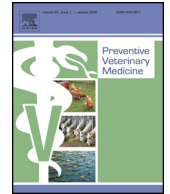




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# Evaluation of a protocol to reduce the incidence of neonatal calf diarrhoea on dairy herds



V. Meganck<sup>a,\*</sup>, G. Hoflack<sup>b</sup>, S. Piepers<sup>a</sup>, G. Opsomer<sup>a</sup>

<sup>a</sup> Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

<sup>b</sup> MSD Animal Health, Lynx Binnenhof 5, 1200 Brussels, Belgium

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## ABSTRACT

Calf diarrhoea causes substantial economic losses in cattle herds worldwide. Neonatal calves are particularly sensitive to infections with enteropathogens. The present study focused on prevention against the main infectious causes of neonatal calf diarrhoea i.e. *Escherichia coli*, rota- and coronavirus, and *Cryptosporidium parvum*. Dairy herds ( $n = 24$ ) with a high percentage of neonatal calves scouring ( $>10\%$ ) were included and calves were sampled for the presence of these four enteropathogens. To decrease diarrhoea problems among neonatal calves, a standard protocol was tested on 13 herds (treatment group) where both *C. parvum* and either *E. coli* or rota- or coronavirus were identified as being involved, the other 11 herds served as control group. The protocol consisted of 2 points of action: preventive vaccination of dams against *E. coli*, rota- and coronavirus, and preventive administration of halofuginone lactate to newborn calves. The average percentage of calves suffering from neonatal diarrhoea (39.7% versus 14.3%,  $P < 0.01$ ) and the average percentage of faecal samples positive for *C. parvum* (34% versus 11%,  $P < 0.05$ ) differed significantly between control herds and treatment herds after implementation of the protocol. No significant differences between control and treatment group were observed in the percentage of calves excreting *E. coli*, rotavirus and coronavirus, both before and at the end of the trial. Furthermore, risk factors potentially associated with the development of neonatal calf scours were determined. Non-significant results were obtained for the effect of the protocol on duration of diarrhoea and the effect of the colostrum IgG quantity on the risk of diarrhoea. Passive immunity transfer status of the calves, measured both before the onset and at the end of the study, were non-significant between groups.

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## 1. Introduction

Neonatal calf diarrhoea (NCD) is one of the major health challenges in both beef and dairy cattle herds (De la Fuente et al., 1999; USDA, 2010). The prevalence and incidence risk for NCD has recently been reported to be 19.1 and 21.2%, respectively (Bartels et al., 2010; Windeyer et al., 2014).

In the USA, diarrhoea in neonatal calves accounts for more than 50% of unweaned dairy heifer deaths (USDA, 2010). Economic losses are also due to reduced growth rates, treatment costs and time spent caring for the affected calves (Anderson et al., 2003; Ok et al., 2009). Moreover, NCD creates a problem in terms of animal welfare and farmer distress (Lorenz et al., 2011; Smith, 2012). The herd veterinarian is the most adequate person to advise farmers how to treat and how to prevent NCD (Smith, 2012).

Diarrhoea in neonatal calves is a complex, multifactorial and dynamic disease with the balance between

\* Corresponding author at: Salisburylaan 133, 9820 Merelbeke, Belgium. Tel.: +32 92647528; fax: +32 92647557.

E-mail address: [vanessa.meganck@ugent.be](mailto:vanessa.meganck@ugent.be) (V. Meganck).

the host's resistance (i.e. active and passive immunity) and the pathogen pressure being cardinal (Lorenz et al., 2011). Enterotoxigenic *Escherichia coli*, rota- and coronavirus and *Cryptosporidium parvum* are the four most important enteropathogens causing NCD worldwide (Santín et al., 2004; Trotz-Williams et al., 2007; Gulliksen et al., 2009; Bartels et al., 2010; Silverlås et al., 2010). Rotavirus and *C. parvum* are most frequently identified in faecal samples from calves with NCD: prevalence in calves with diarrhoea ranges from 2.6 to 45.1%, 17.7 to 79.9%, 3.1 to 21.6% and 27.8 to 58.5%, for *E. coli*, rota- and coronavirus and *C. parvum*, respectively (Geurden et al., 2008; Ok et al., 2009; Bartels et al., 2010; Izzo et al., 2011). The simultaneous or consecutive presence of more than one of these pathogens often causes an increased morbidity and mortality rate (Blanchard, 2012).

Each strategy to prevent NCD should begin with a confirmed diagnosis and the setup of a farm interview. The herd anamnesis addressing young stock management creates a list with potential critical control points. Key questions in this anamnesis should focus on: colostrum management, housing and hygiene, feeding of the calves, periods of stress, and drugs used (Blanchard, 2012; Smith, 2012).

The main objective of the present study was to evaluate the effect of a 2-step approach on the incidence of neonatal calves scouring and on the excretion of *E. coli*, rota- and coronavirus and *C. parvum*. This protocol consisted of vaccinating dams against *E. coli* and rota- and coronavirus and administering halofuginone lactate to newborn calves during their first seven days of life.

The secondary objectives were (1) to determine risk factors for developing NCD, (2) to evaluate the effect of this 2-step preventive approach on duration of diarrhoea and mortality, (3) and to evaluate the influence of the total ingested amount of colostrum IgG within the first 6 and the first 12 h after birth on the risk for developing diarrhoea.

## 2. Materials and methods

### 2.1. Herds and data collection

In total, 79 veterinarians of Flanders and the Netherlands were asked to provide dairy herds with  $\geq 50$  calving's per year in which  $>10\%$  of the calves between 0 and 14 days of age were suffering from neonatal scours and no measures to prevent NCD had been taken yet. To be included in the study, herds had to be positive for both *C. parvum* and either *E. coli* or rota- or coronavirus, in order to justify the protocol used (i.e. vaccination of the dams and halofuginone lactate treatment of all calves). A total of 75 dairy herds were provided by 13 veterinarians of which 24 herds met the inclusion criteria. These herds were subsequently randomly assigned to either the control group ( $n=11$ ) or treated group ( $n=13$ ). The study was carried out from February 2011 until July 2012.

Each farmer was interviewed on several aspects related to colostrum management, feeding practices, hygiene, current diarrhoea problems and preventive and curative treatment of NCD. All interviews were performed by VM. Part of the questionnaire is presented in Table 1.

On each of these herds, both before inclusion and at the end of the study, faecal samples of 5 randomly selected calves between 0 and 14 days of age were analysed with an ELISA (Rainbow Calf Scour 4, Bio-X Diagnostics, Jemelle, Belgium) to test for the presence of *E. coli*, rota- and coronavirus, and *C. parvum*. This ELISA kit detects *Cryptosporidium* spp., but as data worldwide show that *C. parvum* is the most common species found in calves of this age (Geurden et al., 2007; Langkjær et al., 2007; Xiao et al., 2007), we presumed the species to be *C. parvum*. Also, on each of these herds, both before and at the end of the study, blood samples of 5 randomly selected calves between 2 and 5 days old were collected for the determination of the serum IgG concentration using a commercial radial immunodiffusion kit (Bovine IgG Test Kit, Triple J Farms, Bellingham, USA). The herd status for failure of passive immunity transfer (FPT) was defined as: (1) FPT<sub>10</sub> being the % of calves with a serum IgG content of  $<10$  g/l and (2) FPT<sub>15</sub> being the % of calves with a serum IgG content of  $<15$  g/l.

Once included in the study, all herds (treatment and control) were asked to closely monitor the first 20 newborn calves during their first 14 days of life and to daily fill in a form per calf including information on: timing and amount of colostrum ingested, physical appearance of the faeces, appetite, morbidity, demeanour, eventual curative treatments, and mortality. Diarrhoea was defined as faeces with a physical appearance of score 2 or 3 with scores being: 0 = normal firm faeces, 1 = normal soft faeces, 2 = runny faeces, 3 = watery faeces. Duration of diarrhoea was defined as the number of days that a calf had faecal score 2 or 3. Mortality was defined as the percentage of calves that died while having diarrhoea.

Of each dam, a colostrum sample (1.5 ml, mixed four quarter sample) was frozen for later determination of the IgG content using a commercial radial immunodiffusion kit (Bovine IgG Test Kit, Triple J Farms, Bellingham, USA). The IgG data from these colostrum samples were used to estimate the total amount of IgG given to each calf.

### 2.2. Protocol

In the treated herds, extra preventive measures were taken, i.e.:

- (1) vaccination of the dams of the first 20 newborn calves against *E. coli* and rota- and coronavirus with one dose (2 ml IM) of a vaccine specific for *E. coli*, rota- and coronavirus (Rotavec-Corona®, MSD) at 3 months to 3 weeks before the expected calving date;
- (2) administration of halofuginone lactate (Halocur®, MSD, Boxmeer, The Netherlands) to the first 20 newborn calves at a dosage of 100 micrograms/kg per day (=2 ml/10 kg) during the first 7 days of life in the milk as a metaphylactic treatment against *C. parvum*.

### 2.3. Protocol efficacy evaluation

Protocol efficacy was assessed by:

- (1) comparing the incidence and duration of diarrhoea and the mortality between control and treated herds (after

**Table 1**

Univariable associations between herd management practices and the likelihood for neonatal calf diarrhoea (NCD) within 14 days after birth on 18 Flemish and 6 Dutch dairy herds.

Independent variable	$N_{\text{herds}}$	$N_{\text{calves}}$	% NCD	$P_{\text{calves}}$
Presence of calving stable				0.295
No	11	224	21	
Yes	13	299	31	
Cleaning and disinfecting calving stable after calving				0.112
Doing nothing	7	145	36	
Removing dirty straw/faeces	4	94	34	
Removing dirty straw/faeces and cleaning	2	60	7	
No calving stable	11	224	21	
Use of calving stable for sick animals				0.591
No	5	117	31	
Yes	8	182	31	
No calving stable	11	224	21	
Cleaning and disinfecting of obstetric material				0.510
Cleaning	12	258	23	
Cleaning and disinfecting	12	265	30	
Cleaning and disinfecting of hands				0.253
Doing nothing	3	59	19	
Cleaning	10	219	23	
Cleaning and disinfecting	11	245	32	
Cleaning and disinfecting rear of cow				0.313
Doing nothing	9	184	29	
Cleaning	5	114	14	
Cleaning and disinfecting	10	225	31	
Immediately separating calf from cow after calving				0.087
No	14	299	33	
Yes	10	224	17	
Cleaning and disinfecting of calf box after each calf				0.109
Doing nothing	4	93	25	
Removing dirty straw/faeces	6	125	32	
Removing dirty straw/faeces and cleaning	7	140	36	
Removing dirty straw/faeces, cleaning and disinfecting	7	165	13	
Contact between weaned and non-weaned calves is possible				0.108
No	15	328	21	
Yes	9	195	36	
Herd clothes are being used for visitors				0.517
No	15	335	24	
Yes	9	188	30	
Use of one bucket per calf				0.768
No	13	282	27	
Yes	7	160	22	
Use of automate feeder	4	81	32	
Cleaning of buckets after each feeding				0.789
No	14	311	24	
Yes	6	131	29	
Use of automate feeder	4	81	32	
Milk type				0.136
Full fresh milk	14	317	21	
Artificial milk	10	206	34	
Temperature control of milk given				0.102
No	19	421	22	
Yes	2	41	59	
Use of automate feeder	3	61	32	

- full implementation of all preventive measures), based on the data of the individual calf sheets;
- (2) comparing the shedding of *E. coli*, rota- and coronavirus, and *C. parvum* between control and treated herds based on the faecal samples taken at the onset and the end of the study.

#### 2.4. Evaluation of the colostrum management

The herd FPT status of the control versus the treated herds was compared based on the blood samples taken at the onset and the end of the study. Furthermore, the

influence of the amount of IgG administered to each calf within the first 6 and 12 h after birth on the presence of diarrhoea during the study was evaluated irrespective of groups based on the data of the individual calf sheets.

#### 2.5. Statistical analysis

In all models, a variance components correlation structure was used to take into account the clustering of observations within a herd. To evaluate the variance occurring at the herd level, null models were fit with herd as random effect for all dependent variables.

**Table 2**

Overview of the level at which the different dependent variables were measured (calf, observation), type of regression model and variance components at the herd level of the null models using data of 523 calves from 6 Dutch and 18 Flemish dairy herds.

Dependent variable	Level	Variance herd random effect <sup>a</sup>	Type of regression model	SAS <sup>b</sup> PROC function
Likelihood of diarrhoea	Calf	0.99 ± 0.4	Logistic mixed	GLIMMIX
Mortality	Calf	0.02 ± 1.0	Logistic mixed	GLIMMIX
Duration of diarrhoea	Calf	0.09 ± 0.1	Survival frailty	PHREG
Colostrum quality	Calf	192.64 ± 78.3	Linear mixed	MIXED
Amount of IgG given within 12 h	Calf	0.64 ± 0.29	Logistic mixed	GLIMMIX
Shedding of pathogens				
<i>E. coli</i>	Observation	0.12 ± 0.2	Logistic mixed	GLIMMIX
Rotavirus	Observation	0.24 ± 0.2	Logistic mixed	GLIMMIX
Coronavirus	Observation	0.59 ± 0.3	Logistic mixed	GLIMMIX
<i>C. parvum</i>	Observation	0.04 ± 0.1	Logistic mixed	GLIMMIX
Failure of passive transfer				
Serum IgG	Observation	8.48 ± 7.3	Linear mixed	MIXED
FPT10	Observation	0.44 ± 0.5	Logistic mixed	GLIMMIX
FPT15	Observation	0.83 ± 0.5	Logistic mixed	GLIMMIX

<sup>a</sup> Variance estimated at the herd level determined in the model only including the intercept.

<sup>b</sup> SAS Institute Inc., Cary, NC

- (1) Risk factor analysis. To identify herd level risk factors associated with the likelihood of diarrhoea within the first 14 days after birth, a logistic regression model was fit (Table 2). Initially, univariable associations were tested between the binary dependent variable ‘occurrence of diarrhoea’ at the calf level (0=no; 1=yes) and all categorical independent variables at the herd level presented in Table 1. Statistical significance in this step was assessed at  $P < 0.20$ . Secondly, Spearman correlation coefficients were calculated among the significant independent variables to check for multicollinearity. If two independent variables had a correlation coefficient  $\geq 0.6$ , only one was selected for further analysis. Contact between weaned and non-weaned calves and cleaning and disinfecting of the calf box after each calf were correlated, as were milk type and temperature control of milk given. Cleaning and disinfecting of the calf box after each calf and type of milk were selected because these are the biologically most relevant factors. In the third step, a multivariable model was fit using a stepwise backward procedure. A variable was considered to act as a confounder if its removal made the regression coefficients of the remaining variables undergo a relative change  $>25\%$  or in case the regression coefficient ranged between  $-0.4$  and  $0.4$ , if an absolute change  $>0.1$  was observed (Noordhuizen et al., 2001). Statistical significance in this step was assessed at  $P < 0.05$ .
- (2) Protocol efficacy. First, to determine the association between the treatment groups (control or treatment) and the likelihood of diarrhoea within the first 14 days after birth at the calf level and mortality within the first 14 days after birth while having diarrhoea at the calf level, respectively, logistic mixed regression models with herd as random effect were fit (Table 2). The association between groups (control or treated) and the duration of diarrhoea at the calf level was determined using survival analysis. Calves that died while having diarrhoea were censored. A survival model was fit with herd as shared frailty effect to correct for clustering of calves within herds, and group as categorical fixed effect (Table 2).

Secondly, separate logistic mixed regression models were fit (Table 2) to test the association between the binary dependent variables shedding of *E. coli* (0=no and 1=yes), shedding of rotavirus (0=no and 1=yes), shedding of coronavirus (0=no and 1=yes), and shedding of *C. parvum* (0=no and 1=yes) at the observation level, and group (control or treated), time of sampling (0=before the onset of the study; 1=at the end of the study) and the interaction term between group and time of sampling as categorical independent variables.

- (3) Evaluation of the colostrum management. Logistic mixed regression models were fit to determine (a) the association between the amount of IgG given to a calf within the first 6 and 12 h after birth (0: IgG  $< 200$  g and 1: IgG  $\geq 200$ ) as independent variable and the likelihood of diarrhoea within the first 14 days after birth as dependent variable, (b) the association between colostrum quality (0:  $< 50$  g IgG/l and 1:  $\geq 50$  g IgG/l) as independent variable and the likelihood of diarrhoea as dependent variable, and (c) the association between the group (control or treated) as independent variable and the amount of IgG given to a calf (0: IgG  $< 200$  g and 1: IgG  $\geq 200$ ) within the first 6 and 12 h after birth as dependent variable.

Subsequently, separate logistic mixed regression models were fit to test the association between the binary dependent variables FPT<sub>10</sub> (0=serum IgG content  $< 10$  g/l; 1=serum IgG content  $\geq 10$  g/l) and FPT<sub>15</sub> (0=serum IgG content  $< 15$  g/l; 1=serum IgG content  $\geq 15$  g/l) and group (control or treated) as independent variable, time of sampling (0=before the onset of the study; 1=at the end of the study) and the interaction term between group and time of sampling as categorical independent variables (Table 2).

A linear mixed regression model was fit (Table 2) to test the association between group (control or treated) and time of sampling as independent variables, and the serum IgG concentration before the onset of the study and the end of the study as dependent variables.

**Table 3**

Univariable mixed regression models for the dependent variables likelihood of diarrhoea, duration of diarrhoea and mortality, respectively, using data of 523 calves from 6 Dutch and 18 Flemish dairy herds.

Dependent variable	Independent variable	N	Mean	OR or HR	95% CI	P-value
Likelihood of diarrhoea	Group					<0.01
	Control	234	39.7%	Ref.		
	Treated	296	14.3%	0.26	0.12–0.60	
	g IgG < 12 h					0.59
	< 200	231	28.6%	Ref.		
	≥ 200	93	23.7%	0.77	0.51–1.47	
Duration of diarrhoea <sup>a</sup>	Colostrum quality					0.42
	< 50 g IgG/l	60	30.0%	Ref.		
	≥ 50 g IgG/l	322	24.3%	0.75	0.39–1.45	
	Group					0.76
Mortality	Control	234	2.2%	Ref.		
	Treated	296	0.7%	0.30	0.06–1.59	

<sup>a</sup> Within the calves that had at least 1 day of diarrhoea.

### 3. Results

Distribution and univariable analysis outcomes of risk factors extracted from the questionnaire are presented in Table 1. In the univariable step, 5 factors were significantly related to the likelihood of diarrhoea within the first 14 days after birth ( $P < 0.20$ ). In the multivariable model, no significant associations ( $P < 0.05$ ) remained for any of these factors.

The percentage of scouring calves during the study was significantly lower in the treated herds (14.3%) compared to the control herds (39.7%) (OR = 0.26,  $P < 0.01$ , Table 3). No statistically significant difference in the duration of diarrhoea was present between calves of treated and control herds (HR = 0.91; 95% CI [0.57–1.48];  $P = 0.71$ , Table 3). Also the odds of mortality were not statistically significant (OR = 0.30, 95% CI [0.06–1.59],  $P = 0.16$ , Table 3).

Calves of treated herds showed a significantly lower risk of *C. parvum* shedding at the end of the study compared to the start of the study (11% versus 46%,  $P < 0.001$ , Table 4). This risk at the end of the study was also significantly reduced compared to calves of control herds (11% versus 34%,  $P < 0.001$ , Table 4). Occurrence of coronavirus

increased significantly ( $P < 0.05$ , Table 4) during the study period in both groups; no significant differences existed between groups regarding the occurrence of *E. coli* and rotavirus (Table 4).

Calves from both treated and control herds received on average 1.9 (range 0.0–6.0) litres of colostrum at the first colostrum feeding. The average time until a newborn calf received its first colostrum was 2.9 h (range 0.1–20.0) on control herds and 2.0 h (range 0.1–10.5) on treated herds. Within the first 6 h after birth, a calf received on average  $1.8 \pm 1.1$  and  $2.2 \pm 1.2$  litre on control and treated herds, respectively. These differences were not significant. Colostrum quality was significantly lower ( $P < 0.05$ ) on control herds (average 79 g IgG/L; range 1–187) compared to treated herds (average 90 g IgG/L; range 2–241). No significant differences were present between both groups regarding FPT<sub>10</sub> and FPT<sub>15</sub> (Table 4).

The percentage of scouring calves did not significantly differ between calves receiving at least 200 g IgG or less within the first 12 h after birth ( $P = 0.59$ , Table 3) nor between calves receiving colostrum of inferior quality compared to calves receiving high quality colostrum ( $P = 0.42$ , Table 3).

**Table 4**

Univariable mixed regression models to assess the effect of treatment on shedding of pathogens and failure of passive immunity transfer using data of 523 calves from 6 Dutch and 18 Flemish dairy herds.

Dependent variable	Before		After		$P_{\text{between}}$		$P_{\text{within}}$	
	Treated	Control	Treated	Control	Before <sup>a</sup>	After <sup>b</sup>	Treated <sup>c</sup>	Control <sup>d</sup>
Shedding of pathogens (%)								
<i>E. coli</i>	16	15	15	25	0.93	0.19	0.91	0.17
Rotavirus	57	43	44	47	0.20	0.76	0.17	0.63
Coronavirus	14	9	32	34	0.52	0.79	0.02	0.002
<i>C. parvum</i>	46	31	11	34	0.09	0.004	<0.001	0.70
Failure of passive transfer								
Serum IgG (g/l)	24	21	22	25	0.32	0.28	0.49	0.09
FPT <sub>10</sub> (%)	5	12	17	13	0.87	0.14	0.64	0.27
FPT <sub>15</sub> (%)	16	24	27	15	0.15	0.33	0.34	0.33

<sup>a</sup>  $P$ -value to assess statistical significance of differences between control and treated herds before the onset of the study.

<sup>b</sup>  $P$ -value to assess statistical significance of differences between control and treated herds at the end of the study.

<sup>c</sup>  $P$ -value to assess statistical significance of differences between treated herds before the onset of the study and at the end of the study.

<sup>d</sup>  $P$ -value to assess statistical significance of differences between control herds before the onset of the study and at the end of the study.

#### 4. Discussion

Neonatal calf diarrhoea is a disease with a major impact on the economic viability of cattle herds worldwide and can be triggered by both infectious and non-infectious causes. The present study focused on prevention of infectious causes of NCD with main emphasis on *E. coli*, rotavirus and coronavirus and *C. parvum*. Rotavirus and *C. parvum* were the 2 most common enteropathogens found in faeces of calves in the present study, confirming data reported worldwide (Ok et al., 2009; Bartels et al., 2010; Izzo et al., 2011).

No significant associations were present between diarrhoea incidence during the study and the risk factors defined in the questionnaire. This can be explained by a lack of variation in NCD management along with a high variation in the incidence of neonatal diarrhoea among the herds and the relatively small number of herds.

The significantly lower percentage of scouring calves in treated herds in comparison to control herds indicates the efficacy of the preventive 2-step programme. The higher colostrum quality on the treated herds might reflect the success of the vaccination protocol. However, despite the use of a specific vaccine against *E. coli*, rotavirus and coronavirus, no significant differences existed between groups in the occurrence of *E. coli* and rotavirus. As the tetrakit used to determine pathogen excretion is not a quantitative but rather a qualitative to semi-quantitative test, the exact level of pathogen excretion per calf could not be assessed. Hence, we may state that fewer calves excreted enteropathogens following the implementation of the 2 step protocol, but not whether individual excretion was lowered. If so, this potentially also may contribute to fewer calves scouring. Also, calves born from vaccinated dams are significantly less likely to shed *C. parvum* (Trotz-Williams et al., 2007).

The efficacy of halofuginone lactate (a cryptosporidiosis) was evident from the significant reduction of the proportion of calves excreting *C. parvum* after protocol implementation within the treated herds while this did not occur in control herds. A prophylactic or metaphylactic treatment with halofuginone lactate is expected to decrease and delay the peak of oocyst excretion, delaying the onset of diarrhoea to an age when calves are better able to cope with dehydration and acid–base balance disturbances (Silverlås et al., 2009; De Waele et al., 2010; Trotz-Williams et al., 2011).

The overall passive immunity for both treated and control herds was adequate, as indicated by the average IgG serum content which also did not differ significantly between groups. Several other authors reported lower average IgG values ranging from 12 to 19 g/L and FPT<sub>10</sub> percentages up to 50% (Tyler et al., 1996; Calloway et al., 2002; Filteau et al., 2003). McGuirk and Collins (2004) set the alarm level for FPT<sub>10</sub> to be a herd problem at 20%, whilst Chigerwe et al. (2009) consider an FPT prevalence <10% as a rational and achievable goal. Despite the adequate passive immunity in this study, several calves developed diarrhoea which may suggest that the cut off value of 10 g IgG/L is too low, and should be revised (Güngör et al., 2004; Chigerwe et al., 2008; Windeyer et al., 2014). Also, the association

between the level of passive immunity and the occurrence of NCD is less clear in comparison to the occurrence of respiratory disease (Donovan et al., 1998).

#### 5. Conclusions

In herds where the colostrum management suffices, dam vaccination and metaphylactic halofuginone lactate administration can be successful in the reduction of incidence of calf diarrhoea. Implementation of the described protocol in NCD problem herds is straightforward for practitioners.

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