Research paper

Disinfection with hydrated lime may help manage cryptosporidiosis in calves

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A B S T R A C T

Diarrhea is common in young calves and is often caused by *Cryptosporidium parvum* infection. The aim of this study was to investigate if disinfection of calf pens with hydrated lime would reduce contamination of *C. parvum* oocysts and improve calf health in herds with *C. parvum* associated diarrhea problems. Four dairy herds with ongoing *C. parvum* associated calf diarrhea problems each participated in the study over six to seven months. During the study period, all pens/huts for young calves were cleaned according to the usual farm routine before a new calf entered. Hydrated lime was then used to disinfect half of the pens/huts. Diarrhea incidence was recorded by the farmers and by veterinarians, who clinically examined the calves every second month. In total, 402 calves participated in the study. The farmers detected diarrhea in 214 (53%) calves, with similar proportions in calves kept in lime disinfected and control pens. Age at diarrhea onset was significantly higher in lime disinfected pens than in control pens, 9.0 days and 7.6 days, respectively. There was no difference between the groups regarding duration or severity index recorded by the farmers. The body condition score in 6–8 week old calves was significantly higher in calves that had been kept in lime disinfected pens during their first weeks of life, indicating that calves in disinfected pens/huts were less affected by their infections.

Faecal samples from 5 to 21 day old calves, were collected on four occasions at each farm (n = 95). *Cryptosporidium* positive samples were found at all samplings in all four herds. *Cryptosporidium* spp. was detected in 79 (83%) samples with no difference between lime disinfected and control pens. *C. parvum* was the dominant species. Two different *C. parvum* subtypes were found; IIaA16G1R1b in three herds and IIaA16G1R1b_variant in one herd. Only one subtype was found in each herd.

Disinfection of calf pens with slaked lime delayed onset of diarrhea and improved the body condition in the calves, but did not affect diarrhea incidence or duration. Although lime disinfection alone will not be sufficient to control *Cryptosporidium* associated diarrhea in herds with extensive calf diarrhea problems, these results suggest that it can be a valuable complement to other measures.

1. Introduction

Diarrhea is a significant health problem in young calves, and the protozoan parasite *Cryptosporidium parvum* is one of the major infectious causes of calf diarrhea worldwide (Blanchard, 2012; Thomson et al., 2017). Clinical cryptosporidiosis is mostly seen in calves up to six weeks of age and the most prominent clinical symptoms are watery diarrhea, inappetence, depression, and sometimes death (Robertson et al., 2014). Co-infection with other pathogens or presence of non-infectious diarrheal causes can lead to more severe disease. Oocysts, the infective stage of the parasite, are excreted with the faeces and an infected individual can shed millions of oocysts per gram faeces (Uga et al., 2000; Silverlås et al., 2013). Hence, the infection load can rapidly increase and become very high in the herd. In studies comprising dairy calves up to two months of age, 5–93% of investigated calves shed *Cryptosporidium* spp. oocysts (Santín et al., 2004; Silverlås et al., 2009b; Wang et al., 2011; Rieux et al., 2013) and the cumulative prevalence can be 100% in some herds (O'Handley et al., 1999; Santín et al., 2004). *C. parvum* is zoonotic and there are several reports of humans being diseased after contact with infected calves, e.g. (Robertson et al., 2006; Grinberg et al., 2011; Kinross et al., 2015).

Over the years, several substances have been tested for potential anti-cryptosporidial effects in livestock, but with limited success. Halofuginone lactate has shown some beneficial effects but does not

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completely prevent or cure disease (Silverlås et al., 2009a; Almawly et al., 2013; Meganck et al., 2015). Thus, sanitation is still the most important tool in disease prevention. However, the parasite is resistant to all commonly used chemical disinfectants, making it particularly difficult to control (Fayer, 2008). Hydrated lime, or calcium hydroxide, can be used to sanitise animal facilities from bacteria and viruses (Kristula et al., 2008; Anonymous, 2009). It has been shown that hydrated lime decreases the viability of *C. parvum* in the laboratory (Zintl et al., 2010), and when hydrated lime disinfection of the calf pens was used as a complement to the standard cleaning procedures in two Swedish herds with long-lasting cryptosporidiosis problems, the farmers reported a lower incidence and less severe diarrhea in the calves (C. Axén personal communication). Recently, Matsuura et al. (2017) reported about a successful sanitation of a herd that for about 10 years had experienced severe calf diarrhea problems due to mixed *C. parvum* and *Giardia intestinalis* infections. They emptied the calf stable, removed bedding and faecal matter, let the pens dry for several days and then washed them with boiling water, coated them with milk of lime, and exchanged all wooden boards dividing the pens. During the two-year period following the sanitation no calves died and no *C. parvum* oocysts or *G. intestinalis* cysts were found (Matsuura et al., 2017). This indicates that hydrated lime disinfection might be an effective control measure in cattle herds.

The aim of this study was to investigate if disinfection of calf pens with hydrated lime would decrease contamination of *C. parvum* oocysts and improve calf health in herds with *C. parvum* associated diarrhea problems.

2. Material and methods

2.1. Recruitment and study design

Four herds were recruited to this cohort study. Inclusion criteria were that i) the herd had a prevailing calf diarrhea problem for at least two months, ii) more than 20% of the calves developed diarrhea before three weeks of age, and iii) *C. parvum*, but not rotavirus or coronavirus, had been detected in calves from the herd. Furthermore, the herd should not use hydrated lime or other chemicals for disinfection of the calf pens, and the design of the calf facilities should allow arrangement of calf pens into a treatment and a control section. Preferably, the calves should be housed individually until they reached four weeks, but at least until two weeks, of age. Potential experimental herds were identified by field veterinarians at Växa Sverige, an advisory and AI service company with knowledge about calf health in dairy herds in the different regions of Sweden. Based on clinical findings and laboratory diagnostic results, the field veterinarians considered *C. parvum* to be the main cause of the diarrhea problem in these herds. Only herds that fulfilled all requirements were included in the study. Several herds had to be excluded because they already used hydrated lime, and few herds kept their calves in individual pens longer than one week. Owners of potential experimental herds were interviewed by one of the authors (CS) about management and health of the pre-weaned calves using a predefined questionnaire. Before it was finally decided if a herd could be included, faecal samples were collected from five calves under three weeks of age and analysed for presence of *Cryptosporidium* spp., rotavirus and coronavirus. Only herds in which *Cryptosporidium* spp., but no rotavirus or coronavirus, was found were accepted.

Each of the four herds participated in the study over six to seven months and was visited by a project veterinarian every second month, i.e. four times, during this period. At the first visit the magnitude of the calf diarrhea problem and the feeding and cleaning routines were recorded. The veterinarian and the farmer together decided how the pens/huts used for newborn calves should be divided into experimental and control areas, and how the calves should be distributed between the pens. In herds one to three, cleaning routines meant that all pens/huts forming one row in the stable were cleaned at the same time. In these

herds, three to nine consecutively born calves were therefore allocated to experimental and control pens/huts, respectively. In herd four, every other calf born was put in an experimental and a control pen, respectively. The farmers themselves decided which area was used as experimental and control. This was, as far as possible, kept unknown to the veterinarian. The farmers were instructed to clean all pens/huts according to their usual farm routine before a new calf was entered. In the experimental pens/huts a thin layer of hydrated lime should then be spread onto the walls and floor. The recommendation was to use no more than 40 g hydrated lime to coat both floor and walls of a 1.5m² pen. Farmers were instructed to apply the lime while the surface was still damp after washing in order to allow the lime to attach to the walls. If the farm routine did not include cleaning using water, a small volume of water was to be sprinkled onto the walls and floor before the hydrated lime was applied. The lime was allowed to work for a minimum of 24 h and farmers were instructed to brush the walls before the pen/hut was bedded and a new calf introduced. The control pens were to be left empty for at least 24 h after cleaning before a new calf was entered.

Ethics approval for this study was granted by the Regional Ethical Review Board in Uppsala (Reference number C159/14).

2.2. Data collection

For each calf born during the study period, farmers were requested to record the identity number, date of birth, breed, sex, date when the calf was separated from the dam and moved to an individual pen, whether it was kept in an experimental or control pen, and the date the calf was moved from the individual pen to a group pen. If a calf developed diarrhea they were requested to record the date when the diarrhea was first observed, the length of the disease period, if the diarrhea was accompanied by decreased general condition and/or decreased appetite, if the calf received any treatment, and if it was euthanised or died.

At each of the four visits, the project veterinarian performed clinical examinations, scored the body condition and collected faecal samples. All calves up to four weeks of age were clinically examined. General condition was graded from 0 to 3 (0 = normal behavior, alert, gets up when approached, interested in the surroundings; 1 = depressed, must be stimulated to get up; 2 = gets up with help; 3 = unable to stand even with help). Dehydration was graded from 0 to 2 (0 = none; 1 = mildly dehydrated; 2 = moderately to severely dehydrated). Faecal consistency was graded from 0 to 3 (0 = sausage-like; 1 = porridge-like; 2 = gruel-like; 3 = watery). As a measure of disease severity, body condition was scored on a 0.5 scale from 2 to 5 for all two to four and six to eight week old calves based on shape of the lower back (2 = pointed; 3 = rounded, 4 = flat; 5 = inverted). Faecal samples were collected for analysis of *Cryptosporidium* spp., rotavirus and coronavirus from up to five 5–21 day old calves in the experimental and control group, respectively. Samples were taken directly from the rectum. At the first and fourth visits, blood samples were also collected from five 2–7 day old calves for analysis of serum protein as an estimate of the colostrum management in the herd.

2.3. Laboratory methods

One gram of each faecal sample was cleaned and concentrated by a saturated sodium chloride flotation method and analysed for *Cryptosporidium* oocysts by epifluorescence microscopy as described by (Silverlås et al., 2009b)). The entire wells were examined by epifluorescence microscopy, and oocysts enumerated at 200x magnification. An animal was considered *Cryptosporidium* positive if at least one oocyst was detected in the microscope. The lower detection limit of this method is 50 oocysts per gram of faeces (OPG).

All *Cryptosporidium* positive samples collected at the first and fourth visits were analysed further to determine species and *C. parvum* subtype. DNA was extracted using the PowerLyzer® PowerSoil® DNA

Isolation Kit (Mo Bio Laboratories, Cat No. 12855-S) according to the manufacturer's recommendations but with the following modifications: up to 2 ml of each cleaned sample was spun down at 13,000 rpm for three minutes in an Eppendorf centrifuge and the pellet dissolved in 750 μl Bead Solution. The solution was transferred to the Glass Bead tube and 60 μl Solution C1 was added. The tubes were briefly vortexed and incubated at 100 °C in a thermal block for 10 min, followed by bead beating using a MP Fast Prep 24 Bead Beater set at 6.5 M/S for one minute. Finally, the DNA was eluted in 80 μl Solution C6.

A nested PCR protocol for partial amplification of a ~850 bp fragment of the 18S rRNA gene was set up using KAPA2 G Robust HotStart PCR kit (KAPA Biosystems, Cat no KK5517). The reaction mixture for all reactions consisted of 5 μl KAPA buffer A, 0.25 μl of a 20 mM nucleotide mixture, 1.25 μl of forward and reverse primer (10 μM stock), 0.1 μl of KAPA 2 G polymerase and 2 μl template in a total volume of 25 μl. The reactions were run in a Bio-Rad S1000 Thermal cycler. Primers for the 18S amplification were as described in (Santín et al., 2004)). Samples containing *C. parvum* were subtyped through partial amplification (~800 bp) of the 60-kDa glycoprotein (*gp60*) gene using the same PCR mixture as for 18S but with primers described in (Alves et al., 2003). 18S reaction conditions were 95 °C for three minutes followed by 40 cycles of 95 °C for 30 s, 61 °C for 30 s and 72 °C for 30 s, with a two minutes extension at 72 °C after the last cycle. Two μl were used as templates for the second reaction using the same reaction conditions, except that the annealing temperature was raised to 63 °C. The *gp60* annealing temperature was 52 °C in the first PCR and 55 °C in the second. Achieved sequences were compared with sequences deposited in GenBank using Basic Local Alignment Search Tool (BLAST, NCBI http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Analyses for rotavirus and coronavirus were performed at the diagnostic laboratory at the Swedish National Veterinary Institute. Up to five samples are pooled at the laboratory and then rotavirus is detected by antigen-ELISA and coronavirus by PCR. For each sampling, faecal samples from the experimental group and the control group, respectively, were pooled before analyses. Serum protein was analysed by refractometer.

2.4. Data management and statistical analyses

Data were entered and edited in Microsoft Office Excel 2010 spreadsheets (©2010 Microsoft Corporation). Results presented in the text are original, not modeled, data. Statistical analyses were performed with generalised linear mixed models using SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) with the binary variable of whether or not a calf was kept in a hydrated lime disinfected pen as the main predictor. 'Herd' was included as a random variable in all models. 'Sex' was initially included as a fixed variable but had a negligible effect and is therefore not presented in the final results. 'Breed' and 'Housing' (i.e. pen or hut) were excluded from the models as they were associated with 'Herd'. Results were considered significant when p ≤ 0.05.

For farmer recorded data, the association between whether or not a calf was kept in a hydrated lime disinfected pen and the binary outcome variable 'Presence of diarrhea' was investigated by using a generalised linear mixed model with a binomial distribution for the response, i.e. a logistic regression model. The continuous outcome variables 'Age at diarrhea onset' and 'Days with diarrhea' were investigated assuming a normal distribution for the error term. As the residuals for the analysis of 'Days with diarrhea' did not follow a normal distribution, data were log-transformed before analysis. The assumption of normality and equal variances was checked by appropriate residual plots.

A variable 'Severity' was created by combining the farmers´ records of presence of diarrhea and whether the diarrhea had been accompanied by decreased general condition and/or decreased appetite. The 'Severity' variable had three categories; 1 = diarrhea, but general condition or appetite not affected, 2 = diarrhea and decreased general condition or decreased appetite, and 3 = diarrhea and decreased general condition and decreased appetite. Therefore, a proportional odds model was used to model the probabilities to fall in any of these categories when the pen was hydrated lime disinfected or not.

For veterinary recorded data, 'General condition' data were categorised into 0 (normal) and 1 (affected, including all records > 0), 'Dehydration' into 0 (normal) and 1 (dehydrated, including all records > 0), and 'Faecal consistency' into 0 (normal, including records 0 = sausage-like and 1 = porridge-like), and 1 (diarrhea, including records 2 = gruel-like and 3 = watery). These variables were investigated by logistic regression models. Body condition score was investigated by general linear models (GLM), i. e. assuming normally distributed errors.

The variables 'Oocyst concentration', i.e. OPG, and 'Oocyst level ≥ 45,000 OPG' were investigated by GLM on log-transformed data and a logistic regression model, respectively. The second variable was created because it has been suggested that 45,000 OPG might be a suitable cut off to distinguish clinical cryptosporidiosis and subclinical infection (Operario et al., 2015).

Differences between serum protein values in first and fourth visits and between herds, respectively, were tested in a two-way ANOVA without random factors.

3. Results

Information obtained by interviewing the owners of the four herds before the study started is presented in Table 1. The calves were usually kept with their dam for up to 24 h and then moved to individual pens or huts (two herds had pens, one had huts, and one had both pens and huts) where they were kept for two to four weeks until moved and mixed with other calves in group pens or huts. In all four herds the calf diarrhea problems had been ongoing for several years and the farmers estimated the morbidity among 0–60 day old calves to be 33–100% over the last six months. Caretakers gave colostrum manually in all herds except in herd four in which some of the calves suckled their first colostrum.

In total, 422 calves were born during the study period, of which 20 were excluded either because the farmers' records were not complete or because the pen had not been kept empty for 24 h before the calf was introduced. Thus 402 calves were finally included in the study. Of these, 196 and 206 were kept in hydrated lime disinfected pens and control pens, respectively.

The farmers' recordings are presented in Table 2. Overall, the farmers detected diarrhea in 214 (53%) of the calves, with similar proportions in experimental and control pens. The mean age when diarrhea was first seen was 8.3 days (range 1–23 days) and it lasted for 3.9 days on average (median 3 days).

'Age at diarrhea onset' was significantly (p < 0.001) higher in experimental pens than in control pens, 9.0 days and 7.6 days, respectively,. There was no significant difference between calves kept in experimental and control pens for the variables 'Presence of diarrhea', 'Days with diarrhea', or 'Severity' (Table 2; Appendix Table I).

Veterinarians examined 156 calves, i.e. all calves that were up to four weeks old at the time of the visits. There was no significant difference in the veterinary registrations regarding either 'General appearance', 'Dehydration', or 'Faecal consistency' between calves kept in experimental pens or control pens (Appendix Table II).

Body condition was scored for 55 calves at two to four weeks of age and from 54 calves at six to eight weeks of age. Mean scores for calves from experimental and control pens respectively were 3.0 (range: 2.0–4.0) and 2.9 (range: 2.0–4.0) at two to four weeks and 3.1 (range: 2.5–4.0) and 2.9 (range: 2.0–3.5) at six to eight weeks. The six to eight week old calves kept in experimental pens had a significantly higher body condition score (p = 0.0069) than calves in control pens, but the difference between the groups in the younger calves was not significant (Appendix Table III).

Faecal samples were collected from 95 calves. *Cryptosporidium*

Table 1

Background information collected by interviewing four dairy herd owners before the herds were enrolled in a study on the effect of hydrated lime on cryptosporidiosis.

	Herd 1	Herd 2	Herd 3	Herd 4
Herd size (No of cows)	332	222	150	165
Mean annual milk production (ECM)	11960	9156	9000	10381
Time calves spend with their dam (hours)	< 5	4-12	24	5–12
Volume of first meal of colostrum (L)	2-4	4-5	2.5-3	3
Volume of additional colostrum meals on day of birth (L)	5	5-6	3	3
Period of feeding of colostrum or transition milk (days)	> 4	4	4	1.5–2
Type of individual calf housing	Pens indoors	Pens indoors and huts outdoors	Huts outdoors	Pens indoors
Age when calves move to group pens (weeks)	1-2	2-3	1-2	3–4
Interval between cleaning of individual pens or huts	between each calf	between each calf	between each calf	every 7–9 day
Empty period before introduction of new calf (days)	2-3	2-3	7	≤ 1
Washing individual pens or huts with water	briefly between each calf	1-2 times/year	between each calf	1-2 times/year
Drying period after washing with water	2-3 days	2-3 days	4-5 days	1 week
Yearly washing of individual pens or huts	yes	no	yes	yes
Calf mortality 1-60 days – median previous 12 months (%)	4.2	0.6	7.6	8.0
Calf diarrhea during the previous 6 months				
Morbidity (%)	100	33-50	50	50
Proportion of calves with affected appetite or general condition (%)	5-10	10	> 25	> 25
Duration of diarrhea problem	14 years	several years	several years	several years
Where the calves develop diarrhea	individual pens	individual pens	Individual pens not individual huts	calving pen
Age when calves develop diarrhea	2-10 days	1.5-2 weeks	0-2 weeks	0-2 weeks

positive samples were found at all samplings in all four herds. *Cryptosporidium* spp. was detected in 79 (83%) samples and oocyst counts were $50–126 \times 10^6$ OPG, with a median of 52,350 OPG in the positive samples. There was no difference between experimental or control pens in either the variables 'Oocyst concentration' or 'Oocyst level ≥ 45,000 OPG' (Table 3; Appendix Table IV).

When the *Cryptosporidium* positive samples from the first and fourth samplings (n = 52) were analysed by molecular biology methods, the species could be determined in 49 samples. *C. parvum* was the dominant species, found in 45 samples. *C. bovis* was found in four samples. Two different *C. parvum* subtypes were found; IIaA16G1R1b in three herds, and IIaA16G1R1b_variant in one herd (Table 4). Only one subtype was found in each herd.

Rotavirus was found in all the herds and in pools from both

experimental and control calves. In total, 32 pools (8 from each herd) were analysed and rotavirus was found in 11 (34%) of them. Coronavirus was not found in any pool.

The mean serum protein value was 58.4 g/l (range 46–70 g/l), with no significant differences between the first and fourth visits (59.3 and 56.1, p = 0.244) or between herds (p = 0.290).

4. Discussion

In this study, performed in dairy herds with pervasive *C. parvum*–associated calf diarrhea problems, calves kept in the hydrated lime disinfected pens were older when diarrhea was first recorded and had a higher body condition score at six to eight weeks of age. No other significant differences between calves kept in the experimental and

Table 2

Farmer registrations regarding 402 calves born in four Swedish dairy herds participating in a study on the effect of hydrated lime on cryptosporidiosis.

	Herd 1		Herd 2		Herd 3		Herd 4		Overall	
Study period	Nov 2014 – May 2015		Dec 2014 – June 2015		Oct 2015 – May 2016		Dec 2015 – June 2016		Nov 2014 – June 2016	
No of calves	155		112		67		68		402	
	Experimental [1] *(n = 76)*	*Control* [1] *(n = 79)*	*Experimental* [1] *(n = 50)*	*Control* [1] *(n = 62)*	*Experimental* [1] *(n = 35)*	*Control* [1] *(n = 32)*	*Experimental* [1] *(n = 35)*	*Control* [1] *(n = 33)*	*Experimental* [1] *(n = 196)*	*Control* [1] *(n = 206)*
Sex										
Male	38 (50%)	41 (52%)	28 (56%)	37 (60%)	15 (43%)	13 (41%)	15 (43%)	21 (64%)	96 (49%)	112 (54%)
Female	38 (50%)	38 (48%)	22 (44%)	25 (40%)	20 (57%)	19 (59%)	20 (57%)	12 (36%)	100 (51%)	94 (46%)
Presence of diarrhea	53 (70 %)	57 (72%)	19 (38%)	24 (39%)	13 (37%)	12 (38%)	19 (54%)	17 (52%)	104 (53%) [a]	110 (53%) [a]
Age at diarrhea onset										
Mean	7.1	6.4	14.2	11.3	9.2	8.1	9.0	6.2	9.0 [a]	7.6 [b]
95% CI	6.58-7.68	5.57-7.17	11.70-16.72	9.87-12.79	6.69-11.77	6.44-9.90	7.26-10.75	4.13-8.34	8.21-9.85	6.90-8.35
Days with diarrhea										
Median	3	3	5	5	4	3	2	4	3 [a]	4 [a]
25%:75%	2:4	2:4	4:6	4:10	4:6	2:6	2:4	2:7	2:5	2:5

[a,b] = within each row, values followed by different superscripts are significantly different (p < 0.001) in statistical analyses performed with generalised linear mixed models (Appendix Table I).

[1] Experimental = calves kept in individual pens that were cleaned, coated with hydrated lime and left empty for at least 24 h; Control = calves kept in individual pens that were cleaned and left empty for at least 24 h.

Table 3

Presence of *Cryptosporidium* spp. oocysts in faecal samples collected from 5 to 21 day old calves in four dairy herds participating in a study on the effect of hydrated lime on cryptosporidiosis.

	Herd 1		Herd 2		Herd 3		Herd 4		Total	
	Experimenta[1] (n = 16)	Control[1] (n = 13)	Experimental[1] (n = 13)	Control[1] (n = 16)	Experimental[1] (n = 11)	Control[1] (n = 10)	Experimental[1] (n = 9)	Control[1] (n = 7)	Experimental[1] (n = 49)	Control[1] (n = 46)
Oocyst concentration[2] > 45,000 OPG	2.0×10^5 (8.7×10^3- 5.0×10^6) 10 (63%)	1.5×10^4 (200- 3.0×10^4) 3 (23%)	9.0×10^4 (3.3×10^3- 1.2×10^6) 8 (62%)	6.5×10^5 (50- 2.0×10^6) 10 (63%)	2.0×10^4 (1.5×10^3- 3.6×10^6) 5 (45%)	6.8×10^5 (3.0×10^5- 1.5×10^6) 8 (80%)	750 (0-4.5×10^3) 2 (22%)	3.0×10^5 (1.8×10^3- 1.7×10^6) 4 (57%)	4.5×10^4 [a] (1.1×10^3- 1.6×10^6) 25 (51%) [a]	7.5×10^4 [a] (900- 1.5×10^6) 25 (54%) [a]

[1] Experimental = calves kept in individual pens that were cleaned, coated with hydrated lime and left empty for at least 24 h; Control = calves kept in individual pens that were cleaned and left empty for at least 24 h.

[2] Oocysts/gram feces (OPG): median (interquartile range).

[a] = within each row, values are not significantly different (p = 0.85) in statistical analysis performed with generalised linear models (Appendix Table IV).

control pens were observed by farmers or veterinarians.

In disinfected and control pens the mean age at diarrhea onset was 9.0 and 7.6 days, respectively. Although the difference between the groups is small, it is clinically relevant and represents a significant improvement in herds with pervasive diarrhea problems. Body condition score in calves is an easily accessible but rough estimate of body condition and was used as a measure of disease severity. The significantly higher body condition score in the calves in disinfected pens at 6–8 weeks of age indicates that the disease was less severe in these calves. That no difference was recorded at 2–3 weeks of age may be due to the fact that several calves got diarrhea at a late stage; the diarrhea onset varied from 1 and 23 days of age.

C. parvum and rotavirus are the two predominate infectious causes of diarrhea in young calves in Sweden, whereas coronavirus is found sporadically and *E. coli* F5+ is an uncommon pathogen (Björkman et al., 2003; Torsein et al., 2011). All herds were suggested to the study by field veterinarians who had identified *Cryptosporidium* spp. as the main pathogen associated with the diarrhea problems. When we sampled the four herds before including them in the study, we detected no rotavirus or coronavirus in any of them. All four herds did, however, show the presence of *Cryptosporidium* spp. During the study, *Cryptosporidium* spp. oocysts were found in a high proportion of the samples (83%) at all four sampling points for all the herds. Rotavirus was also found, but only a one-third of the sample pools were positive. Together, this suggest that *C. parvum* was the major pathogen, but that coinfection with rotavirus might have contributed to the diarrhea.

Lime has been shown to decrease the viability of oocysts of *C. parvum* (Zintl et al., 2010). However, we found no difference in oocyst shedding between calves kept in the experimental and control pens. This might be due to the study design, in which faecal samples were taken at veterinary visits scheduled every two months. This sampling schedule was therefore not optimised in relation to either diarrhea occurrence or potential oocyst excretion patterns. We also note that for practical reasons, the lime was mostly left to react for only 24 h, whereas the shortest time used by Zintl et al. (2010) was 48 h. Disinfection might have been more effective if the lime had been allowed to react for 48 h in the study pens.

The infection dose of *C. parvum* is very low; 25 oocysts have been reported to induce clinical infections in neonatal calves (Zambriski et al., 2013). In problem herds we can suspect a particularly high infection load, which is a challenge to any disinfectants. Furthermore, Zintl et al. (2010) concluded that in order to avoid reinfection, disinfection would have to be repeated at times when the pens were unoccupied. In the present study, disinfection was repeated, and each pen was unoccupied when coated. However, the barns were not emptied and cleaned before the start of lime disinfection. Lime disinfection was only added as a complement to the ordinary cleaning routines and was started during the housing season, when the infection load was already high. Cleaning routines and the quality of the equipment in the calf barns varied among the experimental herds. For example, in one herd the pens were not cleaned with water between each calf, and in another herd the boards dividing the pens were worn and difficult to clean. It has been suggested that components in cattle faeces enhance the oocysts' resistance to environmental stress (Robertson et al., 1992), and remnants of faecal material might have impaired the effect of the lime. We could speculate that the disinfection might have been more effective if the barn had been vacated, thoroughly cleaned, and allowed to dry for several days before the start of lime disinfection, as described by Matsuura et al. (2017).

Lime has also been found to be effective in reducing rotavirus contamination (Hansen et al., 2007). In the present study, an inclusion criterion was that no rotavirus had been detected on the farm. However, the samplings taken during the course of the study revealed presence of this virus in all herds. The study design did not permit determination of the significance of this infection in the herds.

The passive protection obtained by a high uptake of antibodies from maternal colostrum is essential to combating disease in young calves. Total protein content in blood serum can be used to estimate colostrum uptake; concentrations below 55 g/l are considered to indicate failure of passive transfer (Tyler et al., 1999). In contrast, higher concentrations are associated with improved calf health (Waldner and Rosengren, 2009; Furman-Fratczak et al., 2011). Although the mean serum protein value was above 55 g/l at both serum samplings, all herds had individual calves with lower total protein values, and only 5 of 27 calves had IgG values above 60 g/L, indicating suboptimal colostrum feeding that may have rendered the calves particularly susceptible to cryptosporidial infections.

In agreement with a previous study on calves from Swedish herds with ongoing *Cryptosporidium*-associated calf diarrhea problems (Silverlås et al., 2013), *C. parvum* was the dominant species, and *C. bovis* was only found in a few samples. However, in Swedish herds without diarrhea problems, *C. bovis* is the major species seen in young calves (Silverlås et al., 2010; Silverlås and Blanco-Penedo, 2013). The *C. parvum* subtype IIaA16G1R1b that was found in three of the herds is the most common subtype in Sweden, both in cattle and in humans. It was, for example, the subtype involved in a recent outbreak among veterinary students in Sweden (GenBank: EU647728) (Kinross et al., 2015). The IIaA16G1R1b_variant is not as common and is slightly different from IIaA16G1R1b: the TCG repeat is in a different position in the repeat region. It was first seen in Sweden in 2015 when it was identified in children who had fallen sick after visiting dairy herds at public events held on the occasion of letting the cows out on pasture for the first time in spring (Anonymous, 2016). The sequence is published (GenBank: KT895368.1).

5. Conclusion

Disinfection of calf pens with slaked lime delayed the onset of

diarrhea and improved the body condition of the calves but did not affect diarrhea incidence or duration. Although lime disinfection alone will not be sufficient to control *Cryptosporidium*-associated diarrhea in herds with extensive calf diarrhea problems, these results suggest that it can be a valuable complement to other measures.

Conflict of interest statement

Declarations of interest: none

Acknowledgements

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2018.11.004.

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