Veterinary Parasitology 170 (2010) 149–152

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Evaluation of oral tilmicosin efficacy against severe cryptosporidiosis in neonatal kids under field conditions

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ARTICLE INFO

Article history:
Received 28 October 2009
Received in revised form 5 January 2010
Accepted 15 January 2010

Keywords:
Cryptosporidium parvum
Goat kids
Control method
Macrolide antibiotic

ABSTRACT

Many compounds have been screened for their potential anti-cryptosporidial activity in ruminants but none of them has been totally efficient in controlling the disease. Macrolide antibiotics have demonstrated some activity against *Cryptosporidium* spp. in humans. Tilmicosin is a macrolide antibiotic, available in France in an oral form (Pulmotil® AC, Lilly France). The preventive efficacy of tilmicosin was evaluated in a goat farm experiencing severe clinical cryptosporidiosis in kids. Twenty-two kids were separated from their dams just after birth and placed in a separated pen. They were divided into 3 groups: an untreated group (10 kids), group 1 (6 kids) receiving tilmicosin at 25 mg/kg BW/day and group 2 (6 kids) receiving tilmicosin at 50 mg/kg BW/day. Tilmicosin was individually given by oral route from day 2 of age for 10 days. Three times a week, individual faecal samples were performed to assess the oocyst output. Clinical data, i.e. diarrhea and mortality, were recorded. In control kids, the highest prevalence and intensity of excretion were observed between day 6 and day 16 of age and mortality reached 90%. Excretion dynamic and clinical consequences were similar in both treated kid groups. Finally, tilmicosin did not demonstrate any activity against severe kid cryptosporidiosis in field conditions.

1. Introduction

Cryptosporidium parvum is a highly prevalent protozoal parasite in young ruminants. It is one of the most important agents of neonatal diarrhea in goat kids aged less than 21 days (Muñoz et al., 1996). Morbidity in this species can reach 100% and mortality can be of 50% (Chartier et al., 1996). Many compounds have been tested against cryptosporidiosis in humans and animals (Stockdale et al., 2008). Several of them have received attention for the treatment of this disease in ruminants: paromomycin (Chartier et al., 1996; Grinberg et al., 2002; Viu et al., 2000), halofuginone (Chartier et al., 1999; Giadinis et al., 2008; Klein, 2008; Silverlås et al., 2009), nitazoxanide (Viel et al., 2007; Ollivett et al., 2009; Schnyder et al., 2009). Nevertheless, none of them has demonstrated a clear efficacy for a curative use and when given in a prophylactic way, results were better although are not totally satisfying. Antibiotics of the macrolide family have been tested for the treatment of cryptosporidiosis in humans with some therapeutic or prophylactic effects: spiramycin, azithromycin, clarithromycin, roxithromycin (Stockdale et al., 2008). In veterinary medicine, several antibiotics of this family are available: spiramycin, tylosin, erythromycin, tilmicosin but none of them has been tested in a controlled study against cryptosporidiosis in ruminants (Stockdale et al., 2008). Tilmicosin is available in France through an oral form used for the treatment of respiratory diseases in calves and pigs (Pulmotil®AC, Lilly France). The objective

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doi:10.1016/j.vetpar.2010.01.024

of the present study was to evaluate tilmicosin efficacy administered by oral route for prophylaxis of cryptosporidiosis in naturally infected goat kids.

2. Materials and methods

The study was carried out in March 2009 in a commercial dairy goat farm experiencing severe cryptosporidiosis in kids. At the start of the survey, the kid mortality rate was estimated 60% according to the farmer. Cryptosporidiosis diagnosis was confirmed before the beginning of the study by examination of several faecal smears from ill kids using the Heine staining method.

Twenty-two kids, born on 2 consecutive days, were separated from their dams 12 h after birth and were placed all together in a separated pen. They were fed milk replacer *ad libitum* with a multiteat outlet container. They were randomly divided into 3 groups identified by necklaces of different colours:

- Group 1: 10 kids acted as control ones and did not receive any treatment.
- Group 2: 6 kids received tilmicosin (Pulmotil®AC) with an oral daily dose of 25 mg/kg body weight (BW) from 2 days old for 10 days. This daily dose rate is the standard antibiotic dose rate. The duration of administration was defined in agreement with previous trials performed with paromomycin (Chartier et al., 1996).
- Group 3: 6 kids received tilmicosin (Pulmotil®AC) with an oral daily dose of 50 mg/kg BW from 2 days old for 10 days.

The treatment was conducted by the farmer. The product was diluted in a small amount of milk replacer and distributed individually with a syringe. No other treatment was applied during the experiment.

The farm was visited 3 times a week for 3 weeks. At each date, the following samples and records were done:

- Individual faecal samples with rectal curettes.
- Diarrhea scoring according to the followings: 1: solid faeces, 2: soft faeces, 3: liquid faeces. This record was always done by the same investigator.
- Recording of the dates for each individual treatment, of dead animals and of all the events which could have influenced the results.

Animals were weighed once a week.

The dead animals were necropsied. Faeces were collected and tested for *Cryptosporidium* sp. Appropriate samples were collected for diagnosis of bacterial infections.

Demonstration of oocysts was made by staining faecal smears with Ziehl fuschin and observing at 100× magnification under a phase-contrast microscope according to Heine (1982). The intensity of excretion was evaluated semi-quantitatively according to the average number of oocysts in 10 randomly selected fields. Six categories were defined: 0: absence of oocyst, 1: <1 oocyst per field, 2: 1–10 oocysts per field, 3: 11–20 oocysts per field, 4: 21–30 oocysts per field and 5: >30 oocysts per field. One person performed and read all tests.

The criteria for assessing the development of infection and the efficacy of treatment were: prevalence and intensity of oocyst excretion, presence of diarrhea, mortality rate and weight gain.

DNA was extracted from 7 *Cryptosporidium* positive samples of the control group using the UltraClean Fecal DNA kit (MO BIO Laboratories, Inc.) according to the manufacturer's instructions. Amplification by nested PCR of a part of the 18S rDNA gene followed by sequencing was used to determine the *Cryptosporidium* species of each isolate (Xiao et al., 1999).

Comparison of diarrhea scores between groups was made by a Kruskal–Wallis test at the 0.05 level of confidence at each date (except the 4 last sample dates when there was only one kid in the control group). Correlation between oocyst excretion and faecal consistency in the control group was made by a Spearman correlation rank order test at the 0.05 level of confidence.

A repeated measure analysis of variance model with one grouping factor (group) and one within factor (date) was used to compare oocyst shedding scores between groups ($p < 0.05$) (after exclusion of the 4 last sample dates when there was only one kid in the control group).

Oocyst excretion by goat kids that died during the study was compared between the groups by a Kruskal–Wallis test at the 0.05 level of confidence.

Comparison of mortality rates between control group and treated groups was done using the Fisher's exact test ($p < 0.05$).

Mean weights were compared at the first and second weighing dates by a Kruskal–Wallis test at the 0.05 level of confidence.

Data analyses were performed using Systat 9.1 for Windows 1998, SPSS Inc. (Chicago, USA).

3. Results

In the control group, kids excreted oocysts from the age of 3–4 days to the age of 15–16 days. The prevalence of excretion was maximal between 6 and 16 days when 100% of the sampled kids shed oocysts (Table 1). Intensity of excretion was maximal at the same dates with a mean score varying between 4.8 and 5. During the period of highest excretion, all the kids developed diarrhea. For the sole surviving kid at the end of the study, the diarrhea persisted 10 days continuously (beginning at 5–6 days and end at 15–16 days). Faecal consistency and oocyst excretion were significantly correlated ($p < 0.01$): diarrhea score increased while oocyst shedding increased.

Similar results were obtained in the treated groups. In group 1, kids began to excrete oocysts at age of 3–4 days with a maximal prevalence (100%) from day 6 to day 14 and the highest intensity at 8–9 days (mean score: 5) (Table 1). In group 2, kids began to excrete oocysts at age of 3–4 days with a maximal prevalence (100%) from day 6 to day 16 and the highest level (mean score: 5) between days 8 and 11. No significant difference in the dynamic of oocyst shedding was seen between the two treated and the control groups ($p = 0.142$). Moreover, kids of both the