

## Anti-Cryptosporidium Activity of Albendazole, Metronidazole and Paromomycin in Experimentally Infected Cattle

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**Abstract.-** Efficacy of albendazole, metronidazole and paromomycin was evaluated against *Cryptosporidium* in experimentally infected cattle. *Cryptosporidium* oocysts harvested from cattle reared at different livestock farms identified by microscopic morphology and PCR were used for experimental infection. Oocyst per gram (OPG) count was increased in control (untreated) cattle. A single dose of 10mg/kg body weight of albendazole caused significant reduction in OPG count and efficacy determined was 43.05, 58.7 and 64.6 percents on 13<sup>th</sup>, 20<sup>th</sup> and 27<sup>th</sup> day post treatment. At 7.5mg/kg body weight dose efficacy recorded was 34.8, 57.1 and 62.9 percents, respectively. A single dose of 50mg/kg body weight of metronidazole caused significant decrease in OPG count and efficacy calculated on days 13, 20 and 27 was 32.8, 53.3 and 56.6 percent, respectively. Similar pattern of reduction in oocyst number and efficacy was recorded at higher dose of metronidazole. At dose rate of 25mg/kg body weight of paromomycin used against Cryptosporidiosis under experimental conditions significant reduction in OPG count was observed with percent efficacy of 55.04, 68.5 and 79.4 on different observational days. At 50mg/kg body weight dose rate of paromomycin percent efficacy determined was 48.1, 65 and 69, respectively. On comparison the most effective reduction in OPG was observed by the use of paromomycin at dose rate of 25mg/kg body weight of cattle. Results of paromomycin were better than other two trialed drugs and may be a choice for therapy of, cryptosporidiosis.

**Key words:** Cryptosporidiosis, cattle, efficacy, albendazole, metronidazole, paromomycin.

### INTRODUCTION

*Cryptosporidium*, a unicellular intestinal coccidian, infects microvillus of gastrointestinal tract in wide range of animals (Spano *et al.*, 1998). Higher load of oocysts is excreted in the environment by infected animals. The oocysts are highly resistant to desiccation, disinfectants and other environment stresses. Contaminated environment surrounding the infected animal herds is a constant threat to healthy animals (Chen and Kehe, 2012). Warm and humid monsoon months are more amicable for propagation of the disease (Wang *et al.*, 2011). *Cryptosporidium parvum* is widespread in ruminants for neonatal diarrhea (Lorenz *et al.*, 2011) while *C. andersoni* is responsible for abomasal Cryptosporidiosis in cattle and buffaloes (Putignani and Menichella, 2010). Clinical disease is characterized by mucous to

haemorrhagic diarrhoea, lethargy, pyrexia and loss of condition (Xiao *et al.*, 1999).

Infection is diagnosed by conventional microscopic examination of *Cryptosporidium* oocysts (Fayer *et al.*, 1997). Oocyst of *Cryptosporidium* in different species ranges from 4.5 to 7.9  $\mu\text{m}$  in length and 4.5 to 6.5  $\mu\text{m}$  in width. The shape of *C. parvum* oocyst is ovoid to elliptical depending upon the host of parasite. ELISA has been developed with varying degree of specificity and sensitivity for antigen detection (Elgun and Koltas, 2011). PCR technology is a powerful alternative for the detection of *C. parvum* in both environmental and clinical samples (Ware *et al.*, 2013).

Many vaccines and chemotherapeutic agents have been tested for prophylaxis against the disease (Hueffer *et al.*, 2013). Paromomycin is most commonly used drug against Cryptosporidiosis. Azithromycin show partial results against the disease (Gargala, 2008). In vitro and in vivo effect of nitazoxanide has been demonstrated using different animal models and finally in clinical trials.

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Repeated doses of nitazoxanide and albendazole are also effective against Cryptosporidiosis. Due to environmental contamination and severity of clinical signs (Fayer and Nerad, 1996) protease inhibitors such as saquinavir and ritonavir cause significant decrease in oocyst count. In Pakistan, most of the domesticated livestock is not producing to their potential (Khan and Maqbool, 2012). Major involvement for this lowered performance and economic losses is of different parasitic infections. Therapeutic efficacy of albendazole, metronidazole and paromomycin was evaluated against *Cryptosporidium* in cattle.

## MATERIALS AND METHODS

*Cryptosporidium* oocyst load per gram of collected fecal samples was calculated. Oocysts were identified on the basis of microscopic morphological features and confirmed by polymerase chain reaction (PCR). Efficacy of different anti-parasitic drugs was evaluated on the basis of log reduction in *Cryptosporidium* oocysts.

### Counting of oocysts

Fecal samples (n=720) were collected directly from the rectum of each cattle in a sterilized plastic bag reared at Military dairy farm, Government dairy farm, Gawala colonies and House hold dairies (n=180, each) in and around Lahore city from August, 2007 to July, 2008. Preliminary identification was made by microscopic morphology of *Cryptosporidium* oocysts following the protocol described by Fayer *et al.* (2012). *Cryptosporidium* oocysts were identified at molecular level using PCR as described by Johnson *et al.* (1995). *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.

$$N = S / \text{Vol.} \times \text{wt.} \times \text{pv}$$

N, number of Oocysts per gram of feces; S, number of oocysts counted on the slide; Vol., volume of the sample examined (20  $\mu$ l); Wt., weight of the fecal sample (20 g) and pv, pellet volume (1ml).

### Chemotherapeutic trials

Cattle, 2-3 years of either sex experimentally infected with *Cryptosporidium* were kept at four different farms under similar feeding and management conditions within the radius of 10 kilometer throughout the course of treatment. The negative control group was kept separate in a shed and no one was allowed to make access there. Selected animals (n=150) were divided randomly into five groups (A, B, C, D and E) having 30 herds each. Animals of groups A, B and C were further divided into two subgroups having 15 cattle in each. Prior to start therapeutic trials groups A, B, C and D were infected with *Cryptosporidium* oocyst (n=200) orally whereas group E kept as non-infected control. On 10<sup>th</sup> day of infection oocyst/gm of feces counted as already described. The albendazole (ICI, Pakistan Ltd.), metronidazole (Sonon aventis, Ltd. Pakistan) and paromomycin (Star Laboratory, Ltd. Pakistan) were selected for therapeutic trials against Cryptosporidiosis. Animals in subgroups A, B and C were treated with two different doses of albendazole (7.5 and 10 mg/kg), metronidazole (50 and 100 mg/kg) and paromomycin (25 and 50 mg/kg) orally for five consecutive days. Group D was kept as positive control. Fecal samples were collected from each animal of treated and control groups on 6, 13, 20 and 27 days post treatment and oocysts were counted as described.

### Efficacy of selected drugs

Efficacy of selected drugs against *Cryptosporidium* oocysts was calculated as per formula:

$$\text{Efficacy (\%)} = \frac{(\text{Total oocysts before treatment} - \text{Total oocysts after treatment})}{\text{Total oocysts before treatment}} \times 100$$

Side effects of drugs were recorded. Effects of treatment on body weight and feed intake were observed including randomly selected animals (n=10) from each group/sub group of animals. All readings were documented in triplicate for each animal.

### Hematological data

Blood samples were collected from each

animal of all groups before *Cryptosporidium* infection. After the infection on appearance of oocysts in feces blood samples were collected in similar fashion and complete hemogram was obtained. The leukocyte count particularly of eosinophil was carried out on blood films stained by Giemsa. The comparison of hemoglobin level and leukocyte count among different groups was carried out. Data obtained was analyzed by one way ANOVA using Duncan's Multiple Range test.

## RESULTS

Prevalence of *Cryptosporidium* oocysts in cattle identified by microscopic oocyst morphology was 20.55, 12.77, 6.11 and 3.88 percent at Government dairy farm, Gawala colonies, Military dairy farm and House hold dairies, respectively. Percent prevalence values confirmed by PCR were 22.7, 14.41, 7.7 and 5, respectively.

### *Chemotherapeutic trials*

Efficacy of different selected drugs against Cryptosporidiosis was evaluated on the basis of reduction in the oocyst per gram of feces post treatment in relation to time. The means of reduction in *Cryptosporidium* oocysts of treated and control groups were compared.

Results for efficacy of albedozole, metronidazole and paromomycin at different doses against *Cryptosporidium* oocysts in experimentally infected cattle determine on the basis of reduction in Oocyst per gram of feces (OPG) are presented at Table I. OPG count showed an increasing trend in control (untreated) animals. A single dose of 10mg/kg body weight of albendazole caused a significant decrease in OPG count from 6<sup>th</sup> day post treatment and onward. The efficacy of albendazole observed was 43.05, 58.7 and 64.6 percents on 13<sup>th</sup>, 20<sup>th</sup> and 27<sup>th</sup> day post treatment. At 7.5mg/kg body weight dose of albendazole significant reduction in OPG count of *Cryptosporidium* was recorded from 6<sup>th</sup> post treatment day and onward. The efficacy of albendazole determined at this dose was 34.8, 57.1 and 62.9 percents on days 13, 20 and 27 post therapy.

A single dose of 50mg/kg body weight of metronidazole caused a significant decrease in OPG

count from 6<sup>th</sup> day post treatment and onward ( $P<0.05$ ). Efficacy of drug calculated on days 13, 20 and 27 was 32.8, 53.3 and 56.6 percent, respectively. On statistical analysis by Univariate ANOVA and applying DMR test significant ( $P<0.05$ ) decrease in the OPG count was recorded on 13<sup>th</sup> day post treatment whereas on 20<sup>th</sup> and 27<sup>th</sup> days difference was non-significant. Similar pattern of reduction in oocyst number and efficacy was recorded at higher dose of metronidazole used in infected cattle.

At dose rate of 25mg/kg body weight of paromomycin used against Cryptosporidiosis under experimental conditions significant decrease in OPG count was observed 6<sup>th</sup> day post treatment and onward ( $P<0.05$ ). Percent efficacy of used drug was 55.04, 68.5 and 79.4 on days 13, 20 and 27 post treatment, respectively. On statistical analysis significant difference in relation to reduction in oocyst number was observed on different selected days. At 50mg/kg body weight dose rate of paromomycin significant decrease in OPG count was recorded from 6<sup>th</sup> day post treatment and onward ( $P<0.05$ ). On days 13, 20 and 27 percent efficacy of used drug determined was 48.1, 65 and 69, respectively. On comparison the most effective reduction in OPG was observed by the use of paromomycin at dose rate of 25mg/kg body weight of cattle. Results for efficacy of selected drugs are presented at Table II.

Post treatment side effects observed were sweating and diarrhea. At higher doses three cattle from group A (albendazole), two from B (metronidazole) and only one in C (paromomycin) exhibited the sign of sweating. Diarrhea was noted in the sub group of A and B, which became normal by day 13. No other side effect was recorded during experiments. Increase in body weight of animals in non-infected control and treated groups were in same pattern and differed significantly with animals of infected non-treated group. Significant difference was observed in the values of leukocyte and eosinophil in infected cattle on 6<sup>th</sup> and 13<sup>th</sup> days post inoculation. The values for lymphocytes, monocytes, eosinophiles and basophiles of *Cryptosporidium* non-infected cattle were 58.09, 5.67, 5.33 and 0.59, respectively. Whereas values recorded in infected cattle were 59.09, 5.73, 10.29

**Table I.-** *Cryptosporidium* oocyst per gram of feces in cattle treated with selected doses of different drugs in relation to time

Groups	Subgroups (n=15)	Treatment	Dose (mg/kg.b.wt.)	Oocysts per gram of feces on different days				
				00	06	13	20	27
A	A1	Albendazole	10	878	700 <sup>a</sup>	570 <sup>b</sup>	362 <sup>c</sup>	310 <sup>d</sup>
	A2		7.5	875	740 <sup>a</sup>	570 <sup>b</sup>	375 <sup>c</sup>	324 <sup>d</sup>
B	B1	Metronidazole	100	837	810 <sup>a</sup>	535 <sup>b</sup>	371 <sup>c</sup>	344 <sup>c</sup>
	B2		50	825	755 <sup>a</sup>	554 <sup>b</sup>	385 <sup>c</sup>	358 <sup>c</sup>
C	C1	Paromomycin	25	912	712 <sup>a</sup>	410 <sup>b</sup>	287 <sup>c</sup>	187 <sup>d</sup>
	C2		50	887	700 <sup>a</sup>	460 <sup>b</sup>	310 <sup>c</sup>	270 <sup>d</sup>
D. Group (infected positive control)				878	1045	1170	1304	1429
E. Group (negative non infected control).				0	0	0	0	0

Means carrying same superscript differ non-significantly (P<0.05)

**Table II.-** Comparative percent efficacy of different selected drugs used at different doses against *Cryptosporidium* in cattle

Groups	Subgroups (n=15)	Treatment	Dose (mg/kg.b.wt)	Percent efficacy at different days			
				06	13	20	27
A	A1	Albendazole	10	20.2	43.05	58.7	64.6
	A2		7.5	15.4	34.8	57.1	62.9
B	B1	Metronidazole	100	15.1	36.0	55.6	58.9
	B2		50	8.4	32.8	53.3	56.6
C	C1	Paromomycin	25	21.9	55.04	68.5	79.4
	C2		50	21.0	48.1	65.0	69.5

and 0.69, respectively. Hemoglobin concentrations in infected and non-infected cattle recorded were 11.08 and 11.04 showing non-significant difference statistically.

## DISCUSSION

Cryptosporidiosis is an important zoonotic disease of domestic and wild animals. Infection with *Cryptosporidium parvum* is common in cattle, buffaloes, goats, sheep, horses, cats, human beings and other vertebrates (Leitch and He, 2011). The infection is endemic in ruminants as well as human beings in Pakistan. In present study occurrence of disease in cattle reared at Government dairy farm, Military dairy farm, Household dairy and Gawala colonies was determined. Efficacy of selected drugs against *Cryptosporidium* oocysts was evaluated under experimental conditions in cattle.

### *Efficacy of chemotherapeutic agents*

Oocyst per gram (OPG) count showed an increasing trend in control (untreated) animals. A single dose of 10mg/kg body weight of albendazole

caused a significant decrease in OPG count from 6<sup>th</sup> day post treatment and onward (P<0.05). Similar findings in relation to the efficacy of albendazole at 20mg/kg body weight in calves against Cryptosporidiosis were reported by Xiao *et al.* (1999) and Johny *et al.* (2007). Metronidazole treatment caused a significant decrease in OPG count from 6<sup>th</sup> day post treatment and onward (P<0.05). Paromomycin used against Cryptosporidiosis under experimental conditions showed better results than albendazole and metronidazole.

Although a number of compounds have been tested against Cryptosporidiosis and only limited showed effective results. Kelly *et al.* (1996) observed improvement in symptoms of Cryptosporidiosis and eradication in four Zambian AIDS patients by albendazole used at dose 80mg twice. However control large scale study was recommended. Only paromomycin has been shown to have an anti-cryptosporidial activity (Hahn and Capuano, 2010). Comparable results were documented in relation to efficacy of paromomycin by Leitch and He (2011). Similar findings were

reported by Sharling *et al.* (2010), Tzipori *et al.* (1994) and Verdon *et al.* (1994) while working on animal models regarding efficacy of paromomycin against Cryptosporidiosis. Paromomycin in a dose of 25 to 35 mg/kg/day has a beneficial but limited effect upon oocyst shedding and stool frequency in AIDS patients (Chawla *et al.*, 2011). In conclusion paromomycin is probably the most promising compound for treatment of Cryptosporidiosis in human beings. Paromomycin was suggested to be the most valuable drug for the treatment of *Cryptosporidium* infection based on the clinical trials (Griffiths *et al.*, 1998).

Post treatment side effects observed were sweating and diarrhea. At higher doses three cattle from group A (albendazole), two from B (metronidazole) and only C (paromomycin) exhibited the sign of sweating. Diarrhea was noted in the sub group of A and B, which became normal by day 13. Increase in body weight of animals in non-infected control and treated groups were in same pattern and differed significantly with animals of infected non-treated group. Significant difference was observed in the values of leukocyte and eosinophil in infected cattle on 6<sup>th</sup> and 13<sup>th</sup> days post inoculation. The leukocytes/lymphocytes count of *Cryptosporidium* infected cattle was 58.09 and eosinophil 9.69 percent. The difference noted for hemoglobin concentration was non-significant ( $P>0.05$ ). The rise in eosinophil numbers during parasitic infections is a prominent feature. Eosinophils were found in lungs, skin, intestine and urogenital tract tissues. In agreement higher numbers of eosinophils were observed during infection with *Cryptosporidium* by Hill *et al.* (2008). Out of three drugs tested none got significant side effects and were effective against Cryptosporidiosis.

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