

EPIDEMIOLOGICAL SURVEY OF TRYPANOSOMIASIS IN THE DROMEDARY CAMELS RAISED IN DERA ISMAIL KHAN, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

One-year epidemiological survey was conducted to determine the age, sex and season-wise prevalence of Trypanosomiasis in dromedary camels (*Camelus dromedarius*) at Dera Ismail Khan region of Khyber Pakhtunkhwa, province of Pakistan. A total of 600 blood samples were parasitological examined in which 300 were male and 300 were females. Camels were grouped age-wise as: Adults (<2 years age) and calves (>2 years age), sex-wise as; male and female while ratio of animals in both age and sex group were kept nearly 1:1. Season-wise prevalence was observed by splitting the data in four seasons of the year as Pre-monsoon, Monsoon, Post-Monsoon and winter. The samples were examined microscopically using thin, thick and wet blood film for the identification of *Trypanosoma evansi* based on morphological characteristics. An overall prevalence was determined as 6.67%. Age-wise prevalence was found as high 9.06% in adults and low 4.30% in calves ($P < 0.05$). Sex-wise the infestation was observed as high 7.66% in female and low 5.66% in male camels ($P > 0.05$). Among four seasons of the year, highest prevalence 11.18% was observed in rainy season of monsoon followed by 8.45, 7.85 and 0% in post monsoon, pre-monsoon and winter respectively ($P < 0.05$). All the results were statistically analyzed on SPSS using Pearson Chi-Square test. This survey showed that age and season have significant affect while the sex has non-significant effect on prevalence of trypanosomiasis in dromedary camels.

Keywords: Trypanosomiasis, age, sex, season, dromedary camels.

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1. INTRODUCTION

Livestock industry has a big share in the economy of Pakistan, contributing about 11.5% to the total Gross Domestic Products (GDP). About 8 million families are earning 40% of their livelihood from livestock sector (Anonymous 2020-2021). In Pakistan, small and large ruminants including camels are mainly reared for meat and milk production (Ali et al. 2016; Ali et al. 2017). Camel (*Camelus dromedarius*) is a single humped animal an important multipurpose animal which is raised in arid and semi-arid areas of world. The best source of livelihood of people of desert areas is the camel in Asia, Middle East and Africa (Hussain et al. 2018; AL-Samawy et al. 2019; Gherissi et al. 2020; Hussain et al. 2021). Total camel population of world is about 25.89 million, having 89% single humped animals whereas 11% are double humped (*Camelus bactrianus*) (FAO 2013). Camels in the agriculture-based country like Pakistan are contributing 1.81% towards total milk production by supplying 932000 tons of milk per annum (Economic Survey of Pakistan 2020). Diseases like bacterial, viral and parasitic/protozoal infections have always remained a constant threat to the productivity of animals (Batool et al. 2019; Chemweno et al. 2019).

Trypanosoma evansi (*T. evansi*) is hemoflagellate parasite of order Kinetoplastida and family Trypanosomatidae which replicates in blood and other body fluids (Alsaad 2021). It causes a widely spread endemic disease called "Surra" in camels, various domestic as well as wild animals throughout the world (Elhaig et al. 2013; Rodríguez et al. 2012; Shahid et al. 2013; Chau et al. 2016). Trypanosomiasis was discovered in India more than a hundred years ago by Evans (1880), who detected it in horses, mules and camels causing a disease which was named as "Surra". Including different mammals, humans are also affected (Coura and Borges-Pereira 2010; Dyary et al. 2014). The case of human trypanosomiasis has been reported from Southeast Asia (Chau et al. 2016). It is mechanically transmitted by biting flies (*Tabanus*, *Stomoxys* and *Liperosia*) in which the protozoans undergo a biological cycle (Yadav et al. 2011). Animals suffering from these protozoan parasites usually suffer from induce fever, dullness, weakness, depression, and anemia. Also, these parasites are responsible for economic losses in terms of poor production (low

meat and milk production, low fertility, and less draught power available) and sometimes abortion or death in case of no treatment (Enwezor and Sackey 2005). Trypanosomiasis in camels is characterized by reduced appetite, low water intake, disappearance of hump with disease progress, dull and rough hair coat with loss of hairs at tail. Odema of under belly is visible especially in the morning, pregnant females may abort, and newborn calves of infected calves usually die. There is pallor of mucous membranes of the eyes, a fluctuating temperature with initial peaks of up to 41°C, and urine usually has characteristic smell (Köhler-Rollefson et al. 2001). Animals become emaciated and die within 2-4 months due to anemia, circulatory disturbances and myocardial damages leading to congestive heart failure. The camel may live up to four years with sub-clinical infestation and some may eventually recover and eliminate the parasite. Direct losses due to trypanosomiasis are estimated at US\$ 1-1.2 billion each year. The total losses for the total tsetse fly infested land in terms of Agricultural Gross domestic product (GDP) are US\$ 4.75 billion per year (FAO 2000).

Trypanosomiasis has a wide range of distribution throughout tropical and sub-tropical regions of the world. The disease is most important single cause of economic losses in camel rearing areas (Angara et al. 2012), causing morbidity of up to 30% and mortality of around 3% (Luckins 1988). Surveys in various tropical areas have shown a definite correlation between seasonal outbreaks of *T. evansi* infections and the increase in number of *Tabanus* during rainy season (Mahmoud and Gray 1980). Camel Trypanosomiasis is present in India, Myanmar, Bhutan, Mongolia, Malaysia, Cambodia, China, Thailand, Russia, Nepal, Vietnam, Philippines, Indonesia, South and Latin America and Europe with worldwide distribution (Reid 2002; Mihret and Mano 2007; Konnai et al. 2009; Sood et al. 2011; Salim et al. 2011; Ismael et al. 2014; Kumar et al. 2017; Boushaki et al. 2019). Recently the disease has also been reported in the Canary Island, Spain as well as France (Gutierrez et al. 2000). This disease is a serious concern as it is difficult to treat and non-availability of vaccine (Metwally et al. 2021). The disease replication is supported with environmental and climatic conditions of the regions and also presence of the vectors in the area play an important role in the spread of the disease. Environmental high temperature and relative humidity favors the division of its vectors and thus increases the chances of the trypanosomiasis (Desquesnes et al. 2013).

According to Livestock census conducted in 2006, there are 12930 camels in district Dera Ismail Khan, those are mostly used to pull carts or plough fields. Present study was conducted to determine the age, sex and season wise prevalence of Trypanosomiasis in the dromedary camel population reared in rural areas of District Dera Ismail Khan.

2. MATERIALS AND METHODS

2.1. Sample area

Samples were collected from 20 different villages of D.I. Khan (Fig. 1) including Garrah Rehman, Kulachi, Hathala, Draban Kalan, Chudhwan, Musazzai, Hassani, GarrahJat, Garrah Ali, Garah Meharban, Lar, Kala Ghore, Kot jaiee, Abdul Khel, Shah Hassan Khel, Panyalla, Wanda Madad, Wanda Kali, Yarak and Rori Khel, where dromedary camels are raised. The area covers pane land and somewhere sandy area.



Fig. 1: Map of Pakistan (right side) showing all provinces and on the left side is Khyber Pakhtunkhwa Province and Dera Ismail Khan (study area in red color).

2.2. Sample Collection

During field survey, samples were collected randomly from the animals of respective area including both the healthy as well as morbid animals, if any. For sample collection, regular field visits to the respective villages were conducted on monthly basis. A total of 600 blood samples were collected from Jugular vein/ear vein, after disinfecting the site with 70% alcohol. Punctured into 5mL EDTA coated vacutainer tubes, properly labeled and kept in ice box. Both the thick and thin blood smears were made at the spot of collection. Thin smears were fixed with absolute methanol for 5min. Smear slides were also labeled and placed in ice box. Ice box containing the sealed slides and blood samples were transported to Parasitology laboratory at Veterinary Research and Disease Investigation Center, D.I. Khan for further processing and examination.

2.3. Sample processing

Wet film technique was used to observe Trypanosoma by their waving flagellum movement (Murry et al. 1979). From each camel thin smears of fresh blood were prepared for the microscopic study and identification of flagella of trypanosomes (Hussain et al. 2018). Blood smears prepared were dried, fixed in ethanol (100%) and stained with Giemsa stain. For different morphological changes in erythrocytes and the presence of *Trypanosoma*, all the blood smears were carefully studied under light microscope (Hussain et al. 2018).

Stained Blood Smear (Both thick and thin) were used for detection and identification of Trypanosoma species based on their morphological characters. Samples were parasitologically examined under Oil immersion lens (100X). Morphological characteristics of *T. evansi* recognized from blood samples of infected camels following the methods described by Chandler and Read (1961), while the *Trypanosoma evansi* measurements were carried out following the method of Luckins (1992) and Smyth (1996).

2.4. Seasons Distribution

This study was carried out throughout the year; thus, a year was divided into four seasons, i.e., pre-monsoon (March to May), monsoon (June-September), post-monsoon (October and November) and winter (December-February).

2.5. Statistical Analysis

Data thus collected were subjected to statistical analysis. Camels were categorized into age-wise (>2 years: calves; <2 years: adult camels), sex-wise (male and female) and season-wise (four seasons). Data were reviewed by descriptive statistics using SPSS V21.0, (IBM SPSS Statistics 21, USA). Comparison between groups was evaluated using Pearson Chi-Square test (Petrie and Watson 1999). Any result variable was considered as significant at $P < 0.05$.

3. RESULTS

Out of total camels (n=600) under study, 40 were positive with *T. evansi* showing overall prevalence of 6.67%. During the investigations, *T. evansi* was only species recognized from the samples of infected animals. Age, sex and season-wise results of the study are presented in Tables 1-3, respectively. Despite its low sensitivity, as a routine diagnostic practice, parasitological examination of the samples was conducted due to non-availability of molecular testing facilities at the center. Morphological characteristics of *T. evansi* recognized from blood samples of infected camels. *Trypanosoma evansi* have slender body with centrally located nucleus, and subterminal kinetoplast with the length. *T. evansi* were measured which ranged from 15.30µm in length and 1.5 to 3µm in width.

Table 1: Age-wise prevalence of Trypanosomiasis in dromedary camels

Group	Age	Total camels	Infected Camels		Non-infected camels	
			No	%	No	%
Adult	<2 years	298	27	9.06	271	90.93
Calves	>2 years	302	13	4.30	289	95.70
Total		600	40	6.67	560	93.33

Data analysis by Chi-square test. χ^2 Value=4.772; df=1; P value=0.029.

Table 2: Sex-wise prevalence of Trypanosomiasis in dromedary camels

Sex	Non-infected camels	Infected camels		χ^2 Value	P Value
		No.	%		
Male	283	17	5.66	0.964	0.326
Female	277	23	7.66		
Total	560	40	6.67		

Data analysis by Chi-square test. χ^2 Value=0.964; df=1; P value=0.326.

Table 3: Season-wise prevalence of Trypanosomiasis in dromedary camels

Season	Non-infected Camels	Infected Camels	
		No.	%
Pre-Monsoon	191	15	7.85
Monsoon	170	19	11.18
Post-Monsoon	71	06	8.45
Winter	128	0	0
Total	560	40	6.67

Data analysis by Chi-square test. χ^2 Value=12.908; df=3; P value=0.005.

Prevalence of *T. evansi* infestation in adult camels (<2 years age) was 9.06% while in calves (>2 years age) was 4.30%. The results showed that adult age group was more affected than the calves. The difference in prevalence between age groups was found statistically significant (P<0.029) as shown in Table 1.

Prevalence of *T. evansi* infestation in more in male camels (5.66%) as compared to female camels (7.66%). The difference in prevalence of *T. evansi* between male and female dromedary camels was found non-significantly different (Table 2).

Camels (n=600) from those blood samples collected have distribution the highest in the pre-monsoon (34%) followed by the monsoon (32%), winter (21%) and least (13%) in post-monsoon (Fig. 2). Seasonal prevalence of *T. evansi* was found high 11.18% in monsoon, moderate 8.45% in post monsoon, low 7.85% in pre-monsoon while no case in winter season. The difference in prevalence among four seasons of the year was found statistically significant (P<0.05) as shown in Table 3.

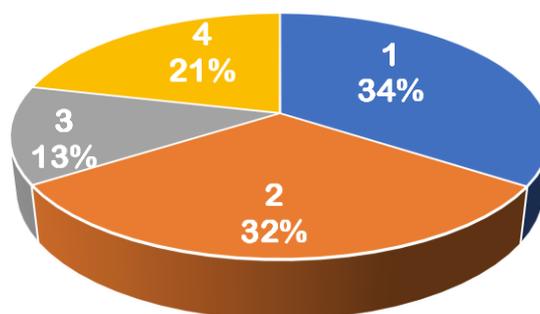


Fig. 2: Camels (n=600) distribution (%) among various seasons, i.e., 1) pre-monsoon, 2) monsoon, 3) post-monsoon and 4) winter from those samples were collected.

4. DISCUSSION

This is the first study on *Trypanosoma evansi* infection in Camelidae using parasitological examination in D.I. Khan, KP Province, Pakistan. Morphologically, *T. evansi* is an elongated blood parasite and measured 14-29µm in length and 1.5 to 3.45µm in width, with a nucleus containing a large central nucleolus (Dávila et al. 1998). According to Khalafalla and Al Mawly (2020), *Trypanosoma evansi* were characterized by slender body, centrally located nucleus, and subterminal kinetoplast with total length including free flagellum of 24.9µm (16.9–31.3µm), the body length of 16.4µm (7.9–25.1µm) and width 2.8µm (0.1–5.7µm). The free flagellum is 28.6µm long (1.1–14.1µm).

The prevalence of “Surra” in Dera Ismail Khan Region as 6.67% can be considered as low compared with other epidemic regions like Somalia (Baumann and Zessin 1992), Kenya (Njiru et al. 2001) and Egypt (Sobhy et al. 2017). Metwally et al. (2021) using molecular method (PCR), reported prevalence of Trypanosomiasis as 42.5% in Kingdom of Saudi Arabia, which is higher in comparison to the result of the present study. Mossaad et al. (2017) and Gerem et al. (2020) in their study reported that the prevalence of Trypanosomiasis is found lower on parasitological examination method as compared to the molecular techniques (PCR). Similar results were reported by Birhanu et al. (2015) and Sadek et al. (2021). Similarly, the prevalence was found 25% in northern part of Kingdom of Saudi Arabia (Al-Joaf Province) (Elwathig et al. 2016). Kasim (1984) and Hussein et al. (1991) reported the prevalence of Trypanosomiasis on microscopic study as 2 to 13.2% in the Saudi Arabia which lies in relation with the findings of this study. Another study conducted by Omer et al. (1998) revealed the prevalence as 5.5% in camel population of Saudi Arabia. Hussain et al. (2021) reported the prevalence as 10.58% from Cholistan region of Province of Punjab, Pakistan which shows more prevalent nature of the disease in Cholistan desert as compared to the present study. This increase can be attributed to the harsh environmental conditions and higher number of vector population in the area as compared to

the present study area. Ahmadi-Hamedani et al. (2014), also concluded that trypanosomiasis was present in 4.76% camels of Semnan, Iran. Khosravi et al. (2011) indicated that *T. evansi* was present in 2.1% camels of Rafsanjani, Kerman province, Iran. The results of this study are closer to the study of Hassan et al. (2006) in which 3.3 to 4% prevalence level was observed in Punjab region of Pakistan and also with Ahmadi-Hamedani et al. (2014) who noted 4.67% in Iran. However, the study revealed lower level of prevalence than earlier studies such as 13.2% by Hussein et al. (1991), 13.72% by Shah et al. (2004) and 11.5% by Bhutto et al. (2010). Factors like ecological variations and vector abundance may lie behind this difference.

This study showed higher rate of infection in adults than calves and the similar difference of 0% prevalence in calf (≤ 2 years) and 7.7% prevalence in young group (> 2 years) was observed in Ethiopia (Kassa et al. 2011). It was also reported from Egypt that prevalence level of *T. evansi* in calves (≤ 4 years) was 14% and in adults ($4 <$ years) was 24-25% (Sobhy et al. 2017) showing higher infection level in adults and lower in calves. Hussain et al. (2016) reported that the difference between the adults and younger camels, but non-significant but older camels were infected more unlike the young camels. They attributed this difference towards the poor management, improper diet and transportation of heavy draught duty. Immunology could also be a factor for protecting the camel from *T. evansi* infestation (Hussen and Schuberth 2021). Similar results were mentioned by other researchers (Shah et al. 2004). Contrary to the results of the present study, relatively higher prevalence of trypanosomiasis has been reported in young camels as compared to adult ones (Kassa et al. 2011). The calves may be less infected due to their confinement at the stall where the vector population could be less as compared to meadow lands where adults camels are grazed by pastoralists.

The higher infection rate in females could be attributed to stress of lactation and successive pregnancies (Bhutto et al. 2010). A higher infection rate (15.68%) in females were relatively lower in males (11.76%) was also found (Shah et al. 2004). Iranian study reflects that in female the loss increases as a result of abortions. The rate of abortion in pregnant females was 16.4% in South Khurasan and 26.3% in Sistan and Baluchistan provinces of Iran (Nazem et al. 2020). Higher infection of *T. evansi* in females (22.9%) and lower in males (14.1%) has also been reported from Egypt (Sobhy et al. 2017). A previous report from Ethiopia (Kassa et al. 2011) showed higher prevalence of *T. evansi* (6.8%) in males than females (4%) which is unlike to the present study. This variation could be due to stress of loading and transportation fatigue acquiring probability of higher infection in male camels. Sobhay et al. (2017) reported higher prevalence in male camels (22.9%) as compared to females (14.1%) on microscopic parasitological examination. Similar data were recorded by Barghash et al. (2014), El-Naga and Barghash (2016) and Sobhy et al. (2017).

It has been unearthing by that the highest prevalence was found in the spring season (26.77%), followed by the summer (23.47%), winter (16%) and autumn (12.6%) using microscopic parasitological techniques. Similar results have also been reported by Sobhy et al. (2017). It was interesting to note that season-wise prevalence of *T. evansi* was related to the biting horseflies (Tabanus species) in the area. These flies are found in abundance during rainy seasons and lower in winter. Dera Ismail Khan District, Khyber Pakhtunkhwa province consists mostly of floodplain land situated at the western bank of the river Indus. The area is with extreme weather conditions during summer and winter. However, rainy season (monsoon) reduces the effect of scorching heat in summer. The high prevalence of Trypanosomiasis in rainy season could be linked with the higher vector population of Tabanus species (Kassa et al. 2011). Luckins (1988) was of the view that season has a direct effect on the distribution of biting flies, which are responsible for the mechanical transmission of *T. evansi*. The prevalence of these flies is higher in spring and summer season; hence the incidence rate is also found higher in these seasons. It was concluded that the best season for Trypanosomiasis is the Spring followed by the Summer that might be related to a higher risk of camels exposure to trypanosome infection due to the increased density of vector populations at this time of the year as reported before and coincides with Barghash et al. (2014). Apart from rainy season, water withholding capacity of soil and surface water pool where Acacia Senegal shrub grows in abundance are considered suitable factors for survival and propagation of vector (Kassa et al. 2011).

Conclusion: The overall prevalence of Trypanosomiasis in dromedary camels was determined to be 6.67% in D.I. Khan, KP Province, Pakistan. It was concluded that the age and seasons of the year significantly ($P < 0.05$) affect the prevalence of Trypanosomiasis in dromedary camels, while the sex has no effect. However, the results cannot be same for all camel producing areas of the country as the ecological and geological factors attributed to the spread of disease vary from area to area within the same country.

Authors Contribution: SZK, UEA, and MR planned the study. AA, IK, Safiullah, Abidullah, SI, Waseemullah and AK collected the samples and analyzed in the Laboratory. SZK interpreted the data and all authors were involved in the revision and approval of final version of the manuscript.

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