Early clinical predictors of diarrhea in the first fifteen days in dairy calves and their relationship with passive transfer status¹⁾

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Summary

The neonatal period is very important in calves. It is extremely important to closely monitor newborn calves with good colostrum management during this period. In this study, the aim was to investigate whether the clinical parameters in the first 15 days after calves are born are correlated with diarrhea and whether those values are the early clinical predictors of the diarrhea that will occur in this period and also to investigate the correlation between diarrhea and the failure of transfer of passive immunity (FTPI). Eighty-two Holstein calves were used in the study. The calves were examined systemically every day and were scored according to a standard form. In the first 15 day period, 64.63% of the calves had diarrhea. In diarrheic calves, the most common findings on the previous day without diarrhea were fetid feces (43.40%), changes in the consistency and color of feces, and a decrease in the temperature of the ears (35.85%). According to non diarrheic calves, fetid feces ($P < 0.01$), changes in the consistency and color of feces, and nasal discharge were more common among diarrheic calves on the day before diarrhea ($p < 0.001$). The duration of diarrhea in calves with FTPI was longer than without FTPI. As a result, it is important for calves to be monitored daily in the first 15 days, looking for early signs of diarrhea in this period, namely fetid feces and changes in the consistency and the color of the feces. In addition, coexistence of any fecal changes with the decrease in the temperature of the ears may be clinical predictors of diarrhea. As a result of the daily monitoring of the calves, although the morbidity rate was high, none of the animals died.

Keywords: calf, clinical predictors, diarrhea, passive transfer immunity

Despite the fact that the neonatal period of calves comprises only a short time in their lives, this is the period of the highest incidence and mortality rates from diseases (16, 22, 35). Calf diseases are very important in terms of both economic and animal welfare concerns because they cause calf deaths, high treatment costs and low efficiency in animal performance (19). The goals of raising calves are health, performance, substitute heifer production and profitability. Investments made in line with these objectives play an important role in ensuring the profitability for livestock owners and businesses economically. Therefore, it is of great importance to prevent illnesses and to improve calf recovery rates by making early diagnosis in this period (24).

During the neonatal period, colisepticemia, enterocolitis, rotavirus, coronavirus and cryptosporidiosis diarrhea are common in calves. Additionally, respira-

tory tract diseases are a problem observed during this period (13, 17, 21, 25). As with other animals, young newborn calves have a high incidence of disease. During the first 15 days in the calves' lives, septicemia and diarrhea are the most common diseases, and it is the period when death is most commonly seen (1, 18, 22). Determination of routine clinical parameters to monitor the health status of animals is a practical method for early diagnosis of diseases (5, 6). It has been observed that the daily monitoring of cows during the postpartum period, a critical period in dairy cattle, is of great importance for the future of the herd (5). It is known that daily assessments are extremely important for calves (23).

Passive transfer immunity is one of the most important factors affecting the health of the calves in the neonatal period (11, 27, 34). The passage of immunoglobulins during pregnancy is minimal because the placenta of ruminants is epitheliochorial, so the

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newborn calves are hypogammaglobulinemic, and they need to consume colostrum as soon as possible after birth (2, 34). Calves that fail to receive this passive transfer are not protected against infectious diseases, and they have higher morbidity and mortality rates (3, 19, 23, 30).

Identification of the early signs of disease by monitoring clinical features daily and correlating the findings with failure of transfer of passive immunity (FTPI) values could be important in the first 15 days of the neonatal period, which is the critical period for the calves.

The aim of this study was to determine the early clinical predictors of diarrhea in calves and also to investigate the correlation between diarrhea and with FTPI.

Material and methods

Animals. The animals used in this study were 82 newborn Holstein calves at a farm. This study was performed

in agreement with the guidelines for animal experimentations (UU Ethics Committee No: 2014-17/09). There were 43 male and 39 female calves. The average live birth weight was 40.07 ± 0.75 kg for males and 38.08 ± 0.82 kg for females. According to the farm management program, 2.5 liters of colostrum was offered to the calves by the employees within 3 hours after birth. They received 2.5 liters of freshly milked colostrum from their dams with nipple twice a day for the first three days of life. The calves were housed in individual boxes from the third day until one month. From the 3rd day onwards, alfalfa hay, calf starter feed and water were given *ad libitum*. On average, 2.5 liters of milk were given twice a day for one month.

Daily clinical monitoring of the calves. General clinical examinations were performed by the same veterinarian between 8:00 am and 11:00 am every day for 15 days from the time they were born, and the data were recorded. The calves were evaluated according to the modified as in Table 1 (5, 6, 22, 28).

Collection of blood samples. Anticoagulant-free blood (8-10 ml) was taken from the calves at the moment of birth

Tab. 1. Daily clinical monitoring form of calves

Calf:	Ear number	Age ... day	Gender F/M	Age of mother
	Clinical parameters	Score 1 Clinically healthy	Score 2 Suspicious health	Score 3 Disease symptoms
Inspection findings	posture	normal	low shoulder	stagnant, back in roundback, slightly raised tail
	eyes looking	live	not alive	dull glance
	suckling reflex	good	medium	weak-no
	movements of the ears	active	decreased	no
	position of the ears	upright	nearly upright, moderate	low
	palpebral reflex	have	slight decrease	significantly reduced-lost
	behavior, lethargy score	0-1	2	3-4
Body temperature parameters	rectal degree (°C)	38.5-39.4	38.0-38.5 39.5-39.7	< 38.0, > 39.7
	muzzle-nose temperature	at body temperature, warm	slight cold	cold
	oral temperature	at body temperature, warm	slight cold	cold
	ears temperature	at body temperature, warm	slight cold	cold
Findings of systemic circulation and dehydration	heart rate/min.	90-130	80-90, 130-140	< 80, > 140
	enophthalmus	no	slight	moderate-severe
	skin elasticity, sec.	1	2-4	> 4
	color of conjunctiva and mucous membranes	normal-pink	slight pale hyperemic	moderate-severe pale, hyperemic cyanotic
	CFT, sec	1-2	> 2	> 3
	muzzle appearance	moist	slightly moist	dry
Respiratory system findings	lacrimation	no	slight	moderate-severe
	nasal discharge	no	slight	moderate-severe
	respiratory freq./min	20-40	16-20, 40-44	< 16, > 44
	cough	no	induced single	spontaneous-induced multiple
Digestive system findings	feces consistency score	1-2	3	4
	the smell of feces	normal	slightly fetid	fetid
	the color of feces	light brown	yellow	grey

(before the calves were given the colostrum) and then on the 1st (24th hour), 3rd and 7th days after birth. Serum was obtained by centrifugation of the non-anticoagulated blood for 5 minutes at 5,000 rpm and was stored in -20°C in 2 ml Eppendorf tubes until analysis.

Evaluation of passive transfer parameters. Immunoglobulin G (IgG) concentrations were measured from the obtained sera. The quantities of IgG in the study were determined by ELISA from serum samples (Bio-X Diagnostic Bovine ELISA Kit) at days 0, 1, 3, and 7. Calves with an IgG concentration < 10 g/L were evaluated as FTPI (23-25). As this classification is based on references (3, 7), calves with an IgG value less than 5 g/L have severe FTPI; calves with the range of 5-10 g/L have partial FTPI; calves with the range of 10-15 g/L have sufficient passive transfer; good passive transfer is between 15-20 g/L; and calves with higher than 20 g/L have very good passive transfer.

Statistical analysis. IBM SPSS 22.0 was used for statistical and descriptive analysis. Whether or not there was any difference between the values of each parameter on different

days was examined by the Friedman test. If a difference was determined, Wilcoxon's test or a Paired Sample T-test was applied, according to whether the groups showed a normal distribution. Categorical variables were compared between diarrheic and non diarrheic groups with Pearson's chi-square test and Fisher's exact test. For all analysis, $P < 0.05$ was determined to be significant.

Results and discussion

In the first 15 days, diarrhea was observed in 53 (64.63%) of the 82 newborn calves that received daily observation and clinical examination. However, there were no health problems in 20 calves. Pneumonia was observed in 6 (7.31%) calves, including one of the animals with diarrhea; 3 calves (3.65%) had septicemia; and 2 calves (2.43%) had omphalophlebitis and diarrhea. In the first 15 days of the neonatal period, 64.63% of the diarrhea that occurred belonged to diarrhea cases as mentioned in the references (18, 31).

Tab. 2. Comparison of the most common findings before diarrhea with non diarrheic calves in first fifteen days

Clinical examination finding	Before the diarrhea (n = 53)/ non-diarrheic (n = 220)*	Score 2 (n)	Score 3 (n)	Score 2 + Score 3 (n)	Score 2 + Score 3 (%)	P value
Fetid feces	diarrhea	15	8	23	43.40	< 0.01
	non-diarrheic	32	13	45	20.45	
Change in the consistency of the feces	diarrhea	19	–	19	35.85	< 0.001
	non-diarrheic	30	–	30	13.63	
Change in feces color	diarrhea	16	3	19	35.85	< 0.001
	non-diarrheic	15	10	25	11.36	
Decrease in the temperature of the ears	diarrhea	15	4	19	35.85	0.147
	non-diarrheic	41	16	57	25.90	
Increase of pulse	diarrhea	8	11	19	35.84	0.361
	non-diarrheic	25	69	94	42.72	
Nasal discharge	diarrhea	11	4	15	28.30	< 0.001
	non-diarrheic	17	2	19	8.63	
Lacrimation	diarrhea	5	5	10	18.87	0.418
	non-diarrheic	38	15	53	24.09	
Extension of CFT time	diarrhea	12	–	12	22.64	0.954
	non-diarrheic	36	13	49	22.27	
Changing color in conjunctiva and mucosa	diarrhea	5	3	8	15.09	0.919
	non-diarrheic	17	15	32	14.54	
Lethargy score 2	diarrhea	7	1	8	15.09	< 0.01
	non-diarrheic	9	–	9	4.09	
Decrease in suckling reflex	diarrhea	7	–	7	13.20	0.781
	non-diarrheic	20	6	26	11.81	
Decrease in the skin elasticity	diarrhea	6	–	6	11.32	0.655
	non-diarrheic	29	1	30	13.63	
Enophthalmus	diarrhea	6	–	6	11.32	0.776
	non-diarrheic	21	1	22	10.00	
Change in posture position	diarrhea	6	–	6	11.32	0.095
	non-diarrheic	9	1	10	4.54	

Explanation: *11 days (in 4-14 days) 11 days × 20 calves = 220

Thirty (56.60%) of the 53 total diarrheic calves were males, and 23 (43.40%) were females. Although the proportion of male calves with diarrhea was not much higher, male calves are heavier than females at birth, which is consistent with the suggestion in the literature (33) that the amount of colostrum male calves receive is insufficient compared to the amount females receive. In addition, the fact that the ratio of IgG in the male calves was lower than that in the females supports this view. Indeed, IgG levels were 13.01 ± 1.82 g/L in female animals with diarrhea on day 3 and 12.63 ± 0.99 g/L in male animals with diarrhea. As a matter of fact, the FTPI rate in a study was 16.0% in female calves and 22.6% in male calves (10).

The diarrhea was classified according to 3 different time periods: 0-5 days, 6-10 days and 11-15 days. Two diarrhea cases were observed in the first period (only on the 5th day) (3.77%), 39 in the 6-10 day period (73.58%) and 12 in the 11-15 day period (22.65%). Notably, 73.58% of them were found between 6 and 10 days after birth, as indicated in other references (14, 21).

Clinical findings were seen the day before diarrhea occurred in more than 10% of 53 diarrheic calves, and the same clinical findings in calves without diarrhea are presented in Table 2. Other findings on the daily clinical monitoring form were not significant.

As diarrhea was seen between days 5 and 15 in the first 15-day period, the number of detections of the same clinical findings in days 4-14 in 20 calves without diarrhea was presented in Table 2. As seen in Table 2, the most common findings on the previous day before diarrhea in the calves were the fetid feces (43.40%), changes in the consistency and color of the feces and a decrease in the temperature of the ears (35.85%). Compared with non diarrheic calves, fetid feces ($P < 0.01$), changes in the consistency and color of the feces and nasal discharge were more common among diarrheic calves on the day before diarrhea ($P < 0.001$). Comparing non diarrheic calves with the diarrheic calves, nasal discharge ($P < 0.001$) and lethargy ($P < 0.01$) were observed more frequently the day before diarrhea. Changes in the feces are expected in the diarrheic calves the day before the onset of diarrhea. This may be a sign of a disrupted digestive activity (22). Comparing non diarrheic calves with the diarrheic calves, nasal discharge and lethargy were observed more frequently the day before diarrhea. Observation of more lethargy may be considered a sign of the onset of the disease (8). More nasal discharge may be due to the stimulation of the parasympathetic nerves (4).

Tab. 3. Co-occurrence of some important findings before diarrhea and comparison with non-diarrheic calves

Some clinical examination finding	Before the diarrhea (n = 53)/ non-diarrheic (n = 220)	n	%	P value
Any two of the three feces scores	diarrhea	20	37.74	< 0.001
	non-diarrheic	21	9.54	
Fetid feces + Change in the consistency of the feces	diarrhea	11	20.75	< 0.05
	non-diarrheic	20	9.09	
Fetid feces + Change in feces color	diarrhea	11	20.75	< 0.001
	non-diarrheic	11	5.00	
Change in the consistency of the feces + Change in feces color	diarrhea	9	16.98	< 0.01
	non-diarrheic	10	4.54	
Three feces scores together	diarrhea	6	11.32	< 0.05
	non-diarrheic	7	3.18	
Finding at least one feces change + Decrease in the temperature of the ears	diarrhea	14	26.41	< 0.01
	non-diarrheic	24	10.90	

Although postural changes on the day before diarrhea were not very common, this finding may be an important predictor when detected because it is close to statistical significance ($P < 0.095$).

Table 3 shows the rate of co-occurrence of the most common findings the day before diarrhea. In diarrheic calves, only 6 had changes in all three feces scores, namely: the fetid of the feces, a change in the consistency of the feces, and a change in feces color, on the day before diarrhea. As shown in Table 3, 37.74% of the cases had two of the 3 fecal changes present together. This suggests that the observation of two of the fecal changes increases the likelihood of diarrhea after one day. In addition, it was observed that it could be an important clinical predictor prior to diarrhea when the temperature of the ears decreased in combination with at least one fecal change ($P < 0.01$). Since these calves have been observed for more than a year, the decrease in the temperature of the ears was not dependent solely on winter conditions.

The mean, minimum and maximum values of the IgG on different days are presented in Table 4 below. IgG level of the calves in the study was highest on the 3rd day, which agrees with the references (12, 34) which indicate that Ig levels reached the highest value in 36-48 hours. It has even been stated that IgG reached the maximum concentration in 2-3 days of age (32).

Significant clinical findings were observed more frequently before diarrhea during the first 15-day period

Tab. 4. Mean, minimum and maximum values of IgG in different days

		Day 0 (n = 63)	Day 1 (n = 82)	Day 3 (n = 82)	Day 7 (n = 82)
		1.27 ± 0.06^a	13.23 ± 0.77^b	13.84 ± 0.75^b	12.48 ± 0.79^c
IgG (g/L)	min	0.93	1.21	2.01	1.88
	max	3.50	45.7	36.51	47.27

Explanation: a, b, c – means with different superscript letters differ significantly at $p \leq 0,05$

Tab. 5. IgG concentrations on different days (n = 82)

Day	Severe FTPI < 5 g/L	Partial FTPI 5-10 g/L	Sufficient PT 10-15 g/L	Good PT 15-20 g/L	Very Good PT > 20 g/L
1	7 (8.53%)	17 (21.25%)	30 (36.58%)	19 (23.17%)	9 (10.97%)
3	6 (7.31%)	18 (21.95%)	28 (34.14%)	19 (23.17%)	11 (13.41%)
7	8 (9.75%)	24 (29.26%)	27 (32.92%)	17 (20.73%)	6 (7.31%)

Explanations: PT – passive transfer; FTPI – failure of transfer of passive immunity

than in those without diarrhea. They were evaluated according to the presence of FTPI, but no significant difference was found. Table 5 shows that FTPI was 29.26% (8.53% + 21.25% and 7.31% + 21.95%) on the 1st and 3rd day and 39.02% (9.75% + 29.26%) on the 7th day. The FTPI was 29.26% on the 1st and 3rd day, when IgG < 10 g/L is taken as a criterion. While this ratio is higher than in some studies (9, 20), it agrees with the FTPI incidence reported in others (3, 8, 34). The high FTPI ratio may be due to the insufficient amount of colostrum given (2.5 liters, 2 times per day for the first 3 days). In fact, higher volumes of colostrum are recommended (26, 34). While this ratio on the 7th day is higher than on the 1st and 3rd days, this increase may be due to the tendency of Ig to decrease (12, 15). The high rate of diarrhea in the first 15 days in the calves may be the result of FTPI on the first and third days being as high as 29% and 26%, respectively.

As noted in Table 6, it was observed that on the 1st and 3rd days diarrhea was seen in 79.16% of the calves with FTPI, whereas 60.34% of the calves without FTPI were diarrheic. The rate of diarrhea in calves with FTPI was high (79.16%), as expected (7). In the calves without FTPI, the rate of diarrhea was also high (60.34%). However, as shown in Table 6, the duration of diarrhea

in calves with FTPI was much longer than without FTPI. For example, the duration of diarrhea in calves with severe FTPI was respectively 5.00 ± 0.81 , 4.80 ± 0.96 and 4.28 ± 0.74 days on the 1st, 3rd and 7th days, whereas 2.08 ± 0.22 , 1.67 ± 0.22 and 1.85 ± 0.26 days in calves with good PT levels. This may be because while FTPI plays an important

role in the pathogenesis of diseases, it is not sufficient on its own, and other factors such as environmental and hygienic conditions are important in pathogenesis (22, 29). On the other hand, although the rate of diarrhea is high, the fact that most of the cases were mild may indicate that they were not exposed to serious infections. In fact, despite the high morbidity rate, the lack of deaths supports this explanation.

Daily inspections of the calves and early interventions, especially in the first 15 days, played their part in the absence of death, because the rate of diarrhea in the calves was very high.

In conclusion, it is important for calves to be monitored daily during the first 15 days, and early clinical predictors of diarrhea in this period are fetid feces and changes in the appearance and color of the feces. The coexistence of any two of these changes in fecal findings further increases the likelihood of diarrhea. In addition, coexistence of any fecal changes with decrease in the temperature of the ears may be clinical predictors of diarrhea. Because of daily monitoring of the calves, it was observed that there was no death, even though the morbidity rate was high. In addition, it was observed once again that the rate of diarrhea was higher in the calves with FTPI.

Tab. 6. Classification of diseased calves in the first 15 days according to IgG concentrations on different days (n = 82)

Day	FTPI		Without FTPI		
	Severe FTPI (< 5 g/L)	Partial FTPI (5-10 g/L)	Sufficient PT (10-15 g/L)	Good PT (15-20 g/L)	Very Good PT (> 20 g/L)
Number of calves at 1 st day IgG level	7	17	30	19	9
Number and ratio of calves with diarrhea and duration (day) of diarrhea	6 85.71% (5.00 ± 0.81)	13 76.47% (2.84 ± 0.50)	17 56.66% (2.11 ± 0.25)	12 63.15% (2.08 ± 0.22)	5 55.55% (2.20 ± 0.33)
Total number and the ratio of diarrheal calves according to FTPI	19/24 79.16%		35/58 60.34%		
Number of calves at 3 rd day IgG level	6	18	28	19	11
Number and ratio of calves with diarrhea and duration (day) of diarrhea	5 83.33% (4.80 ± 0.96)	14 77.77% (3.50 ± 0.43)	19 67.85% (2.05 ± 0.96)	9 47.36% (1.67 ± 0.22)	6 54.54% (1.66 ± 0.32)
Total number and the ratio of diarrheal calves according to FTPI	19/24 79.16%		34/58 58.62%		
Number of calves at 7 th day IgG level	8	24	27	17	6
Number and ratio of calves with diarrhea and duration (day) of diarrhea	7 87.50% (4.28 ± 0.74)	17 70.83% (2.76 ± 0.39)	19 70.37% (2.21 ± 0.31)	7 41.17% (1.85 ± 0.26)	3 60.00% (2.33 ± 0.33)
Total number and the ratio of diarrheal calves according to FTPI	24/32 75.00%		30/50 60.00%		

Explanation: FTPI – failure of transfer of passive immunity

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