JOURNAL OF CLINICAL MICROBIOLOGY, June 1987, p. 1097–1106
0095-1137/87/061097-10$02.00/0

Vol. 25, No. 6

Local and Systemic Antibody Response to Bovine Respiratory Syncytial Virus Infection and Reinfection in Calves with and without Maternal Antibodies

T. G. KIMMAN,[1]* F. WESTENBRINK,[2] B. E. C. SCHREUDER,[1] AND P. J. STRAVER[2]

Department of Herd Health and Pathology[1] and Department of Virology,[2] Central Veterinary Institute, 8200 AB Lelystad, The Netherlands

Received 6 November 1986/Accepted 6 March 1987

Enzyme-linked immunosorbent assays for the detection of immunoglobulin M (IgM), IgA, IgG1, and IgG2 antibodies against bovine respiratory syncytial virus (BRSV) were used to measure antibody responses of calves after experimental or natural infection with BRSV. Serially collected sera, lung lavage samples, nasal and eye secretions, and feces were tested for the presence of these antibodies. Lung lavage fluids and nasal secretions were further examined for the presence of virus. After experimental infection of 3- to 4-week-old, colostrum-deprived (seronegative) calves, the virus was detected from days 3 to 8 post-initial inoculation day (PID). An immune response was first detected 8 to 10 days PID, when BRSV-specific IgM and IgA appeared nearly simultaneously in serum, secretions, and feces. BRSV-specific IgG1 appeared only in serum on days 13 to 17 PID, and IgG2 was first detected in sera from 1 to 3 months PID. Specific IgM and IgA were detectable in the different samples for various periods. In the respiratory and eye secretions, IgA usually remained detectable for long periods, that is, for up to 3.5 months or longer. In lung lavage samples, BRSV-specific IgG1 was only incidentally demonstrated and appeared to be blood derived. The immune response of a 5-month-old calf strongly resembled that of the 3- to 4-week-old calves (feces excepted), indicating that an age effect on the immune response to BRSV is unlikely. After experimental infection of colostrum-fed, seropositive calves, both local and systemic antibody responses were largely or totally suppressed. The degree of suppression seemed to be related to the level of preinoculation virus-specific serum IgG1. Of all isotypes, IgM was least affected. Colostrum-fed animals shed virus in about equal amounts and for the same length of time as colostrum-deprived calves. Clinical signs were mild in both groups. After reinfection, no virus shedding was detected in either colostrum-deprived or colostrum-fed calves. In both groups, a secondary immune response developed, characterized by strong and rapid (from about day 6 PID) mucosal and systemic IgA responses, but reaching higher titers in colostrum-deprived calves. Also, strong mucosal, but not serum, IgM responses were observed, which, however, did not develop faster than those observed after primary infection. Naturally infected calves, showing severe signs of respiratory disease, had various levels of, most likely, maternally derived antibodies on the first day of illness. Mucosal and systemic antibody responses of various heights and durations were observed, but in general these responses were stronger than those observed after experimental infection. The results point to an important role for local IgA, rather than for serum IgG1, in the protection against BRSV infection. The capacity to mount a local memory IgA response seems especially important. Priming for such a mucosal memory response is possible even when the primary immune response is severely suppressed because of the presence of maternal antibodies.

Infections with bovine respiratory syncytial virus (BRSV) and associated clinical signs are observed in calves with or without maternal antibodies (11, 13, 20, 23, 30). Even young calves, from 2 to 3 weeks of age, can exhibit severe signs of disease during BRSV outbreaks (13; T. G. Kimman, F. Westenbrink, P. J. Straver, D. van Zaane, and B. E. C. Schreuder, Res. Vet. Sci., in press). Reinfections with BRSV also occur, but these are usually not accompanied by clinical signs of disease (16). Thus, a natural infection does not prevent reinfection but appears to offer good protection against clinical consequences after reinfection. In contrast, children may exhibit severe disease after the second infection with respiratory syncytial virus (RSV), but subsequent infections result in progressively milder illnesses (8).

Besides affording incomplete protection to BRSV, maternal antibodies are markedly immunosuppressive. In calves with maternal antibodies, not only are serum immunoglobulin G1 (IgG1) and IgG2 responses suppressed, but serum IgM and IgA responses are also decreased or absent (Kimman et al., in press).

Immunity to RSV has been studied in infants (15, 18, 19, 31), cattle (23, 32, 36; Kimman et al., in press), mice (35), cotton rats (25–27, 39), and ferrets (33). These studies sometimes yielded conflicting results, and often the immune response at the sites of infection was not investigated. Especially in cattle, information about the local immune response to BRSV is scarce. The availability of monoclonal antibodies against bovine immunoglobulins (37) made it possible to develop highly specific enzyme-linked immunosorbent assays (ELISAs) for the detection of IgG1, IgG2, IgM, and IgA antibodies against BRSV (Kimman et al., in press). The possibility of collecting repeated lung lavage samples of unanesthetized calves (13) further enabled the study of the local immune response to BRSV.

In the present study, the immune response to BRSV was studied by using serial lung lavages of specific-pathogen-free calves, with or without maternal antibodies, after an experimental infection and reinfection. The infection protocol was

* Corresponding author.

intended to mimic a natural infection and to evoke clinical illness. The results obtained in the experimental infections are compared with results obtained in naturally infected calves.

MATERIALS AND METHODS

Experimental animals. In two experiments, a total of 13 Friesian specific-pathogen-free calves were used. Calves were obtained by cesarean section and reared in isolation. They were housed individually in wooden boxes placed in isolation rooms with positive air pressure. Each experimental group was housed in a separate isolation room. Persons entering the isolation rooms changed clothes beforehand. The calves were fed a commercial milk substitute until 6 weeks of age. Thereafter, they were fed dried grass pellets and concentrates.

During the experiments, the calves remained free of infections with infectious bovine rhinotracheitis virus, bovine viral diarrhea virus, and parainfluenza virus type 3, as determined by serologic examination. Two calves, one colostrum deprived and one colostrum fed, were killed and necropsied because of intercurrent disease (polyarthritis and urinary tract infection, respectively).

Experimental design. In the first experiment, six specific-pathogen-free calves were used. They were raised without colostrum. Four of these calves were infected at an age of 3 to 4 weeks by a combined intranasal and intratracheal route. The inoculum (see below) was administered intratracheally (15 ml) and intranasally (5 ml; 2.5 ml in each nostril) during four consecutive days. Two calves were mock infected with control antigen. Four months later, the calves were reinfected by using the same inoculation protocol, and one of the control calves was then infected for the first time.

In the second experiment, five calves were fed colostrum ad libitum during the first 48 h of life, and two calves did not receive colostrum. Colostrum was obtained from postpartum cows on different farms, stored at −20°C, and pooled before use. The colostrum pool had a BRSV-specific IgG1 titer of 20,480; BRSV-specific IgG2, IgM, and IgA were not detected. Calves ingested 4.5 to 8.5 liters of colostrum. In this experiment, two colostrum-deprived calves and three colostrum-fed calves were infected at 3 weeks of age and two colostrum-fed calves were infected at 10 weeks of age in order to study the effect of different levels of maternal antibodies. Calves were reinfected after 3 months. Four calves (two colostrum fed and two colostrum deprived) were infected a third time, 3 months after the second infection.

Before and after infection, the calves were examined for clinical signs two or three times a week. Rectal temperatures were determined twice daily.

Sampling procedures. During the week of inoculation and for 3 weeks thereafter, blood samples, lung lavage fluids, nasal and eye secretions, and feces were collected three times weekly. Before and after this period, samples were collected at greater intervals.

Blood collected in EDTA-containing tubes was used for determination of hematological parameters (hemoglobin content, hematocrit, number of leukocytes, and differential leukocyte count). Serum was obtained from whole blood by centrifugation and stored until assayed for albumin and BRSV antibodies.

Lung lavage was done as described previously (13), with 60 ml of phosphate-buffered saline (sterile, isotonic, pH 7.2). Usually, 30 to 50 ml of fluid was recovered. After collection, samples were divided into three parts. One part was immediately processed for virologic examination. A second part was used for determination of the total number of cells by using a Coulter Counter (Coulter Electronics, Hialeah, Fla.). If the number of cells was $<0.5 \times 10^5$/ml of lavage fluid, the results obtained with that sample were discarded. The remainder of the lung lavage fluid was centrifuged (1,100 × g, 10 min), and the supernatant was stored until assayed for albumin and antibodies.

Nasal and eye secretions were collected with cotton tips (Medical Wire & Equipment Co. [Bath] Ltd., Potley, Corsham, Wilshire, United Kingdom). One nasal swab was processed for virologic examination immediately. A second nasal swab and an eye swab were suspended in 1 ml of ELISA buffer and stored until assayed for antibodies.

Blood contamination of secretions was assessed by means of a urine test strip (Boehringer GmbH, Mannheim, Federal Republic of Germany).

Feces samples were mixed with four parts phosphate-buffered saline, pH 7.2, containing 0.05% Tween 80, and stored at −70°C. Before being tested, feces samples were centrifuged for 10 min at 1,100 × g. Feces supernatants and nasal or eye swab suspensions were regarded as undiluted test samples. Until being tested, all samples were stored at −70°C.

Inoculum. Calves were inoculated with a BRSV strain isolated from a calf in a herd with severe respiratory tract disease (herd G in reference 13). The virus was isolated and multiplied in monolayers of secondary bovine fetal lung cells. The fifth and sixth passages were used. After a freeze-thaw cycle, cellular lysates were clarified by centrifugation and were stored at −70°C until required for use. Immediately before the lysates were used, virus titrations were done. Inocula contained 10^3 to 10^5 50% tissue culture infective doses of virus per ml. Inocula were checked for the absence of bovine viral diarrhea virus by immunofluorescence (IF) tests on inoculated cell cultures. Uninfected monolayers were treated identically and used as control antigen.

Virologic examinations. Nasal swabs were suspended in 5 ml of Hanks minimal essential medium with 2% fetal calf serum and antibiotics. This medium was also added to lung lavage samples in a 1:1 ratio. After incubation for 30 min at room temperature and centrifugation (1,100 × g for 10 min), 2-ml volumes of these samples were added to monolayers of a fetal bovine trachea cell line, grown in 25-cm^2 disposable polystyrene flasks (Corning Glass Works, Corning, N.Y.). The cultures were incubated at 37°C for up to 3 weeks. Cultures were examined for cytopathic effect every day. When cytopathic effect developed, or else 3 weeks after inoculation, the cultures were examined for BRSV by an indirect IF test by using a specific-pathogen-free calf serum against BRSV.

Virus titrations on positive samples were done in duplicate by using fetal bovine trachea cells. The cells (10^5/ml) were mixed with 0.05-ml volumes of 10-fold dilutions of test samples and incubated for 7 days in 24-well tissue culture cluster plates (Corning). The test was read for cytopathic effect.

Isotype-specific BRSV ELISAs. BRSV-specific IgG1 and IgG2 were detected and titrated in indirect double antibody sandwich assays, as described previously (Kimman et al., in press). Briefly, ELISA plates coated with the immunoglobulin fraction of a horse serum against human RSV (Flow Laboratories, Inc., McLean, Va.) were incubated successively with BRSV antigen (50% ammonium sulfate precipitate of a BRSV-infected cellular lysate), test sample, horse-

radish peroxidase-conjugated monoclonal antibody against bovine IgG1 or IgG2, and finally with substrate. For detection of BRSV-specific IgA and IgM, antibody capture assays were used (Kimman et al., in press). ELISA plates coated with a monoclonal antibody against bovine IgA or IgM were incubated successively with test sample, BRSV antigen, horseradish peroxidase-conjugated horse anti-human RSV IgG, and substrate.

Serial twofold dilutions of samples were made in the ELISA plate starting at a dilution of 1/20, except for the IgG1 ELISA, for which a starting dilution of 1/80 was used. The starting dilution for the other samples (lung lavage fluids, nasal and eye secretions, and feces) was 1/2. Samples obtained during booster reponses were sometimes tested with a starting dilution of 1/80.

Selected lung lavage samples were concentrated. After dialysis against deionized water, the samples were freeze-dried. Subsequently, the residue was dissolved in 500 μl of ELISA buffer and tested as described above.

In each plate, a standard positive and negative sample were included. Each sample was also tested in the lowest dilution with control antigen to check for nonspecific binding. Color development was measured with a Titertek-multiskan (Flow) at 450 nm. The matrix was set per plate according to the maximum E_{450} value obtained with the standard positive serum. As a result, one matrix unit corresponds to 1/10 of the maximum value obtained with the standard positive serum. The titer was taken as the highest dilution of a test sample scoring one matrix unit above the value obtained with the standard negative sample.

Total IgA concentrations. Total IgA concentrations were determined in a double antibody sandwich assay, as described previously (Kimman et al., in press). The starting dilution was 1/10.

Albumin. Albumin was determined in radial immunodiffusion plates.

Field outbreaks. During two field outbreaks (A and B) of severe respiratory tract disease, four and five calves, respectively, were sampled at regular intervals in a manner identical to that for the experimental animals. BRSV involvement was demonstrated by IF on lung lavage cells of all nine calves and by virus isolation from lavage fluid from one calf (outbreak A). BRSV was also demonstrated both by IF and virus isolation in lung tissue of three other calves that died during outbreak A and in one calf from outbreak B that was killed. Serologic examinations failed to demonstrate the involvement of bovine viral diarrhea virus, infectious bovine rhinotracheitis virus, or parainfluenza virus type 3 in these outbreaks.

Calves had the following ages at the onset of disease; 10, 12, and 16 (two calves) weeks (outbreak A); 3 (two calves), 6 (two calves), and 7 weeks (outbreak B).

RESULTS

Clinical, hematological, and postmortem findings. At 5 to 10 days post-initial inoculation day (PID), BRSV-infected animals, both colostrum deprived and colostrum fed, showed mild signs of disease. They had slightly elevated body temperatures (39.3 to 39.4°C), coughed during forced respiration, and had slightly abnormal breath sounds on auscultation. Hematological parameters showed insignificant changes within the normal range.

Two calves, one colostrum deprived and one colostrum fed, were killed on day 8 PID because of intercurrent disease and were examined pathologically. The colostrum-fed calf had a serum IgG1 titer of 1,280 against BRSV on the first day of inoculation. Both calves had several areas (1 to 10 cm^2) of red consolidation in the ventral parts of the lung. Bronchial lymph nodes were enlarged and edematous. Histologically, atelectasis of alveoli and a catarrhal and necrotizing bronchiolitis were observed in these consolidated areas. Syncytial giant cells were occasionally observed. The consolidated areas of both calves were positive for BRSV by the IF test and virus isolation.

Reinfected and control animals did not show clinical signs.

Diffusion of serum proteins into lung lavage fluid. Albumin concentrations in 69 corresponding serum and lung lavage samples, obtained during the first experiment, were determined in order to estimate the contribution made by serum to the lung lavage fluid. The ratio between the albumin concentrations in lung lavage fluid and serum was $4.9 \times 10^{-4} \pm 3.1 \times 10^{-4}$ (mean ± standard deviation). This indicates that the contribution made by serum antibodies to the antibody titers determined in the lung lavage fluids can be neglected.

Antibody responses. (i) Colostrum-deprived animals. The primary antibody response to BRSV of colostrum-deprived (seronegative) calves was first detected at about 8 to 10 days PID, when BRSV-specific IgM and IgA appeared nearly simultaneously in serum, lung lavage fluid, secretions, and feces. BRSV-specific IgG1 appeared in sera on days 13 to 17 PID, whereas IgG2 appeared still later in the sera, varying from nearly 1 to 3 months after inoculation. Low titers of BRSV-specific IgG1 and IgG2 were occasionally detected in secretions and feces, but only in blood-contaminated samples. All colostrum-deprived animals reacted similarly. The immune response of the calf that was 5 months old when first inoculated strongly resembled that seen in the calves inoculated at nearly 1 month of age. The characteristics of the responses of all colostrum-deprived animals are summarized in Table 1. The responses of one animal are illustrated in Fig. 1.

IgM was detectable in the different samples for various lengths of time (Table 1). IgA was found in serum for 12 to 29 days but was not detected in the serum of one animal. This calf was not IgA deficient, since it did show a mucosal IgA response. Most variation between animals was seen in the IgA responses in the lung and nasal or eye secretions. After an initial phase with a peak on day 13 to 14 PID, identical for all animals, IgA usually remained detectable for the total observation period (>2.5 to >3.5 months). In four calves, IgA titers showed a more or less fluctuating and gradually rising course, whereas in two calves a remarkable biphasic pattern was observed. Responses in the lungs, nose, and eyes of individual animals were quite similar. To examine whether variations in total IgA concentrations could explain these patterns (intraisotype competition [37; Kimman et al., in press] or sampling errors), the samples were also tested in the IgA double antibody sandwich assay for total IgA. Some examples of the patterns are shown in Fig. 2. Apparently, variations in total IgA cannot explain the fluctuations seen in BRSV-specific IgA.

In feces, low levels of IgM and IgA were detected for short periods (Table 1), but not in the 5-month-old calf. This was probably due to an age effect, since the total IgA concentrations in feces gradually decreased during aging. IgA was never detected in feces of calves older than 3 months. Proteolytic breakdown of IgA in feces after sampling, as an explanation for this phenomenon, was unlikely, since the BRSV-specific IgA titer of known positive samples was not decreased if they were added to such fecal samples.

TABLE 1. Antibody responses against BRSV after primary infection of colostrum-deprived calves (n = 6)

Specimen and antibody	Mean day PID of first detection (range)	Mean day PID of reaching peak/plateau titer (range)	Mean no. of days detectable (range)	Mean peak/plateau titer (range)
Serum				
IgM	9.2 (8–10)	11 (10–13)	22.7 (15–29)	2,133 (1,280–2,560)
IgA[a]	12 (10–14)	14 (13–16)	20.4 (12–29)	97 (4–160)
IgG1	14.8 (13–17)	33.6 (24–38)	—[b]	2,347 (1,280–2,560)
IgG2	53.2 (25–86)	71.2 (38–90)	—[c]	328 (40–640)
Lung				
IgM	11 (8–18)	11.3 (10–18)	7 (1–17)	41 (2–128)
IgA	11.2 (10–14)	13.8 (10–18)	—[d]	61 (16–128)
Nose				
IgM	10.3 (8–15)	12.2 (10–14)	13.3 (6–24)	25 (8–64)
IgA	12.2 (10–13)	12.6 (11–13)	—[d]	43 (32–64)
Eye				
IgM	10.3 (9–13)	12.5 (10–15)	12.5 (8–22)	39 (4–128)
IgA	13.2 (13–14)	14.2 (13–18)	—[e]	62 (8–128)
Feces[f]				
IgM	10.6 (10–13)	11.2 (10–13)	5.2 (1–8)	57 (4–128)
IgA[g]	11 (10–13)	12 (10–13)	4.3 (1–8)	5 (2–8)

[a] Excluding one nonresponding calf.
[b] >2.5–>4 months.
[c] >2—>4 months.
[d] 2–>3.5 months.
[e] >2.5–3.5 months.
[f] Excluding one 5-month-old calf (see text).
[g] Excluding two nonresponding animals.

After reinfection, the calves developed a secondary immune response characterized by a rapid (4 to 8 days PID) and strong (titers up to 20,480) local IgA response in the respiratory tract. This response was more pronounced in the nasal and eye secretions than in the lung lavage fluid. IgM was detected again in most secretions, but not in the lung lavage samples of three calves. This IgM response did not start earlier than during the primary response, but it lasted longer and reached higher titers (Table 2). In the sera, on the contrary, no IgM was detected, but a secondary IgA response became detectable on day 6 to 7 PID and remained detectable for 3.5 months (one animal) or longer. In the sera, there was a further, rapid increase in BRSV-specific IgG1 and IgG2 (Table 2; Fig. 1B). In feces, no secondary response was detected, which may be explained by the older age of the calves when reinfected (see above).

Two calves were infected a third time. These two animals gave responses similar to those recorded after the second series of inoculations, although one animal with serum IgG1 and IgG2 titers of 10,240 did not have a further increase in these titers.

(ii) Colostrum-fed calves. Colostrum feeding during the first 48 h of life resulted in serum IgG1 titers of 1,280 (three calves) and 2,560 (two calves) 1 week after birth. In feces and secretions, IgG1 was not detectable unless the samples were contaminated with blood.

After infection of these seropositive calves, not only the serum but also the mucosal antibody responses were strongly or totally suppressed. In two calves, which were infected at an age of 3 weeks when serum BRSV IgG1 titers were 1,280 and 2,560, the only response detected was a weak and short-lasting serum IgM response in one of them. The preinoculation BRSV IgG1 serum titer in this calf was 1,280. The results obtained with this animal are shown in Fig. 3. Other responses could not be detected in the normal assays. However, after maximal concentration (i.e., 14 to 40×) of lung lavage fluids, trace amounts of BRSV-specific IgM, but not of IgA, were detected 13 to 17 days PID.

In the two animals infected at 10 weeks of age, when preinoculation BRSV IgG1 serum titers were 640 and 1,280, all responses were still markedly suppressed. Weak and short-lasting IgA responses were detected in serum and respiratory secretions of the calf with the preinoculation titer of 640. In this calf also, IgM could be demonstrated in nasal and eye secretions. In the second calf, only a weak and short-lasting nasal IgA (1 day) and IgM (10 days) response was found.

In general, there was a tendency for responses to be more suppressed when preinoculation serum BRSV-specific IgG1 was higher (Fig. 4).

The half-life for serum BRSV-specific IgG1 in the four colostrum-fed calves was calculated from the moment of inoculation over 2.5 to 3 months. Values were 21.5, 30 (two calves), and 31.5 days.

After reinfection of the colostrum-fed calves, strong and rapid IgA responses were observed, closely resembling those seen in colostrum-deprived animals, though not reaching such high titers (Table 3; Fig. 3B and 5). In the sera of three of four animals, a rapid and significant increase in BRSV-specific IgG1 antibodies was observed, and all four animals had a rapid IgG2 response. A comparison of the BRSV-specific IgA antibody activity in nasal fluids after primary and secondary infection of colostrum-deprived and colostrum-fed calves is shown in Fig. 5.

Two colostrum-fed calves were infected a third time when they were 6 months old. Again, rapid serum and local IgA and serum IgG1 and IgG2 responses were seen. Peak IgA titers in respiratory secretions now reached values nearly as high as those after the second or third inoculation of colostrum-deprived calves. Also, local IgM responses were observed that were similar to those seen after the second inoculation.

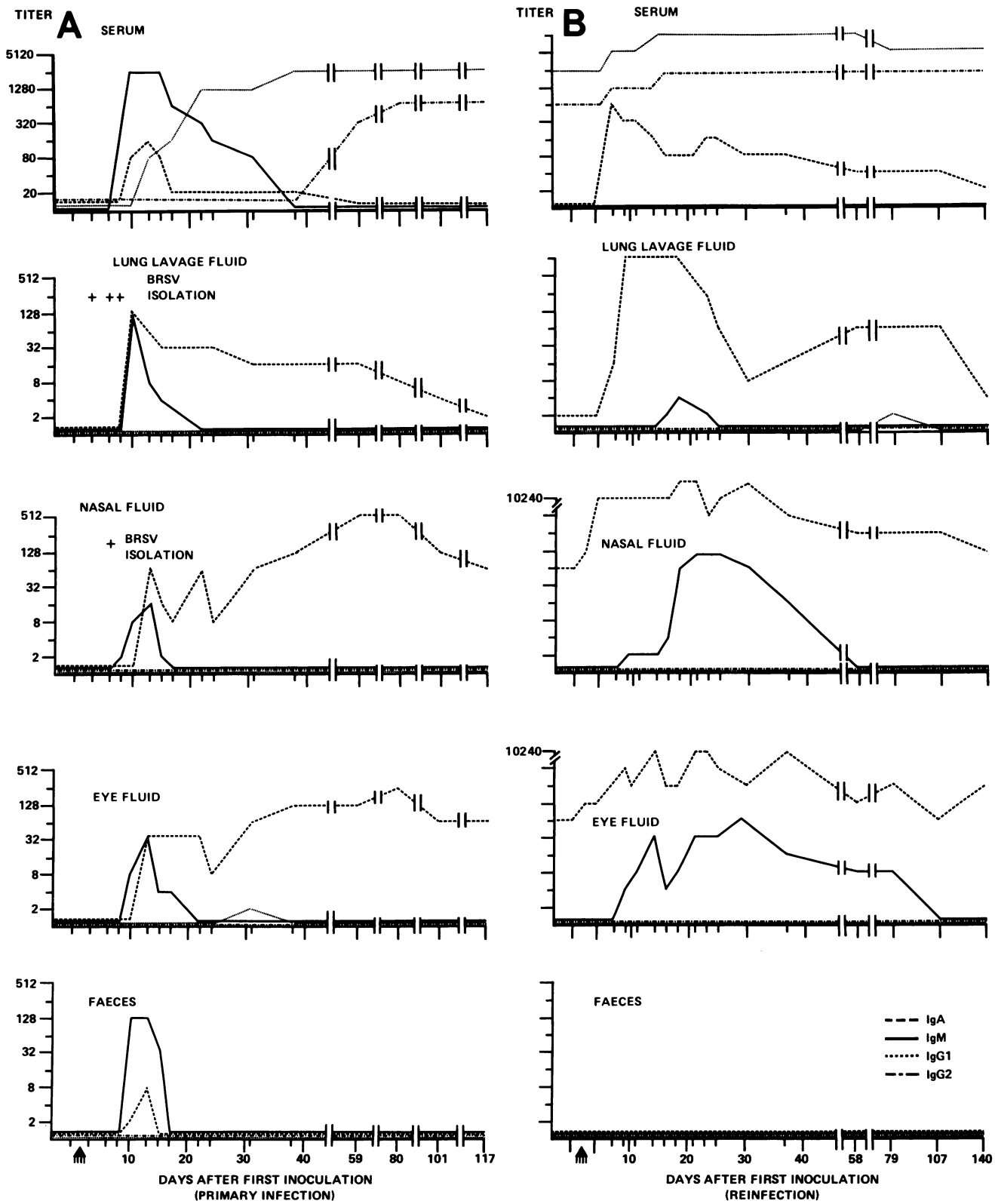


FIG. 1. Kinetics of antibody activity to BRSV in serum, lung lavage fluid, nasal and eye secretions, and feces of a colostrum-deprived calf (no. 1973) after primary (A) and secondary (B) infection with BRSV. Symbols: ⇑, days of inoculation; +, positive virus isolation.

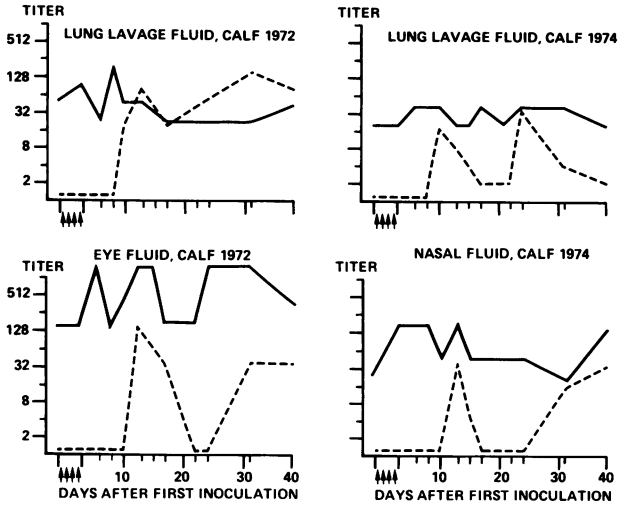


FIG. 2. Comparison of BRSV-specific IgA and total IgA in respiratory secretions of two calves. Symbols: – – –, BRSV-specific IgA; ——, total IgA; ↑ ↑ ↑ ↑, days of inoculation.

Virologic examinations. After primary infection, BRSV was isolated in about equal amounts from the lung lavage fluids of both colostrum-fed and colostrum-deprived animals from days 3 to 8 PID. Results are shown in Table 4. From nasal swabs, the virus was isolated only occasionally and in low titers. After the second and third infections, no BRSV was isolated.

Field observations. Two naturally occurring, severe outbreaks of BRSV-associated disease allowed the study of the local immune response in nine calves from the field. All animals except one had BRSV-specific IgG1 serum titers on the first day of illness. These were most probably maternally derived, because of the age of the calves and because IgM and IgA serum and local responses developed afterward. The IgG1 titers in serum on the first sampling day were <80 and 320 (three calves) in calves from outbreak A and 80, 1,280 (three calves), and 2,560 in calves from outbreak B.

Essential differences between the antibody responses after natural and experimental infection were not found. In general, the responses in calves from the field were stronger. The different levels of preexisting maternal antibodies apparently caused quantitatively different responses between these calves. For example, serum IgG1 responses, defined as a twofold or higher increase in titer, were observed in only three calves. Preexisting BRSV-specific IgG1 titers in sera of these calves were <80, 80, and 320. Serum IgM responses were seen in all nine calves, with peak titers varying from 20 to 10,240. Corresponding IgG1 titers in serum were 2,560 and <80, respectively. Serum IgA responses were observed in only three animals with preexisting BRSV IgG1 titers in serum of <80 and 320 (two calves). Finally, serum IgG2 seroconversion was observed in four animals with corresponding BRSV IgG1 titers in serum of <80, 80, and 320 (two calves).

In nasal, eye, and lung fluids, IgM and IgA responses of various heights and durations were observed in all calves, except the calf with a BRSV IgG1 titer in serum of 2,560. The serum and mucosal responses were often short and insignificant in the animals of outbreak B (younger animals). The animals of outbreak A (older animals) had stronger responses that were of longer duration. For example, the animal without preexisting maternal antibodies had a peak nasal IgA titer of 256, and IgA could still be detected 3 months after the onset of disease.

DISCUSSION

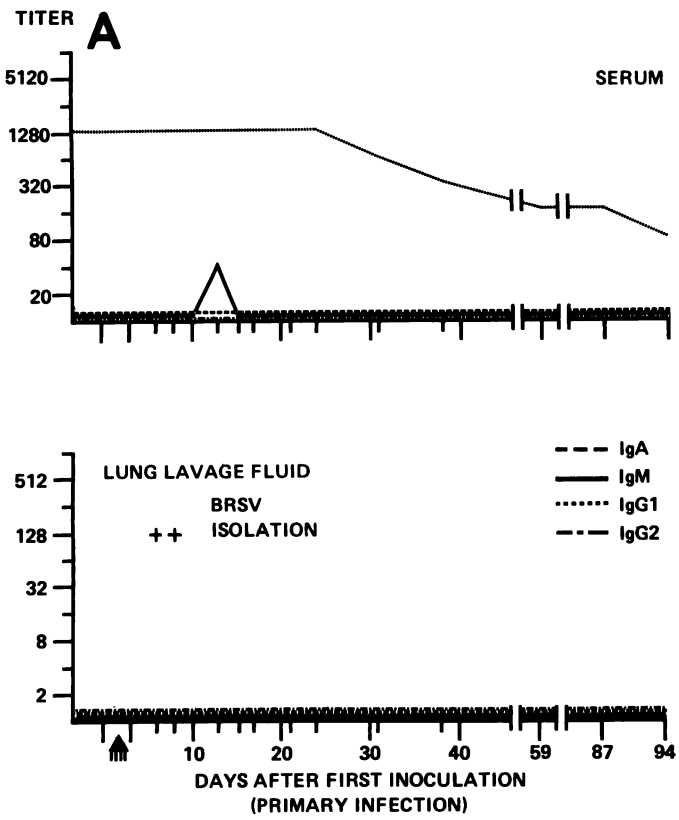
Several studies failed to demonstrate a clear protective activity of maternal antibodies against BRSV in calves (11, 13, 20, 23, 30). This was confirmed in this study. After experimental infection, virus was isolated from lung lavage fluids in about equal amounts and during the same period from colostrum-deprived and colostrum-fed calves. Furthermore, severe clinical symptoms were observed during a natural outbreak of respiratory tract disease, probably due to BRSV, in a herd of young calves with moderate to high levels of maternal antibodies to this virus.

There has been much controversy about this subject (31). Although human infants less than 3 weeks old are relatively spared from severe RSV disease, the correlations between the levels of maternal antibodies and protection are imperfect, and severe disease does occur in the presence of moderate levels of antibody. Protection against virus repli-

TABLE 2. Antibody responses against BRSV after reinfection of colostrum-deprived calves (n = 5)

Specimen and antibody	Mean day PID of first detection (range)	Mean day PID of reaching peak/plateau titer (range)	Mean no. of days detectable (range)	Mean peak/plateau titer (range)
Serum[a]				
IgA	6.6 (6–7)	8.2 (7–11)	—[b]	992 (160–2,560)
IgG1	7 (6–9)[c]	13.2 (9–23)	—[d]	10,240
IgG2	7.8 (6—11)[c]	21.7 (16–24)	—[d]	3,360 (640–5,120)
Lung				
IgM[e]	17 (16–18)	18	4.5 (1–8)	3 (2–4)
IgA	7.5 (6–11)	9 (8–11)	—[d]	582 (32–1,280)
Nose				
IgM	9.8 (9–11)	18 (13–23)	33.4 (15–70)	112 (16–256)
IgA	6 (4–7)	9.4 (8–11)	—[d]	14,336 (10,240–20,480)
Eye				
IgM	9 (8–11)	19 (13–30)	62.2 (27–72)	118.4 (64–128)
IgA	7 (6–8)	9 (8–11)	—[d]	14,592 (1,280–20,480)

[a] No IgM responses were detected for serum.
[b] 3.5–>4 months.
[c] Increase in titer.
[d] >2.5–>4 months.
[e] Excluding three animals for which no IgM was detected in lung lavage samples.



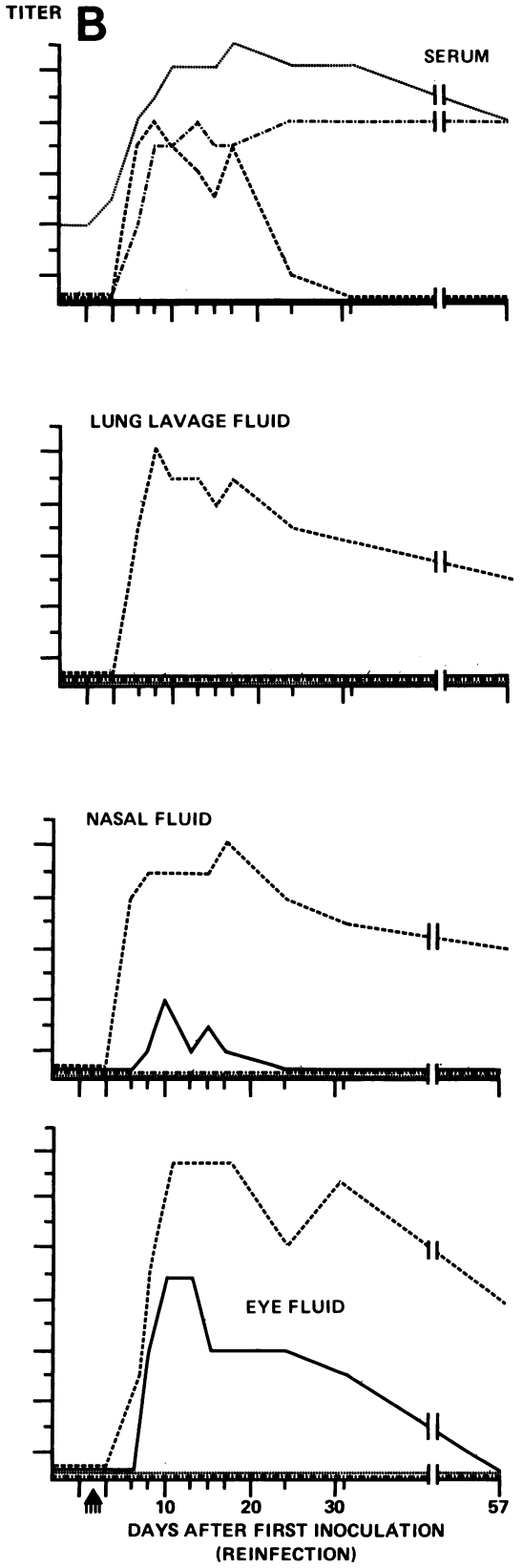


FIG. 3. Kinetics of antibody activity to BRSV of a colostrum-fed calf (no. 2290) after primary (A) and secondary (B) infection with BRSV. After primary infection, responses were not detected in nasal and eye secretions and feces, as shown for the lung lavage fluid. Symbols: ⇑, days of inoculation; +, positive virus isolation.

cation in the lungs, but not in the nasal passages, of mice (35) and cotton rats (25, 27, 39) was afforded by passive transfer of serum and certain monoclonal antibodies directed against the large G and F glycoproteins. Infant ferrets, however, were not protected by passive transfer of serum containing high titers of neutralizing antibody (33). Perhaps these equivocal results can in part be explained by quantitative differences in the amount of passively transferred antibodies. Large quantities of antibodies have to be administered to protect mice and cotton rats against virus multiplication in the lungs (27, 35). Species differences, however, also seem to play a role.

This study clearly demonstrates that IgM and particularly IgA are the predominant antibody isotypes in the respiratory tract after BRSV infection, with IgA especially prominent after reinfection. It also shows that not only serum antibody responses, but also local antibody responses, are strongly suppressed by maternal antibodies. These observations on the mucosal immune response in the bovine agree rather well with those made by Van Zaane et al. (38), studying the intestinal antibody response after rotavirus infection. However, the immune response after rotavirus infection occurred earlier (5 days after inoculation) than after BRSV infection (10 days). Because rotavirus multiplies faster than BRSV, this may be an indication of an important role for virus multiplication and amount of viral antigen in the induction of a mucosal immune response.

A remarkable finding was the nearly simultaneous appear-

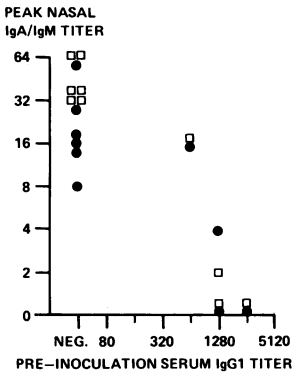


FIG. 4. Correlation between the preinoculation BRSV IgG1 titer in serum (maternally derived) and the peak IgM (●) and IgA (□) titers observed in nasal fluid after primary BRSV infection.

ance of IgM, shortly followed by IgA, in all samples, even feces, after first infection. Although it cannot be excluded that the virus reached the gastrointestinal tract or that the antibodies demonstrated in feces were swallowed or excreted by the liver, the findings suggest circulation of immunocompetent cells to mucosae distant from the site of priming and possibly a tendency to return to the sites of origin, as is the current hypothesis (10, 24). A practical application would be the possibility of priming the mucosae of the intestinal tract, which in ruminants is not easily reached, by antigen presentation via the respiratory route (D. van Zaane, J. IJzerman, and P. W. de Leeuw, Proceedings of the International Conference on Mucosal Immunity, Niagara Falls, in press).

The pattern of antibody response after reinfection yielded clear evidence for mucosal memory, characterized by strong and rapid IgA responses (Fig. 5). This effect was observed even in calves in which a primary IgA response was not detected because of the suppressive action of maternal antibodies. These observations are important, considering the need to protect calves at an early age, when they possess maternal antibodies. The presently available live BRSV vaccines, which are applied intramuscularly, however, are not effective in calves with maternal antibodies (40). The same is true for children (3) and cotton rats (26).

Responses in the lung closely resembled those in the nose and the eye. The site that is sampled by lung lavage is not exactly defined, but probably a mixture of upper and lower respiratory tract material is collected. Apparently, the upper respiratory tract and the eye react as a whole. Our results suggest that sampling of the nose or the eye may give valuable information about immune reactions deeper down the airways.

Quantitative analysis of immunoglobulins recovered from the lower respiratory tract is difficult because of a variable dilution factor and variations in composition (5, 9, 12). Usually, a correction for the contribution made by serum proteins is necessary. Albumin was chosen for this purpose because it is not produced, actively secreted, or degraded in the lung and because its molecular weight is in the middle of the molecular-weight range of the serum proteins (5, 9, 12, 29). In our study, the contribution made by serum appeared to be negligible. This indicates that the BRSV-specific IgA and IgM, demonstrated in our lung lavage samples, were locally produced, selectively secreted, or both.

Although IgG1 has been reported to occur in lower respiratory tract fluids (5, 17, 32), we found BRSV-specific IgG1 only in blood-contaminated samples. There is no active transport of IgG1 from serum to the air spaces of the lung (Kimman et al., in press), and thus the failure to regularly detect BRSV-specific IgG1 in lung lavage samples must probably be explained by the strong dilution of passively diffused serum proteins in the lung lavage fluid.

The close correlation in time between appearance of antibodies and disappearance of virus after first infection suggests that IgM, IgA, or both play an important role in recovery after infection. Similar observations have been made in children (18, 19). On the other hand, calves with maternal antibodies and strongly suppressed IgM and IgA responses did not shed virus for a longer period of time. So it may be that even small quantities of IgM, which were always present, contribute to recovery. Other mechanisms, like cytotoxic cells (2, 14, 34), interferon (7), and maternal serum IgG1, may also have contributed to recovery.

TABLE 3. Antibody responses against BRSV after reinfection of colostrum-fed calves (n = 4)

Specimen and antibody	Mean day PID of first detection (range)	Mean day PID of reaching peak/plateau titer (range)	Mean no. of days detectable (range)	Mean peak/plateau titer (range)
Serum[a]				
IgA	6	8	15.3 (12–19)	600 (160–1,280)
IgG1[b]	6.7 (6–8)[c]	13.7 (10–18)	—[d]	5,973 (2,560–10,240)
IgG2	7.5 (6–8)[c]	20 (10–44)	—[d]	1,280
Lung				
IgM[e]	9 (8–10)	9 (8–10)	2 (1–3)	3 (2–4)
IgA	6	8	—[d]	533 (320–640)
Nose				
IgM	8	12.7 (8–20)	17 (10–24)	18 (8–32)
IgA	6.5 (6–8)	9.2 (8–13)	—[d]	2,080 (640–5,120)
Eye				
IgM	8	11.5 (10–13)	24	164 (16–256)
IgA	7 (6–8)	11.5 (10–13)	—[d]	5,120 (2,560–10,240)

[a] No IgM responses were detected for serum.
[b] Excluding one nonresponding animal.
[c] Increase in titer.
[d] >2.5 months.
[e] Excluding two animals for which no IgM was detected in lung lavage samples.

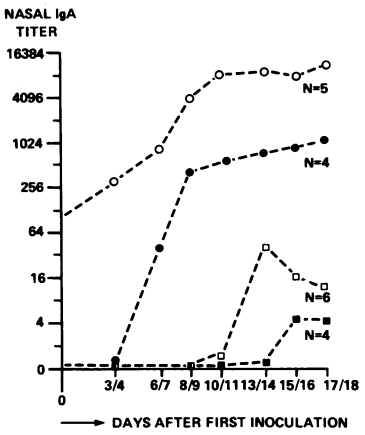


FIG. 5. Comparison of the BRSV-specific IgA activity in nasal fluids after primary infection of colostrum-deprived (□) and colostrum-fed (■) calves and after secondary infection of colostrum-deprived (○) and colostrum-fed (●) calves. Each point represents the mean BRSV-specific IgA titer of the indicated number of calves.

Unsuccessful attempts to recover virus after reinfection of colostrum-fed calves, which had no detectable BRSV-specific IgA levels at the moment of reinfection, suggest that the capacity to mount a mucosal memory IgA response, rather than merely the presence of IgA, may be important in inhibiting virus multiplication. The mechanism by which IgA operates remains to be elucidated, however, because of the observed poor correlation between IgA and virus neutralization, both in calves (Kimman et al., in press) and in children (18).

A peculiarity of the IgA response in the respiratory tract after BRSV infection, when for instance compared with the intestinal IgA response after rotavirus infection (38), was its long duration and course. Responses sometimes steadily increased or showed a second peak. The most probable explanation for these phenomena is persistence or reactivation of the virus, or both. Persistent RSV infections have been reported (1, 6, 21, 22, 28). They could also offer an explanation for the annual recurrence of BRSV infections even in closed herds of cattle.

TABLE 4. Virus titers in lung lavage fluids of colostrum-deprived and colostrum-fed animals after primary BRSV infection

Animal no.	Preinoculation serum IgG1 titer	BRSV log $TCID_{50}$ titer on PID[a]:			
		3	6	8	10
Colostrum deprived					
1972		0.2	3.8		
1973		0.2	1.8	0.2	
1974			1.8	0.2	
1975		0.2			
1981		1.3	3.8		—[b]
2286			1.6	0.2	
2287			3.1	0.2	
Colostrum fed					
2289	2,560		1.6		
2290	1,280		1.6	1.6	
2291	1,280		3.6	2.1	—[b]
2315	640		0.2		
2316	1,280		0.2	1.8	

[a] $TCID_{50}$, 50% tissue culture infective dose. No measurable titers were recorded on PID 1.
[b] Calf was killed.

Of all isotypes, IgM seems least susceptible to suppression by maternal antibodies, as already observed in previous studies (Kimman et al., in press; F. Westenbrink and T. G. Kimman, Am. J. Vet. Res., in press). This makes BRSV-specific serum IgM especially suitable for diagnostic purposes and epidemiologic studies of BRSV infections in young calves. Since IgM was not detected in serum after reinfection, BRSV-specific serum IgA might provide a better tool to monitor reinfections in older animals.

Our experimental infection provoked only mild signs of disease, even though our infection protocol strongly resembled that of Bryson et al. (4). Those authors observed more severe clinical signs. Probably the infective dose was still too low, as suggested by the observation that naturally infected calves had stronger antibody responses than experimentally infected calves. There are also indications that infectivity of BRSV for calves is rapidly lost by only a few passages in vitro (31).

ACKNOWLEDGMENTS

We thank A. Janssen and H. Rutgers for skillfully tending the animals and H. Paal for excellent technical assistance.

LITERATURE CITED

1. **Baldridge, P., and L. B. Senterift.** 1976. Persistent infection of cells in culture by respiratory syncytial virus. Proc. Soc. Exp. Biol. Med. **151**:684–688.
2. **Bangham, C. R. M., M. J. Cannon, D. T. Karzon, and B. A. Askonas.** 1985. Cytotoxic T-cell response to respiratory syncytial virus in mice. J. Virol. **56**:55–59.
3. **Belshe, R. B., L. P. Van Voris, and M. A. Mufson.** 1982. Parenteral administration of live respiratory syncytial virus: results of a field trial. J. Infect. Dis. **145**:311–319.
4. **Bryson, D. G., M. S. McNulty, E. F. Logan, and P. F. Cush.** 1983. Respiratory syncytial virus pneumonia in young calves: clinical and pathologic findings. Am. J. Vet. Res. **44**:1648–1655.
5. **Butler, J. E.** 1983. Bovine immunoglobulins: an augmented review. Vet. Immunol. Immunopathol. **4**:43–152.
6. **Fernie, B. F., and J. L. Gerin.** 1981. The development of Balb/c cells persistently infected with respiratory syncytial virus: presence of ribonucleoprotein on the cell surface. Proc. Soc. Exp. Biol. Med. **167**:83–86.
7. **Fulton, R. W., L. J. Burge, and J. S. McCracken.** 1986. Effect of recombinant DNA-derived bovine and human interferons on replication of bovine herpesvirus-1, parainfluenza-3, and respiratory syncytial viruses. Am. J. Vet. Res. **47**:751–753.
8. **Henderson, F. W., A. M. Collier, W. A. Clyde, and F. W. Denny.** 1979. Respiratory syncytial virus infections, reinfections and immunity. N. Engl. J. Med. **300**:530–534.
9. **Hunninghake, G. W., J. E. Gadek, O. Kawanami, V. J. Ferrans, and R. G. Crystal.** 1979. Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. Am. J. Pathol. **97**:149–206.
10. **Husband, A. J.** 1985. Mucosal immune interactions in intestine, respiratory tract and mammary gland. Prog. Vet. Microbiol. Immunol. **1**:25–57.
11. **Jacobs, J. W., and N. Edington.** 1975. Experimental infection of calves with respiratory syncytial virus. Res. Vet. Sci. **18**: 299–306.
12. **Jansen, H. M., A. J. H. Schutte, J. D. Elema, M. V. D. Giessen, R. Peset Reig, M. A. V. Leeuwen, H. J. Sluiter, and T. H. The.** 1984. Local immune complexes and inflammatory response in patients with chronic interstitial pulmonary disorders associated with collagen vascular diseases. Clin. Exp. Immunol. **56**: 311–320.
13. **Kimman, T. G., G. M. Zimmer, P. J. Straver, and P. W. de Leeuw.** 1986. Diagnosis of bovine respiratory syncytial virus infections improved by virus detection in lung lavage samples.

Am. J. Vet. Res. **47**:143–147.
14. **Kumagai, T., D. T. Wong, and P. L. Ogra.** 1985. Development of cell-mediated cytotoxic activity in the respiratory tract after experimental infection with respiratory syncytial virus. Clin. Exp. Immunol. **61**:351–359.
15. **Lamprecht, C. L., H. E. Krause, and M. A. Mufson.** 1976. Role of maternal antibody in pneumonia and bronchiolitis due to respiratory syncytial virus. J. Infect. Dis. **134**:211–217.
16. **Martin, H. T.** 1983. Indirect haemagglutination test for the detection and assay of antibody to bovine respiratory syncytial virus. Vet. Rec. **113**:290–293.
17. **McDermott, M. R., A. D. Befus, and J. Bienenstock.** 1982. The structural basis for immunity in the respiratory tract. Int. Rev. Exp. Pathol. **23**:47–112.
18. **McIntosh, K., H. B. Masters, I. Orr, R. K. Chao, and R. M. Barkin.** 1978. The immunologic response to infection with respiratory syncytial virus in infants. J. Infect. Dis. **138**:24–32.
19. **McIntosh, K., J. McQuillin, and P. S. Gardner.** 1979. Cell-free and cell-bound antibody in nasal secretions from infants with respiratory syncytial virus infection. Infect. Immun. **23**:276–281.
20. **McNulty, M. S., D. G. Bryson, and G. M. Allan.** 1983. Experimental respiratory syncytial virus pneumonia in young calves: microbiologic and immunofluorescent findings. Am. J. Vet. Res. **44**:1656–1659.
21. **Mills, B. G., F. R. Singer, L. P. Weiner, and P. A. Holst.** 1980. Cell cultures from bone affected by Paget's disease. Arthritis Rheum. **23**:1115–1120.
22. **Mills, B. G., F. R. Singer, L. P. Weiner, and P. A. Holst.** 1981. Immunohistological demonstration of respiratory syncytial virus antigens in Paget's disease of bone. Proc. Natl. Acad. Sci. USA **78**:1209–1213.
23. **Mohanty, S. B., M. G. Lillie, and A. L. Ingling.** 1976. Effect of serum and nasal neutralizing antibodies on respiratory syncytial virus infection in calves. J. Infect. Dis. **134**:409–413.
24. **Pierce, N. F., and W. C. Cray.** 1981. Cellular dissemination of priming for a mucosal immune response to cholera toxin in rats. J. Immunol. **127**:2461–2464.
25. **Prince, G. A., R. L. Horswood, E. Camargo, D. Koenig, and R. M. Chanock.** 1983. Mechanisms of immunity to respiratory syncytial virus in cotton rats. Infect. Immun. **42**:81–87.
26. **Prince, G. A., R. L. Horswood, E. Camargo, S. C. Suffin, and R. M. Chanock.** 1982. Parenteral immunization with live respiratory syncytial virus is blocked in seropositive cotton rats. Infect. Immun. **37**:1074–1078.
27. **Prince, G. A., R. L. Horswood, and R. M. Chanock.** 1985. Quantitative aspects of immunity to respiratory syncytial virus infection in infant cotton rats. J. Virol. **55**:517–520.
28. **Pringle, C. R., P. V. Shirodaria, H. B. Gimenz, and S. Levine.** 1981. Antigen and polypeptide synthesis by temperature-sensitive mutants of respiratory syncytial virus. J. Gen. Virol. **28**:173–183.
29. **Reynolds, H. Y., and H. H. Newball.** 1974. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. J. Lab. Clin. Med. **84**:559–573.
30. **Smith, M. H., M. L. Frey, and R. E. Dierks.** 1975. Isolation, characterization, and pathogenicity studies of a bovine respiratory syncytial virus. Arch. Virol. **47**:237–247.
31. **Stott, E. J., and G. Taylor.** 1985. Respiratory syncytial virus: brief review. Arch. Virol. **84**:1–52.
32. **Stott, E. J., L. H. Thomas, G. Taylor, A. P. Collins, J. Jebbett, and S. Crough.** 1984. A comparison of three vaccines against respiratory syncytial virus in calves. J. Hyg. **93**:251–261.
33. **Suffin, S. C., G. A. Prince, K. B. Muck, and D. D. Porter.** 1979. Immunoprophylaxis of respiratory syncytial virus infection in the infant ferret. J. Immunol. **123**:10–14.
34. **Sun, C., P. R. Wyde, S. Z. Wilson, and V. Knight.** 1983. Cell-mediated cytotoxic responses in lungs of cotton rats infected with respiratory syncytial virus. Am. Rev. Respir. Dis. **127**:460–464.
35. **Taylor, G., E. J. Stott, M. Bew, B. F. Fernie, P. J. Cote, A. P. Collins, M. Hughes, and J. Jebbett.** 1984. Monoclonal antibodies protect against respiratory syncytial virus infection in mice. Immunology **52**:137–142.
36. **Van Nieuwstadt, A. P., and J. Verhoeff.** 1983. Serology for diagnosis and epizootiological studies of bovine respiratory syncytial virus infections. Res. Vet. Sci. **35**:153–159.
37. **Van Zaane, D., and J. IJzerman.** 1984. Monoclonal antibodies against bovine immunoglobulins and their use in isotype-specific ELISAs for rotavirus antibody. J. Immunol. Methods **72**: 427–441.
38. **Van Zaane, D., J. IJzerman, and P. W. de Leeuw.** 1986. Intestinal antibody response after vaccination and infection with rotavirus of calves fed colostrum with or without rotavirus antibody. Vet. Immunol. Immunopathol. **11**:45–63.
39. **Walsh, E. E., J. J. Schlesinger, and M. W. Brandriss.** 1984. Protection from respiratory syncytial virus infection in cotton rats by passive transfer of monoclonal antibodies. Infect. Immun. **43**:756–758.
40. **Zygraich, N.** 1982. Vaccination against RS virus: a five year experience with Rispoval, p. 189–195. In Proceedings of the 12th World Congress on Diseases of Cattle, The Netherlands.