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Prevalence and Risk Factors of Cryptosporidiosis in Cattle in Lashkargah City, Helmand Province of Afghanistan

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ABSTRACT

Cryptosporidiosis is a parasitic zoonotic disease that affects humans and animals and is caused by the protozoa *Cryptosporidium* spp. This was a descriptive study to determine the presence of *Cryptosporidium* oocysts and its associated risk factors in cattle in Lashkargah City, Helmand province of Afghanistan. One hundred and fifty-seven cattle were selected randomly from the 37 areas of 8 districts of Lashkargah. To confirm the presence of *Cryptosporidium* spp., a modified Ziehl-Neelsen staining technique was applied to visualize the oocysts of the protozoa in fecal samples. A pre-designed questionnaire was used to collect additional information regarding demographic, hygienic conditions, and water sources of the animal and human population in the area. From the 157 studied cattle, the oocysts of *Cryptosporidium* spp. were detected in 3 samples (1.9%). At the same time, 56.1% of the studied villages were near the running water, and in 4.5% of cases, animal waste materials or residues ended up in the water sources. In brief, considering the husbandry practices, farm hygienic conditions, and water sources in the study area, there is a very likely higher prevalence of *Cryptosporidium* in Helmand province. High-scale province-wide researches are required to determine the incidence and prevalence of cryptosporidiosis in animal and human populations.

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INTRODUCTION

Cryptosporidiosis is a zoonotic parasitic disease caused by *Cryptosporidium* spp. *Cryptosporidium* is a genus of eukaryotic organisms and intracellular parasites that are typically zoonotic (Quadros et al., 2006). Tyzzer discovered *Cryptosporidium* for the first time in the laboratory mouse's stomach glands in 1907. However, it was long believed to be a harmless parasite. It was only identified as an opportunistic pathogenic parasite in 1976 after two human diarrhea cases were linked to cryptosporidiosis. Nevertheless, *Cryptosporidium* spp. was not identified as a noteworthy human pathogen of acute diarrheal illness until 1982, when it was identified as the cause of self-limiting diarrhea in the general population and a potentially fatal condition for individuals with compromised immune systems (De Graaf et al., 1999; Gerace et al., 2019; Tzipori, 1983).

More than 40 *Cryptosporidium* species have been described in animal and human populations. Among them, four species (*Cryptosporidium parvum, C. andersoni, C. bovis, and C. ryanae*) are the most important *Cryptosporidium* species that cause clinical infections in susceptible hosts (O'Leary et al., 2021; Thomson et al., 2017). They can harm a wide range of hosts, including humans, cattle, sheep, goats, pigs, poultry, pets, and dogs (Pumipuntu & Piratae, 2018; Ramirez et al., 2004; Smith et al., 2021; Velásquez et al., 2018).

Warm-blooded and cold-blooded animals' gastrointestinal tracts are home to the protozoan parasites. Therefore, infected humans and animals speared *Cryptosporidium* oocysts in their feces, contaminating the environment, food, and water. Older livestock species serve as carriers and transmit the disease to other susceptible hosts; however, it has been proven that people may acquire the bovine genotype of *C. parvum* from infected calves. When *Cryptosporidium* is detected in surface water sources, livestock facilities are usually held responsible; however, this is not always verified. Cattle reared near water sources should thus be regarded as possible sources of waterborne contamination since surface runoff carries *Cryptosporidium* oocysts from soil to water source. Poor udder hygiene practices can contaminate milk, and recent reports of outbreaks of human cryptosporidiosis are linked to the use of unpasteurized milk (Innes et al., 2020; Ramirez et al., 2004).

According to the published data, the prevalence of cryptosporidiosis in cattle varies worldwide, from 6.25% to 39.65% (Tarekegn et al., 2021). Cryptosporidiosis in cattle can cause reduced growth rates, weight loss, diarrhea, abdominal pain, dehydration, and reduced milk production, which all have the potential to significantly decrease the farmers' income (Fayer et al., 2010; Santín et al., 2008; Silverlås et al., 2009; Smith et al., 2021; Xiao, 2010).

Cryptosporidiosis is a prevalent waterborne illness in humans, where its prevalence is higher in low and middle-income countries "LMIC" (>5%) than in developed nations (<3%) (Starkey et al., 2006). Humans can contract the infection by consuming contaminated dairy products and meat from infected animals or drinking contaminated water (Kankya et al., 2023; Santín et al., 2008; Silverlås et al., 2009; Smith et al., 2021); in humans, the main symptoms of the disease are mild fever, lethargy, dehydration, loss of appetite, excessive watery bowel movements, gastrointestinal distress, queasiness, and weight loss. These conditions can be serious in individuals with compromised immune systems, like those with cancer or human immunodeficiency virus (HIV) (Diptyanusa & Sari, 2021; Kankya et al., 2023).

Cryptosporidium oocysts can be detected using direct microscopic examination, immunolabelling techniques, PCR, and Ziehl-Neelsen–staining technique. The Ziehl-Neelsen–staining technique is widely used because of its low cost and simple methodology (Ramirez et al., 2004). Despite extensive testing of various treatment strategies, no effective vaccines or treatments are available for cryptosporidiosis in animals or humans (Shahiduzzaman & Daugschies, 2012).

Although many diseases affecting humans and animals are endemic in human and animal populations in Afghanistan (Wallace et al., 2002), currently, there is no published data about cryptosporidiosis incidence and prevalence in the country. Therefore, the research aims to determine the presence of *Cryptosporidium* oocysts and its associated risk factors in cattle in Lashkargah City, Helmand province of Afghanistan.

MATERIALS AND METHOD

Study Area

The study was conducted in Lashkargah, the center of Helmand province. Helmand is the largest province of Afghanistan by area, covering 58,584 square kilometers. Helmand has 15 districts and 119,188 villages. The estimated human population of Helmand is 1,525,188 (Ahmad et al., 2023; Elham et al., 2023; Tamomh et al., 2021). Many livestock species, including cattle, sheep, goats, camel, and poultry, are reared by the people, mostly in an extensive system. Based on the 2003 estimate of the Food and Agriculture Organization (FAO), there are 184,866 in Helmand province (FAO, 2008).

Sample Size and Sampling Design

To detect *Cryptosporidium* in the study population, the sample size was calculated based on the Martine et al. (1987) formula: $n = Z^2pq/r^2$, where Z is the desired confidence level (95%), p is the estimated prevalence (50%), q is 1-p and r is the accepted error (10%). Based on the mentioned specification, the calculated sample size was 157. A two-stage sampling design was applied to detect the oocyst of Cryptosporidium in cattle in Lashkargah. In the first stage, the 37 villages of 8 districts were selected using a convenience sampling design. In the second stage, 157 cattle were selected randomly from the total of animals present in the selected villages.

Sample Collection

To detect *Cryptosporidium* oocysts in selected cattle, approximately 20-30 grams of fresh fecal sample was collected directly from the rectum of each cattle using sterile disposable latex gloves. Each sample was labeled with a unique identification number and then transported to the Veterinary Science Faculty of Helmand University parasitology laboratory for laboratory analysis.

Cryptosporidium Oocysts Identification Process

A modified Ziehl-Neelsen staining technique was applied to visualize the oocysts of *Cryptosporidium* in fecal samples, as described by other researchers (Casemore et al., 1985). To identify the oocyst of *Cryptosporidium*, the following steps were applied; after smears preparation, they were air-dried, fixed in methanol for 3 minutes, stained with strong carbol fuchsin for 15-20 minutes, thoroughly rinsed by tap water, decolorized with acid-alcohol for

15-20 seconds, rinsed by tap water and finally counterstained with 0.4% methylene blue for 30-60 seconds. The stained smears were examined under a bright field microscope using 40x and 100x emersion oil objectives.

Data Collection and Analysis

Besides the laboratory results, other related data about husbandry practices, farm and animal hygiene, farm biosecurity, drinking water sources, presence of running and dam water near the animal stable, history of diarrhea in the animals, and other related factors were collected using a pre-designed questionnaire. The data were coded and analyzed using SPSS (version 20, IBM, USA). Although the chi-square test and logistic regression model were considered to be applied to determine the association between the results and the risk factors, due to low positive cases (3 samples were positive), only descriptive statistics were applied for data analysis and presentation.

RESULTS

Based on the obtained Ziehl-Neelsen staining procedure, the Cryptosporidium oocysts were detected in 3 samples (1.9%). The above three positive samples were of the local breed. Two positive samples were from Little Kariz village, first district, while the other sample was from Bolan village, 5th district of Lashkargah. One of the positives was from a healthy animal, while the other two had diarrhea.

Although the body condition of a small proportion of the studied animals was very fat/fat (26.1%) and thin or very thin (14.7%), almost 60% of the animals had mid-physical conditions (Table 1). Nearly three-fourths of the sampled cattle had no clinical signs during the sampling, while fever (8.3%), diarrhea (23.6%), and cough (9.6%) were seen in some of them; and the body surface of 22.9% of sampled cattle was dirty (Table 1).

Table 1*: Physical, health, and body surface hygienic conditions of the sampled cattle, Lashkargah, 2023*

Variables	Categories	Frequency	Percent	Cumulative Percent
Physical condition	very fat	9	5.7	5.7
	fat	32	20.4	26.1
	mid	93	59.2	85.4
	thin	21	13.4	98.7
	very thin	2	1.3	100.0
Health condition	fever	13	8.3	8.3
	diarrhea	37	23.6	31.8
	cough	15	9.6	41.4
	no signs	92	58.6	100.0
Body surface hygiene	dirty	36	22.9	22.9
	clean	121	77.1	100.0

From the total of 157 cattle sampled, the majority (77.7%) were from local breeds, including Kandhari (20.4%) and mixed breeds (57.3%), where 22.3% of them belonged to foreign breeds, including Holstein Friesian (16.6%) and Jersey (5.7%). The number of cattle in more than half of the selected villages was 10-50 cattle, while 50-100 cattle were present in 25.5% of studied villages, and only in 16.6% of the villages, ≥101 cattle were present during the study period.

The collected data revealed that the most common sources of drinking water for the animals in the studied villages were wells (87.9%), followed by qanats (10.8%), tankers (0.6%), and other sources, including floodgates (0.6%) (Table 2). At the same time, wells were also the most common source of drinking water for humans in the area (90.4%), followed by tankers (4.5%), rivers (3.2%), and other sources including filtered water (1.9%). Moreover, the hygiene status of 78.4% of these resources was in good condition, while 21.6% had poor hygienic conditions (Table 2).

Table 2: *Drinking water sources for animals and humans and their hygienic conditions in selected villages of Lashkargah, 2023*

Variables	Categories	Frequency	Percent	Cumulative Percent
Water Sources for Animals	Qanat	17	10.8	10.8
	Well	138	87.9	98.7
	Tanker	1	.6	99.4
	Others	1	.6	100.0
Water sources for humans	Qanat	5	3.2	3.2
	Well	142	90.4	93.6
	Tanker	7	4.5	98.1
	Others	3	1.9	100.0
Water sources hygienic conditions	Good	123	78.4	78.4
	Poor	34	21.6	100

Interestingly, 56.1% of the studied villages, streams, rivers, or other running water sources were near the farmers' houses. In 4.5% of cases, animal waste material or residue was in these water sources. Meanwhile, 24.2% of the farmers mentioned that their children and other family members swim in these water sources, and 8.9% of the studied farmers used these sources as drinking water for their animals. (Table 3).

Table 3: *Presence of water stream, river, and qanat near animal shelters in studied village of Lashkargah, 2023*

Variables	Categories	Frequency	Percent	Cumulative Percent
Is there a stream, river, or other source of running water near your house?	yes	88	56.1	56.1
	no	69	43.9	100.0
Does animal waste material or residue end up in these water sources?	yes	7	4.5	4.5
	no	150	95.5	100.0
Do children or adults swim in these water sources?	yes	38	24.2	24.2
	no	119	75.8	100.0
Do you use these water sources for any purpose?	animals drink	14	8.9	8.9
	wash the clothes	16	10.2	19.1
	irrigation	76	48.4	67.5
	no	51	32.5	100.0

The study results revealed that 45.28% of the farmers store the manure of the cattle outside the animal shelter, 32.0% of them store the manure inside the stable, and 21.7% store it in the yards of their houses. Meanwhile, 3.2% of the people in the studied villages consumed raw milk, while 16.6% consumed raw and boiled milk interchangeably. After coming into contact with animal feces, 55.4% of farmers wash their hands with soap and water thoroughly, 38.2% wash their hands only with water, and 6.4% do not wash their hands. It has been revealed that 64.3% of the studied farmers kept cats at their houses, and 52.9% of the respondents saw rodents, especially mice, in their animal feed stores (Table 4).

Table 4: *The presence of cats or rats in farmers' houses and feed storage of the studied farms in Lashkargah, 2023*

Variables	Categories	Frequency	Percent	Cumulative Percent
Do you have a cat at your home?	yes	101	64.3	64.3
	no	56	35.7	100.0
Have you seen a rat/mouse in your animal feed storage?	yes	83	52.9	52.9
	some time	54	34.4	87.3
	no	20	12.7	100.0

Most studied villagers (73.9%) feed their animals inside the animal shelters. In comparison, 19.1% graze their animals in private lands, and only 7% of the villagers graze their animals in public pastures. Interestingly, some people in the study area (1.9%) rented their animals to a shepherd, and the shepherd earned the milk of the animals for the sage of animals grazing in the pasture.

Due to weather conditions in Lashkargah, 34.4% of the animal owners kept their animals in the semi-open stable, 26.8% in yards of their houses or fins-free areas outside the stable, and 21.7% interchangeably in both places. It has been found that 62.0% of studied farmers kept other species of animals, including small ruminants, with their cattle in the same stable or shelter (Table 5 below).

More than half of the farmers (56.1%) cleaned the shelter of their animals every day, while 24.2% cleaned it between 2-3 days, 10.2% cleaned it every week, and 9.6% practiced this even once per month. Although 45.28% of the farmers store the manure of the cattle outside the animal shelter, 32.0% of them store the manure inside the stable, and 21.7% store it in the yards of their houses (Table 5).

Almost 80% of the studied farmers gave the anti-parasitic drug to their animals annually (32.5%) or sometimes (46.5%), and almost the same pattern was observed regarding animal vaccination. Although 25.5% of the studied farmers feed enough colostrum to the newly borne calves, a large proportion (74.5%) did not feed the required amount of colostrum to the newly borne calves.

DISCUSSION

To our knowledge, this is the first study detecting *Cryptosporidium* oocysts in Helmand province. There are many studies indicating the high incidence and prevalence of cryptosporidiosis among animals and humans in neighboring countries including Iran, Pakistan, Tajikistan, and Turkmenistan (Abdullaev et al., 2018; Dong et al., 2020; Khalil, 2020; Kumar et al., 2020). According to the published data, the prevalence of cryptosporidiosis in Panjab, Pakistan, was 65% in calves and 42% in cattle (Khan et al., 2018). Meanwhile, it has been stated that 23% of children <5 years old in rural areas of Pakistan are infected with Cryptosporidium (Dong et al., 2020). Meanwhile, the reported prevalence of

Cryptosporidium in calves and adult cattle in Iran ranges from 1.3% to 15.6%, while in humans, it is 4.9% among individuals with gastrointestinal symptoms. The most common isolated species in cattle in this county is *Cryptosporidium parvum*, while *Cryptosporidium hominis* is mostly found in humans (Rasti et al., 2017). In the current study, only the Ziehl-Neelsen staining technique was applied to detect the oocysts of *Cryptosporidium* in fecal samples of cattle, and samples were not collected from the people in the area, so the true prevalence of the infection cannot be determined in the study area.

Table 5: *The shelter cleaning interval and manure storage place, animal shelter types, and grazing areas in the studied village of Lashkargah, 2023*

Variables	Categories	Frequency	Percent	Cumulative Percent
At what interval do you clean the animal shelter and remove the manure?	every day	88	56.1	56.1
	between 2-3 days	38	24.2	80.3
	every week	16	10.2	90.4
	every month	15	9.6	100.0
Where do you store the animal manure?	inside the stable	51	32.5	32.5
	in the yard of the house	34	21.7	54.1
	outside	72	45.28	100.0
Where do you keep your animals?	semi-open stable	54	34.4	34.4
	in the yard of the farmer's house	42	26.8	61.1
	both	60	38.2	99.4
	others	1	.6	100.0
Do you raise or keep other animal species with your cattle?	yes	98	62.4	62.4
	some time	2	1.3	63.7
	no	57	36.3	100.0
Where do you feed or graze your animals?	in the shelter	116	73.9	73.9
	in our private land	30	19.1	93.0
	in public pastures	8	5.1	98.1
	other places	3	1.9	100.0

More than 56% of the studied village was located near running water, and in 4.5% of cases, animal waste material or residue ended up in these sources. They also use this water for various tasks, like swimming, drinking for their animals, and land irrigation. Meanwhile, for 10.8% of the families in the study villages, qanats were used as drinking water sources. Cryptosporidium spp. is proven to be maintained in domestic animal species, including cattle, sheep, goats, camel, and other animals (Pumipuntu & Piratae, 2018). Meanwhile, Ehsan *et al*. (2015) studied the spread of *Cryptosporidium* to humans and cattle in Bangladesh in 2015 and found that *Cryptosporidium* is spread by water between animals and humans (Ehsan et al., 2015; Ramirez et al., 2004).

At the same time, our study results revealed that about 20% of the people in the studied villages consumed raw milk. It has been proven that raw dairy is one of the main sources of *Cryptosporidium* for humans (Ursini et al., 2020), and there are many reported outbreaks of human cryptosporidiosis linked to the use of unpasteurized milk (Cowell et al., 2002). Meanwhile, 38.2% of farm owners did not wash their hands with soap after coming into contact with the waste of animals. Animal feces are the most common way of transmitting Cryptosporidium to humans (Fahey, 2003; Juranek, 1995; Pumipuntu & Piratae, 2018).

The study results also revealed that 9.6% of farm owners feed their newborn calves very little colostrum, which weakens their immune systems and causes them to suffer from various diseases. The calves' immune systems and diarrhea are directly impacted by the quality and quantity of colostrum they consume. Many research results indicated that calves who received adequate colostrum from their mother on their first days of life had lower rates of diarrhea and a lower risk of developing cryptosporidiosis. Furthermore, enough colostrum feeding to the diseased calves could be more effective in controlling the disease than antiprotozoal medications and antibiotics. Additionally, the number of days that calve spread the *Cryptosporidium* through feces could be decreased in infected calve that receive enough colostrum (Arrowood et al., 1989; Arsenopoulos et al., 2017; Fayer et al., 1989).

It is worth mentioning that many limitations were present in the current study. *Cryptosporidium* oocysts in sampled cattle were detected using a modified Ziehl-Neelsen staining technique. Although this technique has accepted sensitivity, it has low specificity. At the same time, samples from the water sources and fields were not tested to identify the sources of the cattle's infection. Meanwhile, samples from diarrheal and apparently healthy farmers and their family members were worth collecting to specify the probability of infection transmission from animals to humans and vice versa. In addition, due to few positive cases, the association between the results and probable risk factors was not determined. So, it is recommended that many samples from animals and humans simultaneously be collected and tested from the area using sensitive and specific diagnostic techniques.

CONCLUSION

Based on the obtained laboratory results, *Cryptosporidium* spp. is present in the cattle population in Helmand province. Considering the high prevalence of the protozoa in the neighboring countries of Afghanistan and the data collected about water sources, hygienic practices, and husbandry systems in the study area, the infection could be prevalent in animal and human populations. To determine the true incidence and prevalence of cryptosporidiosis and identify the *Cryptosporidium* species and associate risk factors in cattle and human populations in Helmand and other provinces of Afghanistan, a large-scale – study using large sample size and sensitive and specific laboratory techniques should be conducted.

Conflict of Interest: The author(s) declared no conflict of interest.

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