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Prevalence and control of bovine cryptosporidiosis in German dairy herds

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Abstract

In a 5-year survey regarding its prevalence and importance in five German state veterinary laboratories *Cryptosporidium* was diagnosed annually in 19–36% of faecal samples either submitted to the laboratories or taken post mortem. In approximately half of the cases no other enteropathogens were detected. However, only 73% of 30 laboratories participating in a questionnaire survey routinely tested for this parasite, and the majority of researchers considered cryptosporidiosis to be of minor importance. In a placebo-controlled field study 152 suckling calves were treated daily against cryptosporidiosis either with sulfadimidine or with halofuginone (Halocur®, Intervet) over 1 week. Treatment by oral drench started at the onset of diarrhoea in the herd. Oocyst excretion, faecal consistency and health status were recorded five times for a 3-week period. Oocyst excretion peaked 7–14 days in the placebo group after the onset of diarrhoea, and during that period prevalence and intensity of excretion were significantly lower in the halofuginone-treated group compared to the sulfadimidine and the placebo control groups. The health status (diarrhoea, dehydration) declined in all groups but was significantly ($P < 0.05$–0.001) better in the halofuginone group in the first 2 weeks. Halofuginone effectively ($P < 0.05$–0.001) reduced oocyst excretion and improved the health status of the treated animals, while sulfadimidine had no effect against *Cryptosporidium*.

Keywords: *Cryptosporidium* sp.; Epidemiology; Cattle-protozoa; Halofuginone

1. Introduction

The intestinal coccidium *Cryptosporidium* is known to cause self-limiting diarrhoea in neonates, especially calves. Animals are infected by oocysts from their environment and

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show clinical signs of disease (pasty to watery diarrhoea with dehydration) within a few days after infection which can last up to 2 weeks (Rommel, 2000). The large numbers of oocysts that are excreted together with the strong resistance of the exogenous stages against disinfection (Blewett, 1989; Fayer et al., 1996) can turn this parasite into a perpetual threat to animal health and productivity on affected farms. The parasite is found worldwide with prevalences in young calves of 20–40% in different European countries (Anon., 1996; Lentze et al., 1999; see Chermette and Boufassa-Ouzrout, 1988; de Graaf et al., 1999). Apart from the veterinary problems that *Cryptosporidium* can cause, it has the potential to cause zoonotic disease, especially in immunocompromised humans (Fayer et al., 2000; Slifko et al., 2000). In the present study we evaluated the prevalence of cryptosporidia in samples examined at veterinary laboratories and the estimation of investigators regarding the importance of this parasite. In a field trial the efficacy of sulfadimidine and halofuginone against cryptosporidiosis was tested.

2. Materials and methods

2.1. Survey of the prevalence of cryptosporidiosis in samples examined at state veterinary laboratories

In a preliminary survey, 30 state veterinary laboratories in Germany participated in a questionnaire regarding the prevalence and importance of bovine cryptosporidiosis. The questionnaire included questions on the routine examination procedure for samples from young calves submitted to the laboratories, the numbers of animals examined for *Cryptosporidium* and its frequency as well as statements on the role of cryptosporidia as primary or secondary pathogens.

Diagnostic results from five state veterinary laboratories in Germany (Stade, Lower Saxony; Stendal, Saxony-Anhalt; Kassel, Hessia; Bonn, North Rhine-Westfalia; Aulendorf, Baden-Wuerttemberg) from examinations of bovine faeces or carcasses between 1993 and 1997 were evaluated. The evaluation included records of diarrhoea and/or enteritis in calves tested for *Cryptosporidium*. All five laboratories routinely tested for the parasite at post mortem examinations of calves up to 4 weeks of age with diarrhoea and in all faecal samples from calves submitted for post mortem examination. Parasitological examination was based on the staining method of direct smears according to Heine (1982), Pohlenz et al. (1978) or Henriksen and Pohlenz (1981) with semi-quantitative estimation of the intensity. In one case samples were examined using a coproantigen ELISA (Digestiv ELISA Kit®; Bio-X, Belgium). Additional tests included flotation of faecal samples (for *Eimeria*) as well as microbiological and virological tests for infectious causes of enteritis.

2.2. Field trial for treatment of cryptosporidiosis in calves with halofuginone-base (Halocur®, intervet)

Seven dairy herds (13–150 cows) in North Rhine-Westfalia with a history of persisting diarrhoea in the presence of *Cryptosporidium* were included in the study. All calves were kept in separate units with straw bedding for the first 3–4 weeks of life. Except for one farm the units were not disinfected. All animals were fed colostrum for the first 4–6 days of life and received commercial milk exchanger thereafter. From day 7 roughage was offered additionally. Cows of three herds were vaccinated against viral diseases (rota-, corona-, parvovirus) and *E. coli* K99 Pilus Antigen (Lactovac®; Hoechst Roussel Vet GmbH).

2.2.1. Study design

Three groups, comprising 50–52 calves each, randomly chosen from the seven herds (with approximately equal numbers of animals from each farm in each group), were treated orally for 7 days. Animals in group 1 (placebo group) received 10 ml of tap water per day. Group 2 calves (sulfadimidine group) were treated with 10 ml of sulfadimidine (3 g sulfadimidin-Na 100%®, Lohmann Animal Health, Cuxhaven). In group 3 animals were treated daily with Halocur® (0.05 g halofuginone-base/100 ml solution, Intervet) according to their weight, i.e. 2 ml/10 kg body weight corresponding to 7–9 ml/animal. The preparations were administered by the farmers themselves who received three colour-coded, otherwise unlabelled bottles together with colour-labelled 20 ml plastic syringes. The farmers were not informed about the contents of the respective bottles. The treatment began when calves (aged 4–17 days) started signs of diarrhoea. All calves borne thereafter were included in the study and treated for 7 days starting within 24–48 h after birth. The study was continued until 152 calves were treated with one of the preparations.

2.2.2. Clinical examination

Each calf was clinically examined five times during the study. On day 0 all newborn calves (first or second day of life) that had received colostrum were examined and randomly assigned to one group (see Section 2.2.1). Weak or sick animals (other than diarrhoeic) were excluded. The recorded data included the general performance, appetite, faecal consistency (normal, semi-liquid or liquid) and contents as well as dehydration on days (d) 0, 4, 7, 14, and 21 of the study.

2.2.3. Examination of faecal samples

Each time the calves were examined clinically (see above) faecal samples were collected and examined for *C. parvum* by phase contrast microscopy (400-fold magnification) of unstained smears (Naciri, personal communication). In short, a drop of saturated sugar solution was mixed with a drop of faecal material, spread on a microscopic slide and covered with a cover slip. This method was used for semi-quantitative evaluation of oocyst excretion. The number of oocysts in 10 randomly chosen fields of vison was determined (400× magnification) and indexed as none, low (1–4 oocysts), medium (5–50 oocysts) or high (>50 oocysts).

Additionally, samples taken on days 0, 7, and 14 were examined for *C. parvum*, rotavirus, coronavirus and *E. coli* K99 using a coproantigen-ELISA (Digestiv ELISA Kit®; Bio-X, Belgium) according to the manufacturer's instructions.

2.2.4. Statistical analysis

Data were analysed with SPSS (SPSS® Inc., Chicago, Illinois) using Spearman's rank correlation coefficient test (for correlations between two observed parameters), Pearson's

χ^2-test (for differences between results observed on different days) and the Mann–Whitney *U*-test (for differences between groups).

3. Results

3.1. Evaluation of test results from state veterinary laboratories

According to the preliminary questionnaire 22 of 30 laboratories regularly examined calf faeces for the presence of *C. parvum*, whereas the other eight only did so in suspected cases. Twenty laboratories described this parasite as common, nine found it only rarely. Six laboratories considered it to be an important primary pathogen, whereas the others did not. While 18 laboratories thought *C. parvum* was a common secondary cause of diarrhoea the rest thought it to be rare. Of the 19 laboratories that answered this part of the questionnaire 11 considered the prevalence of cryptosporidia in faecal samples to be <10%, three laboratories thought it to be 10–20%, two 10–50% and three laboratories 20–50%.

The results of the routine diagnostic tests are described in Table 1. Between 19 and 34% of the faecal samples and 20 and 36% of the post mortem cases tested positive for *C. parvum*,

Table 1
Results of the diagnostic tests of faecal samples (left) or post mortem examinations (right; italics) of calves submitted to five different state veterinary laboratories in Germany

	Stade	Stendal[a]	Kassel	Bonn	Aulendorf[b]	Total (%)
Total number of samples	**1132**, ***1014***	**1141**, ***783***	**157**, ***301***	**96**, ***67***	**1534**, ***1839***	**4060** (**100**), ***4004*** (***100***)
C. parvum-positive	**242**, ***204***	**253**, ***236***	**30**, ***69***	**33**, ***24***	**315**, ***448***	**873** (**21.5**), ***981*** (***24.5***)
C. parvum + rota	19, *21*	36, *13*	5, *20*	4, *10*	155, *261*	219 (5.4), *325* (*8.1*)
C. parvum + rota + *E.c.*	24, *28*	4, *18*	0, *3*	3, *0*	6, *5*	37 (0.9), *54* (*1.4*)
C. parvum + rota + corona	14, *16*	14, *17*	0, *0*	6, *0*	n.d., *n.d.*	34 (0.8), *33* (*0.8*)
C. parvum + rota + corona + *E.c.*	5, *0*	0, *0*	0, *0*	0, *0*	n.d., *n.d.*	5 (0.1), *0* (*0*)
C. parvum + corona	3, *8*	23, *18*	1, *0*	3, *0*	n.d., *n.d.*	30 (0.7), *26* (*0.7*)
C. parvum + corona + *E.c.*	0, *6*	0, *11*	0, *0*	0, *0*	n.d., *n.d.*	0 (0.1), *17* (*0.4*)
C. parvum + *E.c.*	56, *53*	20, *47*	2, *2*	0, *0*	1, *4*	79 (2.0), *106* (*10.9*)
Mixed infections	**121**, ***132***	**100**, ***124***	**8**, ***25***	**16**, ***10***	**162**, ***270***	**407 (10.0)**, ***420 (57.2)***
C. parvum (monoinfection)	**121**, ***72***	**152**, ***112***	**22**, ***44***	**17**, ***14***	**153**, ***178***	**465 (11.5)**, ***561 (42.8)***

rota: rotavirus; corona: coronavirus; *E.c.*: *Escherichia coli*.

[a] *C. parvum* + salmonella: 1.

[b] Coronavirus: not determined.

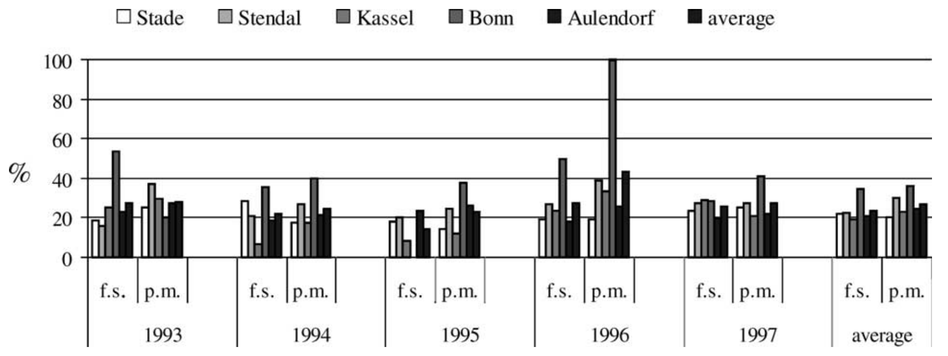

Fig. 1. Annual prevalences of *Cryptosporidium* in submitted faecal samples (f.s.) and samples taken post mortem (p.m.) submitted to five state veterinary laboratories in Germany 1993–1997.

and in 53 and 43% of these cases, respectively, no other pathogens were detected. In mixed infections *C. parvum* was most commonly associated with rotavirus (25 and 33%) followed by *E. coli* (9 and 11%), while other combinations were rare. In Kassel and Bonn where only small numbers of samples were examined the annual prevalence for *Cryptosporidium* varied between 29 and 54% for faecal samples and 12 and 100% in post mortem examinations. The other laboratories reported annual prevalences of 16–28% in faecal samples and 14–40% in post mortem samples (Fig. 1).

3.2. Field trial with halofuginone

Before the trial finished 22 clinically healthy calves (3 animals from group 1, 6 from group 2, and 13 from group 3) were sold before the end of the trial. Fourteen calves died; eight of these had diarrhoea (Table 2).

3.2.1. Oocyst excretion

At the beginning of the trial (d0) no oocysts were detected. On d4, 27.1% of the animals of group 1, 24.0% of group 2, and 11.8% of the calves in group 3 shed *C. parvum* oocysts

Table 2
Changes in group sizes during the field trial

Day	Group 1 (placebo)			Group 2 (sulfadimidine)			Group 3 (halofuginone)		
	Dead	Sold	*n*	Dead	Sold	*n*	Dead	Sold	*n*
d0	–	–	**50**	–	–	**50**	–	–	**52**
d4	2	–	**48**	–	–	**50**	1	–	**51**
d7	1	–	**47**	2[a] (1[b])	–	**48**	1	–	**50**
d14	1[a,b]	4	**42**	2[a] (1[b])	1	**45**	1	10	**39**
d21	1[a,b]	2	**39**	2[a,b]	2	**41**	–	3	**36**

d: day of sampling.
[a] *C. parvum* excretion.
[b] Diarrhoea.

Table 3
Prevalences and intensities of *C. parvum* excretion (%) during the field trial

Excretion intensities						Prevalences
Day	Group	None	Low	Medium	High	
d0	1	100	0	0	0	0
	2	100	0	0	0	0
	3	100	0	0	0	0
d4	1	72.9	10.4	12.5	4.2	27.1
	2	76.0	16.0	8.0	0	24.0
	3	88.2	2.0	5.9	3.9	11.8
d7	1	38.3	19.1	17.0	25.5	61.6
	2	33.3	12.5	25.0	29.2	66.7
	3	70.0	10.0	10.0	10.0	30.0
d14	1	23.8	14.3	40.5	21.4	76.2
	2	26.7	31.1	26.7	15.6	73.4
	3	56.4	20.5	15.4	7.7	43.6
d21	1	71.8	12.8	15.4	0	28.2
	2	80.5	12.2	4.9	2.4	19.5
	3	75.0	8.3	8.3	8.3	24.9

d: day of sampling. Group 1: placebo; group 2: sulfadimidine; group 3: halofuginone. For total numbers of animals in each group at the respective sampling dates refer to Table 2.

(Table 3). On d7, 61.6, 66.7 and 30% of the calves in the respective groups excreted oocysts, while on d14, 76.2, 73.3 and 43.6% did so. At the final sampling (d21) 28.2, 19.5 and 25.0% still shed *C. parvum* (Table 3). There were no significant differences between the groups on d0 and d4 ($P > 0.05$), but on d7 the halofuginone group had a significantly lower extensity than the sulfadimidine ($P < 0.001$) and the placebo ($P < 0.05$) groups. Similarly, on d14 the halofuginone group had a significantly lower ($P < 0.01$) extensity than the other two groups (Table 3).

The intensity of oocyst excretion at the prevalence peaks (d7 and d14) was significantly ($P < 0.05$) lower in the halofuginone group where medium to high numbers of oocysts were shed by 20 and 23.1% compared to 42.5 and 61.9% in the placebo group and 54.2 and 42.3% in the sulfadimidine group, respectively (Fig. 2).

3.2.2. *Clinical findings*

The general performance on d0 was comparable in all three groups; at least 45 calves in each group were in good condition. At the following sampling dates (d4 and d7) the condition declined in all groups, especially 1 and 2 (Fig. 3A) where significant differences were seen ($P < 0.05$) in comparison to the halofuginone group. The average appetite in the groups changed little and differences between groups were insignificant (not shown). In contrast, there was an increase in the prevalence of diarrhoea until d7 to decline again later on (Fig. 3B), and differences between the groups were significant (halofuginone versus placebo on d4: $P < 0.01$; halofuginone versus placebo on d7: $P < 0.001$; halofuginone versus sulfadimidine on d7: $P < 0.01$; halofuginone versus placebo on d21: $P < 0.05$).

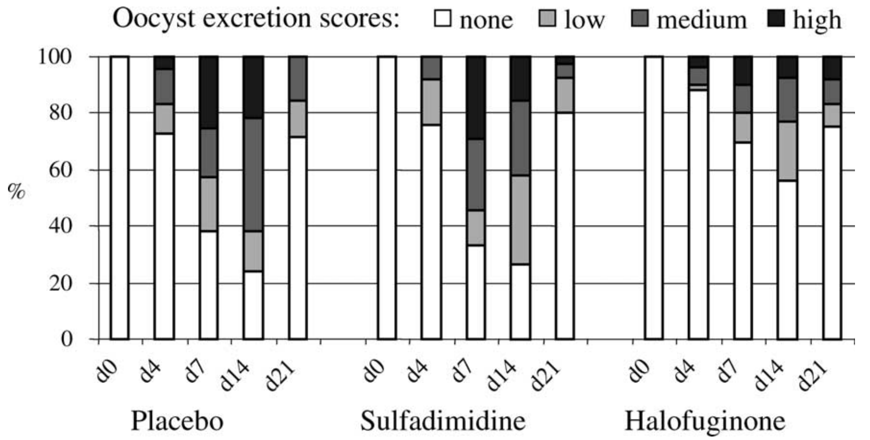

Fig. 2. Prevalence and infection intensities of *C. parvum* during the course of sampling (for scoring, see Section 2).

Blood in the faeces were not very prevalent in the samples and did not differ between groups (maximum prevalences: 10–13% on d4–d14). Mucus appeared at the same time in 10–28% of the samples irrespective of the groups. Dehydration showed similar patterns to diarrhoea (Fig. 3C) and had significantly lower rates in the halofuginone group than in the placebo group on d7 ($P < 0.05$).

3.2.3. Diarrhoea and oocyst excretion

From d4–d14 the number of excreters with diarrhoea was always significantly lower in the halofuginone group. On d7, groups 1 and 2 had the highest prevalences of diarrhoea amongst oocyst-shedding animals (82.8 and 68.8%, respectively; compared to 26.7% in group 3; $P < 0.05$–0.01), while in group 3 this prevalence was highest on d21 (66.7%; $P < 0.05$ compared to group 1 with 18.2%) (Fig. 4).

While in groups 1 and 2 the prevalence of diarrhoea was highest at the excretion peaks (d7 and d14) and declined afterwards, in group 3 it reached its peak on the last sampling day where excretion was declining (Table 3, Figs. 3 and 4). On d4, d7 and d14 the halofuginone group had the highest prevalence of calves that displayed neither oocyst excretion nor diarrhoea (74.4, 46.0 and 38.5%, respectively) compared to the placebo group (45.8, 17.0 and 14.3%) and the sulfadimidine group (60.0, 22.2 and 25.0%). On d21 the three groups had similar prevalences of healthy animals (group 1: 61.5%; group 2: 53.7%; group 3: 52.8%). Significant correlations between oocyst excretion and diarrhoea were found on d4 in group 2 ($P < 0.01$) and on d7 in group 1 ($P < 0.05$) and group 2 ($P < 0.01$), as well as in group 3 on d21 ($P < 0.05$). A significant correlation between oocyst excretion and the severity of diarrhoea could not be found.

Notably, in the halofuginone group 6 out of 9 excreters showed diarrhoea on d21 (four of these had liquid faeces) while in the sulfadimidine group 2 out of 9 and in the placebo group 2 out of 11 excreters were diarrhoeic, significantly ($P < 0.05$) less than in group 3. A comparison of animals from different farms showed that half of the diarrhoeic animals

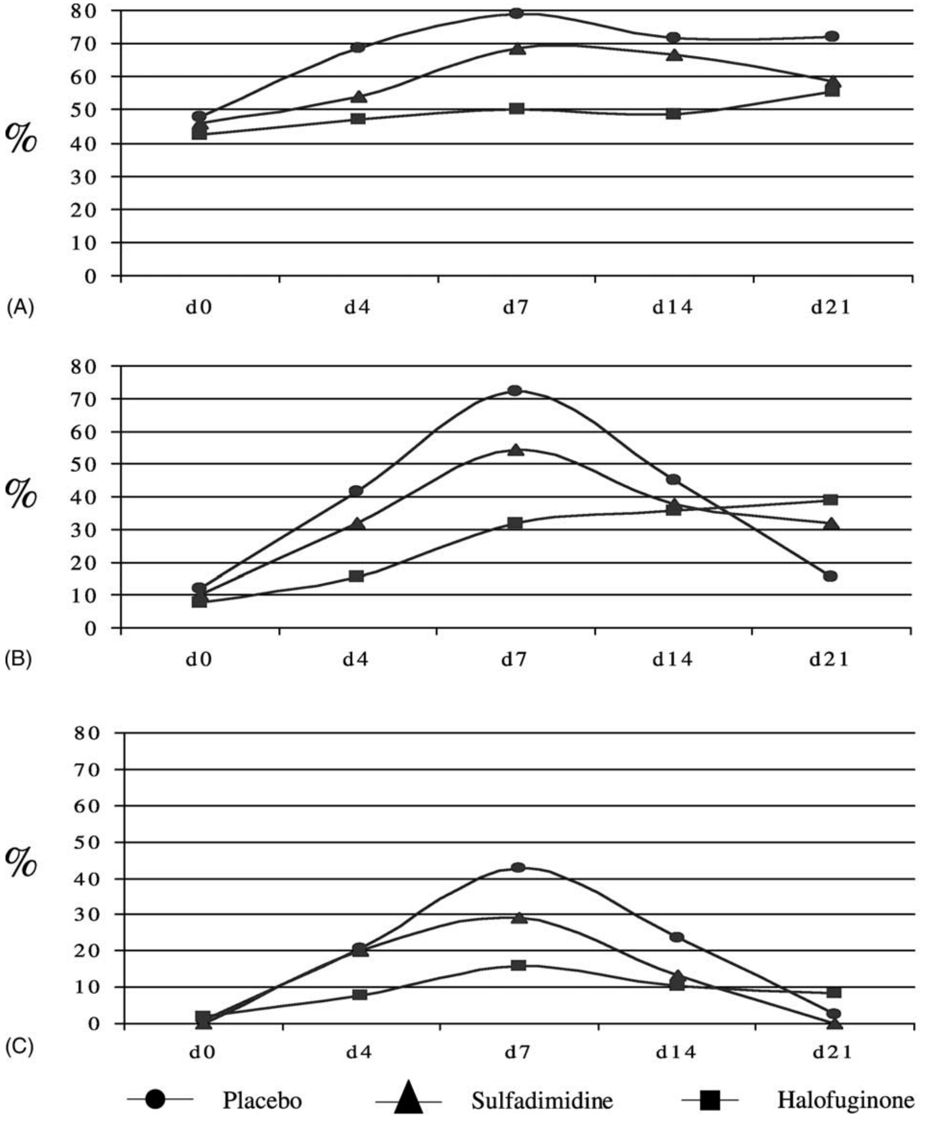

Fig. 3. Clinical findings during the field trial. Prevalences (%) are given for poor general condition (A), diarrhoea (B), and dehydration (C).

came from one farm (no. 7) which also accounted for 9 out of 12 coronavirus-positive calves (d7; see below), the majority of which (5) were assigned to the halofuginone group. When animals from this farm are excluded the differences between the three groups on d21 as well as the correlation between excretion and diarrhoea on d21 for group 1 were statistically insignificant.

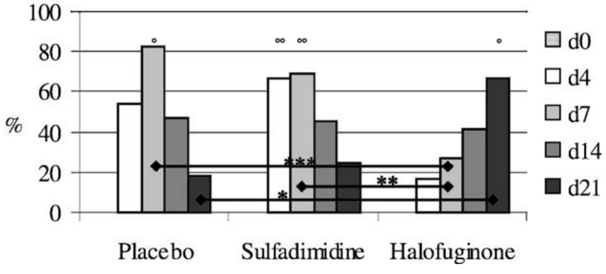

Fig. 4. Prevalences (%) of calves excreting oocysts with diarrhoea. Significant correlations between excretion and diarrhoea: (∘) $P < 0.05$; (∘∘) $P < 0.01$. Significant differences between groups: (∗) $P < 0.05$; (∗∗) $P < 0.01$; (∗∗∗) $P < 0.001$. Group 1: placebo; group 2: sulfadimidine; group 3: halofuginone.

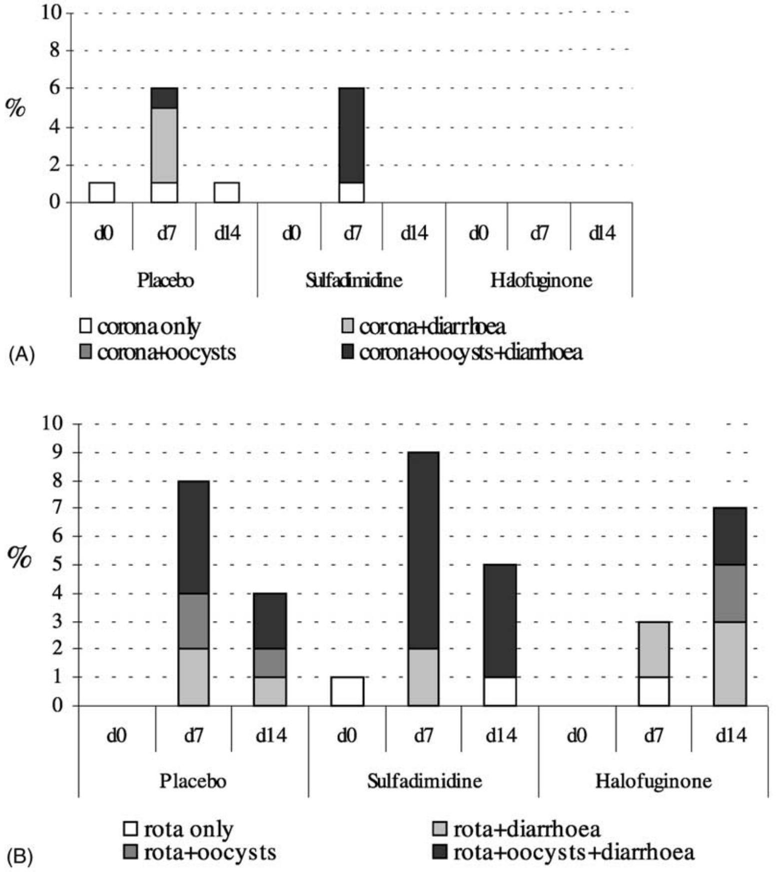

Fig. 5. Prevalences of corona (A) and rotavirus (B) infections during the field trial.

3.2.4. Other enteropathogens

E. coli K99 could not be detected at any of the examination dates (d0, d7, d14). In all, 51 samples were virus-positive, 14 for coronavirus, 37 for rotavirus. Simultaneous infection with both were not detected (Figs. 4 and 5).

4. Discussion

4.1. Estimated and actual prevalence of cryptosporidia

According to the results of the veterinary laboratories around one quarter of the samples from calves (either as faecal samples submitted to the laboratories or as samples taken post mortem) tested positive for *C. parvum* oocysts, with around half of them being monoinfections. This shows the prevalence of this parasite is probably underestimated—only 2/3 of the laboratories included the parasite in their routine testing and considered it to be common; only 26% of the investigators estimated the prevalence to be more than 20%. The role of cryptosporidia as a primary cause of diarrhoea was acknowledged by only 1/5 of the investigators. The bias of samples submitted to the state laboratories does not allow direct comparison between field investigations and our retrospective evaluation. However, the examination of field samples from different parts of Germany shows that the prevalence of cryptosporidia in calves (up to the age of 6 months) ranges mostly from 20 to 30%, with 35–36% monoinfections (Heine and Boch, 1981; Stein et al., 1983; Fiedler, 1985; Schulz et al., 1989). Considering the fact that *C. parvum* is most prevalent in calves up to 4 weeks of age (Krogh and Henriksen, 1985; Schulz et al., 1989; de Graaf et al., 1999) this age group probably has considerably higher prevalences. The present data indicate that although cryptosporidia are acknowledged as primary pathogens (Pohlenz et al., 1978; Stein et al., 1983; Krogh and Henriksen, 1985; de Graaf et al., 1999), not all veterinarians seem to be aware of their prevalence and importance.

4.2. Field trial with halofuginone

Changes in group sizes were mostly due to death in the first half of the trial, while towards the end the sale of calves diminished the groups. Especially in the halofuginone group a significant number of animals (which were clinically healthy at the day of sale according to the owner) was sold and therefore the overall performance of the remaining calves was probably below average. Especially the high number of diarrhoeic calves in group 3 on d21 could be explained with this situation. Additionally, possible effects of coronavirus infections in group 3 might have reduced the health status of some animals (and even increase excretion rates of *Cryptosporidium* oocysts), but since virological examination was not done at that time a definitive cause for this phenomenon cannot be determined.

Although other enteropathogens were present in low prevalences (probably due to vaccination of cows in three of the seven herds) in all groups the correlation between *C. parvum* and diarrhoea was evident in the first week. This was also observed by other workers (Stein et al., 1983; Heine et al., 1984; Krogh and Henriksen, 1985; Schulz et al., 1989; Fayer, 1997; de Graaf et al., 1999). However, a correlation between faecal consistency and excretion rates was not seen, in contrast to observations by Schulz et al. (1989). In our study, diarrhoea was only rarely accompanied by blood or mucus in the faeces. These signs of tissue destructions are not always present in cryptosporidiosis (Nagy and Pohlenz, 1982; Heine et al., 1984; Krogh and Henriksen, 1985; Naciri et al., 1993). As expected, diarrhoea correlated with the general performance of the animals and with clinical signs of dehydration.

As reported earlier (Schulz et al., 1989), treatment with sulfadimidine neither improved oocyst-shedding nor improved the clinical performance of the animals compared to the placebo group. In contrast, daily application of Halocur® in a dose of 7–9 ml/animal for 1 week significantly reduced oocyst excretion and diarrhoea. Under experimental conditions, halofuginone could completely prevent parasite shedding (Naciri et al., 1993; Peeters et al., 1993). In our study a metaphylactic application was chosen with the aim to reduce clinical signs of cryptosporidiosis and excretion of oocysts. A therapeutic application is also possible (Naciri et al., 1993). Although halofuginone could not completely prevent infections under field conditions, it is the only effective drug currently available for the treatment of bovine cryptosporidiosis, and although it is known to be relatively toxic and requires exact dosages, the application by the farmers according to our instructions was save in our study.

4.3. Conclusion

In summary, cryptosporidia are prevalent parasites of calves in Germany and can be considered as an important cause of diarrhoea. Treatment is recommended to reduce disease and contamination of the environment with infective oocysts. However, contamination of the environment cannot be completely prevented with the currently available drugs. It should therefore be emphasised that effective long-term control of the parasite can only be achieved by integrated management including hygienic measures such as regular cleaning and disinfection, e.g. with Neopredisan© (25% chlorocresole, Menno Chemie, Norderstedt, Germany) which also controls other enteropathogens, separation of excreters and the economical use of drugs in combination with other measures are mandatory to prevent the development of resistance.

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