

# Group A Rotavirus as a Cause of Neonatal Calf Enteritis in Sweden

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**Klingenberg K de Verdier, Svensson L: Group A rotavirus as a cause of neonatal calf enteritis in Sweden. Acta vet. scand. 1998, 39, 195-199.** – Faeces samples were collected during outbreaks of neonatal calf diarrhoea in 14 beef and dairy herds. Samples from 33 calves were taken at the onset of diarrhoea as well as from 30 calves with no signs of enteritis. No vaccines or medical treatment had previously been given. The mean age of the calves was 16.8 days (SD 8.2). The clinical evaluation of faeces consistency corresponded well to the dry matter content of the faeces ( $p < 0.001$ ). The samples were analyzed for rotavirus, *Cryptosporidium* species and *Escherichia coli* K99+. Group A rotavirus was detected by ELISA and RNA polyacrylamide gel electrophoresis (RNA-PAGE) in 14/33 (43.8%) of the samples from scouring calves and 1/30 (3.7%) of the samples from non-scouring calves. The correlation between group A rotavirus and diarrhoea was statistically significant ( $p < 0.001$ ). No non-group A rotaviruses were found by RNA-PAGE. *Cryptosporidium* species were detected through demonstration of oocysts in smears from 12/63 (19.0%) of the faecal samples, but no statistically significant correlation between diarrhoea and detection of oocysts was demonstrated. *Escherichia coli* K99+ was not detected in any faeces sample. The clear association between group A rotavirus and diarrhoea is suggested to be due to low pathogenic load in the herds.

***Enteropathogens; Escherichia coli* K99+; *Cryptosporidium* spp; ELISA; RNA-PAGE.**

## Introduction

Neonatal enteritis in calves is a common problem all over the world and its aetiology is complex. Several infectious agents have been isolated in outbreaks of calf diarrhoea, primarily rotavirus, coronavirus, *Cryptosporidium* species (spp) and enterotoxigenic *Escherichia coli* (E coli K99+) (Tzipori 1985). The reported prevalence differs, however, and a clear association between diarrhoea and enteropathogenic agents has often proved difficult to establish. In many surveys, rotavirus has been the most commonly detected agent (Acras *et al.* 1977, Snodgrass *et al.* 1986, Reynolds *et al.* 1986, De

Rycke *et al.* 1986). Infection with rotavirus is a well-known cause of acute diarrhoea in neonatal calves (Flewett & Woode 1978), but subclinical infections are also frequently occurring (De Leeuw *et al.* 1980, McNulty & Logan 1983, Reynolds *et al.* 1985). Rotaviruses of low virulence (Bridger *et al.* 1994) and age-related resistance (Varshney *et al.* 1995, Ball *et al.* 1996) have been suggested to account for subclinical infections.

Based on antigenical differences, rotaviruses are separated into 7 serogroups, named A-G. Group A rotavirus commonly causes neonatal

enteritis in calves and young animals of many other species. Group B and C rotaviruses have also been isolated from cattle (Saif 1990, Tsunemitsu *et al.* 1991), though the knowledge on non-group A rotavirus infections is limited and their prevalence and clinical significance remains unclear (Saif & Jiang 1995).

The aim of this paper was to evaluate the clinical importance of rotavirus infections in diarrhoea outbreaks in Swedish calves.

### Materials and methods

The study was performed in March and April 1992 and comprised 63 calves in 3 dairy and 11 beef suckler herds in the proximity of the Swedish University of Agricultural Sciences, Uppsala, and for which the farmers called for veterinary service for acute calf diarrhoea. Faeces samples from 33 calves with acute diarrhoea and 30 calves with no clinical signs of diarrhoea were collected by veterinarians. None of the calves had been previously treated or immunized against enteropathogenic agents. The average age for all 63 calves was 16.8 days (SD 8.2), ranging from 1 to 30 days old. Diarrhoeic calves averaged 16.8 days (SD 8.7) and calves without diarrhoea 16.7 days (SD 7.8). Thirty-six were heifers and 27 were bull calves. The average herd size was 30 cows (SD 25.5), and the calves were dairy breeds (52%), beef breeds (29%), and dairy/beef crosses (19%).

The faecal samples were collected in plastic bottles and transferred to the laboratory the same day. Examination for rotavirus, *Cryptosporidium* spp. and *E coli* K99+ was performed at the Swedish Institute for Infectious Disease Control and the National Veterinary Institute, Sweden. Rotavirus excretion was detected by group A rotavirus ELISA (Svensson *et al.* 1983, Svensson *et al.* 1986) and by RNA polyacrylamide gel electrophoresis (RNA-PAGE) (Svensson *et al.* 1986). *Cryptosporid-*

ium spp. was detected by parasitological demonstration of oocysts in faecal smears stained with Ziehl-Neelsen (Henriksen & Pohlenz 1981). Faeces samples were cultured on horse-blood agar and lactose agar with bromocresolpurpur, subcultured, and examined for *E coli* K99+ by slide agglutination test, as described by Söderlind *et al.* (1988). The dry matter content in the faeces samples was determined by weighing before and after evaporation at 90 °C over night.

The significance of the relation between diarrhoea and the presence of microorganisms was examined by  $\chi^2$  analysis, and between faeces consistency and dry matter content by the Student's *t* test.

### Results

Faeces from 32 scouring calves had a dry matter content of 14% (SD 4.7) compared to a dry matter content of 26% for normal faeces from 29 calves (SD 6.9). The difference was statistically significant ( $p < 0.001$ ). Group A rotavirus was detected in 15 samples from 8 herds (Table 1), with 100% correlation between ELISA and RNA-PAGE. No nongroup A rotavirus was demonstrated by RNA-PAGE. Fourteen out of the 15 rotavirus-excreting calves had diarrhoea, and the correlation between rotavirus in the faeces and diarrhoea was statistically significant ( $p < 0.001$ ). Rotavirus-excreting calves had an average age of 13.1 days (SD 5.7), ranging from 4 to 25 days.

*Cryptosporidium* spp. was demonstrated in 3 herds, in 12 faeces samples, 4 of which showed mixed infection with rotavirus. Eight out of these 12 calves were diarrhoeic. There was no significant correlation between diarrhoea and the demonstration of oocysts in faeces. The *Cryptosporidia*-shedding calves were from 8 to 21 days old, and on average 15.7 days (SD 4.0). *E coli* K99+ was not detected in any faeces sample.

Table 1. The presence of rotavirus and *Cryptosporidium* spp. in the faeces of 33 diarrhoeic and 30 non-diarrhoeic calves.

Herd no.*	Herd size**	No. scouring calves			No. non-scouring calves		
		Total	Rota +	Crypto+	Total	Rota +	Crypto+
1	7	1	0	0	3	0	0
2	15	2	0	0	3	0	0
3	30	2	1	0	3	0	0
4	70	5	1	5	3	0	3
5	4	2	1	1	4	0	0
6	25	5	4	2	2	0	1
7	30	4	4	0	0	0	0
8	15	2	0	0	2	0	0
9	3	3	0	0	1	0	0
10	17	1	1	0	1	0	0
11	7	1	1	0	0	0	0
12	60	2	0	0	4	0	0
13	70	1	1	0	2	1	0
14	65	2	0	0	2	0	0

\* Nos. 1-11 are beef suckler herds and nos. 12-14 are dairy herds.

\*\* No. of cows.

## Discussion

In this study, a comparison of the presence of microorganisms in faeces from calves with and without diarrhoea was made. The faeces consistency was evaluated clinically, and corresponded well to the dry matter content. According to the classification of diarrhoea by Roy (1990, p.53), i.e., faeces of <120 g dry matter per kg, the severity of scours was in general moderate.

The group A rotavirus ELISA has previously been evaluated in comparison to the reference methods electron microscopy and RNA-PAGE, and the cut-off absorbance value 0.1 was determined by testing human faecal samples. The excellent correlation between the ELISA and the RNA-PAGE results in this study demonstrates that the ELISA is an appropriate test method also for detection of group A rotavirus in bovine faeces samples.

Rotavirus in diarrhoeic calves in Sweden was first reported in 1978 (Moreno-López & Jacobsson), but no conclusions were drawn as to the

aetiological significance. In the present study, group A rotavirus was the most commonly detected agent and clearly associated with diarrhoea. These findings match the results from other studies (Acres *et al.* 1977, Snodgrass *et al.* 1986, Reynolds *et al.* 1986, De Rycke *et al.* 1986, Viring *et al.* 1993). The reason for rotavirus excretion being more frequently demonstrated in the beef suckler herds than the dairy herds is probably that beef suckler calves are more exposed to faeces from herdmates than are dairy calves, which leads to a rapid spread of the infection. The mean age of the rotavirus-shedding calves in this study was similar to that of others (de Leeuw *et al.* 1980, McNulty & Logan 1983, Luchelli *et al.* 1992).

*Cryptosporidium* spp. was only demonstrated in 3 herds, indicating that the clinical importance of *Cryptosporidium* spp. is restricted to certain herds. The local, clinical relevance of *E coli* K99+ remains unclear. *E coli* K99+ was previously detected by Viring *et al.* (1993) in

the faeces from 11.5% of 279 Swedish calves, but no clinical significance was established. In this local study, group A rotavirus appears as the most prominent cause of calf diarrhoea. The clear association between diarrhoea and rotavirus excretion may be explained by a generally low pathogenic load in the herds. To exemplify, the number of reported cases of infection with *Salmonella* spp. in the area was zero, antibodies against bovine herpesvirus-1 (infectious bovine rhinotracheitis) had never been detected in the herds and due to control programmes, the prevalence of bovine viral diarrhoea virus and bovine leukemia virus was steadily declining (the Swedish Board of Agriculture and the National Veterinary Institute, 1993). This provides researchers an opportunity to study specific agents, e.g., rotavirus, exclusively, without major interference from other microorganisms.

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### Sammanfattning

*Grupp A rotavirus som orsak till neonatal enterit hos svenska kalvar.*

Vid utbrott av kalvdiarré insamlades faecesprover i 14 dikalvs- och mjölkproducerande besättningar. Prover togs från 33 kalvar med akut enterit och 30 kalvar utan diarré. Kalvarna var i medeltal 16,8 dagar gamla och hade inte fått någon medicinsk behandling. Den kliniska bedömningen av diarré överensstämde bra med torrsbstanshalten i faeces ( $p < 0.001$ ). Proverna analyserades på förekomst av rotavirus, cryptosporidier och *Escherichia coli* K99+. Grupp A rotavirus påvisades med ELISA och RNA-polyacrylamid-gelelektrofores i 14/33 (43.8%) av proverna från kalvar med diarré och 1/30 (3.7%) av proverna från kalvarna utan diarré. Non-grupp A rotavirus påvisades inte i något prov. Sambandet mellan diarré och grupp A rotavirus var statistiskt signifikant ( $p < 0.001$ ). Oocystor av cryptosporidier diagnostiserades i Ziehl-Neelsen-färgade utstryk från  $12/63$  (19.0%) av träckproverna, men sambandet mellan diarré och påvisande av oocystor var inte statistiskt signifikant. *Escherichia coli* K99+ detekterades inte i något prov. Det klara sambandet mellan rotavirus och diarré kan bero på ett generellt lågt smittryck i besättningarna.

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