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A Longitudinal Study of Bovine Coronavirus Enteric and Respiratory Infections in Dairy Calves in Two Herds in Ohio*

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ABSTRACT

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This prospective longitudinal study examined the epidemiology and disease syndrome associated with bovine coronavirus (BCV) infections in a cohort of 8 conventional calves from 0 to 120 days of age, in two dairy herds in Ohio. The periods of respiratory shedding of BCV were determined by direct immunofluorescent (DIF) staining of nasal epithelial cells and ELISA of nasal swab supernatant fluids. The periods of fecal shedding of BCV were determined by ELISA and immunoelectron microscopy (IEM). The isotype-specific antibody titers to BCV in serum (at selected intervals between 0 and 120 days of age) and the post-suckling (24 to 48 h after birth) total immunoglobulin levels were examined by ELISA and zinc sulfate turbidity tests, respectively.

Of the 8 calves studied, 4 had evidence of BCV respiratory (by DIF or ELISA) or enteric infections (by IEM or ELISA) in association with diarrhea or rhinitis, even though 7 of 8 calves showed increases in one or more serum antibody isotypes to BCV and 6 of 8 calves showed BCV respiratory or enteric antigen shedding by ELISA. Serological antibody titer increases occurred in 3 calves before 30 days of age and in 4 calves after 30 days of age; two of the latter calves had a second rise in serum antibody titers to BCV after the initial rise. A serological antibody titer increase was not observed in one calf. This suggests that BCV infections may be very common in a closed herd and may occur in older calves, although many may be subclinical and some may be recurrent.

There were no statistically significant correlations between total serum immunoglobulin levels or BCV antibody isotype titers in serum (24–48 h after birth) and clinical disease or infection by BCV; however, calves with low levels of IgA BCV antibodies in serum (24–48 h after birth) had a significantly greater average number of days with diarrhea than those calves having high levels of IgA BCV-specific antibodies in serum.

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INTRODUCTION

Bovine coronavirus (BCV) is a well-documented cause of diarrhea in young (1 to 28-day-old) calves (Mebus et al., 1973; Sharpee et al., 1976; Langpap et al., 1979; Reynolds et al., 1986). In addition, BCV also causes infection of the upper respiratory tract inducing mild clinical signs in experimentally or naturally-infected calves (McNulty et al., 1984; Reynolds et al., 1985; Singh et al., 1985; Saif et al., 1986; Heckert et al., 1988; Heckert et al., 1989). There are few published reports describing the prevalence or epidemiology of BCV respiratory or enteric infections in calves in the U.S. Few prospective longitudinal studies of BCV infections have been done; therefore, it is not known if older calves become infected or if reinfections or reactivation of chronic infections are common. Ellens et al. (1979) showed seroconversion in 62% (8/13) of calves less than 3 months old and thereafter significant antibody titer increases in 100% (13/13) of animals studied. In a preliminary study, we found that BCV infections are endemic in some herds and that older calves can have recrudescent respiratory BCV infections at least up to 65 days of age (Heckert et al., 1988).

The temporal occurrence of the respiratory and enteric infections is also unknown. The pathogenesis of BCV has been shown to involve the fecal–oral route of transmission with subsequent enteritis. However, it was proposed recently that aerosol transmission and respiratory replication of BCV may also be important in the pathogenesis of BCV infections in a herd (Saif and Smith, 1985; Saif et al., 1986). If BCV respiratory infections are common they may be important in the pathogenesis of BCV enteritis and BCV respiratory replication may occur prior to clinical outbreaks of diarrhea.

Calves can develop increased antibody titers after infection by BCV (Ellens et al., 1979; Saif, 1987); however, there are no reports on either the antibody isotypes induced following natural infections (except in cows (Crouch, 1985)) or the influence of varying levels of passively acquired antibodies on the induction of an active immune response to BCV in calves. There are reports that the active antibody response is depressed in calves with high levels of passively acquired antibodies after infection with rotavirus, respiratory syncytial virus or parainfluenza virus type 3 (PI-3) (Marshall and Frank, 1975; Saif and Smith, 1985; Kimman et al., 1987). Several authors have shown that higher levels of total passive immunoglobulins (Ig) correlate with reduced risk of neonatal respiratory (Thomas and Swann, 1973; Williams, 1975; Thomas, 1978; Davidson et al., 1981) or enteric disease (Thornton et al., 1972; Boyd and Leyland, 1974; Hancock, 1985). It is not known if passive BCV antibody titers or total serum Ig levels correlate with resistance to BCV infections in calves.

This prospective longitudinal study examines the epidemiology and disease syndrome associated with BCV infections in conventional calves from 0 to 120 days of age. We first determined the period(s) of respiratory shedding of BCV

by immunofluorescent staining of nasal epithelial cells and ELISA of nasal swab supernatant fluid. ELISA and immunoelectron microscopy were used to detect and define the period(s) of BCV fecal shedding. Secondly the isotype-specific antibody titers to BCV and the post-suckling total Ig levels in serum were examined by ELISA and zinc sulfate turbidity tests, respectively.

MATERIALS AND METHODS

Animals and samples

Two closed University research dairy herds housed at separate locations were used in these studies. In herd A (150–180 milking cow herd) calves were removed from the dam 2–3 h after birth, force fed 2 l of colostrum and placed in hutches. Dams in this herd were not vaccinated with BCV and the calves were vaccinated with a modified live infectious bovine rhinotracheitis (IBR) and bovine parainfluenza (PI3) vaccine. In herd B (120–150 milking cow herd) calves remained with their dams for the first 3 days of life and were then moved to individual tie stalls. Dams and calves in this herd were not vaccinated.

A total of eight dairy heifers (Nos. 1–8) in the two herds were randomly selected and sampled from birth to 120 days of age (Table 1). From herd A five calves (Nos. 1–5) were sampled periodically from December 1985 until August 1986 and in herd B three calves (Nos. 6–8) were sampled periodically from December 1986 until August 1987 (Table 1). Rectal swabs/feces and nasal swabs were collected, rectal temperatures were recorded and clinical signs noted three times per week (Saif et al., 1986). Fecal consistency was scored on a scale of 0 to 3, with 0 representing firm feces, 1 pasty feces, 2 semiliquid and 3 liquid. Upper respiratory tract signs were scored on a similar scale with 0 representing a normal amount and consistency of lacrimal, nasal and salivary secretions; 1, excessive clear secretions; and 2, cloudy thick secretions. Blood was drawn from calves every other week in herd A and weekly in herd B.

TABLE 1

A summary of calves studied in herds A and B and the sampling periods

Herd	Calf no. (total)	Ages (days)	Sampling period
A	No. 1-5 (5)	0-120	December 1985 to August 1986
B	No. 6-8 (3)	0-120	December 1986 to August 1987

Immunoelectron microscopy (IEM)

Fecal samples were stored at −20°C after collection and processed for IEM, using procedures similar to those described previously (Saif et al., 1977). IEM was done on all samples, collected from 0 to 120 days of age, which had a fecal score of 2 or 3. Briefly, fecal samples were processed and incubated overnight at 4°C with hyperimmune bovine anti-BCV (1:400). The immune complexes were pelleted twice (69 000×*g*, 35 min, 4°C), resuspended in 20 μl of sterile distilled H_2O and negatively stained with 20 μl of 3% phosphotungstic acid (pH 7.0). A drop of stained sample was applied to an EM grid, blotted dry and examined at 80kv in an electron microscope (Model 201, Philips Norelco, The Netherlands).

Direct immunofluorescent (DIF) staining of nasal epithelial cells

Nasal epithelial cells were collected with 16.5 cm cotton-tipped swabs (one per nostril) washed and fixed onto wells in glass slides for DIF staining as described by Saif et al. (1986). Nasal swab supernatants and fixed cells were stored at −20°C until further testing. Nasal cells were stained using fluorescein-conjugated bovine anti-BCV serum, goat anti-IBR virus serum (Cooper strain), bovine anti-bovine respiratory syncytial virus (RSV) serum, bovine anti-bovine virus diarrhea virus (BVDV) serum, goat anti-bovine PI3 serum (SF4 strain), bovine anti-bovine rotavirus serum (NCDV strain) (control) and PBS. The IBR, RSV, BVD and PI3 FITC conjugates were kindly supplied by NVSL Laboratories, Ames, IA. Wells were considered positive when they contained at least two specific fluorescing cells/well. The number of positive cells was estimated for each well by examining 5 different fields of view (200×) and the results expressed as an average percentage of fluorescing cells.

Antigen detection by ELISA

Nasal swab supernatants and rectal swab/fecal samples collected in the first study were tested for BCV antigen by ELISA using procedures similar to those previously described by Heckert et al. (1989). Briefly, 96-well polystyrene plates were coated with hyperimmune or pre-immune anti-BCV serum, and 100 μl of feces (diluted 1:25 and processed as described previously (Saif et al., 1983)) or undiluted nasal swab supernatants were added. Each sample was added to four wells of positive capture serum and duplicate wells of negative control serum. A set of duplicate samples (on positive capture antibody) received 100 μl of hyperimmune or pre-immune bovine anti-BCV serum, as a blocking test. Bound antigen was detected by addition of hyperimmune guinea pig anti-BCV serum followed by rabbit anti-guinea pig alkaline phosphatase conjugate (ICN ImmunoBiologicals, Lisle, IL). The substrate was p-nitrophenyl phosphate

(Sigma Chemical Company, St. Louis, MO) in 10% diethanolamine. Color development was read at 405 nm (Titertek Multiskan, Flow Laboratories, McLean, VA) when positive control wells reached an absorbance value of approximately 1.0. Fecal and nasal swab supernatants, previously determined as positive or negative for BCV by IEM, were included on all plates as controls. Mean values were determined for each set of duplicate samples tested. A cutoff absorbance value was established for each sample by adding 2 standard deviations (SD) of all negative control samples to the negative control value of the sample. A sample was considered positive if it was above the cutoff value for that sample and if its absorbance value was reduced (in the blocking test) by $>50\%$ of the difference between the unblocked absorbance value and the cutoff absorbance value.

Antibody detection

ELISA. Sera from calves in the first study were analyzed for IgG1, IgG2, IgA and IgM isotype-specific antibody titers to BCV using ELISA as described by Saif (1987). Briefly, microtiter plates were coated with optimally diluted cell culture propagated BCV (NCDV stain) or mock infected cell antigen control and stored at 4°C. Serial 4-fold dilutions of each sample were added to duplicate antigen-coated and control wells. Bound isotypic antibodies to BCV were then detected by the addition of monoclonal antibody to bovine IgG1 (1:1000), IgG2 (1:500) or IgA (1:500) (kindly provided by Dr. Goldsby, Amherst College, Amherst, MA and Dr. Shrikumaran, Univ. Nebraska, Lincoln, NB) or polyclonal rabbit antisera to IgM (1:1000, Cappel Laboratories). Alkaline phosphatase conjugated to goat anti-mouse Ig or goat anti-rabbit Ig, respectively (Boehringer Mannheim, Indianapolis, IN) was then added, followed by the substrate p-nitrophenylphosphate in 10% diethanolamine buffer. Plates were read at an absorbance (A) of 405 nm in a Multiskan microplate ELISA reader. Each plate contained the following controls: (a) positive and negative serum or milk whey standards; (b) sample diluent in antigen and control antigen coated wells; and (c) each specimen in control antigen-coated wells. The A_{405} of duplicate wells were averaged and the antibody titer expressed as the reciprocal of the highest specimen dilution which had an A_{405} of >2 SD above the background control (sample in control antigen coated wells). The test was repeated for each sample and the average (log 10) of the two titers reported, with the error bars indicating the two values determined.

Zinc sulfate turbidity (ZST). Zinc sulfate turbidity determinations of total Ig content were done on serum samples collected from each calf 24–48 h after birth. A procedure similar to that described by McEwan et al. (1970a) was used, but with some modifications. A $ZnSO_4$ solution of 108 mg/ml was prepared in distilled H_2O and kept free of CO_2 by storing the solution over a free

reservoir of soda lime. The $ZnSO_4$ was then warmed to 25°C overnight and 3 ml dispensed into duplicate tubes the day of the test. To each tube, 50 μl of the serum sample was added and the tube was incubated a further 30 min at 25°C. After incubation the tubes were gently inverted and the turbidity measured in a spectrophometer (Spectronic 20, Bausch and Lomb) at 660 nm. Included in each determination were: (a) a standard of pooled adult bovine serum (total Ig content 22.9 mg/ml determined by radial immunodiffusion by W.A. Fleanor, Arizona State Univ., Tuscon, AR); (b) at least 5 intermediate standards made by making incremental dilutions of the known standard with newborn colostrum-deprived calf serum; and (c) $ZnSO_4$ without any serum as a blank.

Data analysis

To determine if any correlation existed between ZST levels, BCV specific antibody isotype titers or number of days sick, a Spearman's rank order correlation coefficient (*rs*) (Colton, 1974) was calculated and the null hypothesis that the population correlation is zero tested in a two-tailed test. With 8 pairs of observations the critical value of *rs* at the 0.05 significance level was 0.738 (Colton, 1974).

To determine if the average number of days with diarrhea was significantly different between calves with greater than or less than the mean BCV specific antibody titer for each isotype, a two tailed Student's *t* test was applied. A P value <0.05 was considered to be significant (Colton, 1974).

RESULTS

Clinical signs

Six of 8 calves showed clinical signs of diarrhea, 3 of 8 had signs of upper respiratory tract disease and all had at least one episode of fever (rectal temperature >39.5°C) during the survey period (Table 2). Clinical signs of diarrhea could be associated with BCV infections as detected by IEM or ELISA in 3 calves (#3, 4 and 6). Clinical signs of respiratory disease could be associated with BCV infection as detected by DIF or ELISA in two animals (#1 and 4).

Immunoelectron microscopy of fecal samples

IEM revealed typical BCV particles, aggregated by the anti-BCV serum, from two animals. Calf #4 (herd A) had shedding of <1 particle/grid square at 25 days of age and 1–10 particles/grid square at 4 weeks of age (Table 2). Calf #6 (herd B) shed <1 particle/grid square at 10 weeks of age (Table 2). Small round structured viruses and rotavirus particles were also observed in some of the fecal samples examined during periods when diarrhea occurred but BCV particles were not detected.

TABLE 2

Age (weeks) of calves at which clinical signs, respiratory and fecal shedding of bovine coronavirus and a 4-fold rise in antibody isotype titers to bovine coronavirus were observed

Calf no.	Clinical signs			Respiratory shedding (age)		Fecal shedding (age)		Antibody isotype to bovine coronavirus and age at titer increase			
	Diarrhea[a] (age)	Rhinitis[b] (age)	Fever[c] (age)	DIF[d]	ELISA	IEM[d]	ELISA	IgG1	IgG2	IgA	IgM
1	none	2,3,5	1,3	none	5	none	none	nr[e]	nr	nr	nr
2	4	none	9,10	none	none	none	9	nr	nr	4,8	2
3	4,5,16	none	8	none	16	none	16	nr	nr	10	6
4	3,4,6	6	2,3,6,9	6	none	4	4	6	6	nr	2
5	6	none	12	none	none	none	none	nr	nr	nr	8
6	1-3,10-13 17,18	none	13-18	9	9	10	none	11	10	8	10
7	10-13,18	2	10,13,14,18	none	none	none	none	13	8,16	5	5,10
8	none	none	15,16,19	none	none	none	18	nr	9	3	3

[a]Diarrhea = fecal score ≥ 2.
[b]Rhinitis = respiratory score ≥ 2.
[c]Fever = rectal temperature $> 39.5\,^{\circ}C$.
[d]DIF = direct immunofluorescence of nasal epithelial cells. IEM = immunoelectron microscopy.
[e]nr = no antibody titer rise.

Direct immunofluorescence (DIF) of nasal epithelial cells

Detection of BCV viral antigens in nasal epithelial cells by DIF revealed respiratory BCV infections in 2 of 8 animals beginning at 6 (calf # 4, herd A) and 9 (calf # 6, herd B) weeks of age (Table 2). BCV antigen detection by DIF was associated with clinical signs of respiratory disease only in calf #4 (Table 2). The infection persisted for a duration of 5 days in calf #4 and the percentage of nasal epithelial cells infected with BCV ranged from 1 to 10%. In calf #6 the infection lasted 4 days and less than 1% of the nasal epithelial cells were infected. Respiratory infections by IBR, RSV, BVD, PI3 or rotavirus were not detected in either herd A or B by DIF.

Antigen detection in feces and nasal swab fluid by ELISA

BCV antigen detection by ELISA revealed respiratory shedding of viral antigens in 3 of 8 animals at 5 (calf # 1, herd A), 9 (calf # 6, herd B) and 16 (calf # 3, herd A) weeks of age. Respiratory shedding of virus by ELISA was associated with rhinitis in calf #1, with positive nasal cells by DIF in calf #6 and with neither in calf #3 (Table 2). Enteric shedding of BCV viral antigens was found in 4 of 8 animals at 4 (calf # 4, herd A), 9 (calf # 2, herd A), 16 (calf # 3, herd A) and 18 (calf # 8, herd B) weeks of age. In two animals (calf # 2 and 8) enteric viral shedding was unassociated with enteritis or other evidence of BCV infection (Table 2). In one animal (calf #3, herd A), enteric and respiratory viral shedding occurred in the same week (Table 2).

Antibody detection by ELISA and zinc sulfate turbidity tests

Passive antibody titers (log 10) to BCV (24–48 h post-suckling) in calves from the first study varied from 4.01 to 2.41 for IgG1, 3.61 to 1.81 for IgG2, 3.41 to <0.6 for IgA and 1.81 to <0.6 for IgM isotypes (Table 3). Most calves began with high passive antibody titers to BCV associated with all isotypes which decayed over time. A $\geqslant 4$-fold rise in antibody titers to BCV with at least one isotype was seen in 7 or 8 animals at some point in the study (Table 2). Increases in antibody titers were generally first observed with IgM and IgA, followed by IgG1 and IgG2 after 1 or 2 weeks. An example of the BCV isotype-specific changes seen is shown for calf #6 (Fig. 1), which is similar to the responses seen in other calves. One calf (#4, herd A) was unique by having low levels of passive antibodies initially, a rapid decline in titers and then a very dramatic increase in all isotype antibody titers except IgA (which showed no change). Two calves (#2 and 7) showed recurrent rises in BCV isotype titers (IgA, IgG2 and IgM) after the initial antibody titer rise (Table 2). This is illustrated by the responses seen in calf #7 (Fig. 2) in which $\geqslant 4$-fold rises were seen with IgM and IgG2 at 10 and 16 weeks of age, respectively.

Levels of total serum Ig content, BCV isotype-specific antibody titers at 24–48 h of age and number of sick days during the study for calves in study 1 are shown in Table 3. The Spearman correlation coefficients between the ZST levels and the BCV specific antibody isotype titers were $rs=0.191$, 0.12, 0.16 and 0.55 for IgG1, IgG2, IgA and IgM, respectively. The Spearman correlation coefficients between the various antibody isotypes and number of days with diarrhea were $rs=0.48$, 0.04, 0.60 and 0.41 for IgG1, IgG2, IgA and IgM, respectively. The Spearman correlation coefficient between the ZST levels and

TABLE 3

A comparison of zinc sulfate turbidity (ZST) values with antibody isotype titers to bovine coronavirus in serum (24–48 h after birth) and the number of sick days (ranked by ZST levels)

Calf #	ZST (mg/ml)	Average BCV antibody titers (log 10)				Aprox. number of sick days	
		IgG1	IgG2	IgA	IgM	Respiratory[a]	Enteric[a]
5	38.2	4.01	3.41	3.01	1.81	0	1
6	12.5	3.01	3.01	1.60	1.20	0	21
8	12.1	3.01	2.41	2.11	1.20	0	0
4	11.5	2.81	1.81	0.48	0.60	3	12
2	11.0	3.94	3.01	3.41	1.00	0	3
1	10.9	3.94	2.81	2.81	1.00	2	0
3	6.90	4.01	3.61	2.74	1.60	0	5
7	1.60	2.41	2.41	1.20	0.48	1	15

[a]Respiratory or fecal score ≥ 2 (including days between observed sick days).

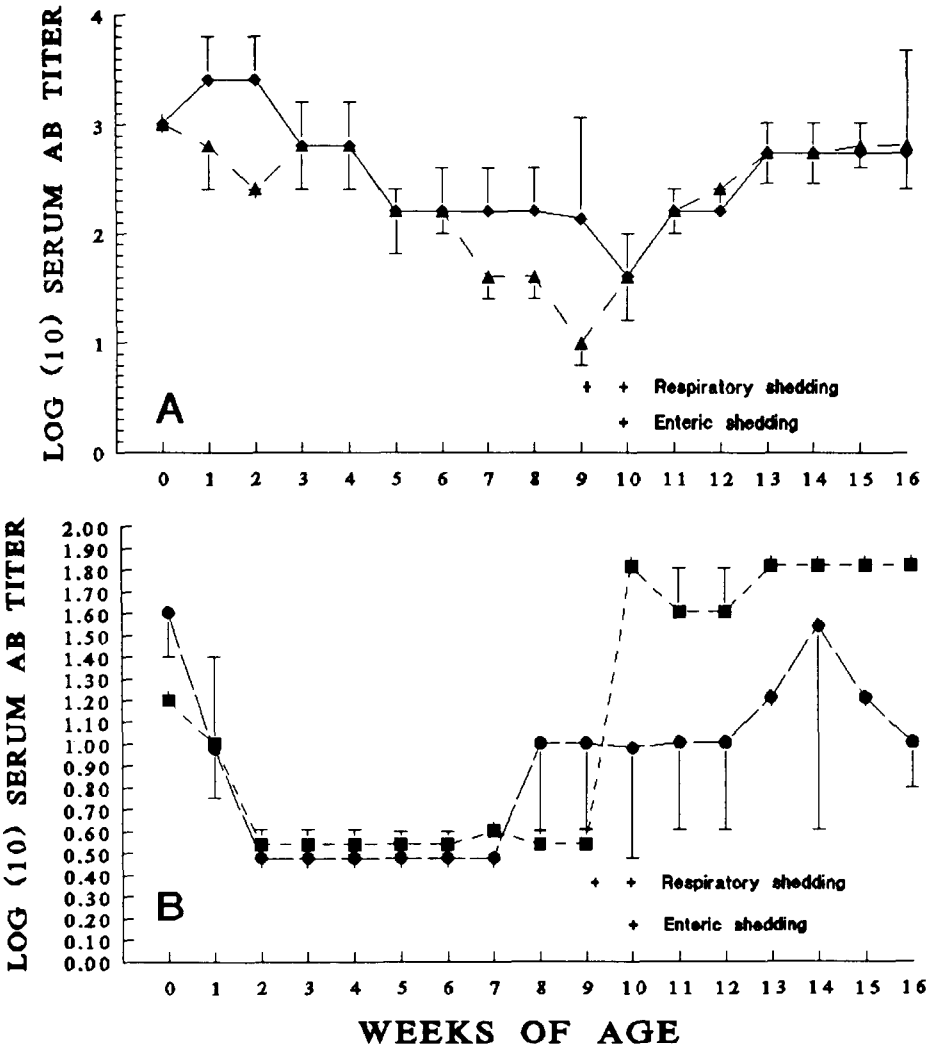

Fig. 1. Antibody isotope titers to BCV from birth to 16 weeks of age in calf #6. Symbols indicate the average titer (log 10) at each time point and the error bars indicate the titers (log 10) of each replicate. Respiratory and enteric virus shedding is indicated by "+" as determined by DIF of nasal epithelial cells or IEM of feces, respectively. (A) Antibody isotype titers of IgG1 ♦ and IgG2 ▲; (B) antibody isotype titers of IgA ● and IgM ■.

the number of days with diarrhea was $rs=0.13$. Correlations were not done between number of days with respiratory disease and ZST or antibody isotype titers because there were only three calves (#1, 4 and 7) which had incidences of respiratory disease with a score ≥ 2. None of these correlations were significantly different from zero in a two tailed test at a significance level of 0.05. There was however, a significantly ($P=0.0009$) greater number of average

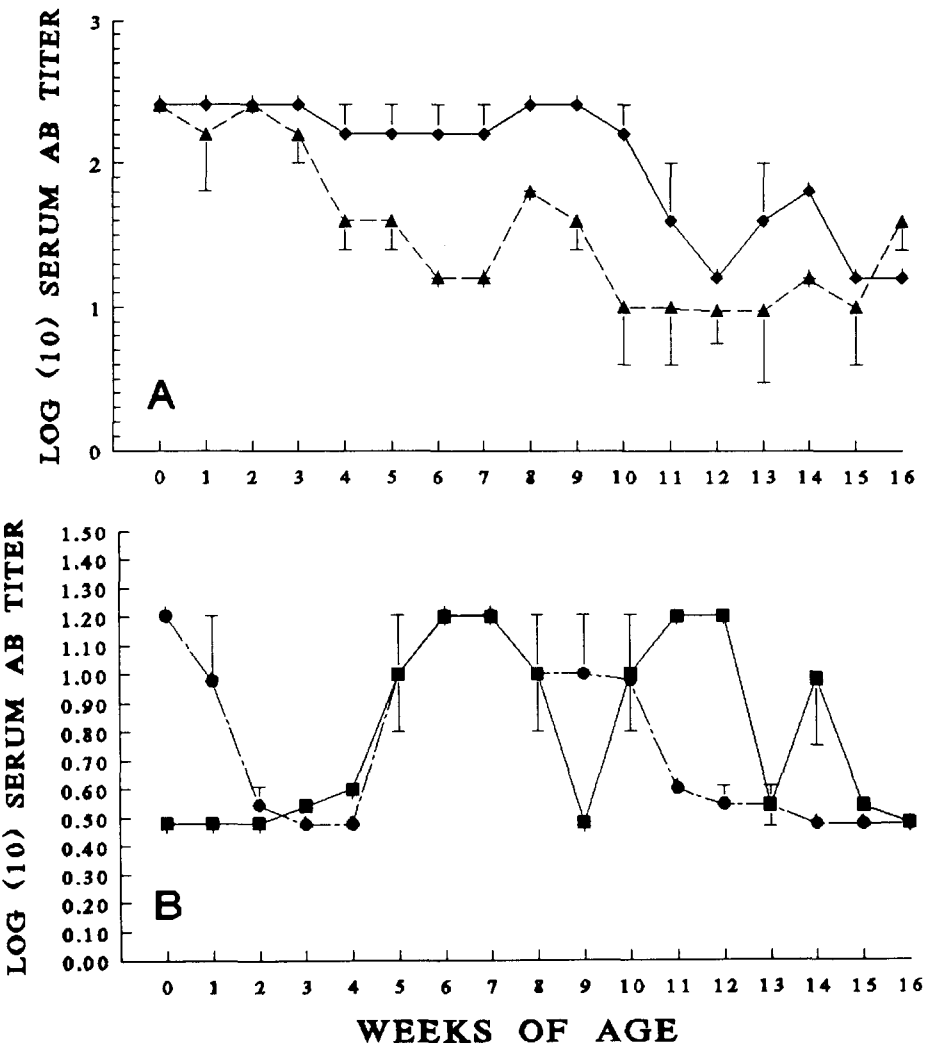

Fig. 2. Antibody isotype titers to BCV from birth to 16 weeks of age in calf #7. Symbols indicate the average titer (log 10) at each time point and the error bars indicate the titers (log 10) of each replicate. Respiratory and enteric virus shedding was not detected in this calf. (A) Antibody isotype titers of IgG1 ♦ and IgG2 ▲; (B) antibody isotype titers of IgA ● and IgM ■.

days with diarrhea in calves which had BCV specific antibody isotype titers less than the mean BCV specific IgA titer (log 10) for the 8 calves. There were no significant differences in the average number of days with diarrhea between calves having greater than or less than the mean IgG1, IgG2 and IgM BCV specific antibody isotype titers, with P values of 0.076, 0.91, and 0.91, respectively (Table 3).

A t-test of association between ZST levels and BCV specific antibody isotype

titers were not done due to the skewed ZST values; however, by inspection the highest ZST values were associated with the highest BCV specific titers of all isotypes (Table 3).

DISCUSSION

Although it is well-established that BCV infects young (1–28-day-old) calves, until now few studies have described detailed observations of respiratory or enteric infections in calves, in particular older calves (>30 days old). This prospective longitudinal study confirms that young calves become infected with BCV and demonstrates the infection in older calves as well.

Six of the 8 calves (75%) shed BCV from the upper respiratory or intestinal tract at various points throughout the study period, as indicated by virus or viral antigen detection. This supports the results of our previous study which showed that BCV respiratory infections occur in older calves (Heckert et al., 1988). Respiratory or enteric BCV viral antigen shedding was detected by ELISA in 4 animals unassociated with detection of BCV infection by other tests but associated with a rise in one or more antibody isotypes to BCV in 3 of the 4 animals. These results may indicate low-grade subclinical infections with little active viral replication.

Serological analysis also indicated that both young and older calves (>30 days old) became infected with BCV. This agrees with the results of previous studies which showed that older calves can have BCV serological antibody titer increases during the first 9 months of life (Ellens et al., 1979). Two calves showed IgM or IgA serum antibody titer increases but little if any IgG1 or IgG2 titer increase (Table 2). These were generally calves which had the highest BCV specific passive antibody titers. It has been shown that high levels of passive antibodies can reduce both the active systemic and local immune responses to bovine RSV (Kimman et al., 1987) or bovine PI3 virus (Marshall and Frank, 1975) and the IgA and IgM active immune responses to rotavirus infection (Saif and Smith, 1985; Van Zaane et al., 1986; Saif et al., 1987). From our limited data this type of suppression appears to be true for active systemic antibody responses to BCV infection as well and may explain why several calves did not show BCV specific antibody titer increases with some isotypes after infection. As suggested for RSV infection (Kimman et al., 1987), only an IgM BCV specific serum titer increase may be a useful indicator of a recent BCV infection in diagnostic serological tests.

Several authors have shown that calves with high (>5–10 mg/ml) (McEwan et al., 1970b; Hancock, 1985) total serum Ig levels in the first week of life tend to experience fewer incidences of pneumonia (Thomas and Swann, 1973; Williams et al., 1975; Thomas, 1978; Davidson et al., 1981) or diarrhea (Thornton et al., 1972; Boyd and Leyland, 1974; Hancock, 1985). In this study there appeared to be no statistically significant Spearman correlation between

clinical disease or infection by BCV and total serum Ig level, nor was there a statistically significant Spearman correlation between BCV isotype specific antibody titers in serum (24–48 h after birth) and clinical disease or infection by BCV. However, all calves except one (#7, herd B) had high (>5 mg/ml) total Ig levels and all had fairly high levels of BCV isotype specific antibody titers (calves # 4 and 7 having some of the lowest). There was however a statistically significant association (by *t* test) between low IgA BCV-specific serum titers (24–48 h after birth) and greater average number of days with diarrhea (Table 3), as shown by others for IgA (Fisher and Martinez, 1978). A larger number of calves may have made the Spearman correlation statistically significant also.

These data suggest that ZST levels or BCV isotype specific antibody titers (except IgA) in serum (24–48 h after birth) may not be good predictors of the probability of BCV infection or disease, which agrees with some reports (Barber, 1978; Hancock, 1985; Caldow et al., 1988) but conflicts with others (Thomas and Swann, 1973; Thomas, 1978; Williams et al., 1975; Davidson et al., 1981). It has been shown that high serum antibody titers to enteric viral pathogens do not always correlate well with protection (Hooper and Haelterman, 1966). The titer of local antibodies in respiratory or intestinal secretions may correlate better with protection from disease. Therefore the poor correlation between serum antibody titers and the frequency of enteric disease was not surprising. In addition, since ELISA is a quantitative measure of antibody binding and not necessarily a measure of protective ability it may be expected that antibody titer assessment by ELISA would correlate poorly with protection from disease, unlike other functional measures like the virus neutralization test, which may correlate better. This demonstrates that determination of risk for disease or infection by BCV in neonatal calves may need to involve more than quantitation of total serum Ig or BCV isotype specific ELISA antibody titers at birth (except IgA). The association between low IgA BCV specific serum titers (at birth) and diarrhea may indicate that IgA titers (at birth) may be good predictors of neonatal enteritis, although a larger number of samples than was used in this study would be needed to confirm these observations.

There was also no statistically significant correlation between BCV specific antibody isotype titers and total Ig levels. This was not surprising as none of these calves' dams had been immunized against BCV; therefore, the fraction of total Ig contributed by BCV specific Ig may be small and within the error limits of the tests involved considering the small sample size.

The natural route of transmission of BCV has been assumed to be fecal–oral. In experimental infections, respiratory replication of virus may be seen before enteritis (Saif et al., 1986). This may further substantiate the speculation that aerosol transmission and respiratory replication may be important parameters in the pathogenesis of BCV in some calves (Saif and Smith, 1985). Calf # 6 showed clinical signs of diarrhea and fecal viral shedding after respi-

ratory infection and therefore may have had a BCV respiratory infection prior to the enteric infection (Table 2). However, calf #4 (herd A) shed BCV in the feces (as determined by EM and ELISA) prior to having positive nasal epithelial cells by DIF (Table 2). Therefore, both portals of entry (respiratory and oral) may be important in the pathogenesis of BCV infections under field conditions. Greater numbers of animals would have to be investigated to elucidate the importance of respiratory BCV infections in the epidemiology of BCV.

Four of the 8 calves studied had evidence of BCV infection in association with abnormal clinical signs, even though 7 of 8 calves showed increases in one or more serum antibody isotypes to BCV. This indicates that BCV infections may be very common in a closed herd but that many may be subclinical. Respiratory infections with BCV may play an important role in the epidemiology and transmission of BCV among susceptible calves. However, in this study of a limited number of calves from two well-managed closed herds, only mild signs of respiratory disease were observed in association with BCV infections of the respiratory tract.

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