Preventive Veterinary Medicine 96 (2010) 143–151

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

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

Control of cryptosporidiosis in neonatal calves: Use of halofuginone lactate in two different calf rearing systems

V. De Waele[a,b,*], N. Speybroeck[c,d], D. Berkvens[c], G. Mulcahy[b], T.M. Murphy[a]

[a] Central Veterinary Research Laboratory, Department of Agriculture, Fisheries & Food, Celbridge, Co. Kildare, Ireland
[b] School of Agriculture, Food Science & Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
[c] Department of Animal Health, Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium
[d] Ecole de santé publique, Université Catholique de Louvain, Clos Chapelle-aux-Champs, 1200 Bruxelles, Belgium

ARTICLE INFO

Article history:
Received 24 July 2009
Received in revised form 30 May 2010
Accepted 27 June 2010

Keywords:
Cryptosporidium
Calves
Diarrhoea
Halofuginone
Rearing system
Generalized estimating equations models

ABSTRACT

To date there is no effective treatment for bovine cryptosporidiosis. This study describes the use of halofuginone lactate in preventing cryptosporidiosis in naturally infected neonatal calves on a dairy farm with a high prevalence of infection. The animals were kept in two different calf rearing systems. A randomized double-blind trial was carried out with 32 naturally infected calves, divided into four groups. The two prophylactic halofuginone lactate treated groups were kept in either individual or group pens. Similarly, the animals receiving the placebo were housed in either individual pens or together in a large pen. A total of ten faecal samples were collected periodically during the 28 days study from each calf and tested for the presence of *Cryptosporidium* spp. using microscopic and molecular methods. Generalized estimating equations models were used to determine if the effects of the various treatments and/or rearing systems on the presence of diarrhoea and infection were statistically significant. Further analysis (classification trees models) was carried out to explore possible risk factors for cryptosporidiosis and interactions between treatments and rearing systems. Halofuginone lactate was shown to be effective in reducing clinical signs of cryptosporidiosis and environmental contamination. However, the treatment did not delay the onset of diarrhoea and did not reduce the risk of infection amongst calves reared together in a highly contaminated environment. The use of halofuginone lactate in combination with good hygienic measures, such as rearing animals in clean individual pens, was the most effective method to reduce the risk of cryptosporidiosis amongst 7–13 days old calves. It was concluded that the control of the parasite could be achieved by the combination of using effective preventive drugs, such as halofuginone lactate and good animal husbandry procedures.

1. Introduction

The zoonotic apicomplexan *Cryptosporidium parvum* is considered the most common enteropathogen of neonatal calves (de la Fuente et al., 1998; Santin et al., 2008). Infected calves can exhibit clinical signs ranging from asymptomatic infection to profuse diarrhoea and dehydration (Fayer et al., 1998; Thompson et al., 2007). These animals readily contaminate their immediate environment as total oocysts

* Corresponding author at: Central Veterinary Research Laboratory, Department of Agriculture, Fisheries & Food, Backweston Campus, Celbridge, Co. Kildare, Ireland. Tel.: +353 16157208; fax: +353 16157253.
E-mail address: valerie.dewaele@agriculture.gov.ie (V. De Waele).

0167-5877/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.prevetmed.2010.06.017

output per infected calf can be up to 10^{10} over a week (Fayer et al., 2004).

A major problem concerning *C. parvum* is the lack of an effective means for controlling infection and decreasing environmental contamination with oocysts. Because oocysts are highly resistant to environmental stresses and to many disinfectants, hygienic measures on their own are not sufficient to avoid infection and long term contamination of calf rearing facilities (O'Donoghue, 1995). In addition, many drugs and vaccines have been evaluated as potential therapeutic or prophylactic agents for cryptosporidiosis but with little success (Santin and Trout, 2008). Halofuginone lactate is a synthetic quinazolinone with cryptosporidiostatic activity on the sporozoite and merozoite stages of *C. parvum* (Jarvie et al., 2005). It has been recommended for both therapeutic and prophylactic use as it delays the onset of infection, reduces shedding of oocysts, and decreases the severity of cryptosporidiosis in calves (Joachim et al., 2003; Jarvie et al., 2005). Its effectiveness as a prophylactic treatment has not been evaluated for the various calf rearing systems used in Ireland. The primary objective of this study was to evaluate the effect of halofuginone lactate in decreasing the number of diarrhoeic calves kept in two rearing systems on a dairy farm with a high prevalence of cryptosporidiosis amongst neonatal calves. The secondary objective was to test the effect treatment and rearing systems may have on the onset of diarrhoeic signs and oocysts shedding, as well as on the number of calves excreting oocysts and the level of this excretion.

2. Materials and methods

2.1. Study design

A randomized double-blind trial was carried out during the period March to May 2005 in a dairy herd of 400 cows situated in Co. Westmeath, Ireland. The herd was selected on the basis of a previous study in which the prevalence of *C. parvum* was estimated by Bayesian analysis to be 98% (credibility interval: 92–100) in 2-week-old calves (unpublished data). In this herd, calving occurred throughout the year. Cows were moved to a calving pen approximately 1 week before calving and re-introduced to the milking herd 24 h after calving. The straw bedding of the maternity pen was changed every 6 weeks. Calves were fed 2 l of their dam's colostrum and separated from their mother within 12 h of birth. Commercial vaccines were not administered to cows or calves.

All Holstein Friesian calves born during the 3-week period, March 29th–April 19th, 2005, were included in the experiment, with the exception of one calf that died a few hours after birth. Newborn calves ($n = 32$) were allocated to their respective experimental groups using a table of random numbers. The first group consisted of calves treated with halofuginone lactate (Halocur, Intervet Ireland Ltd.) and maintained in individual calf pens; the second group was also treated with halofuginone lactate but the calves were allowed to mix freely in a large loose box; the third group consisted of calves treated with a placebo and maintained in individual calf pens; and, the fourth group was treated with a placebo and the animals were allowed to mix freely in a large loose box (Table 1). During the time calves were allocated to their respective experimental groups, the number of calves in the loose boxes increased from one initially to a final population of six and eight in the placebo and halofuginone lactate treatment groups, respectively.

The individual calf pens were made of aluminum with a slatted wooden base. The loose boxes had concrete floors. Both were washed with disinfectant (Hyperox, DuPont, UK) before introduction of the calves. These floors were covered with fresh straw every day. Every 2 weeks, the old bedding was removed; the floor was washed with disinfectant and covered with fresh straw.

The placebo solution was prepared according to the procedure described by Jarvie et al. (2005) and was similar in consistency, color and composition to the commercial Halocur without the active ingredient (halofuginone lactate). An equal volume of either halofuginone lactate (100 μg/kg) or placebo was administered orally to the calves in the morning just before feeding for the first 7 days of their life. The first dose of halofuginone lactate or placebo was given within 12 h of birth and after colostrum was fed. The animal handlers were not informed of the various treatments administered to calves until all the data had been collected. Each calf received 2.5 l of whole milk twice daily. Water was supplied *ad libitum* with a ration containing soya, wheat and citrus pulp which was mixed on the farm.

2.2. Parameters recorded

Serum was collected on one occasion from each 1-week-old calf. This was analyzed for the transfer of maternally-derived immunoglobulins using the zinc sulfate test (ZST) (McEwan et al., 1970). A total of ten faecal samples (2 g) were taken from each calf. The calves were sampled on days 1 and 2, and thereafter every second day on days 4, 6, 8, 10, 12 and 14. A further two samples were collected on days 21 and 28. The consistency of the faeces was recorded at the time of collection using the following scoring system: 0 for solid or pasty sample, 1 for liquid sample and 2 for watery

Table 1
Cumulative geometric mean and range of the oocysts per gram (OPG) of faeces excreted by untreated control calves and calves treated with halofuginone lactate during their first 4 weeks and reared in either individual or group pens.

Rearing system	Treatment	Number of calves	Cumulative geometric mean of OPG	Range of OPG
Individual pen	Halofuginone	9	28	0–200,384
	Placebo	9	7096	0–1,703,267
Group pen	Halofuginone	8	842	0–576,105
	Placebo	6	66,581	0–2,554,901