



# Prevalence and Risk Factors Analysis for *Cryptosporidium* in Apparently Healthy Lambs of Southern Khyber Pakhtunkhwa, Pakistan

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## ABSTRACT

*Cryptosporidium* is considered one of the major gastrointestinal pathogens in young ruminants and has been associated with diarrhea. The current study was conducted in three selected districts (Bannu, Lakki Marwat and Kohat) of southern Khyber Pakhtunkhwa (KPK) to determine the prevalence and risk factors responsible for the occurrence of *Cryptosporidium* in lambs and has been known to cause human infection. Three hundred and sixty fecal samples (n=360) were collected through convenient sampling from apparently healthy lambs and were examined using the modified Ziehl Neelsen acid fast staining. Prevalence was calculated along with different risk factors associated with the disease (month, age, sex and season). Results showed highest prevalence ( $P < 0.05$ ) of the *Cryptosporidium* in Kohat (33.33%) followed by Lakki Marwat (25%), while the lowest percent prevalence was recorded in Bannu (23.33%). Highest month-wise prevalence was recorded in the month of August (46.6%), followed by July (40%), and the lowest during February and December (16.66%). Results showed highest prevalence in summer season (36.66%), while the lowest in the winter season (18.33%). The highest prevalence ( $P < 0.05$ ) was recorded at the age of  $\leq 1-15$  days (38.09%) followed by 16-30 days (29.41%) while the lowest was at the age of  $\geq 31-60$  days or above (15.15%). In lambs, the females had higher ( $P < 0.05$ ) prevalence (31.18%) than males (22.98%). This study addresses the prevalence of infection in lambs, responsible for the spread of etiologic agent in environment.

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### Authors' Contribution

HS, AZD, NA, AH and SA designed the study project. All others collected samples, did lab work and executed the study. MLS statistically analyzed the data and wrote the article.

### Key words

*Cryptosporidium*, *Cryptosporidial* infection, Risk factors, Fecal-oral route.

## INTRODUCTION

**C**ryptosporidial infection is caused by *Cryptosporidium* species, which are enteric protozoan parasites, having global distribution. It belongs to the phylum Apicomplexa and family Cryptosporidiidae (Bouzid *et al.*, 2013). *Cryptosporidium* causes heavy economic losses by infecting humans and animals (Lendner *et al.*, 2011;

Yang *et al.*, 2017). *Cryptosporidium parvum* is the main zoonotic species which principally infects small ruminants and have public health significance (Wang *et al.*, 2014). The *C. parvum* oocysts were so minute that their presence in any acute diarrhea was not detectable until 1970s (Ryan *et al.*, 2014). The transmission of the cryptosporidial infection mainly occurs through fecal-oral route. In lambs, the prevalence is mainly connected with certain risk factors such as early age, sex and seasonal effect (Taylor *et al.*, 2007). Cryptosporidial infection in lambs is one of the main reason of diarrhea in lambs (Ahamed *et al.*, 2015). It affects the epithelial cells of small intestines, however

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it rarely affects stomach, liver, gall bladder, trachea, and lungs (Hunter and Thompson, 2005). Infected animals can shed a huge number (108–109/g) of oocysts (Ortegamora and Wright, 1994), which cause infection in humans and animals by contaminating the surface water of routine usage (Maurya *et al.*, 2013). Cryptosporidial infection causes diarrhea, weight loss and dehydration in humans and clinically characterized by dehydration and is highly fatal for immunocompromised patients (Checkley *et al.*, 2015; Khan *et al.*, 2017). In addition, the cryptosporidial infection has been found to be associated with cancer in humans (Benamrouze *et al.*, 2012). However it is necessary to understand the different environmental risk factors that enhance the susceptibility to the infection (Collinet-Adler *et al.*, 2015). To date, there is dearth of reports regarding prevalence of *Cryptosporidium* in lambs in southern KPK. Therefore, this study was conducted to find out the prevalence of the *Cryptosporidium* in lambs and its association with various risk factors such as age, sex, season and months that effects the occurrence of disease.

## MATERIALS AND METHODS

The present study was conducted in three selected districts of southern Khyber Pakhtunkhwa (KPK), Pakistan *viz.*; Bannu, Lakki Marwat and Kohat.

A total of 360 fecal samples were collected directly from the rectum of the lambs with the help of cotton swab using convenient sampling technique, for a period of one year *i.e.*, January-December, 2016. Observational study was conducted where basic information was entered on a questionnaire to collect useful indistinguishable data such as species, age, sex and season (winter, autumn, spring, summer). All the collected samples were preserved in 10% formalin.

### *Ethical concerns*

Samples were collected after the written formal permission from owners at the time of visit and free adequate veterinary services were provided to all animals.

### *Laboratory analysis of fecal samples*

Fecal samples were analyzed using a Faust modified centrifuge- flotation technique (Leventhal and Cheadle, 1992). Weighed (1-2 grams) fecal material was dissolved in distilled water to make homogenized solution. After homogenization, the solution was centrifuged at 1500 rpm for 60 seconds. As a result of centrifugation, the supernatant was discarded and the sediment was re-

suspended in the flotation solution ( $ZnSO_4$ , 44%). The solution was again centrifuged at 1500 rpm for 60 seconds. Finally, the sediment was examined under microscope. The *Cryptosporidium* oocysts were stained by modified Ziehl-Neelsen (MZN) staining technique (Casemore *et al.*, 1985). All the stained slides were examined with the help of calibrated light microscope for the detection of *Cryptosporidium* oocysts at 100X magnification using oil immersion as reported by Bakiret (2003). *Cryptosporidium* oocysts/eggs were identified on the basis of morphology, size and the key as reported by Wantanbe *et al.* (2005). *Cryptosporidium* oocysts appeared as bright red granules on a blue-green background in MZN stained fecal smears. A fecal sample was considered positive if at least one, clearly identifiable *Cryptosporidium* oocyst was recognized.

### *Statistical analysis*

The data collected were analyzed using Statistical Product and Service Solutions (SPSS) version 20.0 (IBM Corp., Armonk, NY, USA). The prevalence was the proportion of positive animals out of the total animals analyzed and was presented in percentage (%). Statistical differences in the prevalence and other variables were determined using Chi-square test ( $X^2$ ). All values at statistical difference  $P < 0.05$  were considered significant (CI; 95%).

## RESULTS

In the present study, overall prevalence of *Cryptosporidium* infection was 27.22% in lambs in three selected zones of Southern KPK. The highest prevalence of was recorded in Kohat (33.33%), followed by Lakki Marwat (25%) while the lowest prevalence was recorded in Bannu (23.3%). On the basis of statistical analysis of the data, overall there was no significant difference ( $P > 0.05$ ) in three selected zones of study.

Month-wise prevalence showed highest prevalence in the month of August (46.6%), followed by July (40%), April, May and June (30%), September and October (26.66%), March (23.33%), November and January (20%) while the lowest prevalence was recorded in the months of February and December (16.66%) ( $P < 0.05$ ) (Table I).

Overall highest prevalence of *Cryptosporidium* was recorded in the summer season (36.66%), followed by spring and autumn (26.66%) while the lowest prevalence was recorded in winter season (18.33%). On the basis of statistical analysis, significant difference ( $P < 0.05$ ) was recorded in prevalence of *Cryptosporidium* in during different seasons (Table II).

**Table I.- Month wise (Jan- Dec. 2016) prevalence (%) of *Cryptosporidium* in lambs of Southern KPK.**

Factor	District Bannu	District Lakki Marwat	District Kohat	Overall	
				Infected/TE	Prevalence (%)
January	20	10	30	6/30	20 <sup>c</sup>
February	10	30	10	5/30	16.66 <sup>c</sup>
March	20	30	20	7/30	23.33 <sup>c</sup>
April	20	30	40	9/30	30 <sup>abc</sup>
May	30	10	50	9/30	30 <sup>abc</sup>
June	30	30	30	9/30	30 <sup>abc</sup>
July	40	40	50	12/30	40 <sup>ab</sup>
August	40	50	50	14/30	46.66 <sup>a</sup>
September	30	10	40	8/30	26.6 <sup>bc</sup>
October	10	30	40	8/30	26.6 <sup>bc</sup>
November	20	30	10	6/30	20 <sup>c</sup>
December	10	10	30	5/30	16.66 <sup>c</sup>
Total	23.3 (28/120)	25 (30/120)	33.33 (40/120)		27.22 (98/360)
P-value					0.057

<sup>a,b,d,ab,abc,abcd, bcd,cd</sup> mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05); TE, total examined.

**Table II.- Season wise prevalence (%) of *Cryptosporidium* in lambs of Southern KPK.**

Factors	District Bannu	District Lakki Marwat	District Kohat	Overall Prevalence	
				Prevalence (%)	Prevalence (%)
Winter	15	20	20		18.33 <sup>b</sup>
Spring	20	30	30		26.66 <sup>ab</sup>
Summer	35	30	45		36.66 <sup>a</sup>
Autumn	20	20	40		26.66 <sup>ab</sup>
Total	23.33 (28/120)	25 (30/120)	33.33 (40/120)		27.22 (98/360)
P-value					0.102

<sup>a,b,ab</sup> mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05); TE, total examined.

In the current study, the highest percent prevalence was recorded at the age of ≤1-15 days (38.09%), followed by at the age of 16-30 days (29.41%) while the lowest prevalence (15.15%) was recorded at the age of ≥ 31-60 days (P < 0.05) (Table III).

Results showed significantly higher (P < 0.05) prevalence in female lambs (31.18%) as compared to male lambs (22.98%) (Table III).

**Table III.- Age and sex wise prevalence (%) of *Cryptosporidium* in lambs of Southern KPK.**

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall	
	Infected/TE	Prevalence	Infected/TE	Prevalence	Infected/TE	Prevalence	Infected/TE	Prevalence
<b>Age</b>								
≤1-15 days	17/42	40.47	14/40	35	18/44	40.90	49/126	38.88 <sup>a</sup>
16-30 days	7/32	21.87	10/34	29.41	12/36	33.33	29/102	28.43 <sup>a</sup>
≥31-60 days	4/46	8.69	6/46	13.04	10/40	25	20/132	15.15 <sup>b</sup>
Total	28/120	23.3	30/120	25	40/120	33.3	98/360	27.2
P-value								0.011
<b>Sex</b>								
Male	12/56	21.42	12/58	24.13	17/60	28.33	40/174	22.98 <sup>a</sup>
Female	16/64	25	18/62	25.80	23/60	38.33	52/186	31.18 <sup>a</sup>
Total	28/120	23.3	30/120	25	40/120	33.3	98/360	27.2
P-value								0.249

<sup>a,b</sup> mean values carrying same superscript within the row are differ non-significantly (P>0.05) whereas with different superscripts are differ significantly at (P< 0.05); TE, total examined.

## DISCUSSION

The cryptosporidiosis mostly occurs in lambs as compared to the adult sheep and likewise the severity

and intensity of the infection in lambs is more than adult sheep (Majewska *et al.*, 2000). A significant difference in prevalence of the *Cryptosporidium* infection has already been reported in young and older lambs (Noordeen *et al.*,

2000).

In the present study, overall prevalence of *Cryptosporidium* was 27.22% in lambs, in three selected zones of study area. Similar study was also conducted in Spain to find out the prevalence of different enteric pathogens in lambs and goat kids which showed higher prevalence in lambs (45%) as compared to kids (42%) (Munoz *et al.*, 1996). There are certain variations in prevalence in our study and other researchers that might be due to some factors such as study design, breed variation, immune status of the animal, area, hygienic conditions and other environmental conditions such as humidity, temperature and rain fall (Majewska *et al.*, 2000).

The *Cryptosporidium* infection is distributed worldwide and is prevalent in wetter and warmer months of the year with higher ambient temperature, relative humidity and rainfall (Jafari *et al.*, 2013), which is in agreement with the findings of current study. Higher prevalence of *Cryptosporidium* was reported in monsoon season in India due to higher humidity and overcrowding of animals in shelters, which is also in line with the findings of this study (Maurya *et al.*, 2013)

Highest prevalence was observed in summer season and least in winter which is correlated with the temperature and humidity. Similar results were recorded in the autumn season (79.3% and 75%) by Causape *et al.* (2002), which buttress the results of this study. It is the fact that the age related resistance to cryptosporidial infection exists naturally in lambs and kids. During the first two weeks of age, infection is more severe and obvious decrease has been reported in the severity of the clinical signs as the age increases (Giadinis *et al.*, 2007). A study was conducted in Spain by Ortege-Mora *et al.* (1999); and it was estimated that 20,000-444000 oocysts/day can be excreted by an asymptomatic ewe. It was observed that 1 oocyst/lamb can cause the infection which is the minimum infective dose whereas the average numbers of oocysts were 5/lamb to cause infection. Therefore, it was recorded that 4,000-110,000 infective doses per day were shed by ewe without any clinical symptoms (Ortega-Mora *et al.*, 1999). Age is the main risk factor responsible for the spread of cryptosporidiosis in lambs and goat kids (Sari *et al.*, 2009); and high morbidity mainly occurs in neonatal animals such as lambs and kids (Panousis *et al.*, 2008).

Our results are coherent with the findings of Sari *et al.* (2009), where the highest prevalence was recorded at the age of <7 days (44.4%), followed by two weeks of the age (37.5%), 3 weeks (32.3%), while the lowest prevalence was recorded at the age of 4 weeks (22%) in lambs. Ahamed *et al.* (2013) also reported similar findings with highest prevalence in lambs of less than one month of age and decreased infection rate in older animals.

It might be due to certain factors as reported by Fasihi-Harandi and Fotuhi-Ardakani (2008); that at early age, high prevalence of the *Cryptosporidium* infection might be due to poorly developed immune system and their maximum sensitivity to the *Cryptosporidium* infection.

## CONCLUSION

The *Cryptosporidium* is widespread in lambs in spite of the age of animals, breed type, and farm location. The presence of *Cryptosporidium parvum* and *Cryptosporidium hominis* in small ruminants enhance and highlights the importance of lambs because the parasite circulates between humans and animal hosts.

### Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article

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