Research Article

Prevalence of Parasitic Infections among people having close contact with animals in Rural Areas of District Kasur, Pakistan

Asma Adul Latif[1]*, Fakhar un Nisa[1], Shafaq Fatima[1] and Saima Sharif[1]

[1]*Department of Zoology, Faculty of Science and Technology, Lahore College for Women University, Lahore Pakistan.*

Abstract | Close contact with companion animals has been identified as a potential public health issue and has been associated with parasitic illnesses among the poor and disadvantaged populations with inadequate hygiene practices leading to an increased risk of zoonotic transmissions particularly in rural areas. Present study was designed to investigate the prevalence of parasitic infections among individuals having close contact with animals in rural areas of District Kasur, Pakistan. Stool and blood samples were collected and analyzed using standard diagnostic techniques. Results indicated that out of the 360 samples, 57 (16%) individuals tested positive for parasitic infections, while 303 (84%) tested negative. Various parasites identified from samples of infected individuals revealed that *Entamoeba histolytica* (19.3%) was the most prevalent, followed by *Ascaris lumbricoides* (17.5%), *Giardia lamblia* (18.4%), *Cryptosporidium parvum* (11.4%), *Strongyloides stercoralis* (4%), *Blastocystis hominis* (11.4%), *Trichuris trichiura* (3.5%), *Ancylostoma duodenale* (11%), and *Echinococcus granulosus* (3.5%). Adults were found to be marginally more parasitized than children, whereas the male subjects were more infected than the females. Significant associations were observed among different occupational groups, with shepherds exhibiting higher infection rates as compared to the butchers and the dairy farmers.

Received | April 14, 2023; **Accepted** | July 28, 2023; **Published** | December, 2023
***Correspondence** | Dr. Asma Adul Latif, Department of Zoology, Faculty of Science and Technology, Lahore College for Women University, Lahore Pakistan
Email: asma5latif@hotmail.com
Citation | Latif, A.A., un Nisa, F., Fatima, S. and Sharif, S. 2023. Prevalence of parasitic infections among people having close contact with animals in rural areas of District Kasur, Pakistan. *Journal of Innovative Biology and Environmental Sciences*, 4(2): 1-13
Keywords | Parasitic infections, Shepherds, Livestock, ELISA, District Kasur

Introduction

Parasitic infections are the most common global illnesses, and are amongst the main causes of morbidity and mortality among individuals residing in remote regions (Speich *et al*., 2016). The prevalence of parasitic infections, particularly those of zoonotic origin, represent a significant public health concern, notably in rural settings within agrarian economies such as Pakistan (Ulhaq *et al*., 2021;

Talal *et al.*, 2023). With a substantial portion of the populace engaged in agricultural activities, often in close proximity to livestock, the risk of transmission from animals to humans is heightened. This is exacerbated by factors including but not limited to inadequate sanitation infrastructure and limited access to healthcare services (Tauxe, 1997; Khan *et al.*, 2019). Zoonotic parasites are transmitted through various routes, including direct contact with infected animals (Singh *et al.*, 2012), ingestion of contaminated food and water (Slifko *et al.*, 2000; Rahmati *et al.*, 2017), and exposure to contaminated soil or fecal matter (Korkes *et al.*, 2019; Da Silva *et al.*, 2021). Farm animals, chicks, rats, domestic animals, fleas, and lice are some of the organisms which have been recognized as possible carriers (Agunos *et al.*, 2016). Understanding the mode of transmission is pivotal for devising targeted intervention strategies to mitigate disease spread (Wang and Crameri, 2014).

Pakistan has an agrarian economy which relies heavily on farming. About 75% of the country's population is directly or indirectly employed in the agricultural sector (Rehman *et al.*, 2013). The majority of Pakistan's populace (>70%) resides in rural areas and sustains their livelihood through animal husbandry (Habib *et al.*, 2016). Each household typically possesses one or two livestock animals alongside a pet dog (Ahmed *et al.*, 2017), with common domesticated species including buffaloes, cattle, goats, sheep, and poultry (Khan, 2004). Parasitic diseases pose significant challenges, particularly for impoverished farmers in tropical and subtropical climates, such as those prevalent in Pakistan (Abbas *et al.*, 2018). The burden of parasitic infections is further exacerbated by the fact that Pakistan is a developing nation with limited financial resources and inadequate sanitation infrastructure (Zaman *et al.*, 2017).

Efforts to curb the transmission of zoonotic parasites necessitates a comprehensive approach encompassing sanitation improvement initiatives (Youn, 2009), health education campaigns, regular deworming of animals, and stringent monitoring of food processing practices (Mehmood *et al.*, 2000; Ismail *et al.*, 2018). Measures such as vigilant oversight of feedlot operations, proper disposal of animal byproducts, and enhanced health literacy in rural communities are crucial components of disease prevention strategies (Mather and Abdullah, 2015; Shurson *et al.*, 2022). In summary, Pakistan's agrarian landscape, coupled with its rural demographics and reliance on animal husbandry, underscores the prominence of parasitic diseases as a critical public health issue. Addressing these challenges requires concerted efforts aimed at improving sanitation practices, enhancing healthcare infrastructure, and implementing targeted interventions to mitigate the transmission of zoonotic parasites.

2. Materials and Methods

2.1. Study site

District Kasur is 150 to 200 meters above the sea level, located southeast to Lahore at latitude 31012" N and longitude 74044" E (Figure 1). The total area of district Kasur is 3995 km^2 and is surrounded by the Rivers Sutlej and Ravi. Random samples were taken from the remote regions of Bamba Kalan, Usman wala, Pial Kalan, Noor pur, Mahalam Kalan, Salamat Pura, Tatara Kamil, Khudian Khas and Marali Hithar.

2.2. Study design

A cross-sectional study was conducted utilizing recruitment of persons who were in close contact with animals in rural areas of Kasur to determine the prevalence of parasitic diseases. Random sampling technique was used to collect fecal and blood samples from 45 people (aged 5 to 70) every month for an entire year. All subjects included in the study were provided written informed consent with sufficient information to enable the participants to make an informed decision.

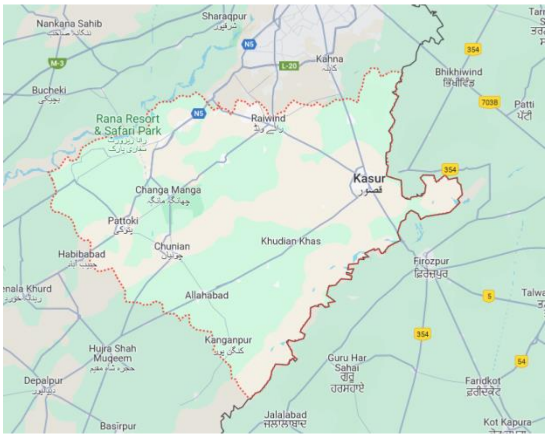

Figure 1: District Kasur

2.3. Studied population

The rural communities selected for inclusion in present study were primarily agricultural economies earning their livelihoods as farmers, animal herders, cattle breeders and dairy farmers. Most residents of these remote areas were not well educated. Animal dung was handled by women and was used as biofuel, whereas the men worked as shepherds, livestock raisers at animal farms, butchers, slaughterhouse workers, dairy farmers, and were often dog owners. The sewerage infrastructure was in disarray and the population's hygienic conditions were poor.

2.4. Questionnaire design

The nature of the questionnaire was descriptive. There were three categories for which data was gathered: 1) Questions for building a personal profile, 2) Questions about current health status, and 3) Questions for assessment of risk factors, such as availability of adequate animal housing, usage of hand gloves, hand cleansing before meals, shoes and protective footwear, water quality, raw vegetable consumption, sanitation, animal faces handling, and overall general awareness.

2.5. Sample collection

A total of 180 fecal and 180 blood samples were collected. Participants were provided disposable gloves along with sterilized 30ml wide-mouth screw-capped storage containers marked with specific labels, as well as instructions to take and deliver the fecal samples properly. The labels contained the participants' name, age, and date of collection. The collected samples were preserved in 10% formalin to prevent any parasite eggs, cysts, and larvae from developing or hatching.

Blood was collected in sterilized vacutainers without anticoagulant, and then transported to the lab in icebox. Blood was allowed to clot by leaving the tubes undisturbed at room temperature for 30 minutes to 1 hour. The tubes were then centrifuged at 4000 rpm for about 10 minutes at room temperature. Serum was separated and kept refrigerated at 2-8°C. All biohazard waste disposal guidelines were followed for fecal and blood samples.

2.6. Parasitological examination

2.6.1. Saline wet mount

Approximately 2 mg of freshly acquired fecal sample was combined with saline solution (0.9%) and mixed well with a sterilized glass rod on a 1mm microscope slide. The saline solution and stool were combined until they formed a uniform solution. Any large chunks of excrement were removed, and the preparation was covered with a cover glass, ensuring that there were no air bubbles or macroscopic material. Three slides on average were made from each sample and then observed under a microscope.

2.6.2. Iodine wet mount

A small amount of fecal suspension was prepared with iodine (Lugol's solution). The specimen was mounted on a microscope slide and sealed with a glass coverslip. The preparation was microscopically examined for distinctive intracellular features.

2.6.3. Zinc-sulphate centrifugation flotation technique

Each fecal specimen suspension was placed in a sterilized tube after being squeezed through a piece of cotton into a cylindrical styrofoam cup (100×16 mm). Subsequently, the mixture was agitated for 5 minutes at 18×100 rotations per minute. The supernatant was then discarded, and the final coarse wet aggregate with zinc sulfate solution was poured onto a clean strip of paper towel. The packed sediment was re-suspended with two applicator sticks until no large particles remained. The mixture was then swirled for 1.5 minutes at 15×100 rotations per minute, transferred to a tray to stabilize it without further stirring, and left to settle for 1.5 minutes to eliminate any destabilizing motion during the spinning process. Two rings of the surface film were deposited onto a dab of 1.18% saltwater and a spray of iodine on a glass slide for wet-mount inspection using a loop. Throughout the experiment, the density of the zinc sulfate solution was regularly determined using a calibrated measuring cylinder for heavier fluids with a coarse aggregate ranging from 1.1 to 1.23.

2.6.4. Formalin-Ethyl Acetate sedimentation concentration technique

A wet cloth was utilized to filter approximately 5ml of each formol (fecal+formalin) specimen into a 15ml conical centrifuge tube. Following the first centrifugation, the quantity was adjusted to achieve a sediment volume of 0.5 to 0.75ml. The suspension was thoroughly mixed with 10ml distilled water, and then centrifuged for 2 minutes at 300×g. The subsequent precipitate was removed and 10ml of distilled water was added, 3ml ethyl acetate was used as a solvent. The tubes were then sealed and vigorously agitated for 30 seconds, after which the mixture was centrifuged for 2 minutes at 300×g (1,500 rpm). This process separated an ethyl acetate top layer, a debris stopper, and a layer of formalin and grit. After removing the detritus block, the topmost three layers were gently drained, and any grit in the effluents was skimmed. The mixture was standardized with gentle stirring, and one drop of saltwater solution and iodine solution were added onto a microscope glass slide. After covering with a glass slide, the slide was observed microscopically for the presence of any parasitic organisms.

2.7. Serological analysis

The serum samples were tested for specific IgG *Toxoplasma* antibodies using the ELISA method (*Toxoplasma* IgG ELISA kit RN-56692, Catalog No: BC-1085, USA). The IgG antibodies were detected in sera to determine the immunological phase of *Toxoplasma gondii* infestation in a qualitative/semi-quantitative manner.

The 96-well (12×8) *Toxoplasma* antigen-coated ELISA plates were utilized as per protocols and criteria provided by the manufacturer entailing preparation of test samples, controls, and calibrators. After dispensing the diluted test solutions (1:40), controls, and calibrators into appropriate wells, the plate was incubated (30 minutes at 37°C), followed by washing steps. The enzyme conjugate was added, followed by incubation and washing. TMB Reagent was then dispensed, incubated, and the reaction was stopped with Stop Solution (1 N HCl). After confirming the absence of air bubbles, the optical density was recorded at 450nm using an ELISA microwell reader.

2.8. Parasitic prevalence

The prevalence of parasitic infection was calculated using a modified formula.

$$\text{Prevalence (\%)} = \frac{\text{Number of infected individuals}}{\text{Number of total individuals}} \times 100$$

2.9. Statistical analysis

Minitab (Version 13.0 for Windows) was used for the statistical analysis. All data was presented as Mean and Standard Deviation (SD). The Chi square test and ANOVA between variables was used to evaluate the percentage of parasite in study subjects. The *p* value <0.05 was considered statistically significant.

3. Results

3.1. Socio-demographic characteristics

Current study was conducted in rural areas of District Kasur, a total of 360 samples were randomly collected, comprising of 180 stool and 180 blood samples, from individuals having close contact with animals. Comprehensive information about the subjects was gathered through questionnaires. Out of the total samples, 57 (16%) tested positive and 303 (84%) tested negative for parasitic infections (Figure 2). Among the stool samples, 43 (24%) tested positive, while 137 (76%) tested negative for parasitic infection. For blood samples, 14 (8%) tested positive and 166 (92%) tested negative for *Toxoplasma gondii* infection. Specifically, among shepherds, 16 (32%) stool samples were positive, with 4 (8%) positive for *Toxoplasma gondii*. Among butchers, 11 (28%) stool samples tested positive, with 2 (5%) positive for *Toxoplasma gondii*. Additionally, 2 (20%) stool samples were positive among dairy farmers, while none tested positive for *Toxoplasma gondii*. Among pet cat owners, 8 (18%) stool samples were positive, with 8 (18%) positive for *Toxoplasma gondii*. Furthermore, 6 (17%) stool samples were positive among pet dog owners.

A total of 180 stool and 180 blood samples were randomly collected from the same individuals in different groups, comprising 162 males and 18 females The highest prevalence of parasitic infections, at 47% and 17% in stool and blood samples, respectively, was observed in the age group of 36-45 years and 26-35 years. Among males, 25% of stool samples tested positive, while 55% of female blood samples tested positive for parasitic infections. Additionally, 42% and 9% of stool and blood samples, respectively, from all participants, were positive for parasitic infection within the income group of 16,000-20,000 RS.

Among individuals categorized as illiterate, a total of 45% and 13% of stool and blood samples, respectively, tested positive for parasitic infections. In each rural area, 20 stool and 20 blood samples were collected. Specifically, 35% of stool samples from Usman Wala tested positive for parasitic infections, while 20% of blood samples from Pial Kalan tested positive for *Toxoplasma gondii*.

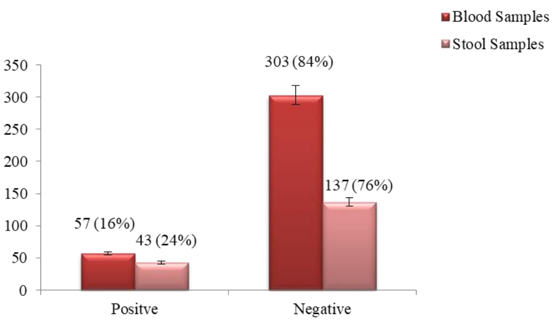

Figure 2: Determination of parasitic infections from blood and stool analysis

3.2. Prevalence of single and multiple parasitic infections

A total of 43 stool samples from participants tested positive (Table 1), with 34 (79%) individuals infected with multiple parasitic infections and 9 (21%) individuals infected with a single parasitic infection. The prevalence of single and multiple parasitic infections were as follows: 19.3% for *Entamoeba* *histolytica*, 17.3% for *Ascaris lumbricoides*, 18.4% for *Giardia lamblia*, 11.4% for *Cryptosporidium parvum*, 4% for *Strongyloides stercoralis*, 11.4% for *Blastocystis hominis*, 3.5% for *Trichuris trichiura*, 11% for *Ancylostoma duodenale*, and 3.5% for *Echinococcus granulosus* among different groups with close contact to animals.

Table 1: Single and multiple parasitic infections among the participants of study groups

Intestinal parasites	Single parasitic infection (%)					Multiple parasitic infections (%)				
	A	B	C	D	E	A	B	C	D	E
Entameoba histolytica	16.67	50	0	0	0	100	89	100	0	0
Giardia lamblia	16.67	0	0	0	0	100	0	100	13	80
Cryptosporidium parvum	16.67	0	0	0	0	100	0	0	25	0
Ascaris lumbricoides	16.67	50	0	0	0	80	33	0	0	80
Strongloies stercoralis	16.67	0	0	0	100	0	0	0	0	100
Blastocystis hominis	16.67	0	0	0	0	90	55	0	0	0
Trichuris trichura	0	0	0	0	0	40	0	0	0	80
Echinoccocus granulosus	0	0	0	0	0	0	0	0	0	80
Ancylostoma duodenale	0	0	0	0	0	50	55	0	25	0

A total of 16 (38%) stool samples tested positive out of 50 randomly collected stool samples from shepherds. Among the 16 positive samples, 10 (62%) individuals were afflicted with multiple parasitic infections, while 6 (38%) individuals had a single parasitic infection. Additionally, 11 (28%) stool samples tested positive out of 40 randomly collected stool samples from butchers. Among these positive samples, 9 (82%) were associated with multiple parasitic infections, while 2 (18%) were associated with single parasitic infections.

Two (20%) positive samples were found in dairy farmers, both of which were associated with multiple parasitic infections. Furthermore, 8 (18%) stool samples tested positive among pet cat owners, with all 8 (100%) associated with multiple parasitic infections and none with single parasitic infections. Lastly, 6 (17%) positive samples were identified in pet dog owners. Among these, 5 (83%) were associated with multiple parasitic infections, while 1 (27%) was associated with a single parasitic infection, specifically *Strongyloides stercoralis*.

3.3. Association of risk factor with parasitic infections

Statistical analysis (Chi square and ANOVA) of risk variables linked with parasite infections revealed that race, schooling, employment, annual income, region, provision of sufficient animal housing, experience/duration of activity, and awareness of zoonotic illnesses, all had non-significant associations with parasite infections (p>0.05). However, univariate analysis considered as risk factors for parasitic infections, including sex and age categories (χ^2=88.56, 50.85 stool and blood samples; p=0.00, 0.042), wearing of protective gloves and footwear (χ2=10, 5 stool and blood samples; p=0.02, 0.02), hand hygiene habit (χ2=10,10 stool and blood samples; p=0.02,0.01), showering and regularly garments changing habit (χ2=15,15 stool and blood samples; p=0.02,0.03), land contact (χ2=12.5, 5.73 stool and blood samples; p=0.00,0.01), household hygiene (χ2=16,13 stool and blood samples; p=0.02,0.04), animal house hygiene (χ2=20,15 stool and blood samples, p=0.02,0.01), access to clean drinking water (χ2=5,5 stool and blood samples, p=0.02,0.01), companion animals in the household (χ2=20,15 stool and blood samples, 0.02,0.01) washing of raw foods, utensil cleaning, properly cooked and covered food (χ2=15,10 stool and blood samples, 0.02,0.01).

4. Discussion

In Pakistan, a considerable portion of the population, mostly poor people, rely on livestock as their primary source of livelihood (Zia, 2009). Punjab is Pakistan's largest metropolitan province, with the majority of its residents reliant on agriculture and livestock. Intestinal parasitic infections have been reported to be widespread in cattle, sheep, and goats, and these animals have long served as major reservoirs for zoonotic parasitic infections and disease transmission among humans (Zafar *et al.*, 2019). Companion animals' role as zoonotic disease carriers has long been recognized as a great significance around the World (Riley *et al*., 2020).

The objective of current study was to evaluate the prevalence and frequency of parasite illnesses among persons in close contact with animals in rural areas of District Kasur. According to the findings, the total prevalence of parasitic illnesses as determined by the stool and blood sample analysis of participants was 16%, whereas 84% of subjects were parasite-free. Individual stool samples revealed that the total prevalence of intestinal parasite illnesses was roughly 24%, with 76% of subjects testing negative. This conclusion was comparable to that of Mahni *et al.* (2016), who found 28% of parasite infestations in stool samples of people living in rural areas with animal contact in Jiroft district, Iran. Barnes *et al.* (2021) did similar work in Mongolia and discovered 20% zoonotic parasite infections among Mongolians.

In current study, the prevalence of single and multiple parasite illnesses was 79% for multiple parasitic infections and 21% for single parasitic infections. Mostly participants were diseased with multi-intestinal protozoans. The prevalence of parasites was found to be highest in *Entameoba histolytica* 19.3% followed by *Ascaris lumbricoides* 17.5%, *Giardia lamblia* 18.4%, *Crytospordium parum* 11.4%, *Strongloides stercoralis* 4%, *Blastocystis hominis* 11.4%, *Trichuris trichiura* 3.5%, *Ancylostoma duodenale* 11% and *Echinococcus granulosus* 3.5%. Khan and Khan (2018) conducted a study in District Sawat and reported that prevalence of *Entamoeba histolytica* was 30.5%, *Giardia lamblia* was around 15.0%, *Ascaris lumbricoides* 17%, *Trichuris trichiura* 11.1%, *Enterobius vermicularis* 9.5%, and *Ancylostoma duodenale* 3.96% in livestock raiser/ shepherds and farmers. These findings are in agreement with results depicted in current work. Additionally, according to Alvi *et al.* (2021), the prevalence of Echinocciasis in Pakistan is 9.61%, however in resent study the prevalence of *Echinococcus granulosus* was found to be 3.5%. This difference may be due to the study area, the study was conducted in slaughterhouses of Faisalabad and Bahawalnagar Districts, whereas current study was conducted in District Kasur.

Current study showed that seroprevalence of IgG antibodies was around 8% in people living in rural areas who had close contact with animals, while 92% of people had no antibodies against *T. gondii*. Ahmad *et al.* (2019) determined that the seroprevalence of *Toxoplasmosis* illness in Pakistan's subtropical areas and regions of the Pothwar was about 20.37%, which was higher than the resent work. The higher seroprevalence may be attributed to a myriad of genetic and environmental factors associated with the studied populations and their geographical locations.

Recent work showed that parasite infections were found in roughly 38% of shepherds or livestock raisers, 11% of butchers and slaughterhouse workers, 20% of dairy farmers, and 18% and 17% in pet owners (cat and dog) respectively. Khan *et al.* (2019) conducted a similar study in District Buner and discovered that farmers (21.9%) and butchers (11.4%) were the most sensitive to Cystic *Echinococcus*. These high rates of parasitic illness may be due to a lack of awareness and unsanitary conditions.

Present study's statistical analysis indicated that there was no substantial gender variation and ratio between males (Mean = 8.00, SD = 4.52) and females (Mean = 3.23, SD = 1.385) with a *p-value* of 0.328. However, men were more infected than women. Males had 25%

higher rate of parasitic illness as compared to women (16%). A study reported in Nepal (Sharchand *et al.* 2016) found that there was no statistically important gender disparity in terms of frequency between males (Average value = 23.64, SD = 9.29) and females (Average = 15.71, SD = 4.51) with a *p-value* of 0.38. However, Abbaszadeh Afshar *et al.* (2020) studied the populations in the remote areas of Iran and noted that most women who engaged in outdoor occupations such as livestock farming, much like males, exposed them to parasitic infections. Similarly women (55%) are reported to have stronger IgG antibodies against *Toxoplasmosis* than men (2%).

In current study, the highest percentage of parasite infection was recorded in patients aged between 36-45 years (47%), followed by the age group 26-35 (31%). Remarkably, 12% of illnesses were discovered in children under the age of 15. According to statistical data, young children are more susceptible to Entameobiasis infection. Hasan *et al.* (2019) discovered that 47% of *Giardia lamblia* infected people were between the ages of 22 and 50 years. The seroprevalence of Toxoplasmosis was higher in the blood samples of participants aged between 26-35 years. Infection was more common in females than males which was in accordance with findings reported by Ahmad *et al.* (2019). Similar findings were recorded by Antolová *et al.* (2018), who found that adult Roma people (45%) had a considerably higher seroprevalence rate than the overall population.

Similarly, Bertoncello *et al.* (2021) did their research in Nepal and discovered that the ratio of gastro-intestinal parasite diseases was much greater in remote areas (52.3%) than in metropolitan cities (32.4%), additionally the school-age girls (55.2%) were more infected than boys (48.6%). Research conducted by Khan *et al.* (2021) in North Pakistan included people living in rural regions who were in direct contact with dogs, which were identified as the main hosts for *E. granulosus*. The risk of infection in humans is increased by improper animal waste disposal. Companion canines were fed contaminated organs from butcher shops. Rural locations and their surrounding vicinities frequently had lower living standards than urban areas. Waste disposal is of particular significance in the majority of Pakistan's rural areas (Mahmood *et al*., 2022).

Higher rates of parasitic infections were diagnosed in people who did not wear protective gloves or shoes and no washing hands. Abbaszadeh Afshar *et al.* (2020) did their research in Iran and found that children's cleanliness and poor personal was linked to their prevalence of intestinal parasites. According to Ahmad *et al.* (2017), the rural lifestyle in Pakistan is highly dangerous for communicable illness. Humans and animals occupy the same living spaces, making humans more vulnerable to zoonotic infections. In rural places, slaughtering animals at home is also a prevalent practice that typically results in dumping of contaminated animal remains into the environment, raising the risk of infection to nearby residents.

Current study found that there was a significant relationship in season wise prevalence of parasite infection. During the spring and summer, the maximum prevalence was seen (32% and 35% respectively). According to Jagai *et al.* (2009), the highest frequency of cryptosporidium was recorded during the warmer months (34.4%). This is due to higher scores of people enjoying swimming in surface water during these months, and the higher temperature aiding the *cryptosporidium spp* cyst survival.

Our findings revealed that 37% (p=0.026) of those who kept pets at home were infected with intestinal parasites. The majority of residents in rural regions of district Kasur were impoverished, and they did not have a separate farm house/Havali for keeping livestock. As a result, most rural people kept two or three livestock at home on a limited scale. Khan *et al.* (2021) conducted a study in District Buner and discovered similar findings.

In our study, we discovered that illiterate and elementary school students had higher parasitic infection rates (45% and 21% respectively), while those with a bachelor's degree had a lower infection rate. Shepherds (animal farm employees) and livestock raisers were the most affected populations. Abbaszadeh Afshar *et al.* (2020) concluded that uneducated persons had a higher ratio of parasitic illness.

5. Conclusion

Findings from current study underscore the importance of improving personal hygiene and raising awareness to reduce parasitic infections. In conclusion, current research sheds light on the prevalence and diversity of zoonotic parasite diseases in rural areas of Pakistan and emphasizes the need for regular surveillance and targeted interventions to mitigate the risk of these potentially fatal infections.

6. Acknowledgments

Authors would like to acknowledge the Department of Zoology, Lahore College for Women University, Lahore Pakistan.

7. Author's Contribution

In current study all authors contributed equally.

8. Conflict of Interest

There is no conflict of interest.

9. Novelty Statement

Current study uniquely explores a wide array of socio-demographic, behavioural, and hygiene-related factors contributing to parasite infections in persons living with companion animals in District Kasur. The study provides comprehensive insights into potential risk factors by offering a robust statistical approach to understanding the complex interplay between these variables.

10. References

Abbas, A., Abbas, R.Z., Masood, S., Iqbal, Z., Khan, M.K., Saleemi, M.K., Raza, M.A., Mahmood, M.S. and Khan, J.A., 2018. Acaricidal and insecticidal effects of essential oils against ectoparasites of veterinary importance. *Boletín Latinoamericano Y Del Caribe De Plantas Medicinales Y Aromáticas*, *17*(5).

Abbaszadeh Afshar, M.J., Barkhori Mehni, M., Rezaeian, M., Mohebali, M., Baigi, V., Amiri, S., Amirshekari, M.B., Hamidinia, R. and Samimi, M., 2020. Prevalence and associated risk factors of human intestinal parasitic infections: a population-based study in the southeast of Kerman province, southeastern Iran. *BMC Infectious Diseases*, *20*, pp.1-8.

Agunos, A., Pierson, F.W., Lungu, B., Dunn, P.A. and Tablante, N., 2016. Review of non-foodborne zoonotic and potentially zoonotic poultry diseases. *Avian diseases*, *60*(3), pp.553-575.

Ahmad, N., Khan, I.A., Iqbal, Z., Naseem, A.A., Kayani, A.R., Afshan, K. and Qayyum, M., 2019.

Seroepidemiology of Toxoplasmosis in Human Population with Reference to Its Zoonotic Potential in Sub-Tropical Areas of Pakistan. *Pakistan Veterinary Journal*, *39*(2).

Ahmed, H., Ali, S., Afzal, M.S., Khan, A.A., Raza, H., Shah, Z.H. and Simsek, S., 2017. Why more research needs to be done on echinococcosis in Pakistan. *Infectious Diseases of Poverty*, *6*(04), pp.113-117.

Alvi, M.A., Li, L., Saqib, M., Ohiolei, J.A., Younas, M.W., Tayyab, M.H., Hassan, A., Qamar, W., Alvi, A.A., Fu, B.Q. and Yan, H.B., 2021. Serologic evidence of *Echinococcus granulosus* in slaughterhouses in Pakistan: global alarm for butchers in developing countries. *The Journal of Infection in Developing Countries*, *15*(06), pp.861-869.

Antolová, D., Janičko, M., Halánová, M., Jarčuška, P., Gecková, A.M., Babinská, I., Kalinová, Z., Pella, D., Mareková, M., Veseliny, E. and HepaMeta Team, 2018. Exposure to *Toxoplasma gondii* in the Roma and non-Roma inhabitants of Slovakia: a cross-sectional seroprevalence study. *International Journal of Environmental Research and Public Health*, *15*(3), p.408.

Barnes, A.N., Davaasuren, A., Baasandavga, U., Lantos, P.M., Gonchigoo, B. and Gray, G.C., 2021. Zoonotic enteric parasites in Mongolian people, animals, and the environment: Using One Health to address shared pathogens. *PLoS neglected tropical diseases*, *15*(7), p.e0009543.

Bertoncello, C., Amoruso, I., Moscardino, U., Fonzo, M., Maharjan, M., Buja, A., Baldo, V., Cocchio, S. and Baldovin, T., 2021. Sex-biased prevalence of intestinal parasitic infections and gender inequality in rural Nepal. *International Journal of Infectious Diseases*, *109*, pp.148-154.

Da Silva, A.M., Bastien, M., Umhang, G., Boué, F., Bastid, V., Boucher, J.M., Caillot, C., de Garam, C.P., Renault, C., Faisse, M. and Courquet, S., 2021. Soil contamination by *Echinococcus* multilocularis in rural and urban vegetable gardens in relation to fox, cat and dog faecal deposits. *Parasite*, *28*.

Habib, G., Khan, M.F.U., Javaid, S. and Saleem, M., 2016. Assessment of feed supply and demand for livestock in Pakistan. *Journal of Agricultural Science and Technology, A*, *6*(2016), pp.191-202.

Hasan, S.M., Shabbir, A., Shaikh, A., Khan, T.N. and Irshad, Z., 2019. Endoscopic biopsy proven co-infection of *Helicobacter pylori* and *Giardia lamblia* in adult population of Karachi city. *Pakistan Journal of Medicine and Dentistry*, *8*(2), pp.4-4.

Ismail, M.A., Eassa, A.H., Mahgoub, A.M. and El-Dib, N., 2018. Review of parasitic zoonotic infections in Egypt. *Kasr Al Ainy Medical Journal*, *24*(3), p.91.

Jagai, J.S., Castronovo, D.A., Monchak, J. and Naumova, E.N., 2009. Seasonality of cryptosporidiosis: A meta-analysis approach.

Environmental Research, *109*(4), pp.465-478.

Khan, A., Shams, S., Khan, S., Khan, M.I., Khan, S. and Ali, A., 2019. Evaluation of prevalence and risk factors associated with Cryptosporidium infection in rural population of district Buner, Pakistan. *PLoS One*, *14*(1), p.e0209188.

Khan, A.G., 2004. The characterization of the agro ecological context in which FAnGR (Farm Animal Genetic Resources) are found. Technical Report, *Development and application of decision support tools to conserve and sustainably use genetic diversity in indigenous livestock and wild relative*. National Agricultural Research Centre Islamabad, Pakistan (2004).

Khan, S.N., Ali, R., Khan, S., Norin, S., Rooman, M., Akbar, N.U., Khan, T.A., Haleem, S., Khan, M.A. and Ali, I., 2021. Cystic echinococcosis: an emerging zoonosis in southern regions of Khyber Pakhtunkhwa, Pakistan. *BMC Veterinary Research*, *17*, pp.1-11.

Khan, W. and Khan, A., 2018. Prevalence of potentially important intestinal pathogenic protozoan parasitic infections in different occupational groups of Swat, Pakistan. *Pakistan Journal of Zoology*, *50*(1).

Korkes, F., Kumagai, F.U., Belfort, R.N., Szejnfeld, D., Abud, T.G., Kleinman, A., Florez, G.M., Szejnfeld, T. and Chieffi, P.P., 2009. Relationship between intestinal parasitic infection in children and soil contamination in an urban slum. *Journal of Tropical Pediatrics*, *55*(1), pp.42-45.

Mahmood, Q., Younus, M., Sadiq, S., Iqbal, S., Idrees, A., Khan, S. and Zia, R., 2022. Prevalence and Associated Risk Factors of Cystic Echinococcosis in Food Animals--A Neglected and Prevailing Zoonosis. *Pakistan Veterinary Journal*, 42(1).

Mahni, M.B., Rezaeian, M., Kia, E.B., Raeisi, A., Khanaliha, K., Tarighi, F. and Kamranrashani, B., 2016. Prevalence of intestinal parasitic infections in Jiroft, Kerman Province, Iran. *Iranian Journal of Parasitology*, *11*(2), p.232.

Mather, T.N. and Abdullah, G.A., 2015. Building molecular biology capacity for preventing tick-transmitted diseases in Pakistan. *Pak-USA Sci Technological Cooperation Program*, *11*, pp.23-15.

Mehmood, N., Arshad, M., Ahmed, H., Simsek, S. and Muqaddas, H., 2020. Comprehensive account on prevalence and characteristics of hydatid cysts in livestock from Pakistan. *The Korean Journal of Parasitology*, *58*(2), p.121.

Rahmati, K., Fallah, M., Maghsood, A.H., Shamsi-Ehsan, T. and Matini, M., 2017. The prevalence of parasitic contamination of vegetables consumed in Malayer City, West of Iran, in 2014. *Avicenna Journal of Clinical Microbiology and Infection*, *4*(2), pp.42380-42380.

Rehman, F., Muhammad, S., Ashraf, I., Mahmood, C.K., Ruby, T. and Bibi, I., 2013. Effect of farmers' socioeconomic characteristics on access to agricultural information: empirical evidence from Pakistan. *The Journal of Animal and Plant Sciences, 23(1)*, pp.324-329.

Riley, T., Lovett, R., Thandrayen, J., Cumming, B. and Thurber, K.A., 2020. Evaluating impacts of a one health approach to companion animal health and management in a remote Aboriginal community in the Northern Territory, Australia. *Animals*, *10*(10), p.1790.

Sherchand, S.P., Joshi, D.R., Adhikari, N., Poudel, R.C., Pant, K.P., KC, M., Shrestha, D. and Sherchan, S., 2016. Prevalence of cryptosporidiosis among school going children in Kathmandu, Nepal. *EC Microbiol*, *4*(1), pp.641-646.

Shurson, G.C., Urriola, P.E. and van de Ligt, J.L., 2022. Can we effectively manage parasites, prions, and pathogens in the global feed industry to achieve One Health?. *Transboundary and Emerging Diseases*, *69*(1), pp.4-30.

Singh, N.K., Singh, H., Jyoti, Haque, M. and Rath, S.S., 2012. Prevalence of parasitic infections in cattle of Ludhiana district, Punjab. *Journal of Parasitic Diseases*, *36*, pp.256-259.

Slifko, T.R., Smith, H.V. and Rose, J.B., 2000. Emerging parasite zoonoses associated with water and food. *International Journal for Parasitology*, *30*(12-13), pp.1379-1393.

Speich, B., Croll, D., Fuerst, T., Utzinger, J. and Keiser, J., 2016. Effect of sanitation and water treatment on intestinal protozoa infection: a systematic review and meta-analysis. *The Lancet Infectious Diseases*, *16*(1), pp.87-99.

Tauxe, R.V., 1997. Emerging foodborne diseases: an evolving public health challenge. *Emerging Infectious Diseases*, *3*(4), p.425.

Ulhaq, Z., Khan, W., Khan, M.F., Kabir, M., Ujjan, A.A., Ullah, W., Masood, Z., Khan, S. and De los Ríos Escalante, P., 2021. Prevalence of intestinal parasitic diseases in school children of rural areas of district Lower Dir, Pakistan. *Brazilian Journal of Biology*, *82*, p.e243150.

Wang, L.F. and Crameri, G., 2014. Emerging zoonotic viral diseases. *Revue Scientifique et Technique*, *33*(2), pp.569-81.

Youn, H., 2009. Review of zoonotic parasites in medical and veterinary fields in the Republic of Korea. *The Korean Journal of Parasitology*, *47*(Suppl), p.S133.

Zafar, A., Khan, M.K., Sindhu, D., Abbas, R.Z., Masood, S., Abbas, Z., Mahmood, M.S., Saleemi, M.K., Khan, J.A., Hussain, R. and Naseer, M.U., 2019. Seroprevalence of *Fasciola hepatica* in Small Ruminants of District Chakwal, Punjab, Pakistan. *Pakistan Veterinary Journal*, *39*(1).

Zafar, M., Malik, M.M., Mushtaq, I., Sajjad, M.T., Ali, S., Masud, S., Latif, M.H., Noor, M.S., Talal, M., Wajid, M. and Younis, W., 2023. Epidemiological factors affecting incidence of parasitic type infections in the school children of rural areas of District Multan (Punjab), Pakistan. *Pure and Applied Biology (PAB)*, *12*(2), pp.1120-1127.

Zaman, M.A., Tauseef-ur-Rehman, T.U.R., Abbas, R.Z., Wasim Babar, W.B., Khan, M.N., Riaz, M.T., Riaz Hussain, R.H., Tasleem Ghauri, T.G. and Muhammad Arif, M.A., 2017. Therapeutic potential of ivermectin, doramectin and trichlorophan against *Psoroptes ovis* in sheep and cattle of cholistan. *Pakistan Veterinary Journal*, pp.233-235.

Zia, U., 2009. Pakistan: A dairy sector at a crossroads. *Small Holder Dairy Development: Lessons Learnt from in Asia*, pp.76-92.