Continental Veterinary Journal
ISSN: 3079-0212 (ONLINE)
www.cvetj.com; editor@cvetj.com

RESEARCH ARTICLE

DOI: 10.71081/cvj/2025.039

Seroprevalence of *Trypanosoma evansi* in Camels using Catt/*t. Evansi* Technique in Lasbella, Noshki and Pishin Districts of Balochistan

Waseem Khan[1], Mian Abdul Hafeez[1], Muhammad Lateef[1], Kamran Ashraf[1], Muhammad Avais[2], Numan Rauf[1], Abdul Wajid[3], Muhammad Farooq[4], Matiullah Sherani[5], Najeeb Kakar[6], Umair Anjum[3], Ajmal Nisar Mengal[6], Niazullah Khan Ayub[5], Muhammad Nazim[6], Hafiz Muhammad Naeem[1] and Naimat Ullah[1*]

[1]Department of Parasitology, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan
[2]Department of Veterinary Medicine, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan
[3]Department of Molecular Biology, Balochistan University of Information Technology, Engineering and Management Sciences, Quetta, Pakistan; [4]University of Calgary, Alberta, Canada
[5]Livestock & Dairy Development (L&DD) Department, Khyber Pakhtunkhwa Province, Pakistan
[6] Livestock & Dairy Development (L&DD) Department, Balochistan Province, Pakistan
*Correspondence: *naimat.ullah879@gmail.com*

ARTICLE INFO

ARTICLE HISTORY: CVJ-25-502

Received: 26 February 2025
Revised: 03 April 2025
Accepted: 30 April 2025
Published online: 03 May 2025

Key words:
Balochistan,
Camel,
CATT/*T. evansi*,
Seroprevalence,
Surra,
Trypanosoma evansi

ABSTRACT

Surra is a zoonotic illness caused by *Trypanosoma evansi* that affects various animal species worldwide. It can cause fatalities and significant financial losses for camels, thereby seriously affecting their productivity, health, and ability to work. A cross-sectional study was conducted, and 393 blood samples were obtained from one-humped camels (*Camelus dromedarius*) in three districts (Pishin, Noshki, and Lasbella) of the Balochistan province, Pakistan (240 Indigenous, 153 imported) to determine the seroprevalence of *T. evansi.* This was the first study to investigate *T. evansi* infection in the Balochistan province using the CATT/*T. evansi* kit method. The overall seroprevalence of *T. evansi* among the examined camel samples was reported to be 50.89%. The seroprevalence of *T. evansi* was found to be higher in animals with poor body condition, based on their health status. Furthermore, it was observed that the seroprevalence of *T. evansi* was much higher in females. Moreover, adults demonstrate a higher seroprevalence than younger camels. During the summer and spring, the likelihood of camels being infected with *T. evansi* was three to five times higher than those sampled in winter. The research conducted indicated that camels in the three districts of Balochistan had a high prevalence of *T. evansi* infection. To ensure efficient management actions, it is crucial to implement a rigorous monitoring program that focuses on risk assessment studies and vectors, as emphasized by this study.

To Cite This Article: Khan W, Hafeez MA, Lateef M, Ashraf K, Avais M, Rauf N, Wajid A, Farooq M, Sherani M, Kakar N, Anjum U, Mengal AN, Ayub NK, Nazim M, Naeem HM and Ullah N, xxxx. Seroprevalence of *Trypanosoma evansi* in camels using catt/*t. Evansi* technique in Lasbella, Noshki, and Pishin Districts of Balochistan. Continental Vet J, x(x): xxx http://dx.doi.org/10.71081/cvj/2025.039

INTRODUCTION

Several animals, including humans, get infected by the significant camel pathogen Trypanosoma evansi, which is a member of the family Trypanosomatidae, genus Trypanosoma, and subgenus Trypanozoon (Desquesnes et al. 2013; Kamidi et al. 2018; Mossaad et al. 2017; Ngaira et al. 2002). In addition, it is a highly pathogenic trypanosome with disastrous consequences for livestock productivity in Africa, Asia, Central America, and South America (Hasan et al. 2006; Muhanguzi et al. 2017; Sánchez et al. 2015). The T. evansi hemoparasite originated in Africa and has since expanded to other continents. It primarily affects camels and horses in Africa and is found in the Sahara zone, above the tsetse fly prevalent belt, where flies (stomoxys and tabanids) mechanically spread it (Eyob and Matios 2013; Thompson et al. 2014). Camels are a significant source of revenue for many African nations (Mohamed et al. 2020). Infected camels and equids in the Dera Ismail Khan Area of Punjab, in what is now Pakistan, were the first animals from which T. evansi was initially isolated in 1880. Following the outbreak of Surra in camels in Balochistan from 1985 to 1986, several investigations on the disease's

incidence in camels have been carried out in various parts of Pakistan (Bhutto et al. 2010). The Food and Agriculture Organization Statistics (FAOSTATS) shows that Pakistan is home to 1.0 million dromedary camels (Delafosse and Doutum 2004). The fact that horses and camels can get serious diseases brings attention to the function of carrier animals (Aregawi et al. 2019). The economic costs of this parasite, which include illness (up to 30%), abortion, and mortality (3%), are frequently underestimated (Abera et al. 2015). Dystocia, poor carcass quality, reduced weight gain, decreased productive and reproductive potential, expensive cost of treatment, and eventually mortality associated with abortion (Elhaig et al. 2013; Aregawi et al. 2019). The persistence of symptoms might be from several months to years in chronic or acute form (Aregawi et al. 2019). Intermittent fever, exhaustion, lethargy, neurological problems, and anemia, primarily brought on by hemolysis and erythrophagocytosis, are among the surra's traditional clinical signs (Amit et al. 2015). Raising small and large ruminants is an essential component of a production system for an agriculture-based economy like Pakistan. Despite the application of appropriate disease prevention and control methods, it is typical for infectious illnesses to recur in both small and large ruminants, which causes later economic losses in terms of morbidity, mortality, and trade embargo (Gwida et al. 2012; Woma et al. 2015). It is important to consider that Pakistan, a country with a population of 1.2 million, is among the top eight countries in the world for raising camels and produces 908,000 tons of milk and 50,603 tons of meat annually for human consumption (Economic Survey of Pakistan 2018–19). The biggest percentage of one-humped camels (dromedaries) is found in Balochistan (41%), followed by Sindh (30%), Punjab (22%), and KPK (7%) (Faraz et al. 2013). Out of the twenty camel breeds of Pakistan, seven breeds are documented in the province of Balochistan, and their names are Kachi, Brahvi, Lassi, Kharani, Pishin, Makrani, and Rodbari. A research study was conducted in the Balochistan region of Pakistan to monitor the prevalence of trypanosomiasis, a serious infectious disease, in camels across a large geographical area using antigen (pathogen) and antibody tests.

The study results are anticipated to determine the essential actions for disease management and control, particularly in a scenario where the nation's vast camel population is frequently in contact with vectors. Due to a lack of diagnostic techniques for determining the degree of the disease's incidence, prevalence, and morbidity in the field, it is currently impossible to estimate the disease's impact on camels in Pakistan and the resulting economic loss. The study's objective was to examine the diagnostic potency of various parasitological and serological diagnoses as well as related risk factors for Trypanosoma by measuring the seroprevalence of camel Trypanosomiasis in three districts (Lasbella, Noshki, and Pishin) of Balochistan province.

MATERIALS AND METHODS

Study Animal

Using information from the owners, the age of the animals was determined. Camels were divided into three categories based on their age: calves, young animals (those between 2 and 4 years), and adults (those beyond 4 years). Each animal had a thorough clinical examination, and data on a variety of factors, including age, gender, breed, the month of a sample, prior trypanocide treatment, history of abortions, and estimated weight, were also collected.

Blood Sample Collection

Blood was drawn at random from each camel's jugular vein from May 2020 until March 2021 and kept in a vacutainer free of EDTA. Three Balochistan districts provided a total of 393 blood samples. Each district (Pishin, Noshki, and Lasbella) yielded about 131 samples. Before being delivered to the University of Veterinary and Animal Sciences Lahore, the sample was stored in an icebox. Animals with clinical signs of parasite infection, such as pyrexia, anemia, productivity loss, poor body condition, and a history of miscarriage, had their ear veins punctured to get samples. The University of Veterinary and Animal Sciences (UVAS), Lahore, parasitology laboratory received samples that were obtained with the assistance of veterinary personnel for additional processing and analysis (Fig. 1).

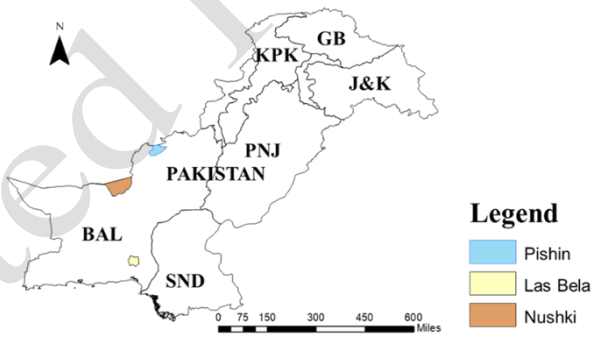

Fig. 1: Map of study areas, districts of Balochistan, where the blood sample was collected. And this map is created by the ArcGIS software version 10.8.1. Abbreviations: KPK, Khyber Pakhtunkhwa; BAL, Balochistan; PNJ, Punjab; SND, Sindh; GB, Gilgit; Balochistan; J&K, Jammu and Kashmir.

Ethical approval

The research was approved by the University of Veterinary and Animal Sciences (UVAS) Lahore, Pakistan Ethical Review Committee (DR-764).

Identification of Trypanosoma through microscopy (Conventional method) Giemsa Staining

To generate thin and thick blood smears on the glass slide using the previously described methods, Trypanosoma-positive blood samples were employed (OIE., 2012; Shah et al. 2004). The smears were fixed in methyl alcohol for three minutes after drying. To identify the Trypanosoma at the genus level, the slides were exposed to 10% Giemsa stain for 30 min. (Sadek et al. 2021). Ten blood samples from camels were positive for *Trypanosoma* throughout the investigation.

Serological analysis

Using the card agglutination test for *T. evansi* (CATT/*T. evansi)*, sera were also examined for the presence of anti-*T. evansi* (Institute of Tropical Medicine, Antwerp,

Belgium). According to the manufacturer's recommendations, 25µl of test sera were diluted at 1/4 with PBS pH 7.2 and added to about 45 µl of the antigen on the test card. After 5 minutes of agitation, the card's response was evaluated in the light of day. When agglutinations were recorded, a positive response was confirmed, and they were blue agglutinations (Kyari et al. 2021).

Statistical Analysis

Data was analyzed by Pearson's Chi-square (**χ2)** test using SPSS version 20.

RESULTS

Prevalence of positive Camels for *T. evansi* through microscopy and CATT/*T. evansi* technique based on District

Based on the district-level study, Lasbella had the highest rate of *Trypanosoma*, as established by microscopic testing, with 20 positive samples and a proportion of 15.26% (95% CI: 1.09-1.22). Noshki came in second with 12 positive samples and a percentage of 9.16% (95% CI: 1.04-1.14). Similarly, the district of Pishin reported 8 positive samples using microscopy, with a prevalence of 6.10% (95% CI: 1.02-1.10) (*p*-value 0.001) (Fig. 2) (Table 1).

According to the results, the rate of seroprevalence as measured by CATT/*T. evansi* was significantly lower in Pishin than in Noshki and Lasbella. In the Lasbella district, 90 samples were evaluated positive, while in Noshki and Pishin, 65 and 45 samples were found positive, respectively. The highest seroprevalence was observed in Lasbella at 64.1% (95% CI: 1.23-1.39), followed by Noshki at 49.61% (95% CI: 1.41 -1.58), and Pishin at 34.35% (95% CI: 1.57 -1.73) (*p*-value =0.000).

Seroprevalence of positive Camels for *T. evansi* through microscopy and CATT/*T. evansi* technique based on Body Condition Score (BCS)

The study suggests that camels with poor body conditions had a higher number of 26 positive samples and 14 positive samples with good body condition camels was found positive for *Trypanosoma* parasites when observed under a microscope, Prevalence with a rate of 14.60% (95% CI:1.08-1.19) this is significantly higher than the prevalence of 6.51% (95% CI: 1.03-1.10) observed in camels with good body conditions (Fig. 3; Table 1).

Table 1: Overall Seroprevalence percentage of *T. evansi* among risk factors by CATT/*T. evansi*

Variables	No. of examined	No. of CATT/ *T. evansi* positive	Prevalence
Districts			
Pishin	131	45	68.1%
Noshki	131	65	49.61%
Lasbella	131	90	34.35%
Body condition score (BCS)			
Good	215	70	32.55%
Poor	178	130	73.03%
Gender			
Female	230	140	60.86%
Male	163	60	37.05%
Age			
Calf	83	25	30.12%
Young	90	40	44.44%
Adult	220	135	61.36%
Breed			
Pishin	98	27	27.55%
Laasi	142	70	49.29%
Crossbreed	153	105	68.62%
Season			
Autumn	48	17	35.41%
Winter	100	52	52.00%
Summer	115	74	64.34%
Spring	130	99	76.15%

All the values were highly significant at 95% confidence interval (*P value< 0.05)*

The CATT/*T. evansi* test results showed a higher seroprevalence of *T. evansi* infection in camels with poor body condition scores, with 130 positive samples and a prevalence percentage of 73.03% (95% CI: 1.26-1.39). In comparison, only 70 positive samples were found in camels with good body condition scores, with a prevalence of 32.55% (95% CI: 1.67-1.80) (*p*-value 0.000). This finding indicates that the likelihood of *T. evansi* infection is greater in camels with poor health compared to those in good health.

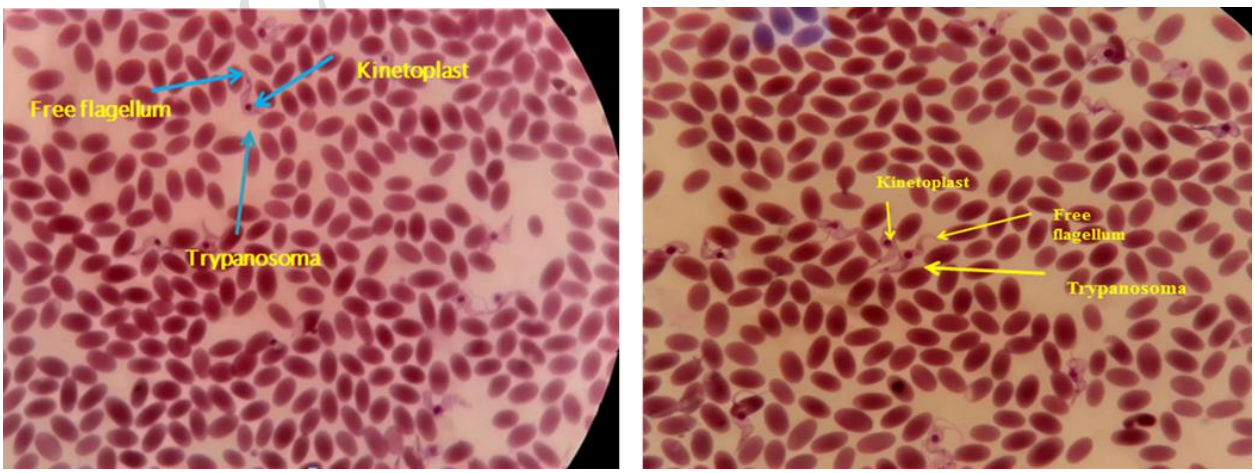

Fig. 2: Field-stained smears from a camel that has evaluated positive for *Trypanosoma* were examined by microscopy at 100 x. The distinctive features of *Trypanosoma* were found for identification, such as the kinetoplast, undulating membrane and free flagellum.

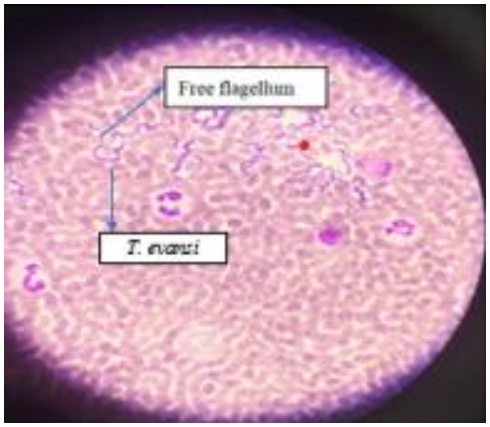

Fig 3: Giemsa-stained blood smears were examined by 100X microscopy, showing *Trypanosoma* positive sample in camel.

Prevalence of positive Camels for *T. evansi* through microscopy and CATT/*T. evansi* technique based on Gender

The data demonstrates a gender disparity in the prevalence of Trypanosomiasis, where females exhibit a higher rate of positive samples at 28, with a percentage of 12.17% (95% CI: 1.08-1.16), while males had 12 positive samples, with a percentage of 7.36% (95% CI: 1.03-1.11) (*p*-value 0.005) (Table 1).

According to the study, the most positive samples were 148 and a higher percentage of women 60.86% (95% CI: 1.55 1.68) evaluated positive for *T. evansi* antibodies by the CATT/*T. evansi* test than males. Among men, there were 60 positive samples, giving a proportion of 37.5% (95% CI: 1.29-1.44) (*p*-value 0.000).

Prevalence of positive Camels for *T. evansi* through microscopy and CATT/*T. evansi* technique based on Age.

The number of positive microscopy samples varied according to age, with the 25 young having 7, and calves having 4 *Trypanosoma* positive samples. Adult camels had the greatest percentage of *Trypanosoma* diagnoses using microscopy, at 11.36% (95% CI: 1.07-1.16), whereas young camels had a ratio of 7.77% (95% CI: 1.05-1.19) this was followed by the calf, which had a 4.81% (95% CI: 1.00-1.10) (*p*-value 0.001) (Table 1).

A serological investigation using the CATT/*T. evansi* kit approach demonstrated a significant statistical variance in the prevalence of *T. evansi* among three age groups: adults, young, and calves. Adults showed the greatest seroprevalence 61.36%, (95% CI: 1.55-1.68), whereas young and calves had a seroprevalence of 44.44% (95% CI: 1.34-1.55) and 30.12% (95% CI: 1.20-1.40) respectively (*p*-value 0.00).

Prevalence of positive Camels for *T. evansi* through microscopy and CATT/*T. evansi* technique based on Breed.

A microscopic study discovered a variation in the prevalence of Trypanosomiasis between Indigenous and cross-boundary camels. When utilizing the microscopic approach on indigenous breeds, the cross-boundary camels showed a greater incidence of *Trypanosoma* positive sample 25 8.45% (95% CI: 1.16-1.32) than the Lassi breed 10 7.04% (95% CI: 1.03-1.11) and Pishin breed 5 5.10% (95% CI: 1.06-1.18) (*p*-value 0.0001) these findings suggest that the percentage of positive microscopic results in cross-boundary camels was greater than in indigenous camels (Table 1).

The serological analysis for Trypanosomiasis by CATT/*T. evansi* kit method revealed differences in the seroprevalence of *T. evansi* between camels in three districts of Balochistan and those from across the border. Among the indigenous breeds, the Laasi breed had the highest seroprevalence at 70, 49.29% (95% CI: 1.42-1.58) while the Pishin breed had a lower rate of 27 27.55% (95% CI: 1.19-1.37) However, when the CATT/*T. evansi* test was conducted on cross-border camels, the seroprevalence was significantly higher 105 with a percentage of 68.62% (95% CI: 1.61-1.76) (*p*-value 0.000), indicating that camels from across the border had a higher seroprevalence of Trypanosomiasis compared to indigenous breed camels.

Prevalence of positive Camels for *T. evansi* through microscopy and CATT/*T. evansi* technique based on Season

The number of microscopic samples varied across different seasons. During the spring season, there were 26, 20.00% (95% CI: 1.14-1.29) microscopic positive samples, while during the summer season, there were 10, 8.69% (95% CI: 1.03-1.14) positive samples. In the winter season, only 2 positive samples were found 3.0%, (95% CI: 1.00-1.08) (Table 1).

Serological analysis by CATT/*T. evansi* showed that the number of seropositive samples was highest during spring, with 99 samples, followed by 74 seropositive samples in summer, 52 samples in winter, and 17 seropositive samples in autumn were observed.

The seroprevalence of *T. evansi*, as measured by the CATT/*T. evansi* kit method, was found to vary across seasons. The highest rate of 76.15% was observed in spring, followed by summer at (64.34%), and winter at (52.00%). The lowest seroprevalence of (35.41%) was recorded in autumn (*p*-value 0.000).

Overall Prevalence

The overall prevalence estimates were statistically significant and varied from one another, according to the findings of the numerous tests performed to check 393 camels for *T. evansi* infection. The prevalence was calculated using the GTS methodology (10.17%), which was much lower than the prevalence calculated using the CATT/*T. evansi* method, which had a percentage of (50.89%) (*p*-value 0.0005) (Table 1).

DISCUSSION

This study involved researching to investigate the prevalence of camel trypanosomiasis in the Balochistan districts of Lasbella, Noshki, and Pishin, utilizing several diagnostic approaches like parasite detection and serological testing.

The CATT/ *T. evansi* method was used in the current investigation, which revealed that more than half of the camels examined in the Lasbella district (68.1%) were seropositive and that *T. evansi* infections also occurred in Noshki (49.61%) and Pishin (34.35%). The current study

indicated that the seroprevalence of trypanosomiasis was higher in the Lasbella district than in the region of Bahawalpur, Pakistan, as Tehseen et al. (2017) observed. As this is the first time CATT/*T. evansi* technique has been used in Balochistan, this study cannot compare current results to those from other seroprevalence studies on Surra in Pakistan, except for those by (Ngaira et al. 2002; Delafosse et al. 2004). In their research, 6% of 200 horses from the Gujranwala area were positive, while 40% of the sample population showed detectable antibodies against *T. evansi* in Bahawalpur, Pakistan.

A higher seroprevalence (65%,70.80%) of *T. evansi* was found in recent research carried out at six farms in Somalia, according to Mohamed et al. (2020). The sero-prevalence observed to be substantially similar between this study and the Somalia survey might be due mostly to the camel density in Somalia. With roughly seven million camels dispersed over the nation, the population is the greatest in the world. This nation's large camel population may favor vector transmissions and encourage *T. evansi* infection (Njiru et al. 2002). Pakistan is ranked eighth worldwide among nations with a large camel population (Aregawi et al. 2019; Tehseen et al. 2015). Similarly, Balochistan has 41% of the one-humped camels (also known as dromedaries). Consequently, trypanosomiasis in Balochistan may be mostly caused by the region's vast camel population, whereby vector transmissions and encouragement of *T. evansi* infection are aided. Research in the Eastern region of Chad found that among 2831 camels examined, the seroprevalence was 30.5% (Delafosse et al. 2004), which is roughly the same as the seroprevalence of the Pishin district of the current study and lower than the Lasbella and Noshki districts. The contrast might be based on variations in population sizes and sampling techniques. The abundance of Surra in transhumant systems in Eastern Chad provided ideal breeding conditions for rapid spread of *T. evansi* among camels (Njiru et al. 2002).

Another study found an overall seroprevalence of 12% in West Niger, with seropositive variations attributable to seasonal herd movements (Nguyen et al. 2021). In contrast to these results, this study demonstrated a higher seroprevalence due to seasonal herd movements since the Noshki and Pishin Districts of Balochistan, Pakistan, share borders with Iran and Afghanistan. The seroprevalence research of *T. evansi* conducted by (Njiru et al. 2002) discovered 44.09% overall seroprevalence (Njiru et al. 2004) revealed (45.9%, 41.77%, and 50.08%) (Baticados et al. 2011) also found anti-*Trypanosoma* antibodies (43.5%, 36.72%, 50.57%) observed comparable results, revealing data that were different from the current investigation.

Likewise, the persistence of antibodies up to several months is associated with the sero-prevalence of *T. evansi* in several types of research. Additionally, other elements like host susceptibility, strain pathogenicity, and nutritional state may be affected by varied seroprevalence (Njiru et al. 2002; Khosravi et al. 2015). Since antibodies may persist in the blood for several months after therapy (Delafosse et al. 2004). It is not unexpected that parasitological prevalence is lower than serological prevalence, similar to earlier research (Atarhouch et al. 2003; Delafosse et al. 2004; Njiru et al. 2004). The high percentage of positive specimens detected by the various tests demonstrates that Surra is quite common in the specified district of Balochistan. The high seroprevalence may be caused by the density of the vectors as well as the fact that camel owners are unaware of the significance and financial consequences of the sickness. Although the sample populations of the two studies are comparable, differences in the seroprevalence percentages might mostly be attributed to camel density in Somalia. The nation is home to the largest number of camels in the world. seven million of them are dispersed over the land. Infection with *T. evansi* may be encouraged by the nation's large camel population and vector transmissions.

Adult camels in the current research displayed greater seroprevalence (61.36%). This is consistent with the findings of Delafosse et al. (2004) and Bhutto et al. (2010), which showed that camels older than 3 years had greater levels of *T. evansi* antibodies than those between 1 and 2 years of age. According to the findings of Atarhouch et al. (2003), the infection rate of *T. evansi* increases with an age of 7-10 years. This may be due to calves avoiding grazing with adults and hence being protected from exposure to abundant vectors of *T. evansi* in pastures. High infection rates were suggested by the seroprevalence of *T. evansi* in adult camels in Lasbella, Noshki, and Pishin. This finding may be consistent with a claim that camels of all ages have been affected by Surra, but a higher sero-prevalence of its symptoms was reported in younger camels (Njiru et al. 2002). However, in this investigation, adults had a greater seroprevalence of Surra than children. As a result, a feasible explanation may be that seroprevalence increases with age (Khosravi et al. 2015; Tehseen et al. 2015). Camel populations in rural regions are under a great deal of strain. To find water and pasture, they must travel a great distance. These demanding activities might damage their quality of life, immunity, nutrition, and susceptibility to *T. evansi* infection. According to Fikru et al. (2015) and (Tehseen et al. 2015), the seroprevalence ratio was found more in the adult camels was significantly higher than in young animals, these results found more similar to the seroprevalence of the adult camels in all three districts of Balochistan in the current study, and this could be due to trans-boundary trading with neighboring countries like Iran and Afghanistan. Additionally, Delafosse et al. (2004) described the association of risk factors in camels in Eastern Chad with *T. evansi* infection and reported an increased risk of infection associated with age. The gender based sero-prevalence of this disease in camels was recorded as 37.5% (male) and 60.86% (female), respectively, in the Balochistan districts of Lasbella, Noshki, and Pishin. In Lasbella, Noshki, and Pishin districts of Balochistan, like in other areas (Kyari et al. 2021; Ngaira et al. 2002; Njiru et al. 2004), a significant percentage of seropositive specimens were found, demonstrating that Surra is a serious problem there. On the other hand, in the Balochistan districts of Lasbella, Noshki, and Pishin, its incidence in male could be explained by the fact of their exhaustion from hard labor and wandering more in quest of food and water, a higher risk of their exposure to vectors. While (Tehseen et al. 2015) indicated that females had a greater seroprevalence than men (50.1%, CI = 45.98%, 54.20%), who had a

seroprevalence of 44.5% (CI = 39.97%, 49.22%) in camels. The camels sampled in summer and spring were three and five times more likely to be infected with *T. evansi* compared to the camels sampled in winter. Camels that have been evaluated positive for *T. evansi*, in the Balochistan districts of Lasbella, Noshki, and Pishin, serve as reservoirs for other animals (other animal species and humans).

Conclusion

Camels were mostly a mix of natives and camels from neighboring countries, including Iran and Afghanistan. To the best of our knowledge, this is the first study of *T. evansi* infection in camels in the Balochistan districts of Lasbella, Noshki, and Pishin disclosed by the CATT/*T. evansi* approach. *T. evansi* was reported in dromedary camels of all ages and genders in the Balochistan districts of Lasbella, Noshki, and Pishin.

To prevent the spread of disease, it is important to conduct continuous monitoring by national veterinary services, as well as control biting insect populations. Additionally, it would be beneficial to conduct studies on various species inhabiting the same area to monitor infection and determine their status as reservoirs for the disease.

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