAA TTT TGT-3' (Holland et al., 2001). PCR conditions were as follows: first, a denaturation step of 4 minutes at 94 °C followed by 35 cycles consisting of 1-minute denaturation at 94 ºC, 1-minute primer-template annealing at 55 °C and 1-minute polymerization at 72 ºC. The last extension step lasted for 5 minutes at 72 °C. The PCR products were analyzed on a 1.5 % agarose gel (1 h at 90 V) along with a 100 bp marker stained with ethidium bromide (2 µL/50 mL gel) on an ultraviolet (UV) trans-illuminator (Dolphin-Doc, Wealtec, USA).

Results

Of the total 160 samples, 103 (64.4%) were male dogs and 57 (35.5%) were females. The dogs recruited in the study belonged to Afghan kochi (n = 1), Barbarian (n = 1), Boxer (n = 1), Bull dog (n = 1), Bull terrier (n = 1) Pakistani bully (n = 19), Cross breed (n = 10), Doberman (n = 4), German shepherd (n = 52), Labrador (n = 37), Pointer (n = 11), Pug (n = 1), Rottweiler (n = 8), Russian husky (n = 7) and local/non-descript (n = 6). Of these 160 blood samples, only 6 samples (3.75%) showed positive by microscopic examination (Fig. 1). By PCR, only 4 (2.5%) samples were found positive (Fig. 2). Among 4 PCR positive dogs, 3 (75%) were males and 1 (25%) was female. Two dogs were from Pakistani bully breed (1.25%) and the other 2 from local/non-descript (1.25%) breeds. These positive dogs were between 2–4 years of age and were raised as pet dogs at homes separate from other domestic animals. These dogs were fed on commercial pet food and received routine vaccination schedule against infectious diseases (e.g., canine distemper, leptospirosis, infectious canine hepatitis, canine parvovirus infection and rabies).

Discussion

Surra is frequently reported in camels and equines and less frequently in bovines, small ruminants, dogs and wildlife in Pakistan. We used microscopy coupled with PCR for species specific detection of trypanosomes. Domestic dogs could possibly get infected and pose threat for zoonotic disease transmission.

In our study, we identified 3.75% (6/160) suspected cases by direct microscopy and 2.5% (4/160) by PCR. Regionally, surra has been reported in dogs as well as other domestic animals from India (up to 19.8%), Iran and Afghanistan (Aref et al., 2013; Hosseininejad et al., 2007; Ravindran et al., 2008). This might be because of hematophagous flies, e.g., *Stomoxys*, *Tabanus* and *Glossina* spp. prevalent in this region (Luckins, 1988). Of the several species of trypanosomes, only *Trypanosoma evansi* has been reported in this region (Ravindran et al., 2008). Infection has not been associated with sex in dogs so far (Chowdhury et al., 2005; Prasad et al., 2015). The 4 PCR positive dogs were between 2–4 years of age, and it is in accordance with previous reports (Lakshmi et al., 2007; Nazifi et al., 2004; Rashid et al., 2008). However, opposing results do exist (Prasad et al., 2015). The other 2 samples might have lost detectable amounts of DNA during the extraction procedure. A higher prevalence of *Trypanosoma evansi* infection was found in Pakistani bully dogs and local/non-descript breeds compared with other selected 13 breeds of dogs. The

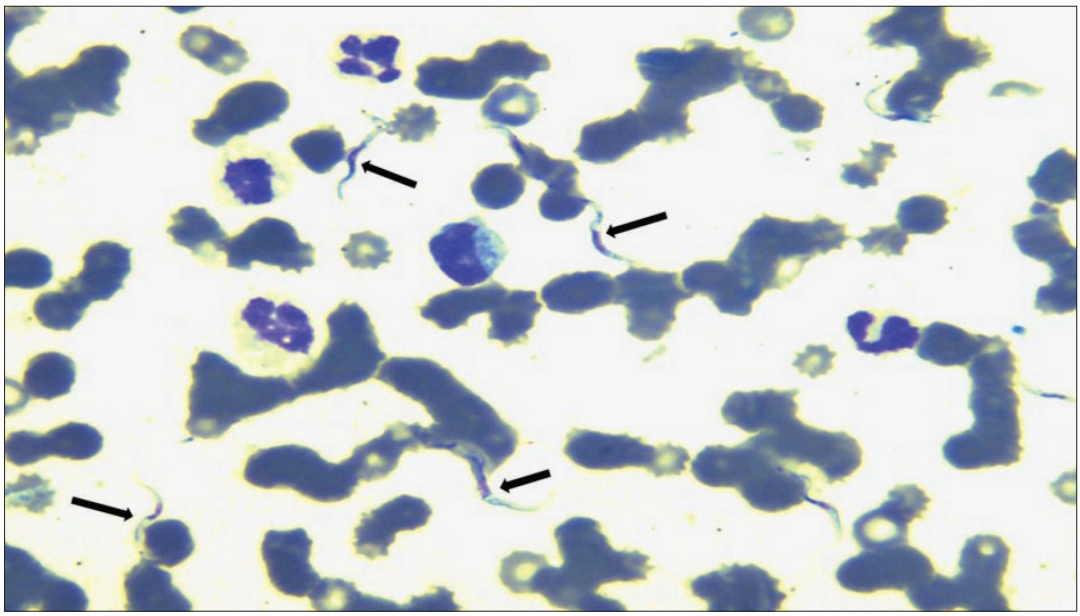

Fig. 1. Trypanosoma in a Giemsa-stained blood smear (Arrows showing *Trypanosoma*)

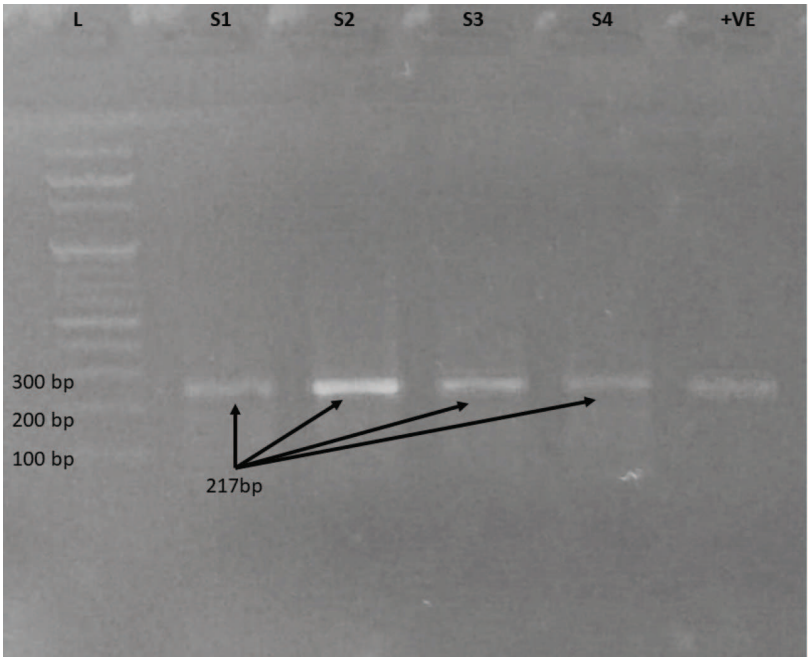

Fig. 2. PCR-based detection of *Trypanosoma evansi*

breed of the dog has not been associated with the infection (Chowdhury et al., 2005). A more precise study would be needed for clarification.

We conclude that *Trypanosoma evansi* infection is present in dogs in Pakistan. Dogs between 2–4 years of age can be more susceptible. Breed does not seem to play a role in susceptibility. The present investigation emphasizes the need for the routine screening of blood samples in dogs with a precise data collection of biologically plausible variables to determine the statistical association and the risk for this infection in future. To the best of our knowledge, this report first presents the prevalence of this infection specifically in dogs in Pakistan.

Ethical statement

The blood samples were collected as per ethical standards of the Research Board of the University of Veterinary and Animal Sciences, Lahore, Pakistan. Verbal consent was taken from the owners before participation in the study.

Competing interests

Authors declare no conflict of competing interests.

Funding

Not applicable.

References

1. Aref M., Yasin S. M., Bahear W., Ghulam Z., Hastie L., Dennison T., Schauwers W., Büscher P., Deborggraeve S. Canine *Trypanosoma evansi* infection in Afghanistan. Veterinary Parasitology. 2013. 197. P. 638-641.
2. Aregawi W. G., Agga G. E., Abdi R. D., Büscher P. Systematic review and meta-analysis on the global distribution, host range, and prevalence of *Trypanosoma evansi*. Parasites & Vectors. 2019. 12. P. 67.
3. Bhutto B., Gadahi J., Shah G., Dewani P., Arijo A. Field investigation on the prevalence of trypanosomiasis in camels in relation to sex, age, breed and herd size. Pakistan Veterinary Journal. 2010. 30. P. 175-177.
4. Britto C., Cardoso M. A., Wincker P., Morel C. M. A simple protocol for the physical cleavage of *Trypanosoma cruzi* kinetoplast DNA present in blood samples and its use in polymerase chain reaction (PCR)-based diagnosis of chronic Chagas disease. Memórias do Instituto Oswaldo Cruz. 1993. 88. P. 171-172.
5. Chowdhury P., Biswas U., Guha C., Jana P. S. Prevalence of canine trypanosomosis in and around Kolkata city. Indian Veterinary Journal. 2005. 82. P. 797-798.
6. Desquesnes M., Dargantes A., Lai D.-H., Lun Z.-R., Holzmuller P., Jittapalapong S. *Trypanosoma evansi* and surra: A review and perspectives on transmission, epidemiology and control, impact, and zoonotic aspects. BioMed Research International 2013. 321237.
7. Gadahi J., Arijo A., Abubakar M., Javaid S., Arshed M. Prevalence of blood parasites in stray and pet dogs in Hyderabad area: Comparative sensitivity of different diagnostic techniqes for the detection of microfilaria. Veterinary World. 2008. 1. P. 229-232.
8. Hasan M. U., Muhammad G., Gutierrez C., Iqbal Z., Shakoor A., Jabbar A. Prevalence of *Trypanosoma evansi* infection in equines and camels in the Punjab region, Pakistan. Annals of the New York Academy of Sciences. 2006. 1081. P. 322-324.
9. Holland W., Claes F., My L., Thanh N., Tam P., Verloo D., Büscher P., Goddeeris B., Vercruysse J. A comparative evaluation of parasitological tests and a PCR for *Trypanosoma evansi* diagnosis in experimentally infected water buffaloes. Veterinary Parasitology. 2001. 97. P. 23-33.
10. Hosseininejad M., Shirani D., Nabian S., Nassiri S. M., Mazaheri R. *Trypanosoma evansi* in three dogs in Iran. Comparative Clinical Pathology. 2007. 16. P. 69-71.
11. Hussain R., Khan A., Abbas R. Z., Ghaffar A., Abbas G., Ali F. Clinico-hematological and biochemical studies on naturally

infected camels with trypanosomiasis. Pakistan Journal of Zoology. 2016. 48. P. 311-316.
12. Jafri S. A., Rabbani M. Prevalence of canine diseases in Lahore area. Pakistan Veterinary Journal. 1999. 19. P. 40-42.
13. Joshi P. P., Shegokar V. R., Powar R. M., Herder S., Katti R., Salkar H. R., Dani V. S., Bhargava A., Jannin J., Truc P. Human trypanosomiasis caused by *Trypanosoma evansi* in India: the first case report. The American Journal of Tropical Medicine and Hygiene. 2005. 73. P. 491-495.
14. Lakshmi R., Suresh K., Srinivas M., Sreenu M. Canine trypanosomiasis. Indian Veterinary Journal. 2007. 84. P. 186-187.
15. Luckins A. G. *Trypanosoma evansi* in Asia. Parasitology Today. 1988. 4. P. 137-142.
16. Muhammad G., Saqib M., Sajid M., Naureen A. *Trypanosoma evansi* infections in Himalayan black bears (*Selenarctos thibetanus*). Journal of Zoo and Wildlife Medicine. 2007. 38. P. 97-100.
17. Nazifi S., Razavi S., Molazem M. Fatal trypanosomiasis in a dog a case report. Indian Veterinary Journal (India). 2004. 81. P. 824.
18. OIE. *Trypanosoma evansi* infection (Surra). In: Manual of diagnostic tests and vaccines for terrestrial animals. Paris, France. 2019. P. 660-674.
19. Prasad K. L., Kondaiah P., Rayulu V., Srilatha C. Prevalence of canine trypanosomiasis in certain areas of Andhra Pradesh. Journal of Parasitic Diseases. 2015. 39. P. 238-240.
20. Rashid A., Rasheed K., Hussain A. Trypanosomiasis in dog; a case report. Journal of Arthropod-Borne Diseases. 2008. 2. P. 48-51.
21. Ravindran R., Rao J. R., Mishra A. K., Pathak K. M. L., Babu N., Satheesh C. C., Rahul S. *Trypanosoma evansi* in camels, donkeys and dogs in India: Comparison of PCR and light microscopy for detection-short communication. Veterinarski Arhiv. 2008. 78. P. 89-94.
22. Shah S., Phulan M., Memon M., Rind R., Bhatti W. Trypanosomes infection in camels. Pakistan Veterinary Journal. 2004. 24. P. 209-210.
23. Shahid M., Janjua S., Burbach J. S. PCR-Based Detection of *Trypanosoma evansi* infection in semi-captive asiatic black bears (*Ursus thibetanus*). Pakistan Veterinary Journal. 2013. 33. P. 442-445.
24. Shahzad W., Munir R., Khan M. S., Ahmad M. D., Ijaz M., Ahmad A., Iqbal M. Prevalence and molecular diagnosis of *Trypanosoma evansi* in Nili-Ravi buffalo (*Bubalus bubalis*) in different districts of Punjab (Pakistan). Tropical Animal Health and Production. 2010. 42. P. 1597-1599.
25. Tehseen S., Jahan N., Desquesnes M., Shahzad M. I., Qamar M. F. Field investigation of *Trypanosoma evansi* and comparative analysis of diagnostic tests in horses from Bahawalpur, Pakistan. Turkish Journal of Veterinary and Animal Sciences. 2017. 41. P. 288-293.
26. Tehseen S., Jahan N., Qamar M. F., Desquesnes M., Shahzad M. I., Deborggraeve S., Büscher P. Parasitological, serological and molecular survey of *Trypanosoma evansi* infection in dromedary camels from Cholistan Desert, Pakistan. Parasites & Vectors. 2015. 8. P. 415.

Received 1 June 2020
Accepted 23 September 2020