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Studies of Enteric Pathogens and γ-Globulin Levels of Neonatal Calves in Sweden

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Viring, S., S.-O. Olsson, S. Alenius, U. Emanuelsson, S.-O. Jacobsson, B. Larsson, N. Linde and A. Uggla: Studies of enteric pathogens and γ-globulin levels of neonatal calves in Sweden. Acta vet. scand. 1993, 34, 271-279. – Faecal and blood samples were taken from 10-30% of calves, 36 hours to 14 days old, in 47 dairy herds in different regions of Sweden from September 1987 to October 1988 (*Olsson et al.* 1993).

Faecal samples from 279 calves were analysed for the presence of *Escherichia* coli ($K99^+$), rotavirus and *Cryptosporidium* sp. Twenty (7.2%) of these samples were from diarrhoeic calves. An ELISA was developed and used for the rotavirus analysis. E. coli $K99^+$ was detected in 11.5%, *Cryptosporidium* sp. in 6.1% and rotavirus in 5.4% of the faecal samples. The presence of rotavirus alone and the combination rotavirus and E. coli ($K99^+$) was found to be associated with diarrhoea ($p<0.001$ and $p<0.01$ respectively).

Blood samples from 327 calves were analysed for the level of total protein and γ-globulin. In 43 of these samples (13%) γ-globulin did not separate from the $ß_2$-region by electrophoresis. The mean total protein concentration was 53.6 g/l in calves free from diarrhoea. The mean γ-globulin concentration, adjusted to 7 days age was 5.9 g/l. The 20 diarrhoeic calves had lower levels of both total protein and γ-globulin, compared with calves without diarrhoea, but the difference was not significant.

One litre more of colostrum at the first feed increased the level of total protein of the calves' sera by 1.4 g/l ($p = 0.05$). Calves born between May and September had a 2.0 g/l *higher ($p<0.001$)* serum concentration of γ-globulin than calves born between October and April.

calf diarrhoea; Cryptosporidium sp.; E. coli ($K99^+$); rotavirus; γ-globulin; total protein; colostrum.

Introduction

Enteritis is one of the most common diseases affecting neonatal calves throughout the world. Enteritis was 7.2% in a Swedish study (*Olsson et al.* 1993) of calves 0-90 days old. In Denmark, enteritis was observed in 44% of calves sent for destruction (*Krogh* 1971), while in Germany, 88% of necropsied calves were found to have a complex of abomasitis, enteritis and pneumonia (*Plöger et al.* 1980). The aetiology of bovine neonatal diarrhoea is complex and includes weak maternal immunity, nutritional factors, unsuitable management and a variety of infectious agents such as rotavirus, coronavirus, bovine virus diar-

rhoea virus, *Escherichia coli* K99+, *Salmonella sp.* and *Cryptosporidium parvum* (*Bridger et al.* 1978; *Tzipori* 1981, *Linthermans & Pohl* 1983, *Baker et al.* 1987, *De Rycke et al.* 1988, *Roy* 1990). All these microorganisms have been detected in Swedish calves. Rotavirus and Cryptosporidium sp. have only been detected in faeces of neonatal calves in a few farms (*Moreno-López & Jacobsson* 1978, *Viring et al.* 1985) and the association between these microorganisms have never been ruled out.

An adequate supply of colostrum and thereby the transfer of maternal antibodies to the newborn calf is most important for the prevention of enteritis and septicaemia (e.g. *Blom* 1982, *Larsson* 1985). Maternal antibodies are absorbed by the intestine and are found in the γ-globulin fraction of blood serum. The quantity of transferred immunoglobulins (Ig) depends on several factors, such as duration between partus and ingestion of colostrum, the content of Ig in colostrum, amount of ingested colostrum, breed, parity and individual factors (*Kruse* 1970 a,b). The concentration of Ig in serum peaks within a few days of birth. The half-life of the predominant Ig in serum, IgG1, is reported to be 17-21 days (*Logan et al.* 1972, *Tizard* 1987). The serum level of Ig in Swedish 2 to 5 week old calves was studied by *Hurvell & Fey* (1971) and *Larsson* (1985), but data is missing for younger calves. Nor have studies been carried out to determine the relation between various enteric pathogens, diarrhoea and serum level of Ig in Swedish dairy calves.

The objectives of this study were (a) to investigate the prevalence of 3 infectious agents (rotavirus, *Cryptosporidium* sp. and *E. coli* (K99+)) in faeces of diarrhoeic and non-diarrhoeic calves in Swedish dairy herds, and (*b*) to correlate the presence of diarrhoea to levels of total protein and γ-globulin concentration in serum, and (*c*) to correlate the levels of total protein and γ-globulin in serum to geographical region of the herd, season of birth, parity of the dam, breed, age at the first colostrum feeding and amount of the first colostrum fed.

Materials and methods

Calves and samples

Forty-seven herds were selected by convenience from a 200-herd survey of diseases in neonatal calves in Sweden, described by *Olsson et al.* (1993). The herds were from 10 regions representing different areas of Sweden. Blood and faecal samples were obtained by practising veterinarians from 327 and 279 calves respectively in the age range 36 h up to 14 days. The samples comprised as a rule 20-30% and at least 10% of the calves born within the herd from September 1987 to October 1988. The calves were classified as diarrhoeic or non-diarrhoeic by the veterinarian, who also received the following information from the farmer: age of the calf at sampling, age at first colostrum given and amount of first colostrum feed.

Examination of faecal samples

For detection of *Cryptosporidium oocysts,* smears were made from non-concentrated faecal samples on glass slides, air dried, fixed in methanol for 3 min and subsequently exposed to formaldehyde vapour at room temperature for 30 min. The smears were then stained by the phenol-auramine technique as described by *Nichols & Thom* (1984) and examined under an incident-light fluorescence microscope (Olympus, Tokyo, Japan) at 200x and 400x magnification.

E. coli strains were isolated from faecal samples with conventional methods (*Cowan & Steel* 1974) at the National Veterinary Insti-

tute. Two colonies from each sample were tested for K99 antigen by slide agglutination (*Smyth et al.* 1981).

For detection of rotavirus antigen in faeces, an enzyme-linked immunosorbent assay (ELISA) was developed. For this purpose, the serum Ig fraction from rabbits immunized 3 times with the rotavirus strain Osu and Gotfried was purified with protein A Sepharose and used as coating antigen at a concentration of 10 µg/ml carbonate buffer (pH 9.6). After incubation overnight, the plates were washed twice with phosphate-buffered saline with 0.5% Tween 20 (PBS-t) and 200-µl volumes of faecal solution (20%) in PBS-t were added per well in duplicate. After an incubation for 2 h at 37°C the plates were washed 3 times with PBS-t. Then 200 µl of an optimal dilution of the conjugate in PBS-t with 5% bovine serum albumin was added per well. The conjugate was the Ig-preparation of the rabbit hyperimmune serum to rotavirus coupled to horse-radish peroxidase according to the method of *Nakane & Kawoi* (1974). The plates were incubated for 30 min at 37°C and washed 3 times with PBS-t. Then 200 µl of a substrate solution (0.1 mg tetramethylbenzidine/ml and 0.05% H_2O_2 in 0.05 M citrate buffer, pH 5.5) was added to each well and the reaction was stopped after 15 min at room temperature by adding 50 µl 2 M H_2SO_4. The absorbance was measured in a Multiscan spectrophotometer (Flow Laboratories, Irvine, Scotland) at 450 nm. Faecal samples were considered positive for rotavirus antigen when the mean optical density was 2.5-fold higher than that of a negative standard.

Analysis of blood samples

Total protein concentration was measured with the biuret reaction performed in a computerized multichannel spectrophotometer (Cobas Mira, Hoffman-La Roche, Switzerland). The coefficient of variation between assays ranged between 1.3% and 2.2%.

Serum protein electrophoresis was performed using cellulose acetate strips as carrier medium and a 0.05% barbiturate buffer solution (pH 8.6). The electrophoresis was run for 20-30 min at 20 V/cm and a current of <10 mA.

The concentration of γ-globulin was adjusted for age to an estimated value corresponding to a calf age of 7 days (γ-globulin7) according to the formula:

$$[\gamma\text{-globulin}]7 = [\gamma\text{-globulin}]_a \times e^{dk} \text{ where}$$

$[\gamma\text{-globulin}]_a$ is the concentration of γ-globulin at the age of sampling in days (a), d is a-7 and k is the quota of ln 2 and the considered half-life of IgG_1, 17 days (*Tizard* 1987).

Statistical methods

The correlation between microorganisms isolated in faecal samples and diagnosis of diarrhoea was calculated using Fisher's Exact test for 2 x 2 tables. Differences between calves with and without diarrhoea with regard to total serum protein, serum γ-globulin, and time and amount of first colostrum feeding were assessed with an ordinary Student's t-test. Least-squares analysis of variance was performed in order to investigate the effects of some factors on the concentrations of total protein and γ-globulin in serum. Independent variables were geographical region of the herd, season of birth, breed and parity of the cow, age in hours at first colostrum feeding and amount of first colostrum fed. Non-significant ($p>0.05$) factors were removed from the model by a backward stepwise procedure. Data from the official milkrecording scheme (Swedish Association for Livestock, Breeding and Production, Eskilstuna, Sweden) and from the information furnished by the farmer were used for this analysis.

Results

Microorganisms in faecal samples

Of the 279 faecal samples bacteriologically examined, 32 (11.5%) proved positive for the presence of *E. coli* (K99+). *Cryptosporidium* was found in 17 (6.1%) and rotavirus antigen in 15 (5.4%) of the samples. Of the faecal samples examined, 20 (7.2%) were from diarrhoeic calves, as determined by the field veterinarian. At least 1 of the 3 pathogens was detected in 11 (55%) of these 20 samples (Table 1), which is a significantly higher detection rate ($p<0.001$) than in non-diarrhoeic samples (18%). Detection of rotavirus antigen, alone or together with *E. coli,* was associated with diarrhoea ($p<0.001$ and $p<0.01$, respectively). In contrast, the presence of *Cryptosporidium* or *E. coli* (K99+) alone was not generally associated with diarrhoea (Table 1).

Total protein and γ-globulin in serum

Blood samples from 327 calves were analysed regarding content of total protein and γ-globulin. The γ-globulin fraction could not be separated from the $ß_2$ fraction in 43 (13%) of the samples. These samples were omitted in the statistics.

The mean total protein and γ-globulin values in relation to age of the calves are shown in Figs. 1 and 2. The concentration of total protein was in the same range in calves from 2 to 14 days old, whereas the γ-globulin concentration decreased with increasing age ($p<0.05$). As shown in Table 2, the mean concentration of total protein in non-diarrhoeic calves was 53.6 g/l (range 39-76 g/l) and that concentration of γ-globulin recalculated to 7 days of age was 5.9 g/l (range 1-16 g/l).

Various factors in relation to diarrhoea

As can be seen from Table 2 the concentrations of total serum protein and γ-globulin were somewhat lower in calves with diarrhoea than in non-diarrhoeic calves, but the differences were not significant. Nor was there any significant difference between diarrhoeic and non-diarrhoeic calves in quantity of first colostrum fed or regarding the time when the first colostrum feed after birth was given (Table 2).

Table 1. The presence of microorganisms in faeces in relation to diarrhoea in 279 dairy calves aged 1½-14 days.

Microorganism			*Diarrhoea*		
E. coli (K99+)	*Cryptosporidium*	rotavirus	Yes	No	Probability*
–	–	–	9	212	
+	–	–	1	25	1.00
–	+	–	1	13	0.47
–	–	+	7	5	<0.001
+	+	–	0	3	1.00
+	–	+	2	1	0.006
–	+	+	0	0	
+	+	+	0	0	

*Degree of association between pathogen status and diarrhoea as estimated with Fisher's exact test.
+, microorganism detected.
–, microorganism not detected.

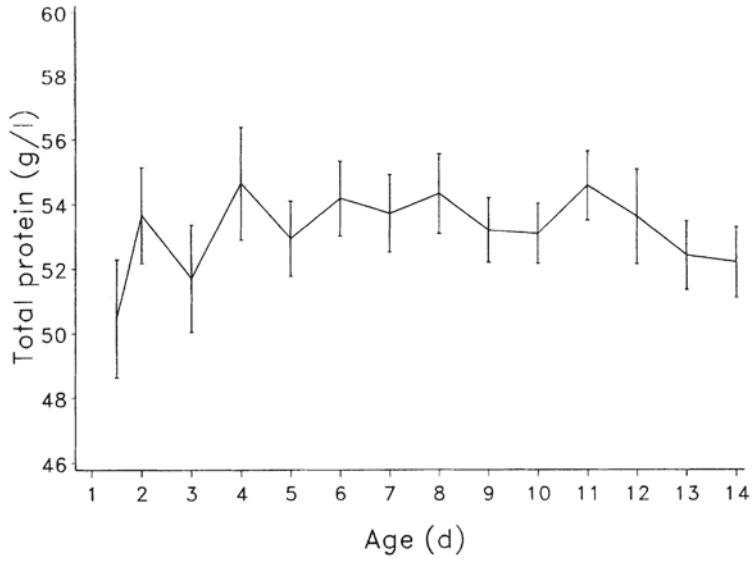

Figure 1. Mean (±SE) total protein level in serum of 327 calves 36 h to 14 days (d) old.

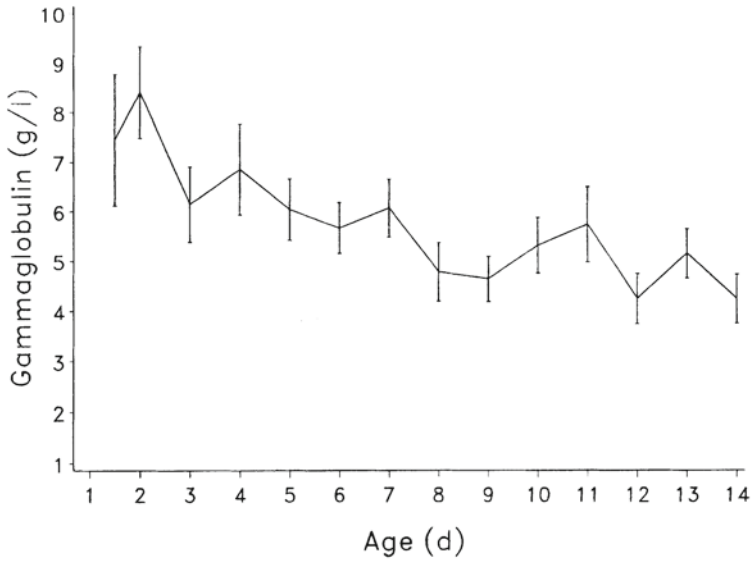

Figure 2. Mean (±SE) gammaglobulin level in serum of 284 calves 36 hours to 14 days (d) old.

Table 2. Overall means (±SE) for recalculated gammaglobulin levels, total protein content and age at first colostrum feeding for calves with and without diarrhoea at the time of sampling (*n*=number of observations).

Variable	*n*	Diarrhoea		Probability*
		Yes	No	
Adjusted gamma-globulin (g/l)	284	5.3 ± 0.6 (n=25)	5.9 ± 0.2 (n=259)	0.373
Total protein (g/l)	327	51.4 ± 1.2 (n=26)	53.6 ± 0.4 (n=301)	0.117
Age at first (hours) colostrum feed	326	4.5 ± 0.4	3.9 ± 0.1	0.207

*Student's *t*-test was used.

Various factors related to concentrations of protein and γ-globulin in serum

Errors in identifications of calves, and thus missing information on variables obtained from the milk recording scheme, reduced the number of observations in the final least-squares analysis for γ-globulin and total protein to 196 and 177 respectively. The concentration of total protein in the serum of calves was significantly ($p \leq 0.05$) influenced by the amount of the first colostrum fed. Thus, each additional litre of colostrum increased the level of serum protein by 1.4 g/l (± SE 0.7). The concentration of γ-globulin was 2.0 g/l (SE ± 0.6) higher in the calving season May to September than in October to April ($p<0.001$). Regarding region, breed, parity and age at first colostrum feed, no significant effect was evident with respect to the concentrations of γ-globulin and total protein in the serum of calves.

Discussion

The prevalence of diarrhoea in this study was 7.2% in 2-15-day-old calves (Table 2). Diarrhoea was diagnosed in 15 of the 47 herds. The diagnosis of diarrhoea was based on clinical observations by a veterinarian at the time faeces were sampled. In a survey of 200 Swedish herds, which included the 47 herds used in this study, the incidence of farmer-diagnosed diarrhoea was 4.5% during the first month of the calves' life (*Olsson et al.* 1993). The lower frequency in the latter study may have been the result of not all cases of diarrhoea having been reported by the farmers. However, in both the study by *Olsson et al.* (1992) and the present one, the frequency of diarrhoea was low in comparison with results from studies in other countries (*Roy* 1990). One possible explanation could be the relatively small size of the farms involved, which are believed to have a lower infection pressure than large farms.

In this investigation, at least 1 of the 3 pathogens studied could be detected in 11 out of 20 (55%) faecal samples from diarrhoeic calves. The fact that no infectious agent was found in the remaining 9 samples may have been due to any of several factors, e.g.: shedding of the agent did not coincide with the sampling occasion, other agents than the 3 examined had caused the diarrhoea, the diarrhoea observed was not caused by an infectious agent, or inadequate diagnostic methods had been employed. However, the detection rate of pathogens in diarrhoeic samples in this study (55%)

is close to results from a Finnish survey, including 8 farms with recurrent outbreaks of enteric disturbances, in which at least 1 of the pathogens rotavirus, enteropathogenic *E. coli* and *Cryptosporidium* sp. was associated with diarrhoea in 75% of the cases (*Pohjola et al.* 1986).

The results indicate that rotavirus is a common finding in connection with diarrhoea in calves also in Sweden. Rotavirus antigen was detected in 9 (45%) of 20 faecal samples from calves with diarrhoea, whereas the virus antigen was only found in 6 (2.3%) of 259 non-diarrhoeic samples. It is not known if any of these 6 calves had recently had diarrhoea or had developed diarrhoea later. Absence of significant association between the microorganisms *E. coli* ($K99^+$) and *Cryptosporidium* sp. and diarrhoea (Table 1) cannot be fully interpreted due to the small number of cases of diarrhoea observed in this investigation and the ages (< 14 days) of calves studied. Cryptosporidiosis is most common in calves 10-20 days old, usually peaking at about 14 days (*Krogh & Henriksen* 1985). Furthermore, both *E. coli* ($K99^+$) and *Cryptosporidium* sp. have been repeatedly documented in Denmark, Finland and Sweden in connection with outbreaks of severe diarrhoea in calves (e.g. *Viring et al.* 1985, *Sihvonen & Miettinen* 1985). In Denmark, Cryptosporidium has been detected in 24.8% of 8-14-day-old calves with diarrhoea (*Krogh & Henriksen* 1985).

The concentrations of total protein and γ-globulin in serum were somewhat lower in calves with diarrhoea than in non-diarrhoeic calves, but the differences were not significant (Table 2). Most of the studies carried out on this subject have observed a close relation between low concentration of γ-globulin and high prevalences of enteritis, septicaemia and mortality among calves (for a review, *Hancock,* 1983). However, in the present study, the incidence of diarrhoea was low, the mean serum concentration of γ-globulin was low (5.9 g/l) and 204 of 284 (72 %) calves had γ-globulin values lower than 8 g/l at 7 days of age and may therefore be considered to have had an inadequate gammaglobulin level. By contrast, *Möllerberg et al.* (1989) reported a mean γ-globulin value of 11.6 g/l in 8 days old calves which had received 2 litres of colostrum within 5 h of birth and a total amount of colostrum during the first day of life corresponding to 15% of their body weight. *Dobbelaar et al.* (1987) also found higher mean γ-globulin concentrations (14.4 g/l) in calves younger than 6 days, than observed in the present study. However, the latter authors found substantial herd-related differences in γ-globulin concentrations. In most herds with low γ-globulin levels in calves, colostrum was served in buckets. This method is most commonly used in Sweden and may help explain the low concentrations of γ-globulin among calves observed in this study.

Calves born between May and September had a 2.0 g/l higher ($p<0.001$) concentration of γ-globulin than calves born between October and April. This seasonal variation in concentrations concurs with results of other authors (*Hurvell & Fey* 1970, *Gay et al.* 1983, *Norheim & Simensen* 1985), but the reason for the variation remains unidentified. We also found a significant ($p=0.05$) correlation between the quantity of first colostrum fed and total protein in calves' serum, which agrees with the results published by *Dobbelaar et al.* (1987) and by *Norheim & Simensen* (1985) - but not with those of *Pivont et al.* (1984).

In conclusion, the present investigation indicates that the prevalence of neonatal diarrhoea is fairly low in Swedish dairy herds in spite of inadequate intake of colostrum as indicated by low levels of γ-globulin in the

calves' sera. The study has also confirmed the importance of rotavirus as a cause of bovine neonatal diarrhoea also under Swedish conditions.

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Sammanfattning

Studier av enteritframkallande mikroorganismer och γ-globulinnivåer hos neonatala kalvar i Sverige.

Från 47 mjölkkobesättningar med 20-50 kor per besättning belägna i olika delar av Sverige togs under perioden september 1987 till oktober 1988 träck- och blodprover från 10-30% av kalvarna i åldern 36 timmar till 14 dagar. Proven togs oavsett om kalvarna hade diarré eller ej.

Av 279 träckprov som analyserades avseende *Escherichia coli* ($K99^+$), *Cryptosporidium sp.* och rotavirus var 20 (7,2%) från kalvar med diarré. Samband förelåg mellan förekomst av rotavirus och diarré ($p<0,001$), och mellan samtidig förekomst av rotavirus och *E. coli* ($K99^+$) och diarré ($p=<0,01$).

Blodprover från 327 kalvar undersöktes med avseende på totalprotein och γ-globulin i serum. I 43 prov (13%) kunde γ-globulin ej skiljas från $ß_2$-globulin med elektrofores. Den genomsnittliga nivån av totalprotein var 53,6 g/l på kalvar som ej hade diarré. Gammaglobulinnivån korrigerad till 7 dygns ålder var 5,9 g/l. De 20 diarrékalvarna hade lägre såväl totalproteinvärden som γ-globulinvärden än kalvar utan diarré, men skillnaderna var ej statistiskt signifikanta.

En ökning av den första råmjölksgivan med 1 liter råmjölk visade sig höja totalproteinvärdet med 1,4 g/l ($p=0,05$). I medeltal var γ-globulin nivån 2,0 g/l högre hos kalvar födda mellan maj och september jämfört med kalvar födda mellan oktober och april.

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