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Short Communication

EFFICACY OF AZITHROMYCIN IN TREATMENT OF CRYPTOSPORIDIOSIS IN NATURALLY INFECTED CATTLE CALVES

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SUMMARY

Large number of chemotherapeutic agents has been screened against cryptosporidiosis but there is no reliable curative treatment for the infection. The objective of this study was to evaluate the therapeutic efficacy of azithromycin against cryptosporidiosis in naturally infected calves under field conditions. Based on the pilot study, the dose of azithromycin for the trial was selected as 25 mg/kg body weight. The therapeutic efficacy was determined in naturally infected cattle calves on the basis of reduction in number of oocysts per gram of faeces excreted in the feces and increase in body weight gain. The study revealed that at 28th day post-treatment, the efficacy of azithromycin was 96.62% against natural infection of *Cryptosporidium* sp. in cattle calves.

Key words: Cryptosporidiosis, Cattle Calf, Treatment, Azithromycin

Cryptosporidiosis is characterized by acute gastrointestinal disturbances, hemorrhagic or mucoid diarrhoea, fever, lethargy, anorexia and loss of condition (Navin and Juranek, 1984) leading to significant economic loss in farm animals especially young or neonates. Till date about 25 species of *Cryptosporidium* have been reported based on host specificity and among them the most important species infecting bovines are *Cryptosporidium parvum, C. andersoni, C. bovis* and *C. ryanae* (Yang *et al*., 2014). In India various studies have reported high prevalence of cryptosporidiosis in young cattle (Paul *et al*., 2008; Yadav *et al*., 2012; Venu *et al*., 2012; Singla *et al*., 2013).

A major problem concerning cryptosporidiosis is the lack of an effective means for prevention and therapy of this disease. More than 200 substances like nitazoxanide, paromomycin, halofuginone etc. have been tested against cryptosporidiosis in cattle calves with variable results (Gargala, 2008). Some of these have exhibited promising effects but none were able to consistently control clinical signs or completely eliminate the infection (Blagburn and Soave, 1997; De Graaf *et al*., 1999). However, use of some drugs can reduce oocyst sheddings, which presumably also reduces environmental pathogen load and subsequent exposure and infection of susceptible hosts. In India except few reports (Randhawa *et al*., 2012; Agrawal, 2013), not much work has been reported against therapeutic management of bovine cryptosporidiosis. The present study determines the efficacy of azithromycin against natural infection of cryptosporidiosis in cattle calves.

Dairy farms were selected having previous history of *Cryptosporidium* in cattle calves in Jammu region. Initially a pilot study was conducted wherein 24 animals naturally infected with *Cryptosporidium* spp. and having oocyst intensity as 2+ (1 to 10 oocysts per field of view observed under x 40 objective lens) and above (OIE, 2008) were divided into six groups (four neonatal calves of 14 to 28 days age in each group). Group I animals were unmedicated and kept as control (administered with distilled water only). Animals from groups II to VI were orally medicated with azithromycin at dose rate of 15, 20, 25, 30, 35 mg/kg body weight, respectively. All the animals were monitored throughout the study and oocyst intensity (OIE, 2008) was determined from day 0 to day 28 post-treatment at weekly interval. The best drug from pilot study was selected for therapeutic trial.

For therapeutic evaluation, 12 calves (aged 14 to 28 days) naturally infected with *Cryptosporidium* spp. and having oocyst intensity as 2+ and above (OIE, 2008) were selected from two different farms not included in the pilot study. These calves were divided into two groups of six animals each. Group I animals were unmedicated and kept as infected control whereas

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animals of group II were orally medicated with best selected dose of azithromycin dehydrate based on the pilot trial. Two parameters *viz.* oocyst per gram of faeces (OPG) and body weight gain were assessed for therapeutic evaluations.

The OPG was calculated using the method of Grinberg *et al*. (2002). As per the method, 1 gram of faecal sample was mixed with 10 ml of tap water, passed through a 100-mesh sieve, the suspension centrifuged and the sediment resuspended in 4 ml of normal saline. Afterwards, 10 microlitre of this suspension was poured as a drop on a slide, air-dried and stained by Modified Ziehl-Neelsen staining (Henricksen and Pohlenz, 1981). The entire area of smear was examined with 40x objective lens and oocysts counted. The OPG was then calculated by multiplying the result by 500. The mean OPG was performed from day 0 to day 28 post treatment at weekly interval. The percent efficacy of azithromycin was assessed as:

Per cent efficacy=

$$\frac{\text{Mean OPG (0 day)} - \text{Mean OPG (Post treatment)}}{\text{Mean OPG (0 day)}} \times 100$$

Similarly body weight of the study animals was measured from day 0 to day 28 post treatment at weekly interval. The body weight gain was assessed as:

Per cent increase in body weight gain =

$$\frac{\text{Weight on concern day - Weight on day 0}}{\text{Weight on day 0}} \times 100$$

Statistical analysis of data was done using two way ANOVA (Snedecor and Cocharan, 1994) and the means having significant difference were ranked as per Duncan's multiple range test (Duncan, 1955)

The study revealed that the mean oocyst intensity in group I of pilot trial was 1.75, while it was 1.25 and 1.0 in groups II & III, respectively. The animals of other three groups (IV to VI) revealed a mean oocyst intensity of 0.75. Thus, keeping in mind the minimum dosage showing maximum reduction in oocyst production, it was planned to carry the final therapeutic trial against natural cryptosporidiosis in cattle calves at dose of 25 mg/kg body weight.

The findings of the therapeutic trial revealed that OPG count at day 28 post-treatment was significantly (p<0.01) lower in azithromycin group (218±34) than in control (2975±142); (Fig. 1); the percent efficacy of azithromycin was 96.62%.

All the bovine calves in the experiment were in between the age group of 14 to 28 days of age and the mean body weight at day 0 varied between 29.83±0.53 to 31.75±0.96 kg. At day 28, percent increase in body weight from the pre-treatment values was 26.51 in azithromycin group as compared to 21.79 in the control group (Table 1). It was observed that the body weight showed significant (p<0.01) periodic increase in both the groups irrespective of the treatment. However, the rate of body weight increase was not significantly (p>0.05) affected by the drug. Nasir *et al.* (2013) reported that calves treated showed 88.2% efficacy of azithromycin (at dose rate of 1500mg/calf/day orally for 7 days) at 21 day post treatment. Agrawal and Shukla (2013) reported that the OPG count at the end of 4th week was significantly lower in azithromycin (orally

Table 1
Body weight (kg, mean ± SE) of calves in control and treatment groups

Groups	Period					P value (period)
	0 day	7th day	14th day	21st day	28th day	Group mean ± SE
Control	29.83±0.53^{a}	31.05±0.62^{ab}	32.18±0.61^{bc}	33.50±0.58^{c}	36.33±0.83^{d}	32.58±0.49
		(4.08%)	(7.87%)	(12.30%)	(21.79%)	(9.21%)
Treatment	31.75±0.96^{a}	33.08±0.96^{ab}	35.58±1.14^{bc}	37.17±1.23^{cd}	40.17±1.25^{d}	35.55±0.72
		(4.18%)	(12.06%)	(17.07%)	(26.51)	(11.96)
Period mean±SE	30.79±0.60^{a}	32.07±0.63^{ab}	33.88±0.80^{bc}	35.33±0.85^{c}	38.25±0.92^{d}	34.07±0.47
						P<0.01
P value					P<0.01	P>0.05

^{abcd} Means bearing different superscripts within a row differ significantly
Values in parenthesis indicate % increase in body weight from day 0 value.

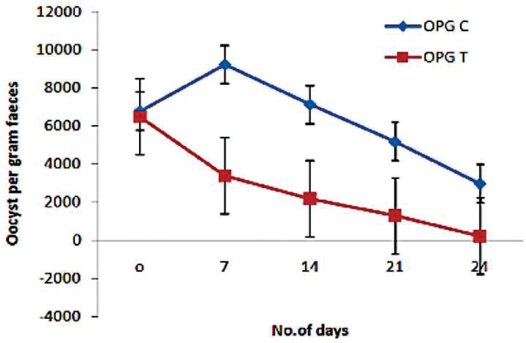

Fig. 1 : Oocyst per gram of faeces in azithromycin treated and untreated group
OPG C=Mean OPG of control group,
OPG T=Mean OPG of treated group

administered at a dose of 20-30 mg/kg b.wt. s.i.d for 7 days) and nitazoxanide (orally @15-20 mg/kg b.wt. b.i.d for 5 days) treated group than control. Like the present study, Elitok *et al*. (2005) also observed greater weight gain in azithromycin (1500 mg/day for 7 days) treated calves. The therapeutic effect of azithromycin may be because it acts by blocking dissociation of ribosomal t-RNA leading to inhibition of protein synthesis (Shahiduzzaman and Daugschies, 2012). The study concludes that azithromycin can be used @ 25 mg/kg body weight against natural cryptosporidiosis in cattle calves.

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