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THE PREVALENCE, EPIDEMIOLOGY AND RISK FACTORS OF TRYPANOSOMIASIS DISEASE IN BUFFALO

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Article Info

Abstract

Trypanosomiasis, a vector-borne parasitic disease caused by Trypanosoma species, poses a major health and economic threat to livestock and human populations in tropical and temperate regions, including Pakistan. This study aimed to determine the prevalence, species identification, clinical signs and associated risk factors of Trypanosoma infection in buffaloes within the Vehari district, a region where disease surveillance is limited. A total of 84 buffaloes, including Nili-Ravi and Kundi breeds, were evaluated using parasitological (microscopic blood smear) and serological (ELISA) diagnostic techniques. The overall prevalence of trypanosomiasis was 13.09%. Among breeds, Kundi buffaloes showed the highest infection rate (16%) compared to Nili-Ravi (11.86%). Gender-based differences were statistically non-significant, though males showed a marginally higher prevalence (13.34%) than females (13.13%). Risk factor analysis revealed that animals housed in herd-based systems (17.94%) and those without vaccination (23.52%) had significantly higher infection rates. Notably, a strong correlation was found between infection and exposure to pets (13.88%) as well as history of exposure to other livestock (17.85%). Age was also a significant determinant, with buffaloes older than five years showing the highest prevalence (15.38%), suggesting age-related susceptibility. Similarly, medicated animals exhibited a higher prevalence (14.28%), possibly due to incomplete or inappropriate treatment protocols. This study underscores the complex interplay of host factors (age, breed), environmental exposures (herd management, contact with other species) and veterinary practices (vaccination, medication) in shaping trypanosomiasis epidemiology. Given its zoonotic potential and impact on productivity, integrated control strategies—incorporating veterinary surveillance, community awareness, vector management and climate-adaptive livestock practices—are essential. The findings emphasize the need for a One Health approach to mitigate the spread of trypanosomiasis and its broader implications for public health, food security and sustainable agriculture in endemic regions.

Keywords: *Trypanosoma Spp., Buffalo, Trypanosomiasis, Epidemiology of Parasitic Diseases, Zoonotic Vector-borne Infections, Livestock.*

INTRODUCTION

Trypanosomes are protozoan parasites that are members of the genus Trypanosoma and the family Trypanosomatidae. Many species, including T. brucei, T. Congolense, T. equipe Dum, T. evansi, T. simiae, T. suis and T. vivax, belong to the genus Trypanosoma and cause diseases known as trypanosomiasis in a variety of animal hosts, including humans (Stevens and Brisse 2004). Trypanosome infections are common in Asia, Latin America and Africa (Luckins and Dwinger 1998). One of the most significant species known to infect both domestic and wild animals is Trypanosoma vivax (Vieiraet al., 2017). According to the Köppen classification, Pakistan is located in the temperate zone and has a warm temperature throughout the majority of its territory. Furthermore, Pakistan is one of the top ten nations in the world that has experienced the worst effects of global warming (Zeb et al., 2019). All taxonomic and eco-epidemiological aspects have been addressed, despite the fact that various ecological and molecular research targeting ticks and tick-borne illnesses in Pakistan during the past few years have been conducted. In Pakistan, for example, the two most common tick species infesting ruminants are Hyalomma anatolicum and Rhipicephalus microplus, according to an assessment of tick distribution across several ecological zones (Zeb et al., 2019). Furthermore, trypanosomiasis is a widespread disease in the desert area of Pakistan particularly Cholistan desert, affecting small ruminants such as sheep and goats along with large ruminants (Sobiaet al. 2018). Cholistan desert, being a desert and remote area of country, the accurate measures of Trypanosomal infection in livestock is not possible. It is very hard to estimate the subsequent economic losses, morbidity and mortality in animals in the field. The present study was designed by keeping in view the importance of livestock as backbone of economy of Pakistan particularly in nomadic form of life and gross domestic product (GDP) of the country. This study aimed to determine the overall prevalence of trypanosomiasis in livestock from different localities of livestock population and to characterize the parasite species associated with infections by using different sets of primers such as genus specific (TBR and TRYP4) or specie specific (Sobia et al., 2018) primers. Trypanosomiasis, also known as African trypanosomiasis or sleeping sickness, is a serious disease caused by the protozoan parasites of the genus Trypanosoma. It is a significant public health and veterinary concern in many parts of the world, particularly in sub-Saharan Africa. This disease affects a wide range of animals, including humans, livestock and wildlife and it poses a risk to public health while causing significant economic losses (Aliet al., 2023).The transmission of trypanosomiasis occurs through tsetse flies, which are blood-feeding insects found in rural areas of sub-Saharan Africa. The infection is started when an infected tsetse fly injects Trypanosoma parasites into the circulation through biting a human or animal host. These parasites then multiply and spread throughout the body, leading to the development of the disease (Batoolet al.,2019).

MATERIALS AND METHODS

3.1 Study area

The study was conducted in District Vehari from its three tehsils of Vehari, Mailsi, and Burewala. The research was conducted on 84 buffalo from three Tehsils of District Vehari.

3.2 Management of animals

The animals on the current trail were managed under an extensive system in which two different breeds of buffaloes were used. Inspection and assessment of body condition were accomplished during sample collection. During sample collection, the body condition was examined and evaluated. In accordance with the needs of the animals, adequate food, medicine, drinking water and other management conditions were given to them.

3.3 Study design

A cross-sectional study was designed to determine the prevalence of trypanosomiasis in the animals and collect data on this most common disease affecting buffaloes through interviews with household owners. Based on the objectives of the research, the study design has been chosen as it includes cross-sectional surveys, longitudinal research and retrospective examinations of available data, which are examples of prevalent designs.

3.4 Animals groups

The first groups of animals included the classification of the two buffalo breeds into two groups: Nili-Ravi and Kundi. The second group was prepared according to the animal's sex: male and female. The third group was formed on the basis of exposure to pets: exposed and non-exposed. The fourth group was prepared according to antibodies in relation to farm type: commercial farm, herd and domestic. The fifth group was prepared according to the vaccination status of animals against other diseases: vaccinated and non-vaccinated. The sixth group was prepared in relation to exposure to other livestock populations: exposed and un-exposed. The seventh group was prepared for trypanosomiasis with reference to previous exposure history, either yes or not. The eighth group prepared the trypanosomiasis relevancy to various age groups: 1-2 years, 2–5 years and < 5 years. The ninth group was prepared for trypanosomiasis by previous history of medication, medicated and non-medicated.

3.5 Sample selection

Define the parameters for the sample selection process and specify the target population (the buffaloes in District Vehari). To ensure accuracy, random sampling techniques might be used in this. Farmers received instructions to bring their buffalo for inspection by authorities in the community and agriculture. Next, a systematic random selection of households that had brought their buffaloes for inspection at specified locations was made among those who had registered on each sampling day. Create a sample plan for collecting blood from the chosen buffaloes. Sample size, sampling method (such venipuncture) and suitable sample storage conditions were specified before blood collection. A random selection of animals was also made from the cattle that were brought to the inspection location.

3.6 Laboratory examination

Parasitological examinations for the detection of Trypanosoma were made in suspected cases based on thin blood smear examination and Buffy coat examination techniques. Putting a drop of blood on a tiny slide, making a film and staining it with Giemsa are the steps in a thin blood smear examination. Subsequently, the slide is cleaned, allowed to dry and examined at a magnification of 100x with the use of immersion oil. The Buffy coat method involves gathering blood into micro-hematocrit centrifuge tubes, caulking them and then centrifuging them for five minutes at 12,000 rpm. Scratching and shattering the tube releases a single drop of Buffy coat onto a microscope slide, which is then spread and viewed at 40x magnification (Singhet al. 2017).

3.7 ELIZA test

The ELISA procedure was derived from a study (Kocheret al. (2015). The procedure is briefly defined in which 100 μL/well of T. evansi soluble antigen (5 μg/mL) was coated on Micro Test 96-well Polysorp Nunc immunoplates (Nunc, Roskilde, Denmark) in carbonate buffer (0.05 M, pH 9.6) and incubated for an overnight period at 4∘C. Blocking buffer (BB) (Phosphate Saline Buffer (PBS), 0.1% Tween 20 (Labchem, Pittsburg, USA) and 7% skim milk powder (ref.: 190-12865, Wako Pure Chemical Industries Ltd., Osaka, Japan) were added to each well of the plate. The plates were then permanently

shaken (at 150 rpm) for 30 minutes at 37°C. The BB was thrown away. Sera that had been diluted 1: 100 in BB were added to the ELISA plates in duplicate. Following incubation, the plates were cleaned eight times using washing buffer (WB) (PBS, 0.1% Tween 20) under the same conditions as before. Afterwards, 100 μL of diluted 1: 25,000 in BB containing peroxides-conjugated anti bovine IgG (ref: A5295, Sigma–Aldrich, USA) was added and the plates were shaken at 150 rpm for 30 minutes at 37∘C. 100 μL of the substrate/chromogen combination(Sure Blue TMB, KPL, Maryland, USA) was added after eight rounds of washing with WB. For thirty minutes, the plates were incubated in a dark room without being shaken. Using an ELISA reader (Dynex Technologies, VA, USA), the optical density (OD) was measured at 620 nm.

Figure 3.1: Animal under the current trail

Figure 3.3: Preparation of Slides at Laboratory

Figure 3.2: Sample collection of Blood from different Animals

3.7 Statistical analysis

The data was established by using MS excel sheet and put carefully to analyzing the prevalence of Trypanosoma in buffalo. Determine the prevalence of Trypanosoma infection in buffaloes by using SPSS (Version.20) to analyze the collected data. Determine the statistical significance ($P<0.05$) and prevalence rates.

3.8 Data Interpretation and Reporting

Analyzed the findings of study. Write that covers the approach, findings and recommendations. It should be noted that performing research on the prevalence of trypanosomiasis necessitates veterinary medicine, Parasitology and research technique skills. Getting advice from subject-matter specialists or examining earlier research might yield insightful information and direction for creating a suitable technique.

Results

The current study was conducted in the three tehsils (Vehari, Mailsi, and Burewala) of District Vehari, whereas the laboratory examinations were completed at the Faculty of Veterinary Sciences, Bahauddin Zakariya University, and Multan.

4.1 Prevalence of Trypanosomiasis according to Breed of Animal

The prevalence of trypanosomiasis according to breed of animal were reported in 84 animals of two different breeds of buffaloes; Nili Ravi and Kundi in the study area (Table 4.1). Out of the total examined (84) animals 59 were of the Nili-Ravi breed and 25 were of the Kundi breed. The results showed that the highest trypanosomiasis prevalence was observed in the Kundi breed (16%) as

compared to Nili-Ravi (11.86%) and the total prevalence of trypanosomiasis recorded was 13.09%. There were 12% trypanosomiasis positive and 88% trypanosomiasis negative animals out of 59 of the Nili-Ravi breed. There were 16% trypanosomiasis positive and 84% trypanosomiasis negative animals out of 25 of the Kundi breed. The disease prevalence was statistically non-significant (P >0.05) between two different breeds of buffaloes; Nili Ravi and Kundi (shown in Figure 4.1).

Animal Breed	Examined (n)	Positive (n)	Negative (n)	Prevalence %	P-Value
Nili Ravi	59	7	52	11.86	P>0.05
Kundi	25	4	21	16	
Total	84	11	73	13.09	

Table 4.1: Prevalence of Trypanosomiasis according to Buffalo Breeds

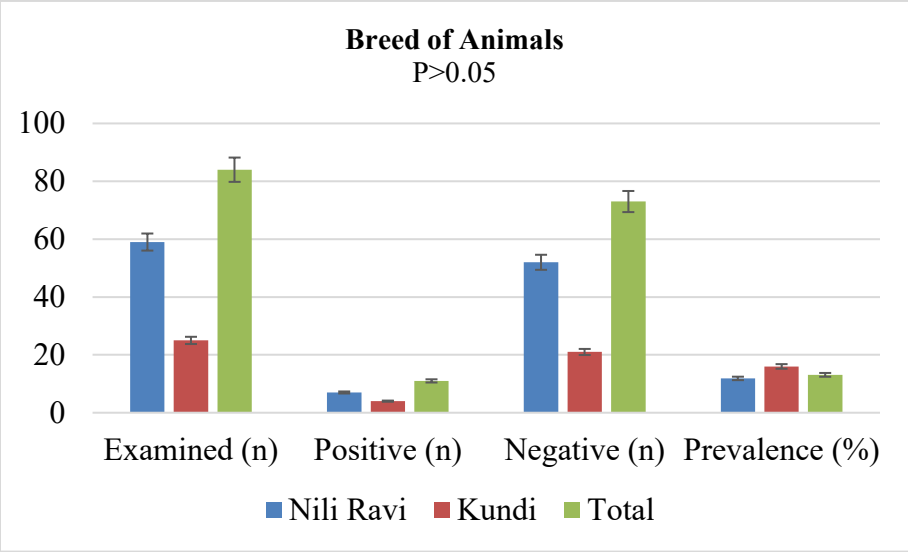

Figure 4.1: Prevalence of Trypanosomiasis according to Breed of Animals

4.2 Prevalence of Trypanosomiasis in Male and Female animals

The prevalence of trypanosomiasis according to sex of animal has been calculated in 84 male and female buffaloes of the study area (Table 4.2) of which 15 were of male and 69 were of female. The results indicated that the highest trypanosomiasis prevalence was observed in male animals (13.34%) as compared to female (13.03%) and the total prevalence of trypanosomiasis recorded was 13.09%. There were 13.3%trypanosomiases positive and 86.7% trypanosomiasis negative animals out of 15 male animals. There were 13%trypanosomiases positive and 87% trypanosomiasis negative female animals out of 69 animals. The disease prevalence was statistically non-significant (P >0.05) between male and female animals two different breeds of buffaloes; Nili Ravi and Kundi (shown in Figure 4.2).

Table 4.2: Prevalence of Trypanosomiasis in Male and Female Animals

Sex	Examined (n)	Positive (n)	Negative (n)	Prevalence %	P-Value
Male	15	2	13	13.34	P>0.05
Female	69	9	60	13.03	
Total	84	11	73	13.09	

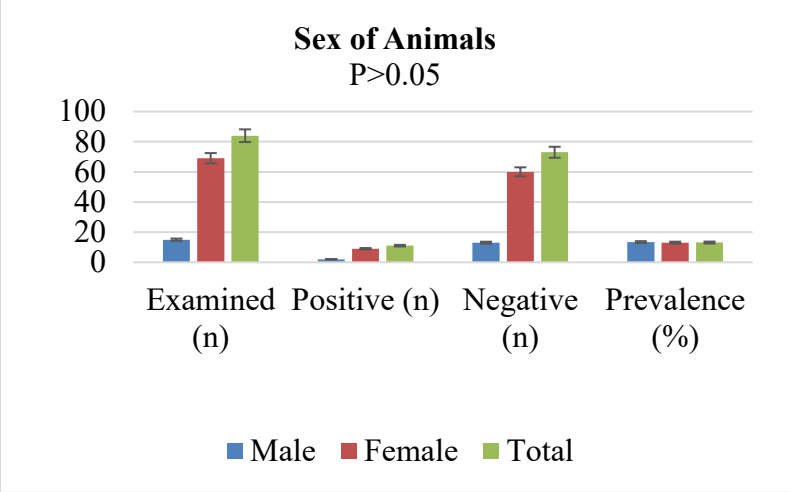

Figure 4.2: Prevalence of Trypanosomiasis in Male and Female Animals

4.3 Prevalence of Trypanosomiasis based on Exposure to Pets

The prevalence of trypanosomiasis based on exposure to pets of animal has been calculated in 84 animals (Table 4.3) of which 72 animals having pets' exposure and 12 animals having no pet's exposure. The consequences of the present study showed that the highest trypanosomiasis prevalence was observed in animals having pets' exposure (13.88%) as compared to having no pet's exposure (8.33%) and the total prevalence of trypanosomiasis recorded was 13.09%. There were 16.1%trypanosomiases positive and 83.9%trypanosomiases negative animals having pets' exposure. There were 8.3% trypanosomiasis positive and 91.7% trypanosomiasis negative in animals having no pet's exposure. The disease prevalence showed statistically significant (P <0.05) differences between the groups having pets' exposure and no pet's exposure (shown in Figure 4.3).

Table 4.3: Prevalence of Trypanosomiasis based on Exposure to Pets

Exposure to Pets	**Examined**	**Positive**	**Negative**	**Prevalence**	**P-Value**
	(n)	**(n)**	**(n)**	**%**	
Pets Exposure	72	10	62	13.88	P<0.05
Without Pets Exposure	12	1	11	8.33	
Total	84	11	73	13.09	

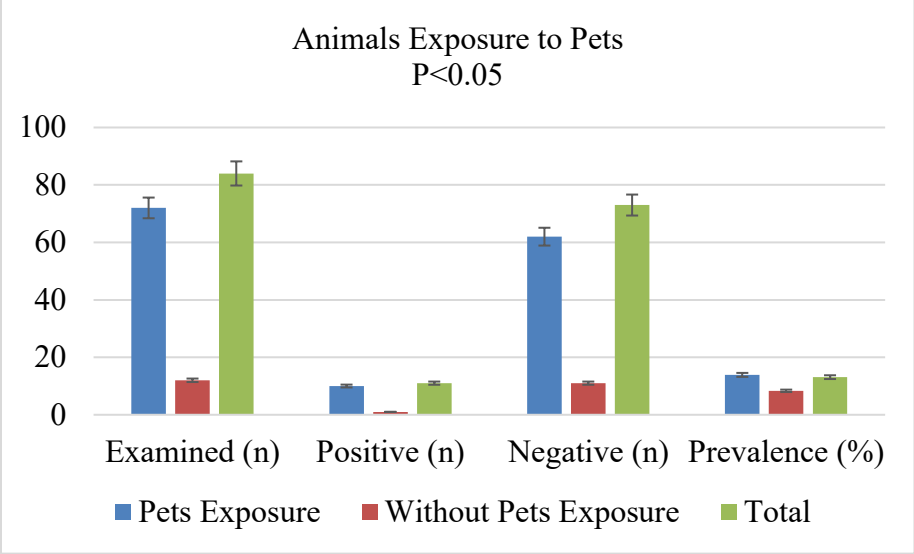

Figure 4.3.Prevalence of Trypanosomiasis based on Exposure to Pets

4.4 Prevalence of Trypanosomiasis Anti-bodies in relation to Farm Type

The prevalence of trypanosomiasis antibodies in relation to farm type has been calculated in 84 animals (Table 4.4) of which 27 animals were from commercial farms, 39 animals from herd and 18 animals at domestic level. The results of the present investigation showed that the highest trypanosomiasis prevalence was observed in animals from herd (17.94%) as compared to commercial farm animals (7.40) and domestic animals(5.55%) and the total prevalence of trypanosomiasis recorded was 7.40%. There were 8%trypanosomiases positive and 92%trypanosomiasis negative animals from commercial farm. There were 18 percent trypanosomiasis positive and 82 percent trypanosomiasis negative animals from herd and 5.5%trypanosomiasispositive and 94.5%trypanosomiases negative animals were from domestic. The disease prevalence showed statistically significant ($P < 0.05$) differences between the groups having pets' exposure and no pet's exposure (shown in Figure 4.4).

Table 4.4: Prevalence of Trypanosomiasis Anti-bodies in relation to Farm Type

Farm Type	Examined (n)	Positive (n)	Negative (n)	Prevalence %	P-Value
Commercial farm	27	2	25	7.40	P<0.05
Herd	39	7	32	17.94	
Domestic	18	1	17	5.55	
Total	84	11	73	13.09	

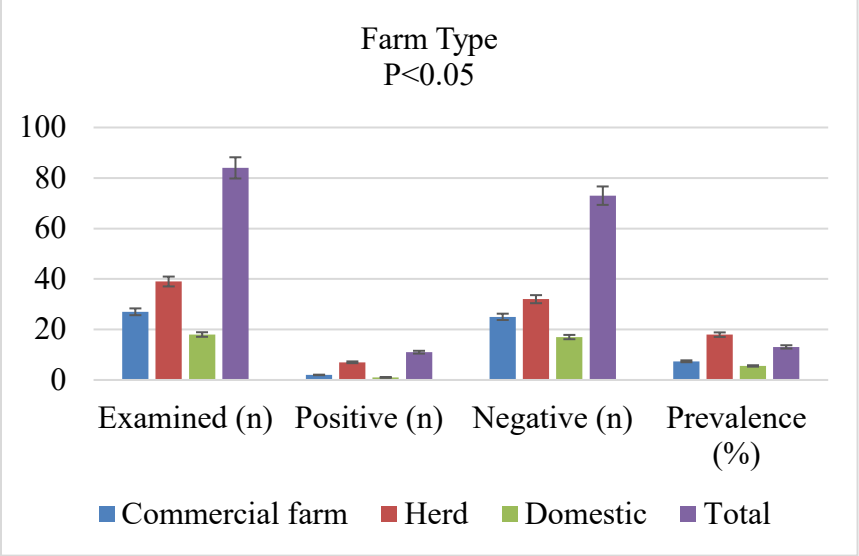

Figure 4.4: Prevalence of trypanosomiasis antibodies in relation to farm type

4.5 Prevalence of Trypanosomiasis by Vaccination Status of Animals against other Diseases

The prevalence of trypanosomiasis by vaccination status of animals against other diseases has been calculated in 84 animals (Table 4.5of which 67 animals were vaccinated; 17 animals non-vaccinated. The results of the current investigation showed that the highest trypanosomiasis prevalence was observed in non-vaccinated animals (23.52%) as compared to vaccinated animals (10.44) and the total prevalence of trypanosomiasis recorded was 13.09%. There were 10.4% trypanosomiasis positive and 88.6%trypanosomiasis negative vaccinated animals. There were 23.5% trypanosomiasis positive and 76.5% trypanosomiasis negative non-vaccinated animals. The disease prevalence showed statistically significant (P <0.05) differences between the vaccinated animals and non-vaccinated (shown in Figure 4.5).

Table 4.5: Prevalence of Trypanosomiasis by Vaccination status of animals against other Diseases

Vaccination Status	Examined (n)	Positive (n)	Negative (n)	Prevalence %	P-Value
Vaccinated	67	7	60	10.44	
Non-vaccinated	17	4	13	23.52	P<0.05
Total	84	11	73	13.09	

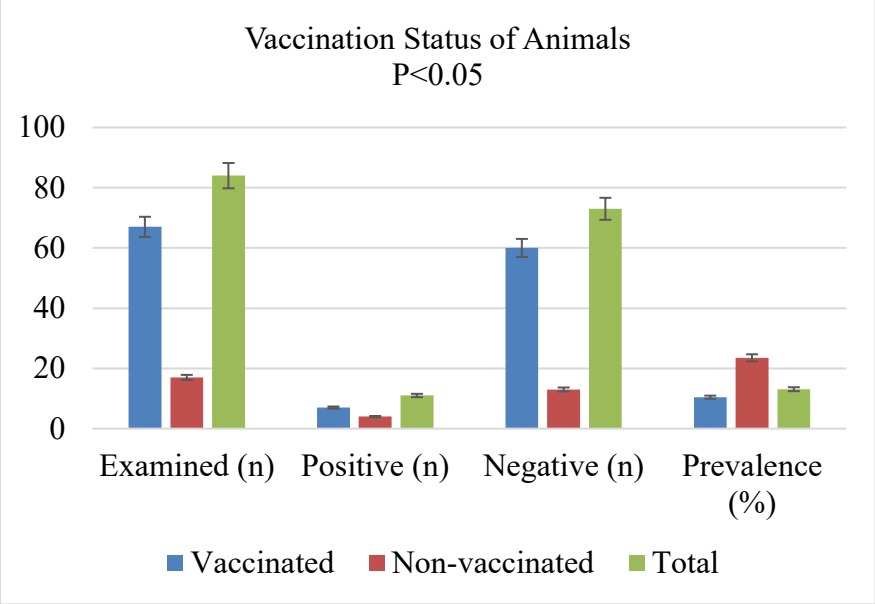

Figure 4.5: Prevalence of Trypanosomiasis by Vaccination status of animals against other Diseases

4.6 Prevalence of Trypanosomiasis in relation to Exposure to other Livestock Population

The prevalence of trypanosomiasis in relation to exposure to other livestock population has been calculated in 84 animals (Table 4.6) of which 28 animals were exposed, 56animalsun-exposed.

The outcomes of the present study showed that the highest trypanosomiasis prevalence was observed in exposed animals (17.85%) as compared to un-exposed animals (10.71%) and the total prevalence of trypanosomiasis recorded was 13.09%. There were 18.5%trypanosomiases positive and 81.5% trypanosomiasis negative exposed animals. There were 10.7% trypanosomiasis positive and 89.3% trypanosomiasis negative un-exposed animals. The disease prevalence showed statistically significant (P <0.05) differences between the exposed animals and un-exposed to other livestock population (shown in Figure 4.6).

Table 4.6: Prevalence of trypanosomiasis in relation to exposure to other Livestock population

Previous exposure history	**Examined (n)**	**Positive (n)**	**Negative (n)**	**Prevalence %**	**P-Value**
Exposed	28	5	23	17.85	
Un-exposed	56	6	50	10.71	P<0.05
Total	84	11	73	13.09	

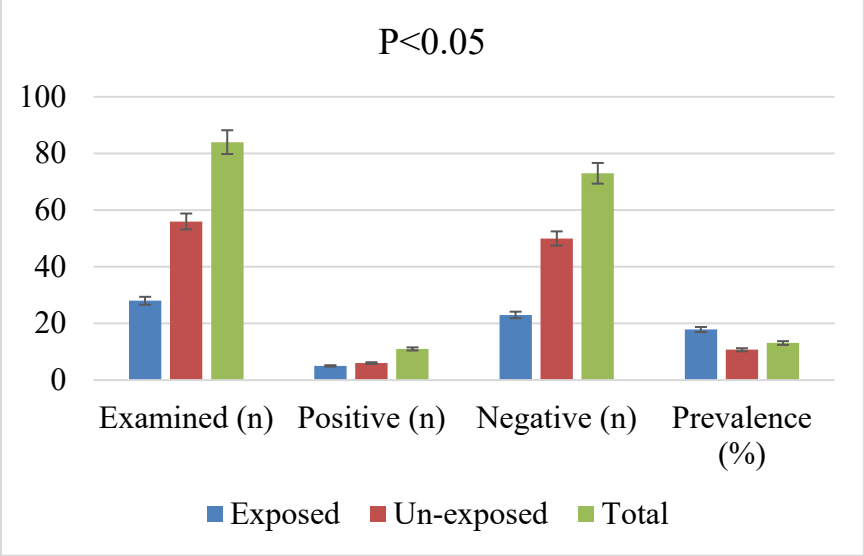

Figure 4.6: Prevalence of trypanosomiasis in relation to exposure to other Livestock population

4.7 Prevalence of trypanosomiasis with reference to previous exposure history

The prevalence of trypanosomiasis with reference to previous exposure history has been calculated in 84 animals (Table 4.7 of which 53 animals were having exposure to other livestock history, 31having no exposure to other livestock history. The results of this study showed that the highest trypanosomiasis prevalence was observed in animals having exposure to other livestock history (15.09%) as compared to animals having no exposure to other livestock history (9.67%) and the total prevalence of trypanosomiasis recorded was 13.09%. There were 15%trypanosomiases positive and 85% trypanosomiasis negative animals having exposure to other livestock history. There were9.6 trypanosomiasis positive and 90.4% trypanosomiasis negative animals having no exposure to other livestock history. The disease prevalence showed statistically significant (P <0.05) differences between the animals having exposure to other livestock history or not. Graphical representations of prevalence of trypanosomiasis with reference to previous exposure history has been shown in Figure 4.7

Table 4.7: Prevalence of trypanosomiasis with reference to previous exposure history

Exposure to other Livestock	Examined (n)	Positive (n)	Negative (n)	Prevalence %	P-Value
Yes	53	8	45	15.09	P<0.05
No	31	3	28	9.67	
Total	84	11	73	13.09	

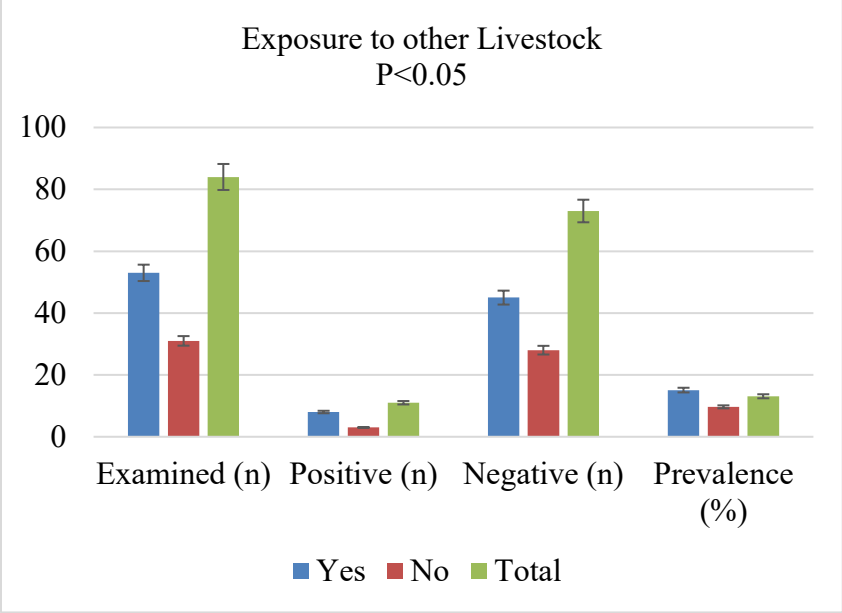

Figure 4.7: Prevalence of trypanosomiasis with reference to previous exposure history

4.8 Prevalence of trypanosomiasis relevancy to various age groups

Trypanosome prevalence, in the older animals would be infected more frequently than younger ones, was significantly correlated with the age of the research animals. The impact of maternal immunity in younger age groups and tsetse flies' predilection for feeding on elderly animals over young ones could be the causes of this. Younger calves are less likely to be exposed to tsetse flies since they are frequently housed on the farm and do not travel far for watering and grazing. Nonetheless, older animals were more susceptible to tsetse infections in the field at grazing and drinking areas. The prevalence of trypanosomiasis relevancy to various age groups has been calculated in 84 animals (Table 4.8) of which 24 animals were aged between 1-2 years, 47 animals were aged between 2-5 years and 13 animals were aged more than 5 years. The results of the current study showed that the highest trypanosomiasis prevalence was observed in more than 5 years aged animals (15.38%) as compared to animals aged between 2-5 years (14.89%) and animals aged between 1-2 years (8.33%), and the total prevalence of trypanosomiasis recorded was 13.09%. There were 8.3%trypanosomiases positive and 91.7% trypanosomiasis negative animals between 1-2 years age. There were 15%trypanosomiases positive and 85% trypanosomiasis negative animals between 2-5 years age. There were 15.3%trypanosomiases positive and 84.7% trypanosomiasis negative animals more than 5 years age. The disease prevalence showed statistically significant (P <0.05) differences among different age groups (10-2 years, 2-5 years and <5 years) (shown in Figure 4.8).

Table 4.8: Prevalence of trypanosomiasis relevancy to various age groups

Age Group	Examined (n)	Positive (n)	Negative (n)	Prevalence %	P-Value
1-2 years	24	2	22	8.33	P<0.05
2-5 years	47	7	40	14.89	

< 5years	13	2	11	15.38	
Total	84	11	73	13.09	

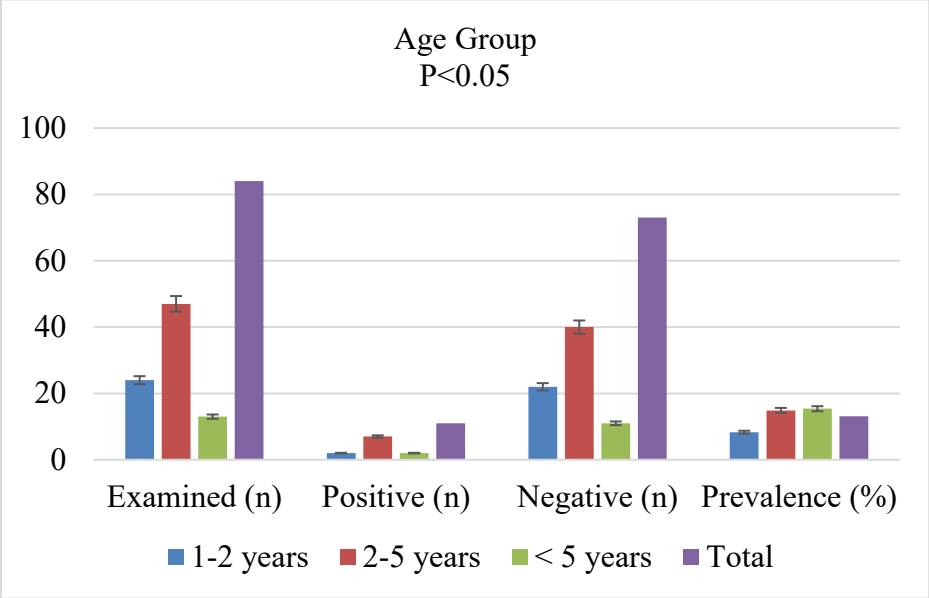

Figure 4.8: Prevalence of trypanosomiasis relevancy to various age groups

4.9 Prevalence of trypanosomiasis by previous history of medication

The prevalence of trypanosomiasis by previous history of medication has been calculated in 84 animals (Table 4.9) which 31 animals were medicated, and63animals were non-medicated. The results of the current study showed that the highest trypanosomiasis prevalence was observed in medicated animals (14.28%) as compared to animals non-medicated (12.69%) and the total prevalence of trypanosomiasis recorded was 13.09%. There were 14.2% trypanosomiasis positive and 85.8% trypanosomiasis negative medicated animals. There were 12.6% trypanosomiasis positive and 87.4 trypanosomiasis negative non-medicated animals. The disease prevalence showed statistically significant (P<0.05) differences between the medicated and non-medicated (shown in Figure 4.9).

Table 4.9: Prevalence of trypanosomiasis in buffaloes by previous history of medication

Medication Status	**Examined (n)**	**Positive (n)**	**Negative (n)**	**Prevalence (%)**	**P-Value**
Medicated	21	3	18	14.28	
Non-medicated	63	8	55	12.69	P<0.05
Total	84	11	73	13.09	

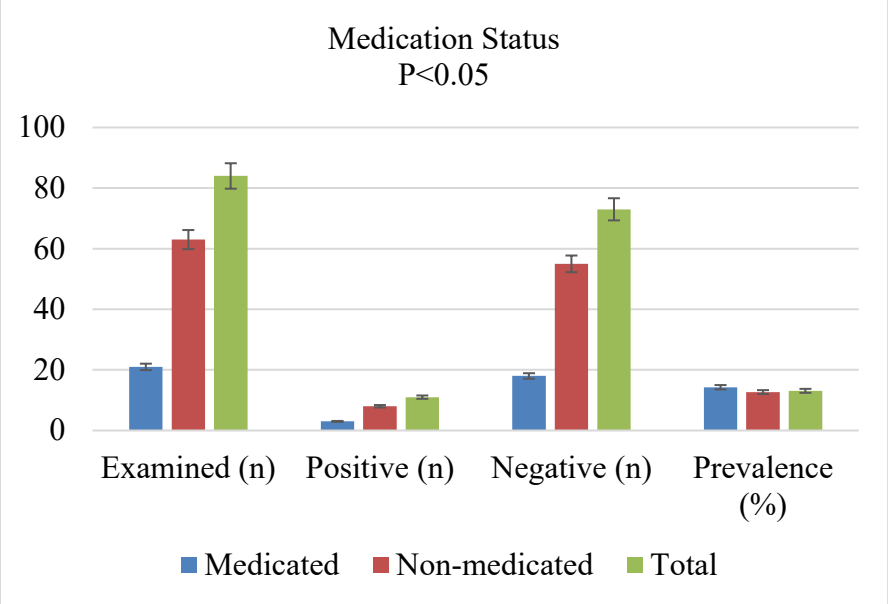

Figure 4.9: Prevalence of Trypanosomiasis by Previous History of Medication

DISCUSSION

This study investigates the prevalence, risk factors and diagnostic methodologies of Trypanosoma annulata and T. evansi in buffaloes from District Vehari, Pakistan, within the broader context of global epidemiology. The findings revealed a 13.9% prevalence rate using microscopy, aligning with similar results from Pakistan (Asif et al., 2022) and highlighting considerable regional variation in prevalence across arid, semi-arid and tropical zones. Comparative analysis indicates substantial geographical and seasonal variability in Trypanosome infections globally, with higher rates reported in regions like Tunisia (61%) and lower in Saudi Arabia (5%) and Turkey (0.59%). Diagnostic tools such as PCR consistently outperformed traditional parasitological methods in sensitivity and specificity, emphasizing the need for cost-effective molecular assays like duplex PCR in low-resource settings. Risk factors including age, sex, body condition, exposure to vectors and management practices significantly influence infection rates. Older and poorly conditioned animals exhibited higher susceptibility, potentially due to weakened immune responses or greater exposure to vectors. Male animals, often used for labor or grazing in vector-prone areas, showed higher prevalence. The presence of tsetse and mechanical vectors like Glossina spp., especially during wet seasons, was strongly linked to disease transmission. Epidemiological models suggest age-dependent infection patterns, with vector attraction influenced by host size and physiology. Additionally, immune evasion mechanisms such as variant surface glycol-proteins (VSG) contribute to chronic infections and complicate control efforts. The global burden of trypanosomiasis, particularly in livestock-dependent economies, underscores the urgency for integrated control strategies. These should include improved surveillance, vector control, drug resistance monitoring and the development of affordable, field-friendly diagnostics. Findings reinforce the "One Health" approach, recognizing the interplay between animal health, vector ecology and socio-environmental factors in managing this economically and medically significant disease.

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