Rotavirus shedding in feces of gnotobiotic calves orally inoculated with a commercial rotavirus-coronavirus vaccine

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Abstract. The purpose of this study was to monitor by negative stain electron microscopy the shedding of rotavirus in the feces of gnotobiotic calves orally inoculated with a commercial modified live bovine rotavirus-bovine coronavirus vaccine. Negative stain electron microscopic examination detected vaccine rotavirus in only 1 of 41 daily fecal specimens collected from 3 gnotobiotic calves during the 2 weeks following oral inoculation with a US Department of Agriculture-licensed modified live bovine rotavirus-bovine coronavirus vaccine. In contrast, rotavirus was demonstrable by the same negative stain electron microscopic examination procedure in 17 of 19 fecal specimens collected from diarrheic gnotobiotic or colostrum-deprived calves during the first 8 days after inoculation with virulent bovine rotavirus field strains. Rotavirus was also detected by this procedure in 4 enzyme-linked immunosorbent assay positive fecal specimens collected from naturally-infected diarrheic dairy calves. These results suggest that fecal shedding of vaccine rotavirus demonstrable by electron microscopic examination is uncommon following oral inoculation of calves with the bovine rotavirus-bovine coronavirus vaccine.

In 1969 a previously unrecognized virus recovered from diarrheic calves in Nebraska was described and shown to induce diarrhea in experimentally inoculated, hysterectomy-derived, colostrum-deprived calves.[12] This virus was initially referred to as neonatal calf diarrhea virus, Nebraska calf diarrhea virus, reo-like virus, or reovirus-like agent but is now known as bovine rotavirus; the name rotavirus was ultimately chosen because of the wheel-like appearance of the virus on transmission electron microscopy (TEM). Further investigations soon established that rotavirus was associated with neonatal calf scours in many midwestern cow-calf and dairy herds.[25] Today rotaviral infections are proven to be a common, economically important cause of calf diarrhea throughout the world.[17]

Rotavirus destroys small intestinal enterocytes resulting in diarrhea which is accompanied by a profuse fecal shedding of virus.[11,12] In fact, such vast numbers of rotavirus particles occur in diarrheal feces that it is feasible to diagnose this infection by negative stain, electron microscopic examination of fecal specimens.[5,10] Although other diagnostic tests are now available for detecting rotaviruses,[2,7] TEM remains the "gold standard" assay used by many research and diagnostic laboratories.

Soon after the discovery of bovine rotavirus, an isolate (the Lincoln strain) was adapted to serial propagation in cell culture,[6,9] which resulted in an attenuated virus for calves.[13] This attenuated strain was incorporated into a vaccine licensed by the US Department of Agriculture (USDA) in 1973 for oral inoculation of newborn calves.[a] In 1976 an attenuated bovine coronavirus was also added to produce a bivalent vaccine licensed for oral inoculation of newborn calves.[b] In 1979 the USDA approved this bivalent vaccine for intramuscular inoculation of pregnant cows to provide passive protection to their calves; the vaccine trade name was changed at this time to reflect the new label indication of the product.[c] Although cow vaccination fits more conveniently into many management programs, oral vaccination of calves is still performed in many dairies and occasionally in beef cow-calf operations.

Challenge inoculation studies using gnotobiotic calves have clearly documented the efficacy of orally inoculated bovine rotavirus vaccine in a controlled setting.[13] In the field, however, the efficacy of the bovine rotavirus vaccine in calves, either by itself or as the bivalent vaccine, has been controversial.[1,3,8,14,15,23,24] Such discrepancies might stem, in part, from the fact that any attempt to obtain an unbiased vaccine field trial evaluation at the herd level is fraught with experimental design limitations.[3] Nonetheless, some field trial results point to neutralization of the orally inoculated rotavirus vaccine by colostral antibodies as the most likely cause for its failure to reduce the herd incidence of scours.[4]

Another confounding factor in determining bovine rotavirus vaccine efficacy in the field is the uncertainty associated with interpreting the infection status of recently vaccinated calves that become diarrheic and shed rotavirus. Published data on vaccine rotavirus shedding in calves after oral inoculation with bivalent vaccine are lacking; hence it is impossible to assess the likelihood of whether the diarrhea was induced by another enteric pathogen coincident with vaccine rotavirus shedding or by virulent rotavirus following vaccine failure. The purpose of this study was to monitor by TEM the shedding of vaccine rotavirus in the feces of gnotobiotic calves orally inoculated with the commercial bovine rotavirus-bovine coronavirus vaccine.

Materials and methods

Vaccine. Single-dose vials of the commercially available bovine rotavirus-bovine coronavirus vaccine[d] with accompanying diluent were purchased and stored at 4 C. Vaccine vials were all from the same serial lot. All studies with this vaccine were conducted within 2 mo of purchase and more than 11 mo remained to the expiration date of this serial when the last gnotobiotic calf was inoculated. One week prior to inoculation of the first gnotobiotic calf, 1 vial of vaccine was reconstituted with diluent and the rotavirus fraction titrated as described below. Later, 1 wk after the last gnotobiotic calf was inoculated, another vaccine vial was similarly reconstituted and titrated.

Vaccination of gnotobiotic calves and experimental design. Three gnotobiotic calves were obtained and maintained in individual isolators as previously described.[18] Upon derivation, each calf was bled by jugular venipuncture and its serum stored at -20 C until tested. The next day, 2 vials each of vaccine and diluent were passed into the calf isolator. One vial of vaccine was reconstituted with 1 vial of diluent as recommended by the manufacturer and quickly used to orally inoculate the calf when it was 24 hr old. Immediately after vaccination, the calf was allowed to drink several ounces of infant formula.[e] The unused vaccine and diluent vials were then passed out of the isolator and held at 4 C until its rotavirus titer was determined. These titrations were performed to confirm that the peracetic acid spray procedure used to sterilize the exterior of the vials during passage into the calf isolators did not affect the vaccine rotavirus. Thereafter, calves were observed several times daily and fecal specimens for TEM were collected daily from each calf for 13 (no. 1) or 14 (nos. 2 and 3) days postinoculation. Each of these fecal specimens also was examined for infectious bovine rotavirus by a cell culture immunofluorescent (CCIF) assay as described previously.[18] In addition, some fecal specimens were examined for bacteria by aerobic incubation at 37 C of streak-inoculated blood agar plates. Between 19 and 21 days postinoculation, serum was collected again from each calf and held at -20 C until tested.

Transmission electron microscopic examination of fecal specimens. Fecal specimens were diluted with 5 volumes of phosphate-buffered saline (pH 7.4) and then prepared for TEM by sonication, clarification, and filtration as previously described.[21] A 400-μl aliquot of each filtrate was pelleted in an air-driven ultracentrifuge as previously described[18] and the pellet suspended in 10 μl of sterile distilled water. Ten microliters of 3% phosphotungstic acid was then added to the suspension and 5 μl of this mixture transferred to a Formvar-coated carbonized 300 mesh copper grid.[f] After excess liquid was removed, the grids were examined by TEM as previously described.[21] For each specimen, the number of virus particles in each of 3 grid squares was determined and the mean number of particles per grid square was calculated. Based upon this mean number, specimens were then categorized from 0 to 5+ according to criteria delineated in Table 1.

For comparative purposes, 19 fecal specimens from diarrheic gnotobiotic or colostrum-deprived calves experimentally inoculated with virulent bovine rotavirus strains were also examined by TEM. All but one of these specimens were collected during the first week postinoculation (Table 1). These gnotobiotic calves were derived and maintained as described above and then were orally inoculated with previously described rotavirus strains Id, In, and Ro.[19] Colostrum-deprived calves were obtained by hysterectomy, housed in isolation rooms receiving filtered positive-pressure air within a biosecurity facility, and fed reconstituted calf milk replacer twice daily; at one day of age, they were orally inoculated with virulent bovine rotavirus NCDV strain. For further comparison, fecal specimens from 4 diarrheic dairy calves, 13 to 28 days old, were similarly examined. These fecal specimens gave positive reactions for group A rotavirus antigen when tested by a previously described enzyme-linked immunosorbent assay (ELISA).[20] Two of these specimens were collected on the day of diarrhea onset, and the other 2 were collected on 2 and 7 days after diarrhea onset.

Serology. Preinoculation sera were tested for IgM and IgG by quantitative radial immunodiffusion assays using commercial kits.[g] All sera were tested for antibody to group A rotavirus by indirect immunofluorescent assay. Briefly, fixed monolayers of rhesus monkey kidney (MA104) cells infected with bovine rotavirus were reacted first with calf sera diluted 1:10 and then with a 1:25 dilution of fluorescein-conjugated antibody to bovine IgG.[h] Stained monolayers were examined by fluorescent microscopy as previously described[22] and sera producing specific reactions, characterized by immunofluorescence of discrete intracytoplasmic inclusions, were considered positive for antibody.

Rotavirus titration. Rotavirus titers in vaccine vials were determined by inoculation of cell monolayers. Briefly, aliquots of serial 10-fold dilutions prepared from single-dose vaccine vials reconstituted with diluent were inoculated onto MA104 cell monolayers maintained in serum-free medium containing 1 μg/ml trypsin.[i] After 5 days of incubation at 37 C the monolayers were fixed and stained with fluorescein-conjugated antibody to bovine rotavirus as described previously[22] The median tissue culture infective dose ($TCID_{50}$) was calculated and titers expressed as $TCID_{50}$ per 3 ml vaccine dose.

Results

Vaccine rotavirus titers in vials assayed before and after the gnotobiotic calf inoculations were $10^{5.23}$ and

Table 1. Electron microscopic detection of rotavirus in fecal specimens from gnotobiotic and colostrum-deprived calves inoculated with virulent bovine rotavirus strains.

Calf	Rotavirus strain	Postinoculation day on which rotavirus detected							
		1	2	3	4	5	6	7	8
Gnotobiotic	In	nd*	5+†	5+	nd	nd	nd	0	0
Gnotobiotic	Ro	nd	5+	4+	nd	5+	nd	3+	nd
Gnotobiotic	Id	nd	5+	4+	nd	5+	nd	nd	nd
Colostrum-deprived	NCDV	nd	2+	5+	3+	5+	3+	nd	nd
Colostrum-deprived	NCDV	3+	4+	4+	nd	nd	nd	nd	nd

* nd = not done.

† Mean number of rotavirus particles detected per grid square; 0 = no rotavirus; 1+ = >0–5 rotavirus particles; 2+ = >5–50 rotavirus particles; 3+ = >50–500 rotavirus particles; 4+ = >500–1,000 rotavirus particles; 5+ = >1,000 rotavirus particles.

$10^{5.98}$ TCID$_{50}$/dose, respectively. Vaccine rotavirus titers in vials passed into and out of isolators of gnotobiotic calves nos. 1, 2, and 3 were $\geq 10^{4.98}$, $10^{5.98}$, and $10^{5.73}$ TCID$_{50}$/dose, respectively.

Prior to inoculation, all gnotobiotic calves were healthy and, as expected, seronegative for IgM and IgG by quantitative radial immunodiffusion assays and for antibody to group A rotavirus by indirect immunofluorescent assay. Several days after oral inoculation with the bivalent vaccine, each gnotobiotic calf developed diarrhea characterized by tan to yellow watery stools. This diarrhea occurred on postinoculation day 3 for gnotobiotic calf no. 1, on postinoculation days 4-6 for gnotobiotic calf no. 2, and on postinoculation days 4 and 5 for gnotobiotic calf no. 3. The feces of each gnotobiotic calf were normal thereafter. Bacteriologic examination of diarrheal feces passed by each gnotobiotic calf on the first day of illness revealed gram-positive, spore-forming rods (*Bacillus* spp.). All gnotobiotic calves were seropositive for bovine rotavirus antibody by indirect immunofluorescent assay by between 19 and 21 days postinoculation.

Only 1 of 24 fecal specimens collected from the 3 gnotobiotic calves during the first 8 days postinoculation with bivalent vaccine contained rotavirus demonstrable by electron microscopy. This specimen, collected from gnotobiotic calf no. 1 on postinoculation day 3, was 4+ and contained only particles with single capsids, many of which were penetrated by the negative stain (Fig. 1B). These particles were scattered individually throughout the specimen and large aggregates were not observed. Furthermore, none of the additional 17 fecal specimens collected from these calves between 9 and 14 days postinoculation contained rotavirus particles by TEM. However, 2 daily fecal specimens collected on postinoculation days 3 and 4 from gnotobiotic calf no. 1 contained infective bovine rotavirus as detected by CCIF assay; both of these specimens produced just a few infected immunofluorescent cells (< 0.1%) within the inoculated monolayer.

In contrast, 17 of 19 fecal specimens collected from diarrheic gnotobiotic or colostrum-deprived calves during the first 8 days postinoculation with virulent bovine rotavirus strains contained virus particles, usually in large numbers, when examined by electron microscopy (Table 1); specimens negative for rotavirus particles were collected after 6 days postinoculation. The majority of these particles possessed double capsids with only a few penetrated by the negative stain (Fig. 1A). Likewise, the 4 ELISA-positive fecal specimens from naturally infected diarrheic dairy calves contained many virus particles with 1 each scoring 5+ and 4+, and 2 specimens scoring 2+. Again, the majority of these particles had double capsids and some were penetrated by the negative stain. Virus particles in specimens from gnotobiotic, colostrum-deprived, and conventionally raised dairy calves were often found in large aggregates.

Discussion

Considering that modified live bovine rotavirus vaccines have been commercially available for over 2 decades, experimental data on vaccine virus shedding from orally inoculated calves under controlled conditions are remarkably scant. Shortly after the vaccine was licensed, the manufacturer declared that vaccinated calves shed vaccine rotavirus at a low rate;[16] in fact, they advocated that all calves in a herd be vaccinated, because this shedding was so low as to afford no practical cross-protection to other nonvaccinated newborn calves. Data supporting this recommendation, however, were not presented.

Fecal shedding of vaccine rotavirus can be determined definitively only by using orally inoculated gnotobiotic calves; this experimental system unequivocally eliminates any spurious results that otherwise might arise from concurrent infection of vaccinates with ubiquitous bovine rotavirus field strains. Only 1 attempt to detect vaccine rotavirus shedding in gnotobiotic calves has been described.[26] In that study, ro-
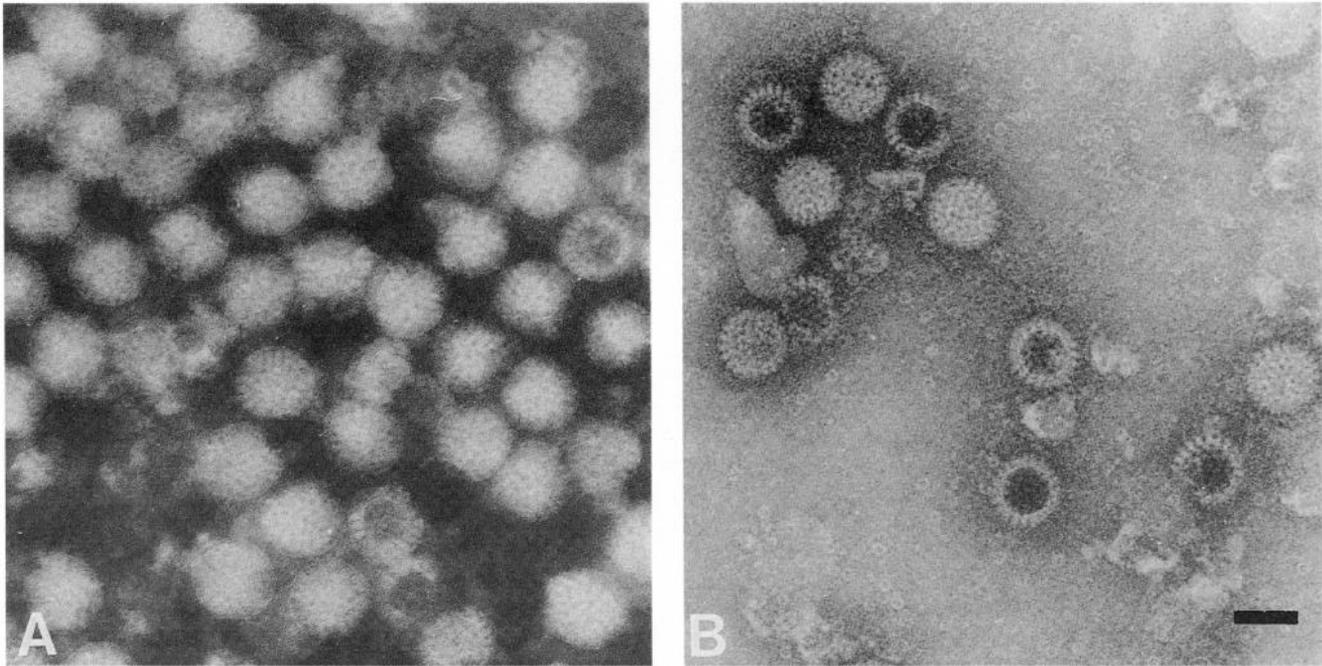
Figure 1. Negative stain electron microscopic detection of bovine rotavirus in fecal specimens. **A.** Aggregate of rotavirus particles with double capsids in diarrheal feces from a gnotobiotic calf 3 days postinoculation with bovine rotavirus IN strain. **B.** Individual rotavirus particles with single capsids in diarrheal feces from gnotobiotic calf no. 13 days postinoculation with bivalent vaccine containing the attenuated bovine rotavirus Lincoln strain and the attenuated bovine coronavirus. Bar = 60 nm.

tavirus was not detected in the feces of gnotobiotic calves after intranasal instillation of the monovalent vaccine. However, the intranasal inoculation used in this study departed from the oral route approved for the vaccine, and therefore may have reduced substantially the quantity of vaccine virus that ultimately reached the small intestine.

Our study reveals that fecal shedding of vaccine rotavirus demonstrable by electron microscopy is uncommon in gnotobiotic calves orally inoculated with a bivalent modified live bovine rotavirus-bovine coronavirus vaccine. Only 1 of 41 daily fecal specimens collected from 3 gnotobiotic calves during the 2 weeks postinoculation contained rotavirus demonstrated by electron microscopy. Our failure to detect vaccine rotavirus in the feces of 2 inoculated gnotiobiotic calves cannot be attributed to mishandling of the vaccine during the experiment. Titers of vaccine rotavirus in the stored vials tested before and after the calf inoculations were high, as were those in vials subjected to passage into and then out of the gnotobiotic calf isolators; all titers were at least 10-fold greater than the $10^{3.9}$ $TCID_{50}$/dose reported to induce protection in gnotobiotic calves.[13] Furthermore, all gnotobiotic calves were free of inhibitory antibodies prior to inoculation as their sera were devoid of immunoglobulins and bovine rotavirus antibody. These gnotobiotic calves, moreover, were fed an infant formula shown to be suitable in this and in previous studies[18] for the passage and recovery of bovine rotavirus field strains in these animals. Indeed, under our experimental conditions sufficient vaccine rotavirus replication did occur in each gnotobiotic calf to induce the appearance of rotavirus antibody in its serum detected by indirect immunofluorescence by about 3 weeks postinoculation.

Procedures used to prepare and examine fecal specimens for viruses by electron microscopy can vary somewhat in different laboratories. Our procedures readily detected rotavirus particles in most of the fecal specimens collected from gnotobiotic and colostrum-deprived calves experimentally infected with virulent rotavirus field strains. Moreover, they effectively demonstrated rotavirus particles in ELISA-positive fecal specimens collected from naturally infected dairy calves with diarrhea. Thus, our inability to find rotavirus in all but one of the daily fecal specimens obtained from the gnotobiotic calves given bivalent vaccine was not a result of inadequate examination procedures. This conclusion is further supported by the CCIF assay results: low levels of infectious bovine rotavirus were found only in the daily fecal specimen from gnotobiotic calf no. 1 that contained vaccine rotavirus particles detected by electron microscopy and in the daily fecal specimen collected from this same gnotobiotic calf on the next day.

The low fecal shedding rate of vaccine rotavirus does

not reflect an intrinsic attribute of the Lincoln strain. Low cell culture passages of the Lincoln strain, prior to its attenuation, induced diarrhea in gnotobiotic calves, and high virus titers were detected in the feces and colonic contents of these infected animals.[9,11] Attenuation of the Lincoln strain involved nearly 200 cell culture passages, of which the final 60 were done at 29-30 C,[13] and this process apparently has diminished the capacity of the vaccine virus to replicate within the intestine. In our study, the virions in the feces of the only gnotobiotic calf inoculated with bivalent vaccine to shed rotavirus were incomplete and often penetrated by the negative stain. This suggests that the vaccine rotavirus undergoes incomplete replication or perhaps has enhanced fragility within the intestinal tract.

The fact that each gnotobiotic calf orally inoculated with the modified live bivalent vaccine developed mild, transitory diarrhea raises the question as to whether this vaccine is completely avirulent for highly susceptible newborn calves. Although no noninoculated gnotobiotic calves were included as simultaneous control animals in this study, our previous experience with similarly maintained noninoculated gnotobiotic calves has shown that they never spontaneously develop diarrhea. Moreover, the *Bacillus* spp. detected in the diarrheal feces from the gnotobiotic calves orally inoculated with the bivalent vaccine were considered inconsequential since they are known common contaminants of gnotobiotic calves,[9,11] and gnotobiotic calves harboring these bacteria remain clinically normal. That all gnotobiotic calves receiving the bivalent vaccine experienced diarrhea, but only one shed vaccine rotavirus demonstrable by electron microscopy and by CCIF assay, suggests that the bovine coronavirus component may be involved in inducing this condition. Detection of fecal shedding of bovine coronavirus by negative stain electron microscopy was not attempted in our study because pleiomorphic coronavirus particles are easily missed amid the cellular membranous debris. Clearly, additional studies are needed to determine if the bovine coronavirus component of the vaccine can induce diarrhea in orally inoculated calves.

In conclusion, our findings indicate that fecal shedding of vaccine rotavirus demonstrable by negative stain electron microscopy seldom occurs after oral inoculation of gnotobiotic calves with a commercial modified live bovine rotavirus-bovine coronavirus vaccine. Given this very low rate of fecal shedding of vaccine rotavirus in highly susceptible gnotobiotic calves devoid of interfering effects due to maternally derived passive antibody, it seems improbable that vaccine rotavirus will be shed in significant quantities from orally vaccinated conventional calves that are also ingesting antibody-laden colostrum and milk. This supposition agrees with our previous inability to detect the vaccine rotavirus genome electropherotype among the rotaviruses in feces from diarrheic neonatal dairy calves in herds in which the vaccine was administered orally to the newborn calves.[18] Overall, these findings should aid veterinary diagnosticians since it is reasonable to conclude that rotavirus detected by negative stain electron microscopy in feces from orally vaccinated neonatal calves is most likely to be virulent field virus rather than vaccine virus.

Acknowledgements

Sources and manufacturers

a. Scourvax-Reo, Norden Laboratories, Lincoln, NE.
b. Scourvax II, Norden Laboratories, Lincoln, NE.
c. Calf-Guard, Norden Laboratories, Lincoln, NE.
d. Calf-Guard, SmithKline Beecham, Lincoln, NE.
e. Similac Low-Iron Infant Formula, Ross Laboratories, Columbus, OH.
f. Ted Pella, Tustin, CA.
g. VMRD, Pullman, WA.
h. ICN Biomedicals, Cosa Mesa, CA.
i. Type IX, Sigma Chemical Co., St. Louis, MO.

References

1. Acres SD, Radostits OM: 1976, The efficacy of a modified live reo-like virus vaccine and an *E. coli* bacterin for prevention of acute undifferentiated neonatal diarrhea of beef calves. Can Vet J 17:197-212.
2. Benfield DA, Stotz IJ, Nelson EA, Groon KS: 1984, Comparison of a commercial enzyme-linked immunosorbent assay with electron microscopy, fluorescent antibody, and virus isolation for the detection of bovine and porcine rotavirus. Am J Vet Res 45: 1998-2002.
3. Blackmer PE: 1976, A practioner's experience with experimental reo-coronavirus calf diarrhea vaccine. Vet Med Sm Anim Clin 71:351-354.
4. De Leeuw PW, Ellens DJ, Talmon FP, et al.: 1980, Rotavirus infections in calves: efficacy of oral vaccination in endemically infected herds. Res Vet Sci 29: 142-147.
5. England JJ, Frye CS, Enright EA: 1976, Negative contrast electron microscopic diagnosis of viruses of neonatal calf diarrhea. Cornell Vet 66:172-182.
6. Fernelius AL, Ritchie AE, Classick LG, et al.: 1972, Cell culture adaption and propagation of a reovirus-like agent of calf diarrhea from a field outbreak in Nebraska. Arch Ges Virusforsch 37:114-130.
7. Goyal SM, Rademacher RA, Pomeroy ISA: 1987, Comparison of electron microscopy with three commercial tests for the detection of rotavirus in animal feces. Diagn Microbiol Infect Dis 6:249-254.

8. Hancock DD: 1983, Studies on the epidemiology of mortality and diarrheal morbidity in heifer calves in northeastern Ohio dairy herds. PhD Thesis, The Ohio State University, Columbus, OH.

9. Mebus CA, Kono K, Underdahl NR, Twiehaus MJ: 1971, Cell culture propagation of neonatal calf diarrhea (scours) virus. Can Vet J 12:69-72.

10. Mebus CA, Rhodes MB, Stair EL: 1972, Laboratory techniques for demonstrating Nebraska calf diarrhea virus. Proc 75th Ann Mtg US Anim Health Assoc, pp. 599-600.

11. Mebus CA, Stair EL, Underdahl NR, Twiehaus MJ: 1971, Pathology of neonatal calf diarrhea induced by a reo-like virus. Vet Pathol 8:490-505.

12. Mebus CA, Underdahl NR, Rhodes MB, Twiehaus MJ: 1969, Calf diarrhea (scours): reproduced with a virus from a field outbreak. U of Neb Ag Exp Station Res Bull 233:1-16.

13. Mebus CA, White RG, Bass EP, Twiehaus MJ: 1973, Immunity to neonatal calf diarrhea virus. J Am Vet Med Assoc 163:880-883.

14. Newman FS, Myers LL, Firehammer BD, Catlin JE: 1974, Licensing and use of the calf scours vaccine. Part II. An analysis of Scourvax-Reo. Proc 75th Ann Mtg US Anim Health Assoc, pp. 59-64.

15. Smith AB: 1974, Economic implications of a calf scours prevention program. Vet Econ 15:38-44.

16. Stear RL: 1975, Calf scours: questions most frequently asked. Norden News 50:4-6.

17. Theil KW: 1990, Group A Rotaviruses. *In:* Viral diarrheas of man and animals, ed. Saif LJ, Theil KW, pp. 35-77. CRC Press, Inc., Boca Raton, FL.

18. Theil KW, McCloskey CM: 1988, Partial characterization of a bovine group A rotavirus with a short genome electropherotype. J Clin Microbiol 26:1094-1099.

19. Theil KW, McCloskey CM: 1989, Molecular epidemiology and subgroup determination of bovine group A rotaviruses associated with diarrhea in dairy and beef calves. J Clin Microbiol 27:126-131.

20. Theil KW, McCloskey CM: 1989, Nonreactivity of American avian group A rotaviruses with subgroup-specific monoclonal antibodies. J Clin Microbiol 27:2846-2848.

21. Theil KW, Reynolds DL, Saif YM: 1986, Comparison of immune electron microscopy and genome electropherotyping techniques for detection of turkey rotaviruses and rotaviruslike viruses in intestinal contents. J Clin Microbiol 23:695-699.

22. Theil KW, Reynolds DL, Saif YM: 1986, Isolation and serial propagation of turkey rotaviruses in a fetal rhesus monkey kidney (MA104) cell line. Avian Dis 30:93-103.

23. Thurber ET, Bass EP, Beckenhauer WH: 1977, Field trial evaluation of a reo-coronavirus calf diarrhea vaccine. Can J Comp Pathol 41:131-136.

24. Twiehaus MJ, Mebus CA, Bass EP: 1975, Survey of the field efficacy of reoviral calf diarrhea vaccine. Vet Med Sm Anim Clin 70:23-25.

25. White RG, Mebus CA, Twiehaus MJ: 1970, Incidence of herds infected with a neonatal calf diarrhea virus (NCDV). Vet Med Sm Anim Clin 65:487-490.

26. Woode GN, Bew ME, Dennis MJ: 1978, Studies on cross protection induced in calves by rotaviruses of calves, children and foals. Vet Rec 103:32-34.