



RESEARCH ARTICLE

Efficacy of Tylosine against Clinical Cryptosporidiosis in Goat Kids

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ABSTRACT

The aim of the present study was to evaluate tylosine efficacy administered by intramuscular for treatment of cryptosporidiosis in naturally infected goat kids. These animals were randomly assigned to test group (n=10) and control (n=10) group after routine clinical examination. All kids showed mild mental depression, decrease in suckling reflex and diarrhea in different severity. Fecal samples were analyzed via virological, bacteriological and coccidial examinations. The consistency of feces was assessed as pastose, semiliquid, or liquid. The rates of infection of the samples were evaluated semi-quantitatively. Tylosine was given by intramuscular route to test group - 2 at a dosage of 10 mg/kg of body weight, twice a day for 5 days. However, isotonic saline solution (1.0 ml) was used by intramuscular route to the control group, once a day for 5 days. Hematological results of all the kids were within normal limits except for hematocrit rates which were mild high in 15 kids as a result of dehydration. However, OPG was not detected in both of group after treatment. It was suggests that tylosine applied animals has been shown more rapid recovery than control group. Tylosine may be useful in order to reduce of treatment period in the disease. In future, more detailed studies which evaluate the effects of tylosine in goat kids with cryptosporidiosis are needed.

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INTRODUCTION

One of the most common problems that occur in lamb, goat kids and dairy calves in the first month of life is enteric infection with *Cryptosporidium parvum* (Delafosse *et al.*, 2006; Sanz *et al.*, 2009; Nasir *et al.*, 2009). *Cryptosporidium parvum* is also common parasite that infects most farms in Turkey (Elitok *et al.*, 2005; Sahal *et al.*, 2005). The main clinical manifestations of cryptosporidiosis in neonate small ruminants are: apathy and depression, anorexia, weight loss, abdominal pain, and mainly diarrhea with yellow, soft or liquid consistency, accompanied by the shedding of a large number of oocysts (Molina *et al.*, 1994).

Up till now, many drugs have been tested for prophylaxis or therapy. A few of them (lasalocid, decoquinate, paromomycin) in calves, goat kids are effective (Mancassola *et al.*, 1995; Mancassola *et al.*, 1997; Sahal *et al.*, 2005). Antibiotics of the macrolide family have been tested for the treatment of cryptosporidiosis in humans and mice with some

therapeutic or prophylactic effects such as spiramycin (Galvagno *et al.*, 1993) and clarithromycin (Cama *et al.*, 1994). Recently, several studies showed the efficiency of azithromycin in calves against cryptosporidiosis (Elitok *et al.*, 2005). Tyrosine alone was apparently successful in blocking oocyst shedding in dogs or cats with cryptosporidiosis (Tams, 1996). Shobhamani (2005) suggested that azithromycin is the drug of choice against cryptosporidiosis followed by tylosine. The present study reports tylosine efficacy for the treatment of cryptosporidiosis in naturally infected goat kids.

MATERIALS AND METHODS

This study was conducted in Saanen breeding flock around the town of Nilufer in Bursa, Turkey. The herd consisted of 35 Saanen goat kids. Of these, 20 kids were found to be suffering from different degrees of cryptosporidiosis. These kids were of different ages (5-15 days), from different sexes (8 males, 12 females) and body weight (6.1±1.5kg). These animals were randomly

assigned to test (n=10) and control (n=10) group after routine clinical examination.

Oocyst count $\geq 10^4$ and diarrhea were also taken as the criteria for the study. All kids showed mild mental depression, decrease in suckling reflex and diarrhea with different severity. The diarrhea was usually detected as very liquid and yellow. Body temperatures, respiration and pulse rates were normal (Table 1). Blood samples with EDTA were collected (HemaandTube®, Turkey) for hematology. Hematological parameters were measured within one hour from sampling using an automatic analyzer (Cell Dyne 3500®, Abott Inc, USA).

Coronavirus and rotavirus antigen detection ELISA test kit (Bio-X Coronavirus and Rotavirus detection Elisa Kit, BIO K344 and 343) was used in according with manufacturer's instructions. Fecal samples were directly streaked onto CHROMagar ECC (M-Tech Diagnostics) containing cefotaxime (1g/ml) using cotton swab and incubated aerobically overnight at 37°C. For the confirmation of negative samples were enriched in buffered peptone water for 18 h at 37°C before being plated onto the same selective medium (CHROMagar ECC).

For parasitological examination, fecal samples were collected directly from rectum and stored on frozen cold packs. Both before and after the treatment, the oocysts were counted on fecal smears that stained with carbol fuchsin (Boch and Supperer, 1992). The consistency of feces was assessed as pastose, semiliquid, or liquid. Feces of every animal were 1/10 diluted with distilled water and consisted fecal suspension was examined under microscope. The rates of infection of the samples were evaluated semi-quantitatively as follows: 0, N/A; 1, fewer than 5-oocysts; 2, between 5 and 25-oocysts; and 3, more than 25-oocysts per field. The complete surface of the slide was examined for each sample. At the same time, numbers of oocysts per visual area (ova) were scored to detect an average OPG count: 1-5 ova= 10^4 - 10^5 , 6-25 ova= 10^5 - 10^6 and >25 ova 10^6 (Villacorta *et al.*, 1991; Lassen and Jarvis, 2009).

Tylosine (Tylan®, Lilly Inc, Istanbul, Turkey) was given by intramuscular route to test group at a dosage of 10 mg/kg BW twice daily for 5 days. However, isotonic saline solution (1.0 ml) was injected intramuscularly to control group, once daily for 5 days. Kids in all two groups were separated during the course of the study. A post-treatment evaluation was performed sixth day after the treatment (Table 2).

Data were analyzed using paired t test and student t test. All statistical analysis was performed using the statistical package SPSS version 13.0 (SPSS Inc., Illinois, USA). $P < 0.05$ and $P < 0.001$ were considered for significant and highly significant differences, respectively.

RESULTS

Hematological results were within normal limits, except for hematocrit rates. Of the 15 kids, 60% control group and 90% treatment group had mild high hematocrit rate (Table 3). Rotavirus, coronavirus and *E. coli* K99+ were not demonstrated in fecal samples, whereas *cryptosporidium spp.* was found in all fecal samples (Table 2). Oocyst was not detected in both of groups after

treatment. Diarrhea completely recovered in all of the treatment group kids with Tylan® application for 5 days as compared with control group. In control group, 5 kids had continued diarrhea.

DISCUSSION

Shobhamani (2005) reported that hematocrit, hemoglobin and total leukocyte counts decreased in cryptosporidic calves. There was no significant difference in total leukocyte and differential count between study and control group in goat kids suffering from cryptosporidiosis. We also found that kids had mild high hematocrit rate due to dehydration. However, hematological findings were not enough to evaluate the effectiveness of treatment in goat kids suffering from cryptosporidiosis.

Tylosine is a member of the macrolide group of antibiotics. Tylosine alone was apparently successful in blocking oocyst shedding in 12 other dogs or cats with cryptosporidiosis (Tams, 1996). It was reported that some macrolides such as spiramycin (Galvagno *et al.*, 1993), clarithromycin (Cama *et al.*, 1994), azithromycin (Elitok *et al.*, 2005) are found to be effective against opportunistic infections associated with immunosuppression like cryptosporidiosis. In the present study, tylosine may be useful in treatment of *cryptosporidia* such as spiramycin (Galvagno *et al.*, 1993), clarithromycin (Cama *et al.*, 1994), azithromycin (Elitok *et al.*, 2005). Mean OPG count after treatment were same with control group because of self-limiting feature of cryptosporidiosis.

Ramirez *et al.* (2004) reported that it depends on the host and the host's immuno-competency pre-patent period ranges from 1 to 3 weeks and patent period can range from several days to months or years. So termination of oocyst shedding of kids both treatment and control groups was almost at about 6th or 7th days, and possibly the control group was on the last days of oocyst shedding. In the present study, it is impossible to detect starting and ending time of the infection due to non-empirical nature of the study. It may be associated with negative results that were detected in control group.

Clinical parameters were observed as more improved after treatment in study group, compared to control group after treatment (Table 1). Also, consistency of feces, condition and dehydration were better than control groups in tylosine applied animals after treatment. The findings may explain the rapid recovery on the clinical parameters with regulatory effect on immune system in addition to antibacterial effect of tylosine in study group.

Cryptosporidiosis is self-limiting infections in immune-competent kids or if management and nutritional status of the goat kids are improved as described in goat kids belong to control group in the present study. However it may lead to severe diarrhea and subsequent death despite aggressive supportive care in immunosuppressive kids. It was suggested that tylosine treated animals has shown more clinically rapid recovery than control group. Tylosine may be useful in order to reduce of treatment period in the disease. In future, more detailed studies which evaluate the effects of tylosine in goat kids with cryptosporidiosis are needed.

Table 1: Clinical finding of goat kids before and after treatment measurements in control and study group.

Parameters	Treatment group		Control Group	
	Before Treatment	Post Treatment	Before Treatment	Post Treatment
Temperature (°C)	39.1±0.06*	38.8±0.06	39.0±0.07	38.9±0.04
Pulsation (/min)	117±3.4*	104.3±0.1	103.9±2.1	102.0±2.0
Respiration (/min)	46.6±1.1**	41±0.9	44.6±1.15	45.4±0.89
Capillary filling time (sec)	4->6	2	4->6	2-4

Figures (mean±SE) bearing single and double asterisks significantly differ between before and after treatment measurements at P<0.05 and P<0.001, respectively.

Table 2: The numbers of oocyst per gram feces in goat kids with cryptosporidiosis before and after tylosine treatment

Parameter (%)	Treatment group (n:10)		Control Group (n:10)	
	Before Treatment	Post Treatment	Before Treatment	Post Treatment
Consistency of feces	80 (SL) 20(L)	100 (P)	80 (SL) 10 (L) 10(P)	50 (P) 30 (SL) 20 (L)
Rate of infection	60 (2) 30 (3) 10 (1)	100 (0)	40 (1) 50 (2) 10 (3)	100 (0)
Mean OPG counts	20 (10 ⁴) 50 (10 ⁵) 30 (10 ⁶)	100 (0)	30 (10 ⁴) 50 (10 ⁵) 20 (10 ⁶)	100 (0)

L: liquid; SL: semiliquid; P: pastose

Table 3: Hematological value in goat kids suffering from cryptosporidiosis before and after tylosine treatment

Parameters	Study group		Control group	
	Before	After	Before	After
Total leukocyte counts (×10 ⁹ /L)	11.7±0.6	12.5±0.7	11.2±0.6	12.8±0.4
Neutrophils (×10 ⁹ /L)	6.3±0.5	6.6±0.7	4.3±0.4	5.8±0.6
Lymphocytes (×10 ⁹ /L)	4.6±0.4	5.2±0.3	6.8±0.6	6.2±0.7
Monocytes (×10 ⁹ /L)	0.73±0.1	0.52±0.2	0.5±0.2	0.4±0.1
Total erythrocyte counts (10 ¹² /L)	10.9±0.33	10.1±0.53	12.8±0.1	11.4±0.1
Hemoglobin (g/L)	133±0.4	85±0.3	124±0.2	101±0.2
Hematocrit (%)	41.5±0.7*	28.1±0.5*	39.4±0.6	34.4±0.6

Values (mean±SE) bearing asterisk differ significantly (P<0.05) between study group and control group.

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