Effect of Buparvaquone on Cryptosporidium parvum Oocysts Shedding in Calf

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INTRODUCTION

Cryptosporidium parvum, a coccidian agent, is now recognized as one of the leading causes of diarrhea in young calves (Anderson, 1998). The frequency of infection is highest in calves at 1-3 months of age. In one study, almost one-half of the calves at this age group were shedding oocysts (NISHMS, 1993). Cryptosporidiosis may appear as an asymptomatic or mild to profuse diarrhoeic form (Fayer and Ungar, 1986) Cryptosporidium parvum can cause excessive fluid and electrolyte losses with subsequent metabolic acidosis, negative energy balance and death (Constable, 2002). A major problem in controlling C. parvum is the lack of an effective means for preventing or treating infection (Harp and Goff, 1998). To date, no agents have been recommended for the prevention or therapy of cryptosporidiosis in calves. Most agents used to treat C. parvum infection have been either ineffective or toxic (Blagburn and Soave, 1997; Moon et al., 1982). Paromomycin and halofuginone lactate (Halocur) have shown efficacy in treating and reducing oocysts shedding, but the first drug is not currently licensed for use in food animals and some research indicating that halofuginone lactate may have negative effects on growth, feed intake and feed efficiency (Harp and Goff, 1998; Committee for Veterinary Medical Products, 1999). Buparvaquone, a new naphthoquinone antibiotic, has recently been considered for Cryptosporidium parvum treatment in human (Armson et al., 1999). To our knowledge, there has not been any report considering the anti-cryptosporidial efficacy of buparvaquone in calf. The objective of this field trial was to assess the effect of buparvaquone on Cryptosporidium oocyst shedding in calf.

MATERIALS AND METHODS

This trial was conducted on dairy farms around Tehran from January 2005 through February 2006. A total number of 18 diarrheic calves of 25-36 day old, shedding Cryptosporidium oocysts in their stool were material for this study. The calves were divided randomly into two equal groups. Animals of the first group were given intramuscular dosages of buparvaquone which was manufactured in a solution of 50 mg mL⁻¹ (Butalex, Arfan Daroo, Tehran Iran) at a recommended dosage (2.50 mg kg⁻¹) while the others received the same volume of saline as sham injections. The two groups were followed up for 1 month while their stool specimens were examined microscopically on
the first, third, sixth, fourteenth and thirtieth days of injection for the number of oocysts per high power field. *Cryptosporidium parvum* oocysts were identified as spherical, pink-tinged, refractive structures approximately 4.5 μm in size. Oocysts were quantified by enumerating the number of oocysts in 10 fields and multiplying the average number per field by the number of fields in the cover-slip area to evaluate potential associations between intensity of *C. parvum* shedding and treatment with buparvaquone during the trial period. The Oocysts Shedding Severity (OSS) was scored for each specimen as following: 0: (without oocysts); 1: (0 < oocysts ≤ 5); 2: (5 < oocysts ≤ 10); 3: (10 < oocysts ≤ 20); 4: (20 < oocysts ≤ 50); 5: (50 > oocysts). The time period of shedding the oocysts as well as the severity of the shedding on each day of examination were the measures utilized to compare these two groups based on. Kolmogorov-Smirnov (KS), Friedman, Wilcoxon, Mann-Whitney U (MWU) and Chi-square tests from SPSS version 12.0 were utilized to analyze data.

RESULTS AND DISCUSSION

KS test showed that data did not follow a normal distribution leading to usage of non-parametric tests for analysis. MWU showed the animals of the two groups had the same OSS on the day of injection (p>0.05) whereas on day 3, OSS of control group was significantly more (p<0.05) than that of the treated group (Table 1). However, OSS of these two groups did not differ significantly in the subsequent days of examination. Friedman test showed improvement by time in both groups (p<0.001); meanwhile, compared with the day of injection, animals of treated group improved significantly on day 3 itself (Wilcoxon, p<0.05) whereas control group improved only on day 6 (Wilcoxon, p<0.05). Chi-square proved no significant differences between the two groups in the proportion of the animals whose stool specimens were positive for oocysts, on different days (p>0.05).

The results of this study showed that in the treated group the length of shedding period was significantly (p<0.05) lower than the control. We also noticed that on the day 3 after treatment with buparvaquone calves had a significant reduction in oocysts shedding. The nature of the disease in our cases was almost self-limited as even calves who received no treatment in the control group, reduced in shedding oocysts. So, when the animals in control group showed a rapid reduction in oocyst shedding, the drug, although reducing the oocysts in number, would not be able to make a statistically significant reduction and this may justify why the drug was not significantly effective after the 3rd day of injection.

In an experimental model of a more severe disease, the drug may prove to be more effective by reducing the oocysts in longer time periods. Also, in our next study, we will examine the effect of multi-dosage vs. single dosage of buparvaquone on experimentally infected calves to evaluate the anti-cryptosporidial efficacy of the medicine.

Table 1: Data representing the number of oocysts per high power field and the time period of shedding oocysts by the calves in control vs. treated group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Oocysts per high power field</th>
<th>Shedding oocysts (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 3</td>
</tr>
<tr>
<td>Control</td>
<td>4.00</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>(3.00)</td>
<td>(3.00)</td>
</tr>
<tr>
<td>Buparvaquone</td>
<td>3.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>(4.00)</td>
<td>(3.00)</td>
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REFERENCES