Pakistan J. Zool., pp 1-4, 2021. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20171007121024

Short Communication

Prevalence and Chemotherapy of Cryptosporidiosis in Goats using Different Herbal and Allopathic Drugs in Southern Khyber Pakhtunkhwa, Pakistan

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Article Information
Received 07 October 2018
Revised 13 May 2018
Accepted 03 September 2019
Available online 08 September 2020

Authors' Contribution
MHS, AZD, NA and AH designed the study plan. NUK collected samples and executed the experimental work. MS, MAKand NR recorded and analyzed the data and wrote the article.

Key words
***Allium sativum*, Small ruminants, Cryptosporidiosis, Diarrhea, KPK**

ABSTRACT

Cryptosporidium is a protozoan parasite causing diarrhea in human and animals. This study has been aimed to find out its prevalence and chemotherapy of cryptosporidiosis in goats in three selected districts of southern Khyber Pakhtunkhwa (KPK), Pakistan. A total of 1440 fecal samples were collected from goats, 120 samples per month from each of 3 districts for twelve months. Identification of oocysts was done through conventional acid fast ZN staining. Prevalence in District Bannu was 48/480 (10%) followed by District Lakki Marwat12.08 % and in Kohat 19.16 %, respectively. Overall prevalence in three Districts was 13.75%. The highest month wise percent prevalence was recorded during July/August 25.83%, 26.66% with lowest in December 0.83%. On seasonal basis overall highest prevalence was during the summer season 21.87 %, followed by autumn 13.75 %, spring (11.66%) and the lowest in winter 6.66 %. Similarly, on age basis, the overall highest percent prevalence was 20.46%, 13.73% and 8.27% at the age of 1, 1-2 and 2-3 years, respectively. While on sex basis, non-significant ($P<0.05$) 15.66 % and 13.91% prevalence was found in female and male animals, respectively. Chemotherapeutic trial of three allopathic (Paromomycin, Metronidazole and Azithromycin) drugs were used against five groups (groups A-E). Paromomycin, Metronidazole, *Allium sativum* and Azithromycin showed 91.77%, 78.20 %, 77% and 59.29 % reduction in oocysts per gram, respectively. This study explores the potential use of *Allium sativum* against crytosporidium infection and also warns about the high prevalence and possible zoonotic transmission of cryptosporidiosis in southern parts of KPK, Pakistan.

Cryptosporidiosis is one of the most important enteric protozoan diseases in many agro-ecological zones of the world and a serious risk to the livestock economy globally (Paul *et al*., 2014). It is the main constraint to the livestock production throughout the world (Akinkuotu and Fagbemi, 2014). The *Cryptosporidium* infection is responsible for high morbidity and sometime causes high mortality in domestic animals resulting in severe economic losses (Ayinmode and Fagbemi., 2012). It is the significant disease affecting wide range of hosts such as mammals, reptiles, birds and fish caused by the small enteric protozoan parasite known as *Cryptosporidium parvum* (Paraud and Chartier, 2012).

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0030-9923/2021/0001-0001 $ 9.00/0

Enteric disorders are mainly caused by the *C. parvum* in various species of mammals population. The disease is diagnosed by various clinical signs such as watery diarrhoea, emaciation, dehydration, loss of appetite, loss of weight and weakness which result in high mortality and poor weight gain (Maurya *et al*., 2013).

Cryptosporidiosis was first reported by Barker and Carbonell (1974) in lambs showing the clinical sign of diarrhoea. Due to zoonotic nature of disease, infected animals are one of the high health risks to human beings particularly those suffering from AIDS/HIV. Transmission mainly occurs through fecal-oral route and low quantity of oocysts ≤ 50, are responsible for causing disease in healthy animals (Fayer *et al*., 2000).

When animals are housed together or overcrowded or when the udder is contaminated with the feces of infected animal, rapid spread of oocyst were recorded

from infected animal to healthy animals (Nasir *et al*., 2009). *Cryptosporidium* oocysts are highly resistive to unfavorable hash environmental conditions such as cool or damp environment and can survive in infective form for months. The disease has been reported in both young and old animals but young animals are highly susceptible in the first two weeks of the age (Olson *et al*., 1997). In Pakistan, although information is available on the prevalence of cryptosporidiosis in bovines but its prevalence in small ruminants has received little attention. However, a recent study conducted in Pakistan recorded 18.66% prevalence of cryptosporidiosis in goats and 21.33% in sheep (Shafiq *et al*., 2015).

Most of the rural farmers of the area treat their animals with some traditional remedies against worm infestations. This study has been designed to determine the prevalence and chemotherapy of*Cryptosporidium* infection using garlic. It has number of active compounds with 17 amino acids, 8 minerals, different enzymes (allinase) and different vitamins. Dietry *Allium sativum* is attributed to allicin (organosulphate) compound with great antimicrobial potential (Ayaz *et al*., 2008).

Materials and methods

Total of 1440 fecal samples (n=40/month/district)) were collected from goats comprising 40 samples per month from each of 3 districts (Bannu, Lakki Marwat and Kohat) of Southern Khyber Pakhtunkhwa for the period of one year. .

Each fecal sample (about 5 g) was directly collected from the rectum of goats using sterile plastic bags in order to ensure the maximum possible sterility. The procedure was approved by institutional ethical committee and care was taken to minimize the pain and number of animals throughout the experiment. All the samples were transported to laboratory and preserved in 10 % formalin at 4 ^{0}C till microscopic examination of *Cryptosporidium* oocysts.

For microscopic examination, about 3 g fecal materials were dissolved in distilled water to make homogenized solution, centrifuged for 1 min at 1500 rpm. Supernatant was removed and sediment was resuspended in the flotation solution of $ZnSO_4$ (44%). The solution was recentrifuged at 1500 rpm for 1 mint. Finally, sediment was examined under microscope and *Cryptosporidium* oocysts were stained by modified Ziehl- Neelsen (MZN) technique (Casemore and Sands, 1985).

For chemotherapeutic trials a total of 50 goats of same age and weight of either sex naturally infected with *Cryptosporidium* infection were randomly selected and divided into five groups (A, B, C, D and E) each of 10 heads. The animals in group A, B C and D were treated with Azithromycin, Metronidazole, *Allium sativum* (garlic) and Paromomycin respectively while Group E was kept as control positive group and no treatment was provided during trial period. All these animals were kept under same management and feeding pattern.

For drug and garlic administration about 1-2 bulbs (50 mg/kg.wt) of *Allium sativum* were fed orally, while other drugs azithromycin, metronidazole and paromomycin were administered orally as 500 mg, 50 mg, 100 mg per kg body weight per day, respectively, for five consective days.

Results and discussion

Overall, 198/1440 (13.75%) prevalence were recorded in study area. Highest prevalence (19.16%) was recorded in district Kohat followed by District Lakki Marwat (12.08%) and lowest in district Bannu (10%) (Table I). The highest month wise prevalence of cryptosporidiosis was recorded during July-August (26%, 27%), followed by June (20%), September (16%), April/May (14%), October (12%), January (11%), March (9%), November (8%), February (7%) and lowest in December (1%).

Our results are in close agreement to a researcher with 17.7% percent prevalence in Nigeria (Danladi *et al*., 2015). While disagree with some findings of 3.3% in Sokoto and 24.0% in Plateau states (Pam *et al*., 2013; Faleke *et al*., 2014). This high prevalence in our study may be due to irregular or limited parasitic infection control program. While, lower prevalence of *Cryptosporidium* infection in goats may be attributed to the environmental and ecological factors where relative low humidity and short period of rainfall may be recorded (Yu and Seo, 2004).

Table I. Sex wise prevalence (%) of cryptosporidiosis in adult goats in three districts of South KPK.

Factors	District Bannu		District Lakki Marwat		District Kohat		Overall	
	Infected/ Total	Prevalence (%)	Infected/ Total	Prevalence (%)	Infected/ Total	Prevalence (%)	Infected/ Total	Prevalence (%)
Male	23/234	9.82	31/247	12.55	38/180	21.11	92/661	13.67[a]
Female	25/246	10.16	27/233	11.58	70/300	23.33	122/779	17.55 [b]

Means with different superscripts differ at ($P < 0.05$)

Table II. Cryptosporidium oocysts/g of feces and % efficacy of cryptosporidiosis in goats treated with different selected allopathic drugs and garlic administered over the period of 28 days.

Groups	Treatment	Dose	Oocyst per gram of feces with efficacy (%)				
			0 day	07 day	14 day	21 day	28 day
A	Azithromycin	10 mg/ kg b.wt	791 (0%)	681(14)^{a}	561(29) ^{b}	436(45) ^{c}	322(59) ^{d}
B	Metronidazole	50 mg/kg b.wt.	835 (0%)	620 (26)^{a}	445(47) ^{b}	266(68) ^{c}	182(78) ^{d}
C	*Allium sativum* (garlic)	50 mg/kg.b.wt.	861(0%)	628(27)^{a}	433(50) ^{b}	239(72) ^{c}	198(77) ^{d}
D	Paromomycin	100 mg/kg.b.wt.	875(0%)	623 (52)^{a}	288(67) ^{b}	109(88) ^{c}	72(92) ^{d}
E. Group (Positive control group)			821(0%)	1091 ^{e}	1192 ^{n}	1360 ^{m}	1463 ^{v}

Means with different superscripts differ at ($P < 0.05$).

Results revealed highest percent prevalence during summer season (22%), followed by autumn (14%), spring (12%) and lowest was (7%) in winter. Overall, age wise percent prevalence of cryptosporidiosis in 1 year old goats was higher than 1-2 (14%) years and 2-3 (8.27%) years or above. Similarly, on sex basis, little higher prevalence (15.66 %) was observed in female than male animals (13.91%) (Table I).

Table II shows efficacies of different chosen drugs on the basis of reduction in the OPG (oocyst per gram) of feces after treatment. The efficacy of Azithromycin was 13.90 %, 29.07 %, 44.87 % and 59.29 % on 7th, 14th, 21th and 28th day post treatment. Statistically, non-significant difference in OPG reduction was recorded in relation to time ($P>0.05$). Similarly, a single dose of Metronidazole caused a significant decrease in oocyst per gram of feces on 7th day post treatment ($P<0.05$). On day 7 post treatment 25.74% decrease was observed. Percent efficacy of Metronidazole was 46.70%, 68.14%, and 78.20 % recorded on 14 th, 21 th and 28 th day respectively. Similarly, a single dose of *Allium sativum* caused a significant decrease in OPG on 7th day post treatment and onward. On day 7th post treatment (27.06%) reduction was recorded in OPG. Similarly, percent efficacy of *Allium sativum* was 49.70 %, 62.24 % and 77.00 % on 14th, 21th and 28th day respectively. Similarly, significant ($P<0.05$) decrease was observed in OPG count on 7th day post treatment. Percent (%) efficacy of paromomycin calculated on 7th, 14th, 21th and 28 th day was 51.77%, 67.08%, 87.54 % and 91.77 %, post treatment, respectively.

Similar findings were reported Masood *et al*. (2013) in calves during summer season followed by autumn, spring and the lowest in winter season. The Cryptosporidiosis is worldwide in distribution and commonly found in all wet and warm seasons (Jafari *et al*., 2012). Our results are consistent with other researchers that strong correlation between the warm and wet seasons of the year and the prevalence of infection rate may be present.

Akinkuotu *et al*. (2015) correlated age of the animals with Cryptosporidiosis infection where highest prevalence was recorded in pre-weaned kids (62.7%) followed by Post weaned kids (30.4%) and lowest in adults (22.5%). Highest percent prevalence was found in young animals of 1 year or below. Our findings contrast to these findings that may be attributed to the differences in hygienic conditions, environmental factors such as heavy rain fall, high relative humidity, high temperature, study design, immune status of animal, feeding and watering management conditions. Although overall infection was observed in both sexes and little higher prevalence (15.66 %) were observed in female than male (13.91%) in three selected districts of Southern KPK. These findings are in line with Pam *et al*. (2013) who recorded 25.3 % prevalence of *Cryptosporidium* in female and 22.7% in male and not in line with (Akinkuotu *et al*., 2015) where 47.5% and 29 % prevalence was recorded in female and male animals. The constancy of higher percent prevalence rates in females than male in goats may be due to hormonal disturbance during pregnancy and lactation stress.

A single dose of Azithromycin (10 mg/ kg b.wt) was used and OPG count was recorded as 13.90 %, 29.07 %, 44.87 % and 59.29 % on 7th, 14th, 21th and 28th day post treatment as supported in previous similar nature of work (Elitok *et al*., 2015).

In current study, a single dose of Metronidazole used against group B naturally infected with Cryptosporidiosis caused a significant decrease in oocyst per gram (OPG) of feces from 7th day post treatment and onward ($P<0.05$). At day 7 post treatment, 25.74% decreases was observed in oocyst count. Our findings are very close to Masood *et al*. (2013) that Metronidazole is highly effective in reducing oocysts burden.

Allium sativum caused significant decrease in OPG of feces on 7th day post treatment and onward. At day 7th post treatment (27.06%) reduction was recorded in OPG. Similarly, percent efficacy of *Allium sativum* was 49.70%,

62.24% and 77 % on days 14, 21 and 28, respectively. Our results corroborate with Masamha *et al*. (2010) that garlic disrupts the normal physiology of the parasite like movement, absorption of food and reproductive activities, resulting in the reduction of burden. Similarly, the phagocytic activities and natural killer cell activity may be increased during the garlic treatment period as reported by (Sutton and Haik, 1999).

Conclusion and recommendation

The current study confirms the *Cryptosporidium* infection in goats in southern parts of KPK, Pakistan and validates the traditional use of *Allium sativum* as therapeutic agent. Furthermore, it might be a good choice against different protozoal/bacterial diseases as local remedy. Further experimentation and investigation may be carried out in combination with different drugs/ plants in order to explore its antimicrobial potentional. Awareness campaign among public about the zoonotic nature of the disease, good management practices including proper hygiene maintenance are more appropriate tools for the control of *Cryptosporidium* infection.

Acknowledgement

The authors declared that this study has received no financial support.

Statement of conflict of interest

The authors declare there is no conflict of interest.

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