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# ROTAVIRUS AND ENTEROTOXIGENIC ESCHERICHIA COLI INFECTIONS OF CALVES ON A CLOSED FINNISH DAIRY FARM

By

*Liisa Sihvonen* and *Paavo Miettinen*

SIHVONEN, L. and P. MIETTINEN: *Rotavirus and enterotoxigenic Escherichia coli infections of calves on a closed Finnish dairy farm.* Acta vet. scand. 1985, *26*, 205—217. — Rotavirus and enterotoxigenic Escherichia coli infections of calves were surveyed during 2 successive years on a closed Finnish dairy farm consisting of 90—105 milking cows. From a clinical standpoint, diarrhoea was of moderate to high severity during the first year, compared to the milder disease in the second year of the study. Diarrhoea or abnormal faeces were found only in calves less than 8 weeks old, with the peak occurring during the first 2 weeks of life.

In the first year, rotavirus was detected throughout the calving season in diarrhoeic or abnormal faeces of calves aged 1 day to 7 weeks. In the second calving season, rotavirus was detected only during the 4 autumn months and in calves aged 11 days to 8 weeks. Rotavirus was detected in only 1 sample of normal faeces in both years. Electron microscopy revealed no enteropathogenic viruses other than rotaviruses. Enterotoxigenic K99 E. coli was found in about half of diarrhoeic or abnormal faeces in both years and throughout the calving seasons. K99 E. coli was also found in 5—10 % of normal faeces.

In the second year of the study, 45 of 101 pregnant dams were vaccinated with 2 doses of E. coli antigen. The vaccination trial did not prevent or reduce altered faeces in calves whose dams had been vaccinated compared with calves whose dams had not been vaccinated in the same year. Comparing the 2 years, the earlier uptake of colostrum together with better cleaning and disinfection of the calf house, contributed to the later and rarer occurrence of rotavirus infection in the second year of the study. The earlier uptake of colostrum together with better cleaning and disinfection of the salf house, in the second year, could not prevent enterotoxigenic E. coli infections in calves but partly prevented and modified the disease.

calf diarrhoea; K99 antigen; colostrum.

Diarrhoea, which frequently occurs in young calves, has been attributed to various causes such as bacteria, viruses, coccidia, nutritional imbalance, and faulty management.

Diarrhoea can be attributed to infection with a single agent or multiple agents. The enteropathogens most commonly found and extensively studied are enterotoxigenic E. coli (ETEC), rotavirus, and coronavirus (*De Leeuw et al.* 1980, *Tzipori* 1981, *Snodgrass et al.* 1982, *McNulty & Logan* 1983, *Sherwood et al.* 1983).

ETEC strains possess a number of virulence attributes that distinguish them from non-pathogenic strains. Adherence to the mucosal cells of the gut and production of toxins are the two main factors essential for enteropathogenicity (*Tzipori* 1981). ETEC overcomes the peristalsis of the small intestine by sticking to the enterocytes with the adhesic antigen K99, an adherence antigen common in calf strains (*Ørskov et al.* 1975). Enterotoxins are produced near the mucosal recoptor sites, which induce leakage of fluid and electrolytes into the lumen causing diarrhoea. ETEC strains are widespread and are detected in faeces from calves with diarrhoea and sometimes also apparently healthy calves.

Rotavirus, which has been recovered from various mammals, has been associated with diarrhoea in young domestic animals. In experimental infections in calves, rotavirus-associated diarrhoea is restricted to the first week of life; but under field conditions, rotavirus is often isolated from diarrhoeic calves up to 8 weeks old (*Tzipori* 1981). Rotaviruses are also sometimes observed in apparently healthy calves.

The purpose of this study was to determine, in a longitudinal survey, the occurrence of rotavirus and ETEC infections of calves on a closed dairy herd during 2 successive years.

## MATERIALS AND METHODS

### *Herd, sampling procedure and clinical surveillance*

The closed dairy herd belonging to an agricultural research station consisted of 90—105 milking cows. The calving season was from April to September, during which time about 95 % of the calves were born. Some calves were transferred from the herd when they were only a couple of weeks old.

Calves were delivered in a cleaned and disinfected stall. The day of delivery was regarded as day 0. During that day, each calf received at least 2000 ml of colostrum from its own dam within 12 h after birth. Thereafter, it was fed 4 l daily, divided between 2 feedings, until day 4. Pooled cows milk was given

from day 4 to day 7 and then milk substitute. During the second year, each calf received at least 2000 ml of colostrum from its own dam as soon as possible after birth, usually within 2 h. Thereafter 4 l, divided between 4 feedings, were fed daily until day 4. Pooled cows milk was given from day 4 to day 7 and then milk substitute.

In the calf house all calves were housed individually, each in a wooden box without bedding, until they were 2—3 months old, at which time they were removed to lairage on the farm or to other farms. The wooden boxes were emptied, cleaned and disinfected after each calf.

The survey of the herd lasted from May 1982 to January 1983 and from May 1983 to December 1983. During the first year, when there were 90 calves on the farm, 82 randomly selected faecal samples from 45 calves, aged from 1 day to 8 weeks, were taken for laboratory testing. During the same year, 10 faecal samples of adult cattle and 38 normal faecal samples of 2—8 month old calves were also tested as controls. During the second year, faecal samples were taken from 101 calves when they were 1—2 days, 1-, 2-, 4- and 8-weeks-old and also between these times if diarrhoea was found. Altogether, 550 faecal samples were taken for laboratory testing.

All calves were examined daily until 2—3-months old, and the consistency of the faeces was recorded. Faeces were classified as: 1) diarrhoeic if both fluid and profuse, 2) abnormal if not obviously diarrhoeic but changed with respect to colour, consistency and/or presence of blood or mucus, or 3) normal.

During the second year, 45 of 101 pregnant dams were vaccinated with 2 doses of E. coli antigen (Coligen, K99, K35, K30, K 85, Fort Dodge, USA). The first dose was given intramuscularly 5 weeks and the second 2 weeks before expected calving date.

Faecal samples were collected and transported to the laboratory in sterile bottles or gloves. All faecal samples were cultured for K99 E. coli and tested using the ELISA test for presence of rotavirus. In the first year, 68 faecal samples were also tested using the latex agglutination test for presence of rotavirus, for comparison with the ELISA test. During both years, 16 faecal samples were examined for enteric viruses using electron microscopy. All faecal samples were also screened for Salmonella species by overnight enrichment in selenite broth (*Edwards & Ewing* 1972). Threehundred of 632 faecal samples that had enough

material for the test were also checked for the presence of parasites by using direct smears and magnesium sulfate flotation-technique (*Coles* 1980).

*Isolation and identification of K99 E. coli*

A loop of faecal sample was inoculated on 5 % calf blood agar and eosin methylene-blue lactose sucrose agar (Merck, W. Germany). After overnight incubation at 37°C, 3—10 representative lactose-fermenting colonies were selected and subcultured on Minca-Isovitalex agar (*Guinee et al.* 1977). After overnight incubation at 37°C, the growth from each colony was tested for K99 antigen by slide agglutination with anti-K99 serum and control serum (supplied by Rijks Institute, The Netherlands). Representative lactose-fermenting colonies were confirmed as E. coli with Micro-ID test (General Diagnostics, USA).

*Virology*

Faecal samples were stored at —20°C, and after thawing they were used for virological tests.

Enzyme-linked immuno-sorbent assay (ELISA) for detection of rotavirus. The method for ELISA assay was the same as that described by *Grauballe et al.* (1981). Briefly, polystyrene microtest plates (A/S Nunc, Denmark) were coated with anti-rotavirus (human) produced in rabbit (immunoglobulin fraction of antiserum, Dako, Denmark) and rotavirus ELISA negative control serum (immunoglobulin fraction of normal rabbit serum, Dako) so that later each specimen could be tested twice with double control. Faecal sample (1—2 g) was homogenized in sufficient saline to make a thin slurry. The suspension was clarified by slow speed centrifugation. The supernatant was diluted 1:10 in buffer and added to wells (2 test wells and 2 control wells). The plates were incubated overnight at 4°C. After removal of non-reacting material by washing, normal rabbit immunoglobulins (Dako) were added to wells to block non-specific reactions. Peroxidase-conjugated anti-rotavirus (human) produced in rabbit (Dako) was added to all wells. After short incubation and removal of conjugate not bound by washing, the reaction was visualized by adding a solution of ortophenylendiaminedihydrochloride (Sigma, USA) and hydrogen peroxide to the wells.

Results were read visually by comparing the colour of the test well with the colour of the corresponding negative control well. Samples which visually showed an obviously more intense staining of the wells than that of the wells with negative control were considered positive for rotavirus antigens. Known rotavirus positive and negative samples were always included in the test.

Latex agglutination test (Rotalex, Orion, Finland) for detection of rotavirus has been described by *Haikala et* al. (1980). One drop of the sensitized latex particles was added to one drop of faecal sample diluted 1:10 with buffer. The drops were carefully mixed, spread, and tilted for 2 min. In a positive case, a distinct agglutination is observed and the test is considered negative if the suspension remains milky.

Electron microscopical detection of viruses. To clarify the faecal suspension, a 20—30 % suspension of faecal sample, prepared in phosphate-buffered saline, was centrifuged twice at 8000 rpm for 30 min. The supernatant fluid was layered onto 5 ml of 40 % (w/w) sucrose solution and centrifured at 20000 rpm for 5—6 h. The pellet was resuspended in 0.2 ml phosphate-buffered saline. A drop of the resuspended pellet was placed on a carbon-fomvar electron microscopic grid. Excess liquid was blotted off, and the grids were stained with 2 % potassium prototungstate and examined with a Jeol JEM-100S transmission electron microscope.

## RESULTS

Diarrhoea was found only in calves less than 8 weeks old. During both years about 2/3 of diarrhoeic or abnormal faeces were detected in calves under 2 weeks of age. In the first year 44 of 90 calves had diarrhoea, and 13 had abnormal faeces during their first weeks of life. Thirty-three calves had no altered faeces (Table 1). Diarrhoea or abnormal faeces, which usually lasted 2—3 days, occurred once in 35 calves, twice in 16 calves, and three times in 6 calves. All calves under 2 weeks of age with diarrhoea had anorexia and were depressed. They were fed an electrolyte mixture instead of milk, and some were given antibiotics.

In the second year 70 of 101 calves had abnormal faeces, and 11 had diarrhoea during their first weeks of life. Twenty calves had no altered faeces (Table 1). In 58 of 81 calves that had

Table 1. Observations on diarrhoea in calves (until 8 weeks after birth) from a closed dairy herd.

| Year | Number of calves | Number of calves with | | | Number of calves sampled | Number of faecal samples examined |
|---|---|---|---|---|---|---|
| | | Diarrhoea | Abnormal faeces | Normal faeces | | |
| 82 | 90 | 44 | 13 | 33 | 45 | 82 |
| 83 | 101 | 11 | 70 | 20 | 101 | 550 |

altered faeces, abnormal faeces or diarrhoea occurred more than once. Abnormal faeces or diarrhoea lasted 1—2 days at a time, and the calves showed no other clinical symptoms. Calves with diarrhoea were fed an electrolyte mixture, but no antibiotics were given.

The prevalence of rotavirus (Table 2) in diarrhoeic or abnormal faeces was high (26 of 35) and was distributed throughout the calving season in the first year. Rotavirus was recovered

Table 2. Detection of rotavirus and K99 E. coli in calves with diarrhoea or abnormal faeces in a closed herd during 2 successive years.

| Age, weeks | Number of faecal samples examined | | Number of faecal samples positive for | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Rotavirus | | K99 E. coli | | Rotavirus and K99 E. coli | |
| | —82 | —83 | —82 | —83 | —82 | —83 | —82 | —83 |
| 0—2 | 25 | 132 | 9 | 7 | 2 | 65 | 11 | 1 |
| 2—4 | 7 | 35 | 2 | 3 | 2 | 15 | 2 | 1 |
| 4—8 | 3 | 29 | 1 | 3 | 1 | 15 | 1 | 2 |
| | 35 | 196 | 12 | 13 | 5 | 95 | 14 | 4 |

Table 3. Detection of rotavirus and K99 E. coli in calves with normal faeces.

| Age, weeks | Number of faecal samples examined | | Number of faecal samples positive for | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Rotavirus | | K99 E. coli | | Rotavirus and K99 E. coli | |
| | —82 | —83 | —82 | —83 | —82 | —83 | —82 | —83 |
| 0—2 | 6 | 175 | 0 | 0 | 0 | 12 | 0 | 0 |
| 2—4 | 15 | 75 | 0 | 1 | 1 | 12 | 0 | 0 |
| 4—8 | 26 | 104 | 0 | 0 | 0 | 11 | 1 | 0 |
| | 47 | 354 | 0 | 1 | 1 | 35 | 1 | 0 |

in calves aged 1 day to 7 weeks. In the second calving season, the situation was quite different; the detection of rotavirus (Table 2) was low (17 of 196) and limited to the 4 autumn months and to calved aged 11 days to 8 weeks. In both years, rotavirus was detected in 1 sample of normal faeces (Table 3). Electron microscopy revealed no enteric viruses other than rotaviruses.

Comparison of electron microscopy and Rota ELISA results for 32 faecal samples is shown in Table 4. The 2 tests agreed well, only 1 sample, positive in Rota ELISA, revealed no rotavirus in electron microscopy. Comparison of Rota ELISA and latex agglutination results for 68 faecal samples from the first year is shown in Table 5. Clear-cut positive or negative agglutination reactions of the latex test were reliable, but 6 of the 68 samples gave questionable or unspecific reactions.

Table 4. Comparison of electron microscopy and Rota ELISA results for 32 faecal samples.

| EM | — | + | — | + | |
|---|---|---|---|---|---|
| ELISA | — | + | + | — | Agreement |
| | 16 | 15 | 1 | 0 | 31/32 (97 %) |

Table 5. Comparison of Rota ELISA and latex agglutination results for 68 faecal samples.

| Latex agglutination | — | + | + | — | +* | +** | |
|---|---|---|---|---|---|---|---|
| | | | | | — | + | |
| ELISA | — | + | — | + | — | — | Agreement |
| | 37 | 23 | 1 | 1 | 2 | 4 | 60/68 (88 %) |

* questionable reaction
** unspecific reaction (control agglutination)

Enterotoxigenic K99 E. coli was found in 19 of 35 diarrhoeic or abnormal faeces samples in the first year and in 99 of 196 diarrhoeic or abnormal faeces samples in the second year (Table 2). K99 E. coli was detected in 2 of 47 samles of normal faeces in the first year and in 35 of 354 normal samples in the second year (Table 3). No other pathogenic bacteria were detected in the faeces of the calves except in a one-day-old calf, which excreted

Salmonella agona in the second year. The next 6 feacal samples (taken 1 week apart) from that calf revealed no salmonellas, nor did other faecal samples from the whole herd cortain salmonellas.

In the first year, all 22 sampled calves with diarrhoea or abnormal faeces were positive for rotavirus, K99 E. coli or both in at least one sample. In the second year, 71 of 80 calves with abnormal or diarrhoeic faeces were positive for rotavirus, K99 E. coli or both in at least one sample.

In the first year, 38 normal faecal samples of 2- to 8-month-old calves and 10 faecal samples of adult cattle were surveyed for comparison, but no rotavirus or pathogenic bacteria were detected.

The results of parasitological studies revealed only minute numbers of oocysts of Eimeria spp.

In the second year, 45 of 101 pregnant dams were vaccinated with 2 doses of E. coli antigen. The effect of this vaccination trial on the occurrence of diarrhoea, abnormal or normal faeces in calves is shown in Table 6. There was no significant difference between the calves of vaccinated and nonvaccinated dams in the second year.

Table 6. Effect of vaccination of dams with E. coli antigen on consistency of faeces of calves.

| Calves with | 1—2-day-old calves | | 1-week-old calves | | 2-week-old calves | |
|---|---|---|---|---|---|---|
| | vaccinated | not vaccinated | vaccinated | not vaccinated | vaccinated | not vaccinated |
| *Normal faeces* | 27 | 34 | 15 | 30 | 29 | 38 |
| no bacteria, no virus | 22 | 29 | 15 | 29 | 26 | 38 |
| K99 E. coli | 5 | 5 | 0 | 1 | 3 | 0 |
| *Abnormal faeces* | 18 | 21 | 28 | 25 | 15 | 14 |
| no bacteria, no virus | 6 | 7 | 14 | 16 | 8 | 6 |
| K99 E. coli | 12 | 14 | 14 | 9 | 3 | 5 |
| rotavirus | 0 | 0 | 0 | 0 | 3 | 3 |
| rotavirus and K99 E. coli | 0 | 0 | 0 | 0 | 1 | 0 |
| *Diarrhoeic faeces* | 0 | 1 | 0 | 1 | 0 | 1 |
| K 99 E. coli | 0 | 1 | 0 | 1 | 0 | 1 |

## DISCUSSION

Diagnosis of enterotoxigenic E. coli infections in calves may be based on the detection of K99 antigen. Good correlation has been shown between possession of K99 antigen, production of heat stable enterotoxin, and ability to dilate intestinal loops (*Guinee et al.* 1981, *Sherwood et al.* 1983). In our survey of a closed dairy herd, we detected no enteropathogenic bacteria other than K99 E. coli. The only exception was one case of Salmonella agona in a healthy one-day-old calf. All animals on the farm were tested twice and found to be negative for salmonellas. The origin of Salmonella agona remained unclear but it had no connection with diarrhoea.

The double-antibody sandwich Rota ELISA, described earlier by *Grauballe et al.* (1981) and used in this experiment, was easy to perform, reproduced well, and was sensitive. Positive reactions were easily recognised with the naked eye, and the results were highly correlated (97 %) with electron microscopy, the reference method for diagnosing rotaviruses. The latex agglutination method was used in the first year and compared with the ELISA technique. Eight of 68 first year samples investigated with the latex agglutination test gave questionable or unspecific reactions. When investigated by Rota ELISA, they were found to be negative. The overall agreement between Rota ELISA and latex agglutination results was quite good (88 %). In conclusion, Rota ELISA was the most suitable test for diagnosing calf rotavirus infections when larger amounts of faecal samples were tested; therefore, in the second year we used only this test to determine the presence of rotavirus. In both years, some faecal samples were tested using electron microscopy to reveal possible enteric viruses other than rotaviruses. The presence of other viruses could not be confirmed.

From a clinical point of view, diarrhoea was moderate to severe during the first year compared to the milder disease in the second year. During both years, faeces were most frequently altered during the first 2 weeks of life. Some retardation in growth, extra work with feeding and nursing obviously accounted for the major economic loss due to diarrhoea in calves.

In the first year, all calves were exposed to rotavirus starting immediately after birth. The proportion of altered faeces samples found to be positive for rotavirus (26 of 35) was quite high. The proportion of faeces samples with dual infection (both K99 E.

coli and rotavirus) was also moderate (14 of 35). All tested calves that had diarrhoea or abnormal faeces were also found to excrete rotavirus, K99 E. coli or both in at least one of the sequentially tested faecal samples. These observations suggest that in the first year both rotavirus and K99 E. coli helped cause diarrhoea in 1-day-to 8-week-old calves. In the second year, through the calving season, apparently only K99 E. coli caused diarrhoeic and abnormal faeces in calves aged 1 day to 8 weeks. The involvement of rotavirus was limited to the 4 autumn months and to calves aged 11 days to 8 weeks.

Some earlier studies have indicated that co-infection of calves with enterotoxigenic E. coli and rotavirus can induce diarrhoea in circumstances where one agent alone does not (*Snodgrass et al.* 1982, *Tzipori et al.* 1983). Our results also suggest that dual infection with enterotoxigenic E. coli and rotavirus may effect the severity of the clinical disease, as seen in the first year compared to the milder disease in the second year when dual infection was an exception. Large numbers of rotaviruses are excreted in infected faeces and are comparatively resistant to inactivation (*Woode* 1978). Environmental contamination with rotaviruses is heavy and persistent. Rotavirus surviving in a contaminated environment from one calving season to the next could be the source of infection in an outbreak. Another possibility may be that adults are initially the major source of infection for calves (*Woode* 1978). In our survey, however, we detected no rotavirus in the faeces of the older calves or adult cows tested. Once an outbreak is underway, as in our first year of study, the major source of rotavirus infection is apparently environmental. The cycle of rotavirus infection can be broken by thorough cleaning and disinfection of the calf house (*McNulty & Logan* 1983). Cleaning and disinfection of the calf house was emphasized in the second year; this apparently was reflected in later and rarer occurrence of rotavirus infection in that year.

The interval between birth and first uptake of colostrum is critical (*Tzipori* 1981). In the first year, colostrum uptake could be delayed until 12 h after birth, which differed from the situation in the second year when all calves were given colostrum as soon as possible after birth, usually within 2 h. The delay of colostrum uptake apparently contributed to the more severe clinical disease in the first year. During that year, the calves could easily have been expossed to enterotoxigenic E. coli and

rotavirus before consumption of colostrum, and therefore inhibitors in the milk could not prevent infections, but only modify the disease. In the second year, early uptake of colostrum, together with better cleaning and disinfection of the calf house, probably contributed to the later and rarer occurrence of rotavirus infection. Early uptake of colostrum in the second year did not prevent enterotoxigenic E. coli infections in calves but partly prevented and modified the disease.

Earlier experiments have indicated that lacteal immunity against the K99 antigen could prevent severe diarrhoea and death in calves experimentally challenged with E. coli strains B41 or B44 (*Acres et al.* 1979, *Nagy 1980)*. Our data indicated no differences between those calves whose dams were vaccinated with E. coli antigen and those whose dams had not been vaccinated in the second year. The role of maternal antibodies is essential for prevention of diarrhoea in calves. On our farm, enterotoxigenic E. coli infections were common in the first year; so cows apparently could have had high enough titres of antibodies against enterotoxigenic E. coli without vaccination in the the second year. For the calves the critical point was the timing of the uptake of colostrum.

The results obtained in this study indicate that both K99 E. coli and rotavirus were involved in causing diarrhoea on a closed Finnish dairy farm. Earlier uptake of colostrum and better cleaning and disinfection of the calf house in the second year than in the first year contributed to rarer occurrence of diarrhoea and milder disease in calves.

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SAMMANFATTNING

*Rotavirus och enterotoxigena Escherichia coli infektioner hos kalvar i en sluten mjölkkobesättning.*

En långvaring undersökning av rotavirus och enterotoxigena Escherichia coli infektioner under 2 påvarandra följande år utfördes hos kalvar i en sluten mjölkkobesättning bestående av 90—105 mjölkkor. Kliniskt sett var det första årets diarre'er moderata till ytterst allvarliga i jämförelse med ett lindrigare förlopp under det andra året. Diarre konstaterades bara hos kalvar under 8 veckor, och främst under de 2 första veckorna.

Rotavirus påvisades i avföring från kalvar med diarre eller onormal avföring, vid en ålder av en dag upp till 7 veckor, och fynden fördelades över hela kalvningsäsongen under det första året. Situationen var annorlunda under det andra årets kalvningar, rotavirus påvisades bara under 4 höstmånader och hos kalvar med en ålder från 11 daga rtill 8 veckor. Rotavirus påvisades bara i en normal avföring under de båda åren. Elektronmikroskopi avslöjade inga andra virus än rotavirus. Enterotoksigena K99 E. coli påvisades i ungefär hälften av avföringarna från kalvar med diarre eller onormal avföring under de båda åren. K99 E. coli åvisades också i 5—10 % av normala avföringsprov under de båda åren.

Fyrtiofem av 101 dräktiga kor vaccinerades under det andra året med 2 doser av E. coli antigen. Vaccineringen kunde inte förhindra eller förminska förekomsten av onormal avföring hos deras kalvar jämfört med de kalvar vilkas mödrar inte var vaccinerade under samma år. En jämförelse mellan dessa 2 år, visade att möjligheten att tidigare få colostrum kombinerad med en bättre rengöring och desinfektion av kalvboxarna tydligt medverkade till en senare och sällsyntere förekomst av rotavirus infektioner under det andra året. En tidigare upptagning av colostrum och en bättre rengöring och desinfektion av kalvboxarna kunde inte förhindra enterotoxigena E. coli infektioner hos kalvar det andra året, men kunde delvis hämma och modifiera sjukdomen.

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Reprints may be requested from: Liisa Sihvonen, the National Veterinary Institute, P. O. Box 92, 70700 Kuopio, Finland.