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Research Article

COMPARATIVE THERAPEUTIC EVALUATION OF AZITHROMYCIN AND NITAZOXANIDE IN CRYPTOSPORIDIOSIS AFFECTED CROSSBRED DAIRY CALVES

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ABSTRACT

Cryptosporidiosis, an emerging zoonotic disease of young ruminants is associated with significant economic losses. The present study aimed to evaluate azithromycin and nitazoxanide therapy in cryptosporidiosis affected crossbred dairy calves. Eighteen diarrhoeic calves aged 8-15 days and found *Cryptosporidium* positive by modified Ziehl-Neelsen staining (mZN) and formal ether concentration techniques, were placed randomly in three groups of six each. Group I served as infected untreated control. In Group II, Azithromycin dehydrate @ 30 mg/kg b.wt. once daily orally for 7 days and in Group III, Nitazoxanide @ 15 mg/kg b.wt. twice daily orally for 5 days were given. Ringer's lactate was instituted as supportive therapy in calves of all the groups for 2-3 days. Clinical examination of all calves was conducted daily till day 42. Assessment of faecal consistency, dehydration and oocyst counting was done on alternate days. Significantly ($p<0.05$) lesser days were taken in azithromycin group to attain normal temperature, pulse, faecal consistency and recovery from dehydration than control. Nitazoxanide did not show a positive response in coping up with diarrhoea and dehydration. Oocyst shedding ceased in all the groups before the end of observation period. Azithromycin treatment significantly ($p<0.05$) reduced the duration of oocyst shedding and mean oocysts shed. The OPG count at the end of 4th week suggested significant ($p<0.05$) treatment effects; the counts being lower in azithromycin and nitazoxanide group than control.

Keywords: Azithromycin, Cryptosporidiosis, Dairy calves, Nitazoxanide

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Cryptosporidium is a protozoan parasite, inhabiting the gastrointestinal and respiratory tracts of wide variety of vertebrate hosts including humans, and is responsible for causing significant morbidity and mortality (Agrawal *et al.*, 2018). It is recognized as third major cause of scours in calves resulting in significant direct and indirect economic losses. High environmental stability of the cryptosporidium oocysts (Castro-Hermida *et al.*, 2002), low dose infectivity and high excretion level of sporulated oocysts favour the quick transmission of infection (Ramirez *et al.*, 2004). Resistance to common disinfectants (Chalmers and Giles, 2010) and non-availability of effective vaccine along with limited treatment options lead to increased mortality rates (O'Donoghue, 1995). The treatment options are limited and often rely on rehydration therapy (Meganck *et al*., 2014). Azithromycin dihydrate has been reported to effectively suppress oocyst shedding, reduce the environmental contamination by oocysts in addition to improvement in clinical signs (Elitok *et al.*, 2005). Nitazoxanide (NTZ), an antimicrobial substance approved by FDA for human cryptosporidiosis has been used in experimentally induced calf cryptosporidiosis; and found to be effective in reducing the duration of oocyst shedding and improvement in fecal consistency (Ollivett *et al.*, 2009).The aim of present study was to investigate the comparative effect of Azithromycin and Nitazoxanide in the treatment of calf cryptosporidiosis.

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MATERIALS AND METHODS

Selection of animals and design of treatment : The study was conducted in government and private dairy farms of Jabalpur (M.P.). A total of 52 faecal samples were collected from crossbred female diarrhoeic calves of 8-15 days age. Faecal samples were tested for *Cryptosporidium* spp. oocyst using modified Ziehl-Neelsen staining technique (mZN) (Henricksen and Pohlenz, 1981) and formal ether concentration techniques (WHO, 1994). Twenty three (44.2%) calves tested positive for natural *Cryptosporidium* spp. oocyst infection. Scoring for intensity of infection in positive faecal samples (by acid fast staining) with *Cryptosporidium* spp. oocyst shedding was done semi quantitatively as per Castro-Hermida *et al.* (2002) by finding the average number of oocysts in 20 randomly selected fields at 100x. Eighteen naturally infected calves whose oocyst intensity score ≥ +2 were placed randomly into three groups of six calves each. Rehydration therapy (Ringer's lactate) was given in Group I which served as infected untreated control. Calves in Group II were treated with Azithromycin dehydrate (AZAX®-500, Ranbaxy Laboratories Limited, New Delhi) @ 30 mg/kg b.wt. s.i.d for 7 days orally. Group III calves were given Nitazoxanide (Nizonide® 500, Lupin Ltd., Mumbai) @ 15 mg/kg b.wt. b.i.d for 5 days orally. Ringer's lactate i.v. was instituted as supportive therapy to all the calves as per the level of dehydration for 2-3 consecutive days.

Faecal samples from all the 52 calves were additionally analysed for *E. coli*, *Salmonella* spp. and coccidian oocysts as per the methods described by Cheesbrough (2006) and Zajac and Conboy (2006), respectively.

Assessment of drug efficacy: Clinical examination of all calves was carried out during inclusion and thereafter daily for 42 days from the commencement of therapy (day 0). Drug efficacy was assessed by evaluating the following parameters:

i. **Faecal consistency:** Changes in faecal consistency from diarrhoeic to normal status was assessed as described by Larson *et al.* (1977).

ii. **Level of dehydration:** Improvements in clinical signs of dehydration were assessed by using cervical skin tent time as described by Naylor (1989).

iii. **Oocyst counting:** The number of cryptosporidial oocysts per gram (OPG) of faeces was counted on alternate days by the method described by Grinberg *et al.* (2002).

iv. **Body weight:** Body weight of calves was measured with a weighing balance on day 0, 14, 28 and 42.

Statistical analysis: The data obtained from the study were analyzed statistically by analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) using SPSS 15.0 software.

RESULTS AND DISCUSSION

Forty four per cent faecal samples tested positive for *Cryptosporidium* spp. oocysts. None of the samples tested positive for coccidia oocyst or *Salmonella* spp. All 52 faecal samples were positive for *E. coli* but since non pathogenic *E. coli* are extremely common, faecal cultures results are not diagnostic as a cause for diarrhoea (Foster and Smith, 2009). The main focus of the study was to evaluate the effect of Azithromycin and Nitazoxanide in *Cryptosporidium* positive calves, and since infection is self limiting, the calves with intensity score ≥ +2 were selected for drug therapy.

Clinical parameters: At presentation (day '0'), seventeen (94.4%) calves had temperature and pulse rate higher than normal. Rectal temperature 104.4±0.19 ºF (103-105.8 ºF) and the pulse rate was 126.7±1.01 per minute (120-134 per minute) on day 0. As the calves were between 8-15 days old, the normal range considered for temperature was between 101.4-102.8 ºF and that for pulse was 100-120 per minute. Das *et al.* (2006) observed that the body temperature increased at the onset and declined with the advancement of infection whereas pulse rates were higher at early stages and became thready subsequently. Similarly, Shobhamani *et al.* (2007) recorded mild to moderate rise in rectal temperature in cryptosporidic calves which in later stages, with the onset of diarrhoea became subnormal.

The azithromycin treated calves attained normal temperatures much earlier (by day 2 to 3 of treatment) than the nitazoxanide treated (by day 4 to 14) and control (by day 6 to 17) calves. Similarly, the pulse rate in azithromycin group was normal by second or third day, in contrast with nitazoxanide treated and control group, where 8.17±0.79 and 7.33±0.71 days were required to attain normalcy (Table 1). Similarly, Shobhamani *et al.* (2007) reported that in all azithromycin treated calves rectal temperature came to normal by second day, while in untreated calves, it required 9 days. They reported normal pulse rate reached on day 8 in azithromycin treated calves in contrast to untreated calves where they remained at a lower level till end of observation.

Calves are not as effective in compensating a hypovolemic shock and also their urine concentrating capacity is low, which makes them susceptible to rapid dehydration (Koch, 2004). Therefore, fluid therapy was instituted in all the groups as a basic therapy since fluid losses in calves with diarrhoea can reach 13 to 18% of body weight per day (Berchtold, 2009). Altered faecal consistency (diarrhoea) and dehydration was noted on day 0 in all cryptosporidic calves of our study with difference in duration and severity. Initially the consistency of diarrhoeic faeces was liquid in nature, which subsequently changed to semisolid and normal in varying duration. Change in faecal consistency towards normal was significantly ($p<0.05$) earlier in azithromycin treated calves as compared to control and nitazoxanide group (Table 1). Mild to moderate dehydration as evidenced by skin tenting method was recorded in control calves for significantly ($p<0.05$) longer duration in contrast the azithromycin treated calves (Table 1). The results of the present study coincide with findings of Elitok *et al.*, 2005 and Shobhamani *et al.* (2007) showing resolution of diarrhoea was recorded by day 7 in azithromycin treated calves. The improvement recorded in the control group receiving supportive fluid therapy in the present study well corroborated with earlier reports indicating that the parenteral fluid therapy may be beneficial to combat dehydration to restore electrolyte balance and to provide a source of energy in severe cases (Constable *et al.*, 2017).

The nitazoxanide treatment did not show the expected positive response in resolution of diarrhoea and dehydration (Table 1). In fact, diarrhoea, dehydration and oocyst excretion persisted longer in nitazoxanide group

Table 1

Duration of manifestation of clinical signs and oocyst excretion in control and treated Cryptosporidic calves (Days, Mean±SE)

Groups	Number of days					Mean
	Pyrexia/fever	Tachycardia	Diarrhoea	Dehydration	Oocyst excretion	OPG $\times 10^3$
Control	9.0 ± 1.65^{p}	8.17 ± 0.79^{p}	16.67 ± 1.49^{p}	13.83 ± 1.54^{p}	27.50 ± 1.36^{p}	7.64 ± 0.12^{p}
Azithromycin treated	1.2 ± 0.20^{q}	1.6 ± 0.24^{q}	11.17 ± 0.17^{q}	7.17 ± 0.48^{q}	23.33 ± 1.47^{q}	3.42 ± 0.11^{q}
Nitazoxanide treated	6.17 ± 1.54^{p}	7.33 ± 0.71^{p}	19.17 ± 0.65^{p}	16.83 ± 0.70^{p}	29.00 ± 0.68^{p}	5.53 ± 0.43^{r}

Values with different superscripts ^{p,q,r}vary significantly ($p<0.05$) within a column

Table 2

Oocyst count ($\times 10^3$ per gram of faeces, Mean±SE) in calves of control and treatment groups

Groups	day 0	day 7	day 14	day 21	day 28
Control (n=6)	7.92 ± 0.56^{bp}	10.08 ± 0.45^{ap}	8.33 ± 0.69^{bp}	5.17 ± 0.42^{cp}	3.58 ± 0.68^{dp}
Azithromycin treated (n=6)	7.50 ± 0.53^{ap}	4.33 ± 0.15^{br}	2.67 ± 0.15^{cr}	1.17 ± 0.19^{dr}	0.25 ± 0.16^{dr}
Nitazoxanide treated (n=6)	8.08 ± 0.40^{ap}	7.83 ± 0.51^{aq}	6.75 ± 0.70^{aq}	3.42 ± 0.343^{bq}	1.67 ± 0.28^{cq}

Values with different superscripts ^{a,b,c,d}vary significantly ($p<0.05$) within a row; Values with different superscripts ^{p,q,r}vary significantly ($p<0.05$) within a column

Table 3

Body weight (Kg, Mean±SE) of calves in control and treatment groups

Groups	Body weight (kg)				
	day 0	day 7	day 14	day 28	day 42
Control (n=6)	34.23 ± 2.37^{ap}	35.03 ± 2.42^{ap} (2.3%)	35.62 ± 2.40^{ap} (4.0%)	37.05 ± 2.36^{bp} (8.2%)	40.38 ± 2.46^{cp} (17.9%)
Azithromycintreated (n=6)	35.05 ± 2.39^{ap}	38.25 ± 2.53^{bq} (9.1%)	43.75 ± 2.75^{cq} (24.8%)	47.45 ± 3.09^{dq} (35.4%)	56.42 ± 3.36^{eq} (60.9%)
Nitazoxanide treated (n=6)	34.72 ± 2.43^{ap}	35.92 ± 2.44^{ap} (3.5%)	38.35 ± 2.55^{br} (9.5%)	40.08 ± 2.59^{cr} (15.4%)	43.78 ± 2.70^{dr} (26.1%)

Values with different superscripts ^{a,b,c,d,e}vary significantly ($p<0.05$) within a row; Values with different superscripts ^{p,q,r}vary significantly ($p<0.05$) within a column; Values in parenthesis indicate % increase in body weight from day 0 value

than control. Longer diarrhoeic episodes with altered fecal consistency was also reported by Ollivett *et al.* (2009) and Schnyder *et al.* (2009) and it might be due to direct negative effect of nitazoxanide on GIT mucous membrane (Theodos *et al.*, 1998) or flora (Hemphill *et al.*, 2006).

Oocyst shedding: The excretion of oocyst in faeces ceased in all the groups before day 42 which indicated that *Cryptosporidium* infection is self-limiting. Our findings can be supported by those of Swain *et al.* (2019) who found decreased oocyst shedding with increasing age in buffalo calves. The duration of oocyst shedding was significantly ($p<0.05$) lesser in azithromycin group as compare to control group (Table 1). The mean oocyst per gram of faeces counted on alternate days till ceasing of shedding was significantly ($p<0.05$) lower in azithromycin and nitazoxanide groups than control (Table 1). Similar to present study, Elitok *et al.* (2005) and Shobhamani *et al.* (2007) observed marked decline in the magnitude of oocyst excretion in azithromycin treated calves.

Significantly ($p<0.05$) lower OPG count at the end of 4th week in azithromycin and nitazoxanide group than control suggested significant ($p<0.05$) treatment effects (Table 2). Our results are quite similar to that of Schnyder *et al.* (2009) who observed significantly lower mean OPG in nitazoxanide group inspite of non-significant difference in the number of days of oocyst excretion when compared to control. They opined that oocyst determinations may have been altered by the severe diarrhoea in nitazoxanide group thus diluting oocyst densities in the faecal samples. Ollivett *et al.* (2009) found that by the end of the observation period, 85% of the nitazoxanide treated calves stopped shedding oocysts in contrast to the control group where 85% continued oocyst shedding.

Body weight: Initial mean body weight of calves in three groups varied non-significantly between 34.23±2.37 to 35.05±2.39 kg. At the end of observation period (day 42),

the per cent increase in body weight from the pre-treatment values was 60.9 per cent in azithromycin group, 26.1 per cent in nitazoxanide group and 17.9 per cent in the control group (Table 3); the difference was significant ($p<0.05$) at day 7 in azithromycin group. Significantly greater body weight gain in cryptosporidic calves medicated with azithromycin was also reported by Elitok *et al.* (2005).

At day 7, we found similar weight gain in nitazoxanide group as control. Later we observed a significant ($p<0.05$) difference on day 14 and 28 as compared to control group. Ollivett *et al.* (2009) on the other hand found no significant difference in body weight between nitazoxanide treated and control calves.

Azithromycin proved to be the most promising drug for decreasing the intensity and duration of oocyst excretion thereby significantly decreasing environmental contamination and increasing calf welfare as evidenced by early recovery of clinical indices.

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