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Prevalence, Water Borne Transmission and Chemotherapy of Cryptosporidiosis in Small Ruminants

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Abstract.- The present study was conducted to record the prevalence of *Cryptosporidium* in small ruminants with relation to age, diarrhoea in animals and water borne transmission. *C. parvum* is a zoonotic protozoan of all mammals, causes severe diarrhea that leads to great economic losses as small ruminants are a major source of cash for many rural populations, especially in semi-arid and arid regions of developing countries. Transmission occurs by direct contact with infected animals and contaminated food or water. To determine the prevalence of *C. parvum* a total of 300 animals (n=150 sheep and n=150 goats) with or without diarrheoa were monitored. Prevalence was recorded as 18.66% in goats and 21.33% in sheep. It was more prevalent (40%) in lambs. Out of positive animals 75% goats and 71.87% sheep were with diarrhoea. Chemotherapeutic trial of two anti parasitic drugs Paromomycine and Metronidazole were used to check their efficacy against *Cryptosporidium* in positive animals. Both showed significant results against positive control and reduction in egg count at different days. Metronidazole was found to be more efficacious.To check the waterborne transmission of *Cryptosporidium* a total of 200 water samples were collected from different water sources *i.e.,* 50 tap water, 50 mineral bottle water, 50 underground (tube well) and 50 canal water. *C. parvum* was identified by measuring the size using stage micrometry and morphology. Overall 10.5% prevalence was observed in water samples containing 28% *C. parvum* oocysts in canal water, 8% in tap water and 4% in underground water, whereas no oocyst was found in mineral water bottles.

Key Words: *Cryptosporidium parvum,* chemotherapeutic trial, small ruminants, water borne transmission.

INTRODUCTION

Cryptosporidiosis is the disease caused by small enteric protozoan parasites belonging to the genus *Cryptosporidium* which is responsible for a potentially severe infection in an extensive range of hosts including mammals, birds, reptiles and fish (Paraud and Chartier, 2012; Fayer and Ungar, 1986; Angus, 1983). Cryptosporidiosis in goats was first observed in Australia in 1981 in a 2 week old goat kid (Mason *et al*., 1981; de Graff *et al*., 1999). Many species of *Cryptosporidum* are present, out of which 20 are known as valid species (Xiao *et al*., 2012; Xiao, 2010). Main source of transmission of cryptosporidiosis is water and food borne contamination. The parasite has a global distribution with capacity to reproduce and disseminate and is recognized to be an emerging zoonotic disease (Mead, 2002). From the economical point of view cryptosporidiosis is a serious disease causing morbidity and mortality in domestic animals (Ramirez *et al*., 2004). Cryptosporidiosis gained maximum attention as a human public health parasitoid (Chalmers and Davies, 2010).

C. parvum infection is very much prevalent in small ruminants all over the world, especially in pre-weaned kids and lambs (Causape *et al*., 2002). The life cycle of *Cryptosporidum* is direct and oocysts are the infective stage of this parasite which is released in feces. Infections oocysts rapidly accumulate in environment and remain infectious till about a month in cool and humid place. Prepatent period in sheep is 10-21 days.Studies of cryptosporidiosis shows that jejunum and ileum is affected by this parasite and diarrhea is characterized by two mechanisms. One is secretions, which prevents the absorption of sodium and the higher production of prostaglandins in the intestinal mucosa or the increase in the interferon level by increasing the permeability of mucosa (Foster and Smith, 2009). *Cryptosporidium parvum* infection is transmitted by ingesting the thick walled resistant oocysts from different sources which are confined to

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the host epithelial cells microvillus region as it resides in parasitophorous vacuoles of the host (Elliott and Clark, 2000). *C parvum* is commonly present in surface, drinking, canal and swimming water used for recreation and even treated or untreated sewerage water (Fayer and Santin, 2009; Hunter and Thompson, 2005; Slifko *et al.,* 2000).

Cryptosporidium parvum may probably be more frequent in children attending the day care centers and preschool age (Heijbel *et al*., 1987). Cryptosporidiosis is mechanically transmitted from the infected animals or even birds (Graczyk *et al*., 1999). It is a severe disease in immune compromised humans and AIDS patients. It causes severe diarrhoea, enteric infection and dehydration which become difficult to treat. This disease can lasts from 3 days to few weeks. In immune disorder patients other diseases can also occur which may lead to death (Diaz lee *et al*., 2011; Lucio *et al*., 2010).

There is no exact treatment for cryptosporidiosis because of the lack of actual efficacious drugs. Chemotherapeutic trial of more than 140 molecules in different drugs are conducted on different animals but there are no entirely significant and satisfactory results (Stockdale, 2008). Studies have shown the reduction in eggs per gram feces in Cryptosporidia calves by using metronidazole and paromomycine with different dose rates (Masood *et al*., 2013). Paromomycine administration for consecutive two or three days clearly shows the reduction in oocysts output in small ruminants (Blagburn and Soave, 1997). Clinical signs and symptoms are identical to the neonatal diarrhoea and dehydration in small ruminants. Due to unavailability of any specific treatment, sanitary and manage mental recommendations and preventive measures are necessary to control this disease (Pam *et al*., 2013). In Pakistan, most of the domesticated livestock is not producing to their potential that leads to lowered performance and major economic losses (Khan and Maqbool, 2012). Therefore, present study was designed to evaluate the waterborne transmission of *Cryptospridium* and therapeutic efficacy of metronidazole and paromomycin against it in small ruminants to counteract the problem effectively.

MATERIALS AND METHODS

Present study was designed to assess prevalence of *cryptosporidium parvum* in sheep goats and its association with diarrhoea and waterborne transmission. *Cryptosporidium* oocyst load per gram of collected fecal samples was calculated. Oocysts were identified on the basis of microscopic morphological features. Efficacy of two different anti-parasitic drugs was evaluated on the basis of log reduction in *Cryptosporidium* oocysts.

Experimental sampling plan

A total of 300 fecal samples (n=150 sheep and n = 150 goats) with or without diarrhoea were collected directly from the rectum of each animal in a sterilized plastic bag reared at farm at UVAS, Ravi Campus Patoki and different house hold animals in and around Lahore. Preliminary identification was made by microscopic morphology of *Cryptosporidium* oocysts following the protocol described by Fayer *et al.* (2012). *Cryptosporidium* oocyst load per gram of feces was calculated. Known weight by volume suspension of each collected fecal sample was prepared. Following formula was used for calculation of oocysts per gram of feces.

$$N= S/Vol. \times wt, \times pv$$

N, number of oocysts per gram of feces; S, number of oocysts counted on the slide; Vol., volume of the sample examined (20 µl); Wt., weight of the fecal sample (20 g) and pv, pellet volume (1ml). Observational study was carried out by recording related information on a questionnaire developed to gather desired data during visit to selected animal sites. Samples were collected according to age and diarrhoeic or non diarrhoeic condition as mentioned in the developed questionnaire.

Prevalence

The prevalence of the cryptosporidiosis was recorded as per formula described by Thrusfield *et al*. (2007).

$$\text{Prevalence} = \frac{\text{Number of cases of disease}}{\text{Population at risk}} \times 10^n$$

Identification of egg / oocyst

Oocyst/eggs were identified on the basis of size and morphology and key as described by Wantanbe *et al.* (2005).

Chemotherapeutic trial

A total of 40 sheep and goats mostly lamb and kids, infected with cryptosporidiosis were divided into 4 groups *i.e.*, A, B, C and D. Animals in groups A and B were treated with paromomycin (Star Laboratory, Ltd. Pakistan) and metronidazole (Sonon Aventis, Ltd. Pakistan) at their recommended dose rate of 25 and 50mg/kg.b.wt respectively whereas in group C infected animals were not given any drug and served as positive control. Group D animals were negative for cryptosporidiosis and kept as negative control. Faecal samples were examined on zero,3rd,7th and 18th day post treatment.

Efficacy of selected drugs

Efficacy of selected drugs against *Cryptosporidium* oocysts was calculated as per formula described by (Moskey and Hardwood, 1941).

$$\text{Efficacy(\%)} = \frac{\text{No of oocyte before treatment – no of oocyte after treatment}}{\text{No of oocyte before treatment}} \times 100$$

Collection and examination of water samples

To check the waterborne transmission of *C. parvum*, a total of 200 water samples were collected from different water sources *i.e.,* 50 tap water, 50 mineral water bottles, 50 underground (tube well) and 50 from canals water. Each sample was centrifuged at 2500 rpm to collect concentrate pellet of 0.5 ml. Oocysts of *Cryptosporidium* species were identified using smears stained with the modified Ziehl-Neelsen method following concentration by the formol ether oocysts concentration technique as described by Henriksen and Pohlenz (1981).

Statistical design

Data thus obtained was subjected to statistical analysis by using one way and two way analysis of variance and independent sample test through SPSS software version 16.0.

RESULTS

Prevalence of cryptosporidiosis in goats and sheep

Prevalence of *Cryptosporidium* oocysts in goats was 18.66%. Data was analyzed in relation to age of the animals. The highest prevalence in lambs of less than 3 months of age was recorded as 35%. It was most prevalent in neonatal kids, prevalence in age of less than 6 months was 15% followed by 12.5% in less than 1 year of age. Animals of more than 1 year showed 10% prevalence (Table I).

Table I.- **Age wise prevalence (%) of cryptosporidiosis in goats and sheep.**

Age	Examined	Goat Positive		Sheep Positive	
		No.	%	No.	%
0-3 months	40	14	35%	16	40%
4-6 months	40	6	15%	9	22.5%
7-12 months	40	5	12.5%	5	12.5%
1 year	30	3	10%	2	6.67%
Total	150	28	18.66%	32	21.33%

Table I also shows that prevalence of *C. parvum* was higher in sheep than in goats. Over all prevalence of cryptosporidiosis recorded in sheep was 21.33%. Age wise prevalence indicated that it was highest 40% in lambs under 3 months of age, followed by 22.5% in animals from 4-6 months of age groups then 20% in animals less than 1 year of age. So studies have shown that *Cryptosporidium* is much prevalent in goats and lambs of age less than 3 month than the other age groups.

Prevalence of cryptosporidiosis in association with diarrhea

C. parvum is much related to diarrhoeic condition of the host. It was observed that both shedding and intensity of oocyst shedding was significantly higher in diarrheic than in non-diarrhoeic animals.

In goats, 75% positive animals were suffering from diarrhoea and 25% animals positive for cryptosporidiosis were without diarrhoea. In sheep, 71.87% animals positive were in diarrhoeic condition and 28.12% were not diarrhoeic. Over all, prevalence of Cryptosporidiosis in association with diarrhoea in sheep and goat was recorded as73.33% (Table II).

Table II.- Association of diarrhoea with cryptosporidiosis in sheep and goat.

Group	Positive with diarrhoea		Positive without diarrhoea		Total Positive
	No.	%	No.	%	
Goats	21	75%	7	25%	28
Sheep	23	71.87%	9	28.12%	32
Total	44	73.33%	16	26.66%	60

Waterborne transmission in cryptosporidiosis

To check the waterborne transmission, 200 samples from different sources *i.e.* tap water, canal water, underground water and mineral water samples, were examined for *C. parvum* oocysts. It was observed that 8% samples from tap water, 4% from underground water and 28% samples from canal water showed positive results whereas no oocysts was found in mineral water bottles. Overall total 10.5% prevalence of *C. parvum* oocysts was recorded from 200 samples of water. Statistically data showed that canal water positive percentage was significantly higher compared to other water sources (Table III).

Table III.- Water borne transmission of *Cryptosoridium parvum* in relation to different sources.

Source	Examined	Positive	Percentage (%)
Tap Water	50	5	8%
Tube well	50	2	4%
Canal water	50	14	28%
Mineral water bottle	50	0	0%
Total	200	21	10.5%

Chemotherapeutic trials

Results for efficacy of Metronidazole (50mg/kg.b.wt) and Paromomycin (25mg/kg.b.wt) were used for chemotherapeutic trials on positive animals against *Cryptosporidium* oocysts on the basis of reduction in oocyst per gram of feces (OPG) (Table IV). For this 40 animals were arranged in 4 groups A, B, C and D. Each group of study included 10 animals, both sheep and goats randomly. Group A and B included positive animals and treated with Paromomycine and Metronidazole. Group C was control positive *i.e.,* animals positive with *C. parvum* and were untreated and group D was negative control which included animals negative for this parasite and remained untreated with any drug. OPG was observed after 0, 3rd, 7th and 18th day of post treatment. At dose rate of 25mg/kg body weight of paromomycin used against cryptosporidiosis under experimental conditions showed significant decrease in OPG count which was observed 3rd day post treatment and onward ($P<0.05$). A single dose of 50mg/kg body weight of metronidazole caused a significant decrease in OPG count from 3rd day post treatment and onward ($P<0.05$). After OPG count the results were evaluated through statistical design and it was observed that both drugs showed significant efficacy compared to non treated one but were non significant in comparison with each other. Metronidazole showed more reduction in egg count than the paromomycine (Table IV).

DISCUSSION

Cryptosporidium has been reported as a major enteric parasite associated with neonatal diarrhoea and mortality in lamb and goat kids (de Graaf *et al*., 1999; Elwin *et al*., 2001). *Cryptosporidium* infection is highly prevalent throughout the world, especially in pre-weaned lambs and kids. There may be the possibility to support the transmission of zoonosis from host to human.This infection is characterized by severe diarrhea (Paraud and Chartier, 2012). In the present study, prevalence of *Cryptosporidium* was recorded as 18.66% in goats and 21.33% in sheep (n=150 sheep and n=150 goats). These results are supported by Majewska *et al*.(2000) who reported 10.1% *C. parvum* infection in sheep from Poland with high intensity in lambs. Santin *et al*. (2007) observed prevalence of *C. parvum* in ewes and lambs. He reported 20.6% prevalence with IFA technique and 50.8% with PCR. Cryptosporidiosis is highly prevalent in pre-weaned goats and prevalence is recorded 38% in kids in France by Hermida-Castro *et al*. (2005). Very high prevalence 59% was reported by Causape *et al*. (2002) in Spain with 86% diarrhoea in lambs. Similar findings were reported by Nasir *et al*. (2009) and Masood *et al*. (2013) in calves of dairy cows and buffaloes.

Table IV.- *Cryptosporidium parvum* oocysts per gram of feces in sheep and goat treated with two different drugs in relation to time.

Groups	Treatment	Dose (mg/kg.b.wt)	Oocysts per gram of feces			
			0 day	3rd day	7th day	18th day
A	Paromomycin	25	930±109.59	770±113.57	600±97.75	490±94.81
B	Metronidazole	50	690±90.00	610±97.12	490±83.60	350±77.81
C	Infected positive control		840±110.75	990±116.85	1200±121.10	1478±117
D	Negative control	0				

Values in bold letters are showing Standard deviation SD.

In small and large ruminants this disease causes chronic diarrhoea. This observation is supported by the present study which has shown significant results in association with diarrhoea in animals. Out of positive animals 75% goats and 71.87% sheep were in diarrhoeic condition which was more prevalent in neonatal kids and lambs. These results are in accordance with Masood *et al*. (2013) and Nasir *et al.* (2009) who reported the association of diarrhoea with *C. parvum* in calves. Similar results were recorded by Paraud and Chartier (2012) in small ruminants.

Although a number of compounds have been tested against cryptosporidiosis but only limited showed effective results (Regh, 1994; Verdon *et al.,* 1994; Masood *et al*., 2013). In the present study anti cryptosporidial activity of Paromomoycin and Metronidazole was observed after 0, 3rd, 7th and 18th day of post treatment. At dose rate of 25mg/kg body weight of paromomycin used against cryptosporidiosis under experimental conditions, significant decrease in OPG count was observed 3 day post treatment and onward ($P<0.05$). Similar findings were reported by Sharling *et al*. (2010), Tzipori *et al*. (1994), (Verdon *et al*. (1994) and Leitch and He (2011). In the present study single dose of 50mg/kg body weight of metronidazole caused a significant decrease in OPG count from 3 day post treatment and onward ($P<0.05$). Metronidazole was found to be more efficient in reducing OPG in faecal samples of positive animals which are in accordance of the observations of Masood *et al*. (2013) who suggested Paromomycin to be the most valuable drug for the treatment of *Cryptosporidium* infection based on the clinical trials (Griffiths, 1998). Both drugs shown significant results against untreated positive animals.

Earlier studies confirmed the transmission of cryptosporidiosis through water, environment and food items. In this study occurrence of oocysts from different water sources was observed which was highest in canal water 28% and minimum in mineral water bottles. Over all total positive samples showed 10.5% prevalence. These results are supported by Fayer (2004) and Heijbel *et al.* (1987), who confirmed waterborne transmission of cryptosporidiosis in human beings through environment, contaminated water and food (Slifko *et al*., 2000). The observation of present study is also supported by Ongerth and Stibbs (1987) who examined the water samples from 11 different rivers in America and found them contaminated with the oocysts of *C. parvum*.

The occurrence of this infection in ruminants as revealed by this study may be attributed to factors such as poor sanitation and unhygienic management of farms in the study area, thus exposing these animals to the risk of infection (Graczyk *et al*., 1999). Unavailability of portable water for these animals as well as contamination of grazing pasture by feces of carrier animals and humans which is a common practice in developing countries might also be additional factors that contributed to this prevalence.

The contamination of the environment, water and grazing pasture by carrier animals might also be possible reasons for these parasitic infestations. Environmental factors such as temperature, humidity and moisture are essential for the survival of eggs and oocysts of parasites; this might have also influence survival of these parasites and their subsequent infection of these animals. To prevent the transmission of zoonotic gastro-intestinal parasites such as *Cryptosporidium* from animals to humans, standard sanitation and hygienic practices must be embraced.

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