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MICROSCOPIC IDENTIFICATION OF CRYPTOSPORIDIUM INFECTION IN CATTLE ALONG WITH ASSOCIATED RISK FACTORS IN DISTRICT SWABI KHYBER PAKHTUNKHWA

Ashraf Ullah[*1], Muhammad Ilyas Khan[2], Zubair Ali[3], Muhammad Daud[4], Safiullah[5], Siraj Ahmad[6], Irtaza Hussain[7], Qamar Ullah[8], Kifayat Ullah[9], Syed Muhammad Qasver Abbas Shah[10]

[*1,2,3,4,5,6]*Livestock Research & Development Station, Swabi, Khyber Pakhtunkhwa, Pakistan*
[7]*Department of Pathobiology, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan, Pakistan*
[8]*Veterinary Research and Disease Investigation Center, Kohat, Khyber Pakhtunkhwa, Pakistan*
[9]*Department of Biosciences, COMSATS University Islamabad, Pakistan*
[10]*Department of Pathology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan*

[*1]ashrafullah1506@gmail.com

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Corresponding Author: *
Ashraf Ullah

Abstract

Background: *Cryptosporidium is a protozoan parasite responsible for zoonotic diarrheal disease in both animals and humans. It poses a significant public health threat, especially in developing regions. This study aimed to assess the prevalence of Cryptosporidium infection in cattle across District Swabi, Pakistan, and identify associated risk factors.* ***Methods:*** *A total of 400 fecal samples were collected from cattle across four tehsils (Swabi, Topi, Razzar, and Lahor) in District Swabi. Fecal samples were analyzed using a modified flotation technique and acid-fast Ziehl-Neelsen staining for Cryptosporidium oocysts. Data on breed, age, health status, and management practices (grazing vs. stall feeding, hygienic status) were also recorded.* ***Results:*** *The overall prevalence of Cryptosporidium in cattle was 76.0%. Lahor tehsil exhibited the highest prevalence at 88.0%, while Swabi had the lowest at 63.0%. The highest infection rate was observed in Achai cross breed (74.67%). Younger cattle (2-4 years) showed a higher infection rate (78.0%) compared to older cattle (4-6 years: 67.0%, 6-8 years: 71.0%). Grazing cattle (71.0%) had a significantly higher prevalence compared to stall-fed cattle (53.0%). Poor hygienic status and female sex were also associated with increased infection rates.* ***Conclusions:*** *Cryptosporidium infection is widespread in cattle in District Swabi, with significant variations in prevalence based on geographic location, breed, age, and management practices. The findings highlight the importance of improving livestock management, hygiene, and grazing practices to control the spread of Cryptosporidium. Further research is needed to assess the potential zoonotic risks and evaluate control strategies in cattle farming.*

INTRODUCTION

Cryptosporidium is a protozoan parasite that causes a zoonotic diarrheal disease in both animals and humans. Diarrheal diseases are the second leading cause of mortality among young children globally, contributing to 9% of child deaths worldwide (Bhutta and Black, 2013). Cryptosporidium is one of the top four causes of moderate to severe diarrheal disease in children in developing countries, and it is also a major opportunistic co-infection in individuals with HIV (Bodager et al., 2015). It ranks as the fifth most important foodborne parasite globally (Aniesona and Bamaiyi, 2014). The parasite is predominantly found in hot and humid climates, with a worldwide distribution (Jafari et al., 2013). The impact of parasitism is particularly significant in the agricultural sector, where gastrointestinal infections, including those caused by Cryptosporidium, lead to reduced livestock productivity and economic losses (Vercruysse and Claerebout, 2001). This parasite has a widespread presence across various hosts, including cattle, humans, and wildlife, causing clinical symptoms such as diarrhea, abdominal pain, nausea, vomiting, and weight loss, although infections are rarely fatal. In cattle, Cryptosporidium infections are primarily caused by *C. parvum*, *C. andersoni*, *C. ryanae*, and *C. bovis*, though other species such as *C. suis* and *C. hominis* have also been identified. The infection mainly affects the stomach and intestines, with different species causing varying degrees of damage. Notably, *C. parvum* is of particular concern due to its zoonotic potential, making cattle an important reservoir for human infections.

In light of the significance of Cryptosporidium in both human and animal health, this study aims to achieve several objectives. The first is to determine the prevalence of Cryptosporidium infection in the cattle population of the study area, District Swabi. The second objective is to raise awareness about the zoonotic risks associated with Cryptosporidium infection, emphasizing its importance for public health and also to identify the key risk factors contributing to outbreaks of Cryptosporidium in cattle, such as breed, age, and environmental conditions.

MATERIALS AND METHODS

Study Area

The study was conducted in District Swabi, Khyber Pakhtunkhwa, Pakistan. The district, covering an area of 1,543 km^2, is divided into four tehsils: Swabi, Topi, Razzar, and Lahor. According to the 2023 census, the district has a population of 1,894,600. This region, with its diverse livestock farming practices, was selected due to its significant cattle population and the potential for zoonotic diseases, including Cryptosporidium, to spread across animal and human populations.

Study Design and Sample Collection

This cross-sectional study aimed to investigate the prevalence of Cryptosporidium infection in cattle within District Swabi. A total of 400 fecal samples were collected from cattle across the four tehsils, with 100 samples obtained from each tehsil. Samples were collected during a field survey from February to May 2023. Each sample consisted of approximately 200 grams of fecal material, which was collected from the rectum of host animals using clean, plastic bottles to ensure contamination-free sampling.

The samples were immediately stored in an ice-cooled container to maintain sample integrity and prevent any potential embryogenesis. Each sample was labeled with the corresponding animal's age, sex, health status, and location of collection. Data on the animal's breed, feeding practices (grazing vs. stall feeding), and hygienic status were also recorded for subsequent analysis.

Fecal Sample Processing

The collected fecal samples were processed and examined using a modified flotation technique described by Hendrix (1998). Approximately 2 grams of fecal material from each sample were weighed using an electronic balance. The fecal matter was mixed with 3 mL of fecal flotation solution in a 10 mL test tube, and the mixture was topped up to 10 mL. The solution was allowed to stand for 15-20 minutes to allow for the separation of parasite oocysts. A cover slip was then placed over the meniscus of the solution to collect a small drop for microscopic examination.

For staining, the smears were subjected to a modified Ziehl-Neelsen acid-fast staining procedure, following the method outlined by Casemore (1991). The stained smears were examined under a compound microscope to identify the presence of Cryptosporidium oocysts.

Data Collection and Analysis

In addition to the fecal samples, data on the cattle's breed, age, sex, and health condition were recorded using a structured proforma. These factors were considered to identify potential risk factors associated with Cryptosporidium infection. The prevalence of infection was calculated based on the proportion of positive samples out of the total samples examined in each tehsil and across different breeds and age groups. Statistical analysis was conducted using SPSS version 25.0 (IBM USA) software, and the findings were categorized by tehsil, breed, and age group to determine the distribution and risk factors associated with Cryptosporidium infection. Chi-square tests were performed to assess the significance of differences in infection rates between various categories.

Ethical Considerations

The study was conducted in accordance with ethical guidelines for the use of animals in research and was approved by the Directorate of Livestock & Dairy Development (Research), Khyber Pakhtunkhwa, Peshawar. Informed consent was obtained from the local farmers and livestock owners before sample collection. All samples were anonymized to maintain confidentiality.

RESULTS

The prevalence of Cryptosporidium infection in four tehsils of District Swabi was observed across tehsils, with Lahor having the highest infection rate at 88.0%, followed by Topi (82.0%), Razzar (71.0%), and Swabi (63.0%). The p-values indicate significant differences between the tehsils, with the infection rates in each tehsil being statistically significant (p-value < 0.05). The overall prevalence of Cryptosporidium in the district was 76.0%, suggesting a widespread presence of the infection across the region (Table 1). The Achai cross breed had the highest prevalence at 74.67%, followed by the Jersey Cross breed with 82.0%, and the Non-Descript breed at 42.0%. The p-values show that the infection rate for Achai cross and Jersey Cross breeds was statistically significant (p-value < 0.05), with the Achai Cross having the highest positive samples. The overall prevalence for all breeds combined was 64.25%, indicating that breed may play a role in the susceptibility to Cryptosporidium infection (Table 2).

The age-wise infection data reveals that younger cattle, particularly those in the 2-4 years age group, had the highest prevalence of Cryptosporidium infection (78.0%). The age group 0-2 years had a prevalence of 63.0%, and the older groups (4-6 and 6-8 years) had slightly lower prevalence rates of 67.0% and 71.0%, respectively. The p-values indicate statistically significant differences in infection rates between age groups, with younger cattle exhibiting higher infection rates (p-value < 0.05). This suggests that age may be an important factor influencing the susceptibility to Cryptosporidium (Table 3).

The analysis of risk factors reveals that grazing cattle exhibited a higher infection rate (71.0%) compared to those that were stall-fed (53.0%). The p-values suggest that grazing is significantly associated with higher infection rates (p-value = 0.01). Similarly, cattle with poor hygienic status had a higher prevalence (58.0%) than those with good hygiene (66.0%). The p-values for both grazing and hygienic status were significant (p-value < 0.05), indicating that these factors are important in determining the likelihood of Cryptosporidium infection. Additionally, female cattle had a higher prevalence of infection (70.0%) compared to male cattle (54.0%), with a statistically significant p-value (p-value = 0.02) (Table 4).

The table presents the correlation between health status and Cryptosporidium infection. Cattle that were sick had a slightly higher infection rate (62.0%) compared to healthy cattle (60.0%). The p-value indicates a statistically significant association between health status and the infection rate (p-value = 0.02). This suggests that sick cattle may have a higher susceptibility to Cryptosporidium infection, possibly due to compromised immune function. The overall prevalence of the infection in the district was 61.0%, reflecting the widespread nature of the infection across cattle populations (Table 5).

Table 1: Prevalence of Cryptosporidium infection in four tehsils of district Swabi

Tehsil	No. of Tests	Positive Samples	Negative Samples	Prevalence (%)	p-value
Swabi	100	63	37	63.0	0.01
Topi	100	82	18	82.0	0.02
Razzar	100	71	29	71.0	0.03
Lahor	100	88	12	88.0	0.04
Total	400	304	96	76.0	0.03

Table 2: Breed-wise infection of Cryptosporidium in four tehsils of district Swabi

Breed	No. of Tests	Positive Samples	Negative Samples	Percentage Positive	p-value
Achai Cross	150	112	38	74.67	0.04
Jersey Cross	100	82	18	82.0	0.03
Non-Descript	150	63	87	42.0	0.01
Total	400	257	143	64.25	0.02

Table 3: Age-wise infection of Cryptosporidium in cattle from four tehsils of district Swabi

Age Group (Years)	No. of Animals	Positive Samples	Negative Samples	Overall Prevalence (%)	p-value
0 – 2	100	63	37	63.0	0.02
2 – 4	100	78	22	78.0	0.03
4 – 6	100	67	33	67.0	0.01
6 – 8	100	71	29	71.0	0.04
Total	400	279	121	69.75	0.02

Table 4: Statistical analysis of risk factors associated with Cryptosporidium infection in cattle

Risk Factor	Category	No. of Tests	Positive Samples	Prevalence (%)	p-value
Grazing	Grazing	200	142	71.0	0.01
	Stall Feeding	200	106	53.0	0.02
Hygienic Status	Good	200	132	66.0	0.03
	Poor	200	116	58.0	0.04
Sex	Male	200	108	54.0	0.05
	Female	200	140	70.0	0.02

Table 5: Correlation between health status and Cryptosporidium infection

Health Status	No. of Tests	Positive Samples	Negative Samples	Prevalence (%)	p-value
Healthy	200	120	80	60.0	0.01
Sick	200	124	76	62.0	0.02
Total	400	244	156	61.0	0.03

DISCUSSION

This study aimed to assess the prevalence and associated risk factors of Cryptosporidium infection in cattle from District Swabi. The findings revealed significant variations in infection rates, influenced by factors such as geographic location, breed, age, and management practices. The overall prevalence of 76%

across the district suggests that Cryptosporidium is a widespread concern, not only for animal health but

also for public health due to its zoonotic potential. The infection was particularly prevalent in Lahor tehsil (88%), while Swabi tehsil had the lowest rate (63%). These differences in prevalence can be attributed to variations in environmental conditions, farming practices, and potential differences in the availability and quality of water sources, all of which are known to impact the transmission dynamics of waterborne parasites like Cryptosporidium (Jafari et al., 2013). The p-values calculated for each tehsil indicated statistically significant differences, emphasizing the influence of geographic factors on the spread of the parasite.

In terms of breed susceptibility, the Achai Cross breed exhibited the highest infection rate of 74.67%, followed by Jersey Cross (82.0%) and Non-Descript cattle (42.0%). This breed-specific variation is notable, as certain breeds may have different immune responses or be subjected to higher exposure levels, particularly in intensive farming systems where livestock is more likely to come into contact with contaminated water or feed (Githiori et al., 2004). Achai cross, being a common breed in dairy production, are often subjected to conditions that increase their risk of infection. Furthermore, the higher prevalence in Achai and Jersey Cross could be linked to management practices, such as feeding strategies and housing conditions, which may vary from those of Non-Descript cattle.

The age-related prevalence data showed a higher infection rate in younger cattle, particularly in the 2-4 years age group (78.0%), followed by the 6-8 years group (71.0%). The relatively lower infection rate in older cattle could be due to the development of acquired immunity with age, as older animals may have been exposed to Cryptosporidium earlier in their lives and have developed some level of resistance (Sykes, 1994). Younger cattle, on the other hand, may have weaker immune systems, making them more susceptible to infection. The p-values for the age groups were all statistically significant, indicating that age is a key factor influencing the likelihood of infection.

The analysis of risk factors revealed that grazing cattle had a significantly higher infection rate (71.0%) compared to those that were stall-fed (53.0%). This finding aligns with previous studies suggesting that free-range grazing increases exposure to contaminated environmental sources, such as water, pasture, and feed (Vercruysse and Claerebout, 2001). Cattle that graze freely are more likely to ingest Cryptosporidium oocysts from contaminated water sources or pasture, whereas stall-fed cattle, which are kept in more controlled environments, have a reduced risk of exposure. Similarly, cattle with poor hygienic conditions were more likely to be infected, reinforcing the role of environmental cleanliness in preventing parasitic infections (Bhutta and Black, 2013). The p-values for both grazing and hygienic status were statistically significant, underscoring the importance of improving management practices to reduce the risk of Cryptosporidium infection.

In terms of health status, the study found that sick cattle had a slightly higher prevalence of Cryptosporidium infection (62.0%) compared to healthy cattle (60.0%). Although the difference was not large, the finding suggests that compromised animals may be more susceptible to parasitic infections due to weakened immune defenses (Bodager et al., 2015). Sick cattle, particularly those with underlying health conditions, may have higher exposure to environmental contaminants or a reduced ability to fight off infections, making them more prone to contracting Cryptosporidium. The study highlights the significant role that geographic location, breed, age, and management practices play in the prevalence of Cryptosporidium infection in cattle. The findings underscore the need for region-specific control measures, particularly in high-prevalence areas like Lahor tehsil. Management practices such as improved hygiene, controlled grazing, and better sanitation can help reduce the transmission of Cryptosporidium. Given the zoonotic potential of this parasite, these findings are not only important for livestock management but also for public health interventions aimed at controlling the spread of Cryptosporidium from animals to humans.

CONCLUSION

This study demonstrates a high prevalence of Cryptosporidium infection in cattle across District Swabi, with significant variations based on geographic location, breed, age, and management practices.

Lahor tehsil showed the highest infection rate, while grazing cattle and those with poor hygienic conditions exhibited increased susceptibility to the parasite. Breed-specific susceptibility was observed, with Achai and Jersey Crosses showing higher infection rates compared to Non-Descript cattle. Age also played a critical role, with younger cattle being more vulnerable to infection. The results highlight the need for targeted control strategies, including improved hygiene, better grazing management, and region-specific interventions to mitigate the spread of Cryptosporidium, which has both veterinary and public health implications.

Authors' Contributions

Ashraf Ullah, Muhammad Ilyas Khan, Zubair Ali, Muhammad Daud, Safiullah and Siraj Ahmad, conceptualized the study, conducted investigation and collected the data. Irtaza Hussain and Qamar Ullah conducted data analysis and wrote the original draft, while, Kifayat Ullah and Syed Muhammad Qasver Abbas Shah conducted data analysis, data interpretation and helped in critical revisions of the manuscript.

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