



RESEARCH ARTICLE

Evaluation of clinical efficacy of gamithromycin in the treatment of naturally infected neonatal calves with cryptosporidiosis

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Cryptosporidiosis ile doğal olarak enfekte olan neonatal buzağların tedavisinde gamitromisin klinik etkinliğinin değerlendirilmesi

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Öz

Amaç: Bu çalışmada kriptosporidiozis ile doğal olarak enfekte olmuş neonatal buzağların tedavisinde gamitromisinin klinik etkinliğinin belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: 5-20 günlük 20 diyareli neonatal buzağı oluşturdu. Kriptosporidiozis tanısı mikroskopik inceleme ve ELISA testi ile konuldu. Dışkıda gram başına 4×10^4 ile 15×10^6 arasında kriptosporidium ookisti görülen buzağlar çalışmaya dahil edildi. Hemogram ve kan gazları tedavinin başlangıcında ve tedaviden sonra ölçüldü. Buzağlara subkutan olarak tek doz 6 mg/kg dozunda gamitromisin uygulandı. İlacın etkinliği, 5. güne kadar ishalin durumu, ookist saçılımı ve vücut durumunun değerlendirilmesi ile takip edildi.

Bulgular: Beşinci günde, altı tane buzağı dışkısında ookist saptanmazken, 11 buzağı dışkısında ookist sayısı önemli oranda azaldı. Tedavinin başlangıcında ve tedaviden sonra kan değerlerinde istatistiksel olarak belirgin fark ($p<0.05$) gözlemlendi.

Öneri: Hem klinik iyileşmeye hem de dışkıdaki ookist sayısındaki azalmaya bağlı olarak, gamitromisinin kriptosporidiozis ile doğal olarak enfekte olmuş buzağların tedavisinde orta derecede etkiye sahip olduğu belirlendi.

Anahtar kelimeler: Buzağı, kriptosporidiozis, tedavi, gamitromisin

Abstract

Aim: The purpose of this study was to investigate the clinical efficacy of gamithromycin in the treatment of naturally infected neonatal calves with cryptosporidiosis.

Materials and Methods: 5-20 days old 20 diarrheic neonatal calves were used as material in this study. Diagnosis of cryptosporidiosis was made by microscopic examination and ELISA test. Calves shedding *Cryptosporidium* oocysts between 4×10^4 and 15×10^6 per gram in feces were included in the study. Hemogram and blood gases were measured at the beginning of the treatment and after the treatment. A single dose of 6 mg/kg body weight of gamithromycin was administered subcutaneously. Drug efficacy was assessed by evaluating the existence of diarrhea, oocyst shedding and body condition from day 1 to 5.

Results: On 5th day, the medicated 6 calves had no oocysts, and number of oocysts in feces had been decreased in 11 calves on 5th day. Statistically significant difference ($p<0.05$) was observed in the blood values at the beginning of the treatment and after the treatment.

Conclusion: It has been determined that based on both clinical improvement and decrease in oocyst count in feces, gamithromycin was found to have moderate effect in the treatment of cryptosporidiosis in naturally infected neonatal calves.

Keywords: Calf, cryptosporidiosis, treatment, gamithromycin



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Introduction

Diarrhea is one of the most significant diseases of neonatal calves. As the results of infection in calves, high mortality and morbidity, expenditure of treatment, growth rate reduction occur. Neonatal enteritis is an economically important problem in calves worldwide (Ok et al 2009, Sen et al 2009, Coşkun et al 2010). *Cryptosporidium* spp. play an important role as an etiological agent in neonatal diarrhea (Santin 2013, Garro et al 2016, Thakre et al 2016, Cenesiz et al 2017, Aydogdu et al 2018, Stebbins et al 2018). The disease is widespread among animals and humans (Sarı and Arslan 2012, Askari et al 2016, Mekonnen et al 2016, Dinler et al 2017). *Cryptosporidium parvum* is the main causative agent of cryptosporidiosis (Santin 2013, Askari et al 2016, Ok et al 2020). Cryptosporidiosis is observed in 5-20 days old calves. *Cryptosporidium* spp. causes severe villi damages in the intestines by multiplying at the microvillus borders of enteric epithelium (Gookin et al 2006).

Many therapeutic and protective drugs have been developed for cryptosporidiosis in calves (Jarvie et al 2005, De Waele et al 2010, Masood et al 2013, Connor et al 2017). However, two of these drugs, halofuginon lactate and paromomycin, have been licenced for calves in some countries (Pines and Nagler 1998). It has been determined that halofuginon lactate and paromomycin is effective on calves with cryptosporidiosis (Viu et al 2000, Jarvie et al 2005, Klein 2008, Masood et al 2013, Aydogdu et al 2018). In addition, monensin, toltrazuril, azithromycin, decoquinate, albendazole, metronidazole and nitazoxanide are being used (Fathi et al 2011, Masood et al 2013, Yagci et al 2017). In the treatment of cryptosporidiosis, macrolides are mostly used as antibiotic group. Gamithromycin, a long-acting macrolide, is used for the treatment and control of Bovine Respiratory Disease of cattle. Therefore, we suggest that gamithromycin may be effective in the treatment of cryptosporidiosis.

Several macrolide antibiotics are effective against cryptosporidiosis, therefore gamithromycin, which is a longer-acting macrolide, is also thought to be effective in the treatment of calves with cryptosporidiosis. The goal of the present study was to determine the clinical efficacy of gamithromycin in the treatment of cryptosporidiosis in naturally infected neonatal calves.

Material and Methods

Animals

Twenty neonatal diarrheic calves infected with cryptosporidiosis (13 Holstein breed, 4 Simmental and 3 Brown Swiss) were admitted to the clinics of Sencuk University, Faculty of Veterinary Medicine. The ages of calves were between 5 to 20 days. The diarrheic calves had anorexia, general weakness,

loss of condition, moderate dehydration and yellow mucoid watery diarrhea. Calves had diarrhea for 2-3 days. The infected calves were diarrheic and moderately dehydrated (moderate acidemia). Calves with cryptosporidiosis infected with *E.coli*, *Rotavirus* and *Coronavirus* were not included, and only calves with cryptosporidiosis were involved in the study.

Clinical examination and diagnosis of cryptosporidiosis

A routine clinical examination was performed to all diarrheic calves. Clinical examination was consisted of evaluation of diarrhea, dehydration, and measurement of body temperature, heart and respiratory rate. The diagnosis of cryptosporidiosis was made by microscopic examination followed by enzyme linked immunosorbent assay (ELISA) (BovID-5 Ag®, Bio-X Diagnostics, Belgium) technique to detect *Cryptosporidium* oocysts and parasite antigens in fecal samples. Calves shedding *Cryptosporidium* oocysts between 4×10^4 and 15×10^6 (mean score: 3.87 million) per gram feces were included in the study.

Sample collection and blood analysis

The blood samples were obtained by jugular venipuncture. Venous blood pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), oxygen saturation ($SatO_2$), base excess (BE), lactate, sodium (Na), potassium (K) and chlorine (Cl) levels were measured immediately after blood sampling before initiating to treatment and at the end of treatment using an automatic blood gas analyser (GEM Premier 3000, Instrumentation Laboratory, Lexington). Complete blood count was measured immediately after blood sampling before initiating to treatment and at the end of treatment using a MS4e-Vet hematology analyzer (Melet Schlosing Laboratories, France).

Treatment protocol

Single dose of gamithromycin (6 mg/kg/SQ, Zactran, Boehringer®, Austria) was administered to each infected calf. In addition, standard diarrhea treatment which was consisted of intravenous fluids (1.3% $NaHCO_3$, Ulugay®, Turkey, and 5% dextrose, Desktrosol, Vilsan®, Turkey) were administered slowly as required, individually. Colloid fluid (hydroxyethyl starch, Voluven®, Fresenius Kabi Deutschland GmbH, Germany) was also used at doses of 5-10 mL/kg/hr as in cases of need. Single dose vitamin A, D, E (1 mL/day/IM, Ademin®, Ceva-Dif, Turkey) and vitamin C (3 mL/day/SQ, Cevit®, Biovita, Turkey) were administered during treatment protocol every day for 5 consecutive days.

Cryptosporidium oocysts count

The fecal samples were taken from infected calves 3 times a day to count oocyst numbers per gram of feces during 5

days of hospitalization. Collected fecal specimens, from twenty calves which were diarrheic and infected with cryptosporidiosis were examined by a quantitative technique to estimate the *Cryptosporidium* oocyst number. Briefly, 1 g of feces mixed with 1 mL phosphate buffer saline (PBS, pH: 7.4) to obtain a suspension, then the suspension was re-suspended with PBS at 1/1000 dilution. 10 µL aliquots of this suspension was dropped on a slide using a micropipette, air dried and methanol was used for fixation. Fixed drops were stained with Modified Ziehl Neelson (MZN) method. The stained preparations were examined by light microscope at 100 x magnification with immersion oil, and the oocyst number in 1 g of feces was calculated.

Statistical analysis

All data were submitted as mean values and standard errors (mean±SEM). Student's t-test and Wilcoxon test were used for parameters and oocyst numbers, respectively. SPSS software for Windows, version 14.01 was employed for the statistical analysis.

Results

In this study, 16 of 20 calves with cryptosporidiosis responded to the therapy (80%). Four of infected calves did not respond to the treatment. Two calves died despite treatment. Loss of appetite, dehydration, depression, decrease in suckling reflex and weight loss were observed in calves with cryptosporidiosis. After 2 days of treatment, increase in appetite, partial recovery in the suckling reflex and improvement of general clinical appearance were observed. On the 3rd day of treatment period, the feces of the calves began to be solidify, and their feces became fully normalized on 4th day of the treatment period. 16 of the 20 calves with cryptosporidiosis clinically recovered on 5th day of treatment.

The parameters of blood gases and hemogram are submitted in Table 1. Statistically significant ($p<0.05$) difference was observed in the blood pH, $p\text{CO}_2$, BE, HCO_3^- , K, Na and Cl values at the beginning of the treatment and after the treatment. Statistically significant ($p<0.05$) difference in white blood count (WBC) was detected at the beginning of the treatment and after the treatment.

Table 1. Hemogram and blood gases values in the all calves with cryptosporidiosis (mean±SEM)

Parameters	Before treatment (n:20)	After treatment (n:20)	p value
pH	7.26±0.14	7.37±0.07	0.001
$p\text{CO}_2$ (mmHg)	38.1±6.87	48.0±7.88	0.000
$p\text{O}_2$ (mmHg)	28.5±7.98	27.1±4.89	0.167
SatO ₂ (%)	57.5±14.1	51.6±14.0	0.153
K (mmol/L)	4.91±0.21	4.37±0.14	0.039
Na (mmol/L)	129.7±12.0	140.9±11.2	0.000
Cl (mmol/L)	96.1±10.0	101.3±9.02	0.001
Lactate (mmol/L)	2.15±2.56	1.84±1.32	0.446
BE	-8.43±9.73	2.63±5.16	0.000
HCO_3^- (mmol/L)	18.0±6.92	25.8±4.02	0.000
WBC ($\times 10^9/\text{L}$)	15.4±7.84	11.5±5.82	0.024
RBC ($\times 10^{12}/\text{L}$)	9.55±1.93	9.39±2.08	0.512
HTC (%)	34.9±8.91	34.4±9.71	0.627
Hb (g/dL)	11.2±2.59	11.0±2.85	0.568
PLT ($10^9/\text{L}$)	438.4±189.7	530.1±261.8	0.132

$p\text{CO}_2$ = partial pressure of carbon dioxide; $p\text{O}_2$ = partial pressure of oxygen; SatO₂(%) = oxygen saturation; K = potassium; Na = sodium; Cl = chlorine; BE = base excess; HCO_3^- = bicarbonate; WBC = white blood count; RBC = red blood cells; HTC = hematocrit; Hb = hemoglobin; PLT = platelet



In fecal samples obtained from all calves included in this study, the presence of *Cryptosporidium* oocysts detected at 100 x magnification with immersion oil by light microscopy was confirmed by ELISA test.

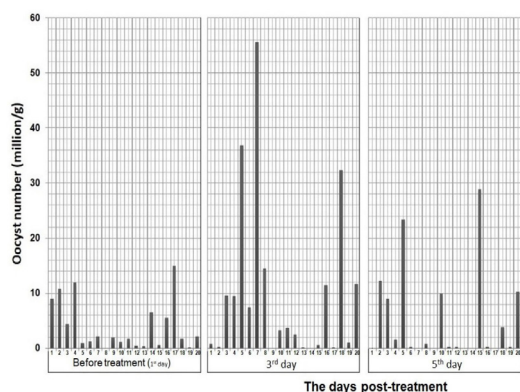


Figure 1. Each column shows naturally infected calves with cryptosporidiosis in order from 1 to 20 in different days of study

As shown in (Figure 1) oocyst number was ranging from 0.04 million to 14.90 million in 1 g feces (mean score: 3.87) at the beginning of the study trials. In the middle of the treatment (approximately 3rd day of treatment) the oocyst number was counted between zero and 55.60 million (mean score: 10.07). On 5th day post-treatment, the oocyst number ranged from zero to 28.86 million (mean score: 5.06). During the study trials, two of twenty calves could not survive despite treatment, and died between 4th and 5th days of the treatment period. On the 3rd day of treatment period an increase of oocyst number in feces was detected and it was thought to be due to life cycle of the parasite. Also it was evaluated that gamithromycin had a slight effect on the oocyst quantity in feces which was determined by the decreased number of the oocysts in the 11 of 20 calves at the end of study. Two of infected calves died due to diarrhea. Even though seven calves had high level of oocyst number in feces, improvement of clinical conditions were observed in 5 of 7 calves at the end of the study. However, the other two calves could not recovered.

The number of oocysts on the day of treatment and 5th day after treatment were analyzed with the Wilcoxon test, but no statistically significant difference was found ($p>0.05$).

Discussion

In the study, the clinical efficacy of gamithromycin in the treatment of cryptosporidiosis was evaluated in naturally infected neonatal calves, and the results were found to be moderately successful. Cryptosporidiosis is one of the life-threatening parasitic diseases affecting neonatal calves.

Also it is an important zoonotic disease of domestic and wild animals. Cryptosporidiosis causes diarrhea, weight loss, dehydration and even death in neonatal calves, and significant economic losses can be occurred (Sarı and Arslan 2012, Mekonnen et al 2016, Yagci et al 2017, Aydogdu et al 2018, Ok et al 2020). Infected calves may display clinical symptoms ranging from asymptomatic clinical course to diarrhea and dehydration (Connor et al 2017). In the present study, the clinical signs such as loss of appetite, diarrhea, dehydration, depression, decrease in suckling reflex and weight loss were observed in calves with cryptosporidiosis. During the treatment protocol, increase in appetite, partial recovery in their suckling reflex and partial improvement of general clinical appearance were monitored in infected calves after the 2nd day of the treatment. On 3rd day of treatment period, the feces of the infected calves began to be solidify and became fully normalized on the 4th day of the treatment. Our findings were found to be consistent with earlier reports (Connor et al 2017, Aydogdu et al 2018).

Several pharmaceuticals have been used for cryptosporidiosis in animals. However, treatment of cryptosporidiosis is tedious since a safe and effective treatment protocol has not been constituted yet (Yagci et al 2017). Drugs used in the treatment and prevention of cryptosporidiosis can be listed as halofuginone lactate, paromomycin, azithromycin and nitazoxanide (Viu et al 2000, Jarvie et al 2005, Klein 2008, Fathi et al 2011, Masood et al 2013, Yagci et al 2017, Aydogdu et al 2018). De Waele et al (2010) reported that the control of cryptosporidiosis could be succeeded by using preventive halofuginone lactate. Fathi et al (2011) stated that monensin and toltrazuril were effective on *C. parvum* oocysts, and monensin was more effective than toltrazuril.

In the present study, clinical improvements were observed in 16 of 20 calves. Gamithromycin was found to be moderately effective on the quantity output of *Cryptosporidium* oocysts in 6 calves which had no oocysts in their feces on the 5th day of treatment. Additionally, it was also recorded that oocyst quantity output was decreased in 11 calves prominently. However, a statistically significant difference could not be determined. It is considered that this may be due to the high number of oocysts detected in some animals on day 5 or the short observation period applied as 5 days in the study. Villacorta et al (1991) were also reported similar findings in a study regarding the efficacy of halofuginone lactate against cryptosporidiosis. Clinical, hematological and blood gas findings showed that clinical improvements occurred in the infected calves following by gamithromycin and fluid therapy administration. When compared with current agents used in the treatment of cryptosporidiosis which are usually administered orally for 5 or 7 consecutive days, the advantage of gamithromycin is sufficient single parenteral dose administration. Therefore, treatment resulted in recovery of clinical symptoms, and diarrhea stopped in most of the medicated



calves. Gamithromycin was shown to be effective in clinical improvement of calves infected with cryptosporidiosis.

Dehydration, metabolic acidosis and electrolyte imbalance are common findings in diarrheic calves with cryptosporidiosis (Yagci et al 2017, Aydogdu et al 2018). Thakre et al (2016) reported that leucocyte counts increased significantly in calves with cryptosporidiosis. In the calves with cryptosporidiosis, hyperkalemia was detected due to decreased bicarbonate level and metabolic acidosis. Therefore, fluid treatment (1.3% NaHCO₃ and 5% dextrose) and gamithromycin were administered to all of the infected calves. Clinical improvement was recorded with the treatment protocol in 16 of 20 calves with cryptosporidiosis. Four calves did not respond to treatment, and two of them died despite treatment. In addition, we detected significant leukocytosis in calves with cryptosporidiosis. Our results were consistent with earlier researchers' results (Thakre et al 2016, Yagci et al 2017, Aydogdu et al 2018).

Conclusion

As a result of the study, based on both clinical improvement and decrease in oocyst count in feces, gamithromycin was found to have moderate effect on the treatment of cryptosporidiosis in neonatal diarrheic calves.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

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Critical Review: Mahmut Ok, Ferda Sevinç

Ethical Approval

Approval of the study was obtained from institutional ethics board of the Veterinary Faculty of Selcuk University (No: 2020/52)

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