JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1984, p. 388–393
0095-1137/84/030388-06$02.00/0

Vol. 19, No. 3

# Monoclonal Antibody Capture Enzyme-Linked Immunosorbent Assay for Detection of Bovine Enteric Coronavirus

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Received 19 August 1983/Accepted 18 November 1983

Monoclonal antibodies reactive with three different viral polypeptides were evaluated singly and in combination as the capture antibody(s) in an enzyme-linked immunosorbent assay system for the detection of bovine enteric coronavirus. Similar levels of sensitivity were found for all combinations tested. A sensitive, highly specific, and reproducible assay for the detection of bovine enteric coronavirus was developed, using a mixture of two of these monoclonal antibodies reactive with antigenic components either external or internal to the virion. These monoclonal antibodies were bound indirectly to 96-well plates via rabbit anti-mouse immunoglobulin. After sample application and incubation, virus was detected by using rabbit anti-coronavirus peroxidase conjugate followed by enzyme substrate and chromagen. Fecal samples from a single herd of cows were screened for the presence of coronavirus by this assay. Five percent of clinically normal cows were found to be shedding coronavirus.

Bovine enteric coronavirus (BEC) has been shown to be a primary pathogen in neonatal calf diarrhea (4, 22). Some studies on the association of BEC with field outbreaks of neonatal calf diarrhea have been performed (1, 19, 20, 23–25), but diagnostic tests were limited to electron microscopy of fecal samples and immunofluorescent staining of intestinal sections. Electron microscopy is time consuming, and care must be taken to distinguish between coronaviruses and the fringed particles seen in normal feces. The immunofluorescent histopathology technique is limited to those animals that can be sacrificed. Alternative diagnostic methods have been developed including a hemadsorption-elution-hemagglutination assay and a capture enzyme-linked immunosorbent assay (ELISA) (9, 30). Previous attempts by this laboratory to develop a capture ELISA for BEC have been unsuccessful due to a lack of specificity of the system, primarily due to the presence of naturally occurring rotavirus antibody in the hyperimmune capture antibody. This report describes the development of a highly specific capture ELISA with monoclonal antibodies for the detection of BEC.

## MATERIALS AND METHODS

**Preparation of purified BEC.** The P.Q. isolate of BEC (originally obtained from S. Dea, Department of Pathology and Microbiology, Faculty of Veterinary Medicine, University of Montreal, St. Hyacinthe, Quebec, Canada) grown in Madin Darby bovine kidney cells was purified as described previously (7). Tissue culture fluid was clarified by centrifugation at 3,500 × *g* for 20 min. Virus present in the supernatant fluid was pelleted at 54,500 × *g* for 4 h (L5-65 centrifuge; Beckman Instruments, Inc.) and resuspended in 1 to 2 ml of TNE buffer (pH 7.6; 0.05 M Tris-hydrochloride, 0.1 M NaCl, 1 mM EDTA). This virus suspension was carefully layered onto a 30-ml 20 to 60% (wt/vol) linear sucrose gradient prepared in TNE buffer and centrifuged at 81,500 × *g* for 2 h. The visible virus band was collected, diluted in 0.15 M phosphate-buffered saline (PBS; pH 7.2), and the virus was pelleted at 54,500 × *g* for 4 h. This pellet was resuspended in PBS and stored at −70°C until used.

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**Preparation of antisera.** Standard positive bovine antiserum (ELISA titer, 1 in 64,000) for use in the blocking assay was prepared by hyperimmunization with coronavirus of a gnotobiotic calf which had previously been orally infected. Bovine antiserum negative for coronavirus (ELISA titer, <1 in 5) was obtained from a gnotobiotic calf which had been hyperimmunized with rotavirus.

Rabbits were hyperimmunized with coronavirus by giving them two consecutive injections 2 weeks apart of 0.5 ml of purified coronavirus (35 μg of protein) emulsified 1:1 with Freund adjuvant (the first injection was in Freund complete and the second was in Freund incomplete adjuvant) into each of two footpads, followed 2 weeks later by a single intraperitoneal injection of 1 ml of purified virus (70 μg of protein). Sera collected between 10 and 24 days after the final injection were pooled and used for preparation of a rabbit anti-coronavirus peroxidase conjugate.

**Preparation of rabbit anti-coronavirus peroxidase conjugate.** The globulin fraction of the serum pool was prepared by the procedure of Schultze and Heremans (28). Globulin protein (8 mg) was conjugated with 4 mg of horseradish peroxidase (no. P-8375; Sigma Chemical Co.) by the sodium metaperiodate oxidation method of Wilson and Nakane (32). Unreacted enzyme was separated from the mixture by gel chromatography on a 50-cm Sephadex G-200 (Pharmacia Fine Chemicals) column. The ratio of optical densities (ODs) at 403 to 280 nm of the final conjugate was 0.41. Ten milligrams of bovine serum albumin (BSA) was added per ml of conjugate before storage at −70°C.

**Preparation of mouse hybridoma cell lines.** Eight-week-old BALB/c mice were given five 50-μl intravenous injections of purified coronavirus, each containing 3.5 μg of protein, at 3-day intervals. Three days after the last injection the mice were sacrificed, and their spleen cells were mixed with Sp2/0 Ag14 myeloma cells (kindly provided by W. E. Rawls, Department of Pathology, McMaster University, Hamilton, Ontario, Canada) (29) and fused, using 40% polyethylene glycol 1450 (lot no. 052332; J. T. Baker Chemical Co.) following a modification (T. J. G. Raybould, B. E. Duncan, and D. H. Watson, Abstr. IV Int. Conf. Comp. Virol. 1982, P-28, p. 243) of the method described by Kennet et al. (15). The cells were dispensed into 24-well tissue culture plates (Linbro) and incubated at 37°C in an atmosphere containing

7.5% carbon dioxide. Screening of cell culture supernatant fluids was performed by ELISA, using microtiter plates sensitized with a whole BEC preparation (7).

Positive hybridoma cultures were cloned in 96-well microtiter plates by limiting dilution. After 14 days, the supernatant fluids from growing clones were rescreened by ELISA, and subcultures from positive monoclones were stored in liquid nitrogen. Cloned hybridoma cell lines were subsequently cultured in RPMI 1640 medium (GIBCO Laboratories) containing 10% fetal bovine serum. Monoclonal antibody for capture ELISA was collected after a 24-h yield of selected clones into serum-free RPMI 1640.

**Immune precipitation and polyacrylamide gel electrophoresis (PAGE).** Purified BEC was solubilized in 1% Triton X-100 and incubated with 1.0 or 2.5 ml of serum-free culture supernatant fluid containing monoclonal antibody for 18 h at room temperature (20°C). One hundred microliters of a 20% suspension of protein A–Sepharose CL-4B (Pharmacia) was added and incubated for 1 h at room temperature (20°C) with constant mixing. The protein A-Sepharose-bound immune complex was pelleted and washed twice with PBS before resuspension in 100 μl of sample buffer (0.0625 M Tris-hydrochloride [pH 6.8] containing 5.0 M urea, 2% sodium dodecyl sulfate, 10% glycerol, and 0.001% bromphenol blue). After the protein A-Sepharose was boiled for 2 min it was pelleted, and the clear supernatant fluid was removed and reboiled for 2 min before electrophoresis. Controls composed of either protein A-Sepharose plus monoclonal antibody or protein A-Sepharose plus virus were included in each run.

Electrophoresis was performed in slab gels (length, 10 cm; thickness, 1.5 mm) by the Laemmli discontinuous buffer system (18). Proteins were separated in a resolving gel containing 10% polyacrylamide after migration through a stacking gel containing 4% polyacrylamide. After electrophoresis individual polypeptides were detected by Coomassie brilliant blue staining.

**Methods for binding monoclonal antibodies to microtiter plates.** Optimal conditions for the binding methods outlined below were determined in preliminary studies in which the ELISA procedure described below was used (data not shown). All studies were carried out with Immulon 2 96-well plates (Dynatech Laboratories, Inc.).

**(i) Direct binding of monoclonal antibodies by passive adsorption.** Monoclonal antibodies E7, G9, and H10 were diluted in 0.06 M phosphate buffer at optimal pH, and 100 μl was added to each well. Passive adsorption was carried out at 37°C for 3 h. Antibodies E7 or G9 bound optimally at pH 8.0, whereas antibody H10 bound optimally at pH 6.0.

**(ii) Indirect binding of monoclonal antibody via poly-L-lysine.** Poly-L-lysine (PLL; 100 μl per well) (P1274; Sigma) at a concentration of 0.63 μg/ml in 0.1 PBS was adsorbed for 0.5 h at room temperature (20°C). PLL was shaken from the plates, and 100 μl of monoclonal antibody E7, G9, or H10 was added to each well and allowed to bind for 1 h at room temperature.

**(iii) Indirect binding of monoclonal antibody via anti-mouse immunoglobulin.** Rabbit anti-mouse immunoglobulin G (IgG) (H + L) (Zymed Laboratories) was passively adsorbed to Immulon 2 plates at pH 9.6 (0.05 M carbonate-bicarbonate buffer) at 37°C for 3 h. After the plates were washed six times with distilled water, 100 μl of monoclonal antibody E7, G9, or H10 was added to each well and allowed to bind for 1 h at room temperature.

**ELISA procedure.** ELISA tests were performed by the microplate modification (31) of the method of Engvall and Perlmann (10). A standard washing procedure of six washes in distilled water was adopted (7). Incubations were performed at room temperature (20°C) for 1 h unless stated otherwise.

**(i) ELISA for determination of the amount of antibody bound to the solid phase.** Antibody was passively adsorbed to 96-well microtiter plates with or without pretreatment of the plates with PLL or anti-mouse immunoglobulin. The plates were then washed, and nonreacted binding sites were blocked by incubation after the addition of 100 μl of 1% BSA in 0.01 M PBS to each well. After washing, 100 μl of an optimal dilution (previously determined by titration) of affinity-purified peroxidase-conjugated rabbit anti-mouse IgG (H + L) (Zymed) diluted in 0.01 M PBS containing 0.05% Tween 20 was added to each well, and the plates were further incubated. Finally, the plates were again washed, and bound conjugate was reacted with chromagen and enzyme substrate (100 μl of recrystallized 5 amino-salicyclic acid per well [Aldrich Chemical Co., Inc.] diluted to 1 mg/ml in 0.01 M phosphate buffer containing 0.01 M $Na_2$ EDTA [pH 5.95] to which 0.005% hydrogen peroxide was added immediately before use [12]). After 30 min at room temperature, the OD at 450 nm of each well was determined with a micro-ELISA reader (MR580; Dynatech).

**(ii) ELISA for determination of monoclonal antibody isotype.** Each well of 96-well microtiter plates was sensitized with 100 μl of an optimal dilution (previously determined by titration) of rabbit anti-mouse IgG1, IgG2a, IgG2b, IgG3, IgM, or IgA sera (Miles Laboratories, Inc.) diluted in 0.05 M carbonate-bicarbonate buffer (pH 9.6), by incubation overnight at 4°C. After nonreacted sites were blocked with 1% BSA (see above), plates were washed, and 100 μl of culture supernatant fluid containing monoclonal antibody was added to each well. Plates were incubated and washed, and then 100 μl of an optimal dilution (previously determined by titration) of affinity-purified, peroxidase-conjugated rabbit anti-mouse IgG (H + L) (Zymed) was added to each well. After one more incubation, plates were washed, and bound conjugate was detected as described above.

**(iii) Capture ELISA for the detection of BEC.** A modification of the above procedure adapted for the detection of microbial antigens (33) was used. Microtiter plates (96-well) were sensitized with the appropriate capture antibodies (see below). After incubation the plates were washed, and nonreacted sites were blocked with 1% BSA (see above). Test samples were diluted 2- or 10-fold in 0.01 M PBS containing 0.05% Tween 20, and 100 μl of each dilution was added to duplicate wells. Control fecal samples positive or negative for coronavirus were included in each series of tests. Plates were incubated, washed, and then further incubated after the addition of an optimal dilution of rabbit anti-coronavirus peroxidase conjugate. Bound conjugate was detected as above. Negative controls had an OD of <0.01 U. Test samples were therefore considered positive if both duplicates had an OD of <0.02 units (see below).

**Blocking assay.** Specificity testing was performed by a blocking assay in which the reaction between the test sample and the peroxidase conjugate was blocked in one of a pair of duplicate sample wells by a 1:10 dilution of bovine anti-coronavirus serum and in the second by a 1:10 dilution of a bovine serum negative for coronavirus antibody. After the plates were washed, peroxidase conjugate was added and the standard capture ELISA procedure was continued as outlined above. A positive reaction was considered specific if the absorbance value was reduced by >50% in the well treated with bovine anti-coronavirus serum.

**Experimental infections.** Colostrum-deprived calves, delivered by Caesarian section and housed in individual isolators equipped with filtered air supplies, were used. Calves were infected orally, and fecal samples were collected at least twice per day for the duration of the experiment.

Calf 1 received 8 ml of a fecal sample from a colostrum-deprived calf which contained coronavirus 011483D (obtained from L. Saif, Ohio State University, Columbus) diluted in 2 liters of homogenized milk. Calf 2 received 6 ml of a 1 in 2 dilution of fecal material (0.45-μm [pore size] membrane filter) from calf 1 diluted in 1 liter of homogenized milk. Calf 3 received 10 ml of a fecal sample from calf 2 diluted in 1 liter of homogenized milk. Calves 1 and 2 were infected 2 days after birth, and calf 3 was infected 1 day after birth.

**Preparation of fecal samples.** Fecal samples were stored at −70°C until diluted in 0.01 M PBS containing 0.05% Tween 20. After low speed centrifugation at 3,000 rpm (MSE Chilspin) for 20 min, the partially clarified supernatant fluids were carefully removed and stored at −20°C until tested.

## RESULTS

Hybridoma monoclonal antibodies which exhibited a strong positive reaction by ELISA, using microtiter plates sensitized with a whole coronavirus preparation (7), were screened by immune precipitation and PAGE. Four major structural polypeptides with apparent molecular weights of 120,000, 100,000, 52,000, and 26,000 could be resolved by PAGE of Triton X-100 extracts from purified virus preparations. Monoclonal antibodies from hybridoma cell lines E7, G9, and H10 specifically reacted with the 120,000-, 26,000-, and 52,000-molecular-weight polypeptides, respectively (Fig. 1), and were selected for evaluation as capture antibodies in ELISA for the detection of BEC. The isotypes of monoclonal antibodies E7, G9, and H10 were IgG2a, IgM, and IgG3, respectively.

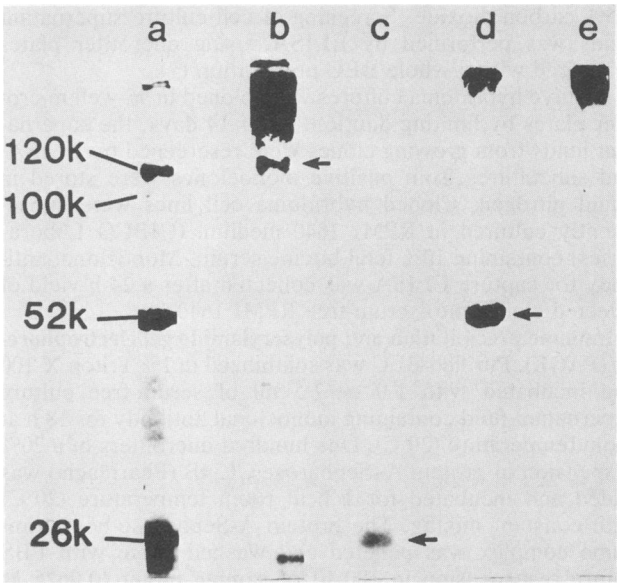


FIG. 1. PAGE of immune precipitates of BEC. Polypeptides were visualized by using Coomassie brilliant blue. The major BEC polypeptides are designated by their molecular weights as previously determined from their migration in PAGE relative to that of standard proteins. Arrows indicate viral polypeptides specifically precipitated by monoclonal antibody. The 23,000-molecular-weight polypeptide visible in track b is probably immunoglobulin light chain and was present to a varying degree in all preparations. Track a is purified virus; tracks b through d are immune precipitates of detergent-solubilized virus, using monoclonal antibodies E7, G9, and H10, respectively; and track e is the control (protein A–Sepharose CL-4B plus monoclonal antibody E7).

**Comparison of the sensitivity of capture ELISA for the detection of BEC, using plates sensitized by different methods with single or combinations of monoclonal antibodies.** Plates (96-well) were sensitized with an optimal dilution of monoclonal antibodies E7, G9, and H10 (previously determined by titration) by each of the three binding methods described above (antibody G9 bound poorly by passive adsorption but bound to the same extent as antibodies E7 or H10, using the two indirect binding methods). The following samples were then titrated by serial twofold dilutions in these sensitized plates (Table 1): fecal samples from two calves which became diarrheic after experimental infections with coronavirus (A and B), a fecal sample obtained from calf A before infection (C), tissue culture-grown coronavirus from cell culture fluid (D), tissue culture-grown coronavirus from (D) concentrated ca. 100-fold by pelleting (E), and a cell culture fluid from mock-infected cells (F).

Microtiter plates were sensitized by the three binding methods previously described with single monoclonal antibodies and with combinations in 1:1 or 1:1:1 ratios (Table 1). All negative samples (Table 1; C and F) gave background readings (OD, ≤0.01). There was no significant difference in the sensitivity of ELISA, using plates coated with antibodies, either singly or in various monoclonal combinations, by any of the three binding methods described.

The need for blocking plates with 1% BSA after sensitization was investigated. When ELISA tests were performed with unblocked plates to which monoclonal antibody had been bound by passive adsorption or via anti-mouse immunoglobulin no increase in nonspecific reactivity was observed. However, when blocking was omitted with plates to which monoclonal antibody had been bound via PLL, increased background OD readings (0.05 OD units compared with <0.01 OD units) were observed. Since it was desirable to include at least two capture monoclonal antibodies of different specificities in the ELISA system (see below), plates to which antibodies E7 and H10 had been bound via anti-mouse immunoglobulin were used, without blocking, for further studies.

**Reproducibility and specificity of capture ELISA.** The reproducibility of the assay system was evaluated by testing the six standard samples in a twofold dilution series a minimum of five times on separate occasions. The range of titers obtained for the five replicates did not exceed one twofold dilution for any of the six samples tested. Sensitizing different batches of microtiter plates with optimal dilutions of monoclonal antibodies prepared on different occasions gave identical results. The absence of nonspecific reactivity was confirmed by the blocking assay.

Twenty fecal samples from Caesarian-derived specific pathogen (coronavirus)-free calves maintained under strict isolation conditions were tested. These gave ODs in the range of 0.002 and 0.008 which were not reduced by the blocking assay. The mean OD of 0.005 plus three times the standard deviation resulted in an OD of 0.011. Samples giving ODs of ≥0.02 were therefore considered positive. When tissue culture-derived rotaviruses were tested in the coronavirus capture ELISA, ODs of <0.01 were obtained, showing that this assay does not detect rotavirus antigens.

**Patterns of virus shedding in experimentally infected calves.** Both calves infected at 48 h postpartum (calves 1 and 2)

TABLE 1. Dilution endpoints of samples positive or negative for coronavirus

| Monoclonal antibody bound to microtiter plate | Titers of sample[a] | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | F |
| Passive adsorption[b] | | | | | | |
| E7 | 256 | 128 | – | 32 | 4,096 | – |
| H10 | 512 | 1,024 | – | 64 | 2,048 | – |
| G9 | NT | NT | NT | NT | NT | NT |
| PLL[b] | | | | | | |
| E7 | 64 | 128 | – | 8 | 2,048 | – |
| H10 | 256 | 512 | – | 32 | 2,048 | – |
| G9 | 256 | 128 | – | 32 | 1,024 | – |
| E7, G9 | 256 | 128 | – | 8 | 2,048 | – |
| E7, H10 | 512 | 512 | – | 8 | 1,024 | – |
| G9, H10 | 256 | 128 | – | 8 | 1,024 | – |
| E7, G9, H10 | 128 | 128 | – | 8 | 512 | – |
| Anti-mouse IgG[b] | | | | | | |
| E7 | 512 | 128 | – | 16 | 2,048 | – |
| H10 | 512 | 512 | – | 16 | 256 | – |
| G9 | 512 | 128 | – | 32 | 1,024 | – |
| E7, G9 | 512 | 256 | – | 16 | 1,024 | – |
| E7, H10 | 512 | 512 | – | 32 | 2,048 | – |
| G9, H10 | 256 | 256 | – | 8 | 1,024 | – |
| E7, G9, H10 | 256 | 128 | – | 8 | 512 | – |

[a] Titers expressed as highest dilutions positive for coronavirus (OD, >0.02). –, Indicates undiluted sample negative for coronavirus (OD, <0.02); NT, not tested. A and B, Positive fecal samples; C, negative fecal samples; D, positive tissue culture fluid; E, pelleted virus from tissue culture; F, control tissue culture fluid.

[b] Method of binding monoclonal antibody to microtiter plate.

developed symptoms within 40 to 48 h postinfection (p.i.). These symptoms included a mild-to-severe watery diarrhea, accompanied by a slightly depressed appearance and reluctance to move, soon followed by slight dehydration. In calf 1, which was allowed to survive until fully recovered, these symptoms persisted until ca. 150 h p.i. Calf 3, infected at 24 h of age, developed a severe watery diarrhea at 20 h p.i. accompanied by severe depression and increasing dehydration, culminating in death 72 h later. In all three animals, commencement of virus shedding was found to coincide with the onset of diarrhea (Fig. 2). High titers of virus were present in feces until at least 90 h p.i., followed in calf 1 by a gradual reduction in shedding, with no virus detectable after 170 h p.i.

**Incidence of BEC in diarrheic and nondiarrheic cattle.** Fecal samples from a total of 80 diarrheic calves in the field and 121 clinically normal cows from a single herd were tested for the presence of BEC by ELISA. Of the 80 fecal samples from diarrheic calves, 16 (20%) were positive, whereas 6 (5%) of the samples from clinically normal cows were also positive. The specificity of these positive reactions was confirmed by the blocking assay. No false-positive reactions were detected, since in all cases the absorbance values were reduced by more than 50% after blocking by anti-coronavirus serum.

## DISCUSSION

Passive adsorption of proteins to solid phases depends not only on the type of solid phase but also on the conditions under which binding occurs (16, 21). With the three monoclonal antibodies utilized in this study, a significant variation in the pH at which optimal binding occurred was noticeable. Although this is not a problem when the binding of only one monoclonal antibody is required, the use of a combination of monoclonal antibodies could result in the selective binding of certain antibodies from that pool at different pHs. Further, it is apparent that some monoclonal antibodies (e.g., G9) bind poorly by passive adsorption. It is not known whether the poor binding observed with antibody G9 is characteristic of IgM molecules or whether it is a function of that particular monoclonal antibody.

A potential problem associated with the use of monoclonal antibodies in capture ELISAs is that they may possess low affinity constants (11, 26). This can lead to dissociation of the antigen-antibody complex during the multiple washing steps of the assay and the consequent loss of bound antigen from the system. Incorporation of antibodies reactive with different sites on the antigen concerned should improve the binding of antigen to antibody, such that the avidity of the pooled system is greater than that of the individual monoclonal antibodies. Since a monoclonal antibody is directed against a single antigenic determinant, it is possible that some strains of BEC may lack this determinant and thus go undetected in an ELISA. Use of a pooled system will reduce the specificity of the assay and consequently reduce the chances of failing to detect the presence of virus.

Of the two monoclonal antibodies used in this study, H10 reacts with the 52,000-molecular-weight polypeptide which is probably an internal protein, whereas E7 reacts with a polypeptide of a molecular weight of 120,000 which is probably a peplomer (17). This may increase the reactivity of the assay system since both degraded and complete virions can be bound.

Since the conditions under which optimal passive adsorption occurs vary between different monoclonal antibodies, an alternative method for binding combinations of antibodies to the solid phase was required. Of the two indirect methods investigated, neither PLL nor anti-mouse immunoglobulin significantly increased the amount of antibody from cell line E7 or H10 that could be bound to the plates (data not shown); however, the amount of antibody from cell line G9 that could be bound was greatly increased. The major disadvantage of using PLL to bind antibody to the solid phase was an increased nonspecific absorption of immunoreactants leading to a reduction in sensitivity due to increased nonspecific reactivity. Although this could be blocked with 1% BSA, it was felt that the use of an anti-mouse immunoglobulin to bind antibody to the solid phase was the method of choice.

There have been many reports of false-positive results when solid-phase immunosassays have been used for the detection of viruses in feces (3, 5, 8, 13, 34). The causes of such nonspecific reactions and methods of minimizing them have been reviewed by Yolken (35). Most nonspecific reactivity is probably due to the specificity of the antisera used. Contaminants may persist in the immunogen despite rigorous purification. Alternatively preexisting antibody resulting from previous natural infection of the hyperimmune animal may be present in the serum produced. The use of monoclonal antibodies in the ELISA system described ensures highly specific reactivity, as demonstrated by the absence of false-positive results. Antibodies secreted into serum-free tissue culture fluid were used in preference to mouse ascitic fluid to maintain a high level of specificity; ascitic fluids contain, in addition to high titers of monoclonal antibody, preexisting antibody from natural infections, as well as antibody induced by tissue culture fluid components (e.g., fetal bovine serum) in which the hybridoma cells were grown (T. J. G. Raybould, unpublished data).

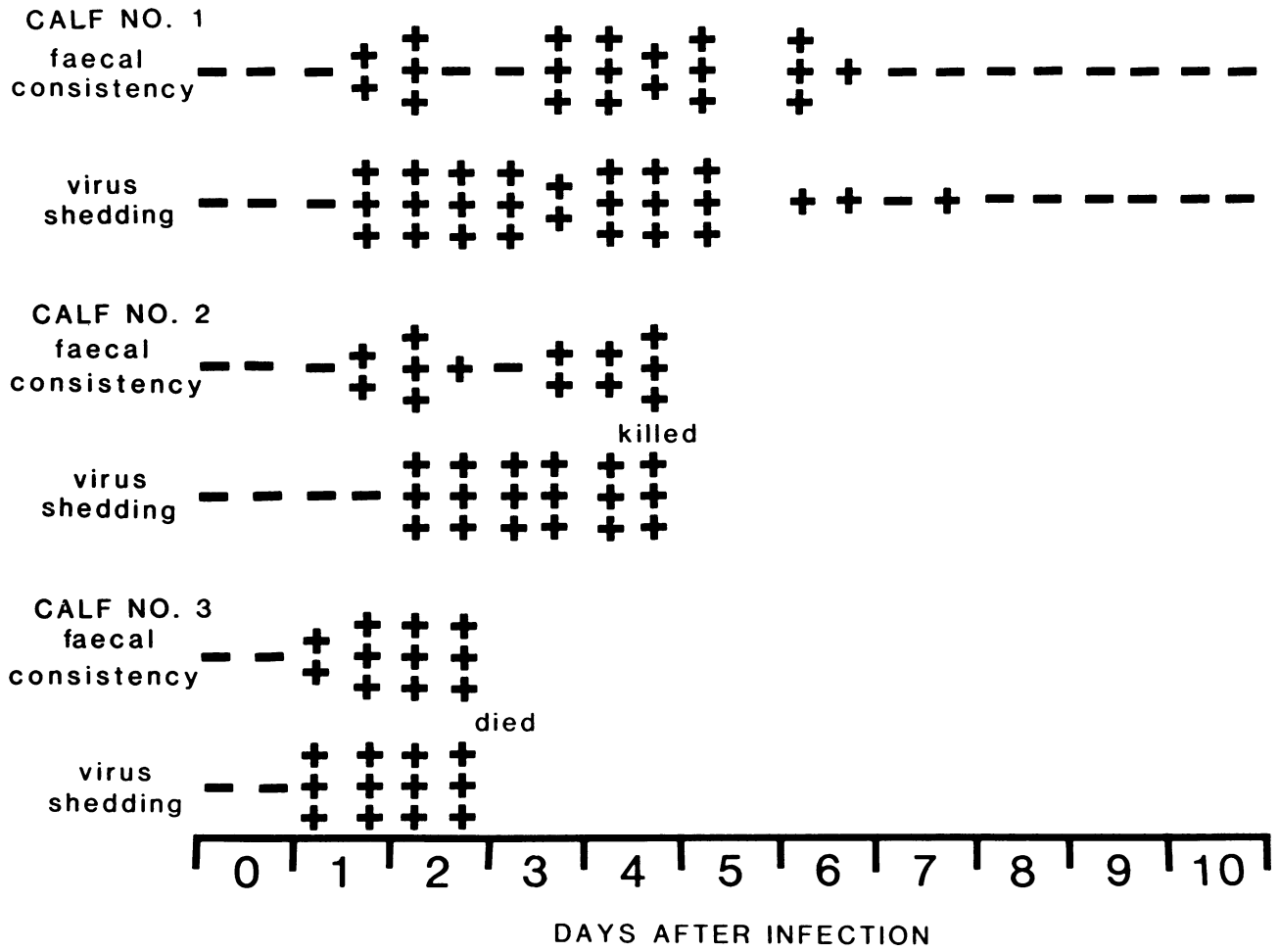


FIG. 2. Detection of BEC in fecal samples from three experimentally infected gnotobiotic calves. Fecal consistency: −, clinically normal; +, abnormal; ‡ mild diarrhea; ‡, severe diarrhea. Virus shedding (dilution of fecal sample in which virus remains detectable): −, no virus detectable; + undiluted; ‡, 1:10; ‡, 1:100.

The patterns of virus shedding obtained from experimentally infected calves indicates that coronavirus antigen can be detected throughout the period of diarrhea. This close correlation between diarrhea and virus shedding may not be demonstrable in field cases since the presence of maternal antibody in the milk consumed by the calf will result in the formation of antigen-antibody complexes, thus inhibiting the reactivity of the antigen in ELISA. This does suggest that diarrheic material, even when obtained late in the infection, has potential for the detection of BEC. The efficacy of this ELISA for the diagnosis of BEC infection is further proven by the results obtained from naturally infected animals, in particular the detection of virus shedding in clinically normal cows. This observation suggests a possible role for these animals as natural reservoirs of the virus. Transmissible gastroenteritis virus (a coronavirus) has been isolated from apparently normal sows (14), and asymptomatic shedding of rotavirus from adult animals has also been reported (2, 6, 27). Further studies are required to determine the patterns of coronavirus shedding in asymptomatic animals to determine whether a carrier state exists.

The use of monoclonal antibodies in an ELISA for BEC has overcome former problems associated with the specificity of antisera used in the test. This is shown by the absence of cross-reactivity with tissue culture-derived rotaviruses and the failure of the coronavirus-negative (rotavirus-positive) bovine serum to significantly reduce the ODs obtained in the blocking assay. The assay has proved to be sensitive, highly specific, reproducible, and capable of testing large numbers of samples.

## ACKNOWLEDGMENTS

We wish to thank Trent Watts and his assistants for the animal support services they provided and to acknowledge the technical assistance of Donna Dent and Molly Denson. Thanks are also due to Phyllis Zoerb for her extreme patience while typing this manuscript.

This project was supported by the Agricultural Research Council of Alberta—Farming for the Future.

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