Passive Immunity in Calf Diarrhea: Vaccination with K99 Antigen of Enterotoxigenic *Escherichia coli* and Rotavirus

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Twenty-four pregnant cows were vaccinated intramuscularly with K99 extract from enterotoxigenic *Escherichia coli* and inactivated rotavirus as follows: six cows were injected with 2 ml of oil-adjuvanted vaccine; six cows were injected with 0.5 ml of oil-adjuvanted vaccine; six cows were injected with 4 ml of aluminum hydroxide-adjuvanted vaccine twice with a four-week interval; and six cows were unvaccinated as controls. Calves born to these cows were challenged with enterotoxigenic *E. coli* at 6 to 18 h after birth. Serum and milk antibodies to K99 and rotavirus in cows vaccinated with either dose of oil vaccine were significantly increased until at least 28 days after calving. In cows vaccinated with alhydrogel vaccine, there was a significant K99 antibody increase in serum and in colostrum but not in milk and a significant rotavirus antibody increase only in colostrum. Five of six calves born to unvaccinated cows developed enterotoxic colibacillosis after challenge, and all excreted the challenge strain of enterotoxigenic *E. coli*. None of the 18 calves in the three vaccinated groups developed clinical colibacillosis, and fecal excretion of the challenge organism was reduced. A combined enterotoxigenic *E. coli*-rotavirus vaccine may prove useful in preventing some outbreaks of calf diarrhea.

Although many infectious agents have been implicated in the etiology of diarrhea in young calves, four microorganisms stand out as being of widespread occurrence and proven enteropathogenicity: rotavirus, coronavirus, enterotoxigenic *Escherichia coli* (ETEC), and cryptosporidia (2, 8, 10, 11, 23).

Control of diarrhea has been attempted with a live attenuated rotavirus-coronavirus vaccine for oral inoculation of newborn calves (Scourvax-II; Norden Laboratories) but has not been proven effective in blind field trials (3, 9). Control can also be attempted through dam vaccination to elevate the titers of specific antibody ingested by the calf in colostrum and milk. Such an approach has been used successfully with both bacterins and with K99 pili from ETEC (1, 14, 15), although live attenuated rotavirus-coronavirus vaccination of pregnant cows (Calf Guard; Norden Laboratories) does not significantly raise milk antibody titers (L. L. Myers and D. R. Snodgrass, J. Am. Vet. Med. Assoc., in press). The use of inactivated adjuvanted rotavirus vaccine results in greatly increased colostrum and milk antibody production (20, 21).

The objectives of this study were to combine K99 pili from ETEC with a rotavirus vaccine for pregnant cows and to assess the efficacy by serology and by challenging newborn calves with ETEC.

MATERIALS AND METHODS

Animals. A total of 24 pregnant hill suckler cows from 7 to 15 years of age were allocated to treatment groups at 4 to 10 weeks before calving. Six cows were vaccinated once with 2 ml of oil-adjuvanted vaccine by deep intramuscular injection in the neck; six cows were similarly vaccinated once with 0.5 ml of the same oil-adjuvanted vaccine; six cows were vaccinated twice with a 4-week interval with 4 ml of aluminum hydroxide-adjuvanted vaccine by deep intramuscular injection in the neck; and six cows were not vaccinated. Four of the cows allocated to the control group had been vaccinated with rotavirus-coronavirus vaccine in their previous pregnancy and were included as controls only for the ETEC component.

The cows were housed before calving for the duration of the experiment. Calves were challenged with ETEC between 6 and 18 h after birth. After challenge, cow-calf pairs from the different treatment groups were kept apart for at least 3 days to prevent cross-suckling and then were moved to a pen separate from that of the uncalved cows.

E. coli cultures. The following serotypes of *E. coli* were used: O101:K-:K99 (designated ETEC 1); O9:K30(B):K99 (designated ETEC 2); O9:K35(A):K99 (designated ETEC 3); and O8:K85ab:K99 (designated ETEC 4).

Vaccines. For the preparation of alhydrogel-adjuvanted vaccine, the K99 component was derived from

TABLE 1. Mean K99 antibody titers (measured by ELISA) in cow serum, cow whey, and calf serum after K99-rotavirus vaccination

Vaccine[a]	Titer ($\log_{10}$) in cow serum			Titer ($\log_{10}$) in whey at following day after calving:						Titer ($\log_{10}$) in calf serum at following day of age	
	Before vaccination	4 Wk after vaccination	At calving	1	3	7	14	28	90	3	28
None (control)	1.9	1.8	1.8	2.0	1.7	1.7	1.7	1.7	1.7	1.8	3.2
Oil (2 ml)	1.5	4.2[b]	4.5[b]	4.3[b]	4.1[b]	3.6[b]	3.5[b]	3.1[b]	2.9[b]	4.3[b]	4.0[b]
Oil (0.5 ml)	2.0	3.9[b]	4.0[b]	4.3[c]	3.9[b]	3.3[b]	2.8[b]	2.3[c]	2.6[b]	4.2[b]	3.6
Alhydrogel	1.7	2.1	3.1[b]	3.1[b]	2.6[b]	1.9	1.9	1.7	1.7	2.3[c]	2.6

[a] There were six cows in each vaccine group.
[b] Differs significantly from control ($P < 0.01$).
[c] Differs significantly from control ($P < 0.05$).

the culture supernatant of ETEC 1, which was grown in a synthetic medium containing selected amino acids, trace salts, and lactose and was buffered with phosphates to pH 7.5. After 8 to 10 h of incubation at 37°C in an aerated vessel, the culture was inactivated in situ at 60°C for 30 min. The cells were separated aseptically from the supernatant by centrifugation. After measurement of K99 antigen in the sterile culture supernatant, 20% alhydrogel was added.

Tissue culture-adapted calf rotavirus was prepared and inactivated as previously described (20). The rotavirus and K99 components were blended aseptically with alhydrogel in the proportions 1:1:8 so that each milliliter of the combined vaccine contained 15 U of K99 and $10^{5.4}$ 50% tissue culture infective doses of rotavirus before inactivation.

For the preparation of oil-adjuvanted vaccine, the K99 component was derived from ETEC 1 grown on 5% horse blood agar at 37°C for 18 h. The growth was harvested in sterile saline and concentrated by centrifugation so that it contained 2.50×10^{11} cells per ml. Portions (20 ml each) of the concentrate were homogenized in a Silverson homogenizer for 4 min at 0°C. The cells were separated from the supernatant at 20,000 × g for 30 min; then 0.1% Merthiolate was added, and the preparation was heated at 60°C for 30 min. The sterility of this crude K99 extract was checked, and its K99 content was measured before use.

Rotavirus and K99 were mixed with 0.2% Tween 80, and this aqueous phase was emulsified with 2 volumes of oil adjuvant (90% Marcol 52 [Esso], 10% Arlacel A [Sandria Chemicals]). Each milliliter of vaccine contained 60 U of K99 and $10^{5.2}$ 50% tissue culture infective doses of rotavirus before inactivation.

ETEC challenge. Strain B44 (ETEC 2) was grown in Trypticase soy broth (BBL Microbiology Systems) for 8 h and then on Minca-IsoVitaleX (BBL) (6) agar for 18 h at 37°C. The bacteria were suspended in phosphate-buffered saline with 10% dimethyl sulfoxide and stored in 10-ml aliquots at −70°C.

To inoculate each calf, one 10-ml aliquot was thawed and given orally by syringe. The mean inoculum titer was 4.1×10^{10} colony-forming units per 10 ml (range, 3.0×10^{10} to 6.8×10^{10} colony-forming units per 10 ml). No decrease in the inoculum titer occurred over the 2-month experimental period. The enteropathogenicity of the stored organisms was confirmed periodically by slide agglutination for K99 and the

infant mouse test for heat-stable toxin production (4).

K99 serology. Antibodies to K99 were assayed by enzyme-linked immunosorbent assay (ELISA) or passive hemagglutination (PHA). The ELISA utilized rabbit anti-K99 immunoglobulin G (kindly supplied by W. H. Jansen) as capture antibody, followed successively by K99 antigen, test serum or whey, and rabbit anti-bovine immunoglobulin G (Miles Laboratories, Inc.) conjugated with alkaline phosphatase. The final phosphatase substrate (Sigma Chemical Co.) reaction was read at 405 nm after 2 h at room temperature. In each test, doubling dilutions of a standard bovine anti-K99 serum were included. The titers of the test samples are expressed in relation to a calibration curve calculated from the standards.

In the PHA assay, pyruvic acid-stabilized sheep erythrocytes (7) were sensitized with K99 antigen from ETEC 4. A suspension of erythrocytes in 0.1 M acetate buffer (pH 4.5) was coated to saturation with K99 derived by the method of Morris et al. (12), washed five times in phosphate-buffered saline (pH 7.5), and resuspended to 1% (vol/vol). Test sera and whey samples were adsorbed with an equal volume of packed unsensitized erythrocytes for 18 h at 4°C to remove nonspecific hemagglutinins. Serial doubling dilutions of serum or whey samples in 0.3% Formol saline were prepared in microtiter plates, and an equal volume (0.025 ml) of sensitized erythrocytes was added. The agglutination pattern was read after 18 h of incubation at 37°C. All of the samples were tested on one occasion, although repeat tests on selected samples yielded the same titers.

Rotavirus serology. Serum samples, whey from colostrum samples, and whey from milk samples were tested for the presence of neutralizing antibody to tissue culture-adapted calf rotavirus on bovine embryo kidney cells or MA104 cells grown in microtiter plates.

Titration of K99 antigen. The titration method used is based on the capacity of the K99 antigen to adsorb K99 antibodies from a standard antiserum of known titer, which is then titrated for residual antibody by PHA. This method is based on an in vitro assay designed for quantitating K88 antigens of E. coli (16). The results are expressed as agglutinin absorbing units. The standard antiserum used was produced in a pig vaccinated with a sterile culture supernatant of ETEC 3. The serum was adsorbed to remove all detectable O9 and K35(A) agglutinins.

Toxicity of K99 components in combined vaccines. The toxicity of the K99 vaccine preparations was examined by injecting groups of 10 mice intraperitoneally with 15 U of alhydrogel-adsorbed K99 and 180 U of crude K99 extract.

Feces examination. Calf feces samples were examined for rotavirus by ELISA (5) and for cryptosporidia by examination of Giemsa-stained fecal smears (19). Samples were cultured on Minca-IsoVitaleX (6) and MacConkey agars overnight aerobically at 37°C. Five colonies grown on Minca-IsoVitaleX agar were tested for K99 by slide agglutination with rabbit antiserum to strain K12:K99 adsorbed with the K12 strain.

At intervals, fecal swabs from all calves were tested for the presence of *Salmonella* spp. after overnight enrichment in Selenite broth and for *Campylobacter* spp. by growth on 5% sheep blood agar plus Skirrows antibiotic supplement (Oxoid Ltd.) under microaerophilic conditions at 37°C.

Observations. Each cow was bled for serum at vaccination, 4 weeks after vaccination, at parturition, and 28 days after parturition. Colostrum and milk samples were collected at 1, 3, 7, 14, 28, and 90 days after calving. The calves were bled for serum at 3 and 28 days of age. Feces samples were collected from the calves daily for 6 days. A sample of these feces was taken for microbiological examination, and the remainder was dried to constant weight for dry-matter estimation. The calves were weighed at 1, 2, and 3 days of age. All calves were examined clinically at least once a day for 6 days and assigned a clinical score on a subjective scale similar to that used by Myers (14): (i) normal, feces firm; (ii) transient diarrhea within 24 h of inoculation, lasting only a few hours; (iii) severe watery diarrhea, calf becoming dehydrated and dull; (iv) severe watery diarrhea, calf too weak to stand, with death ensuing.

RESULTS

K99 immunological response measured by ELISA. Six cows had low preexisting serum antibody (mean titer, 158), whereas all other cows were negative (titer < 100). All vaccine regimes significantly increased serum antibody titers ($P < 0.001$) (Table 1), although oil vaccines produced significantly higher titers at calving (18,900 and 8,830 for 2- and 0.5-ml doses, respectively) than did the alhydrogel vaccine (1,190) ($P < 0.001$).

Both 2- and 0.5-ml doses of oil vaccine produced very high titers in colostral whey (32,100 and 19,800, respectively) compared with controls (108) ($P < 0.001$). These colostral antibody levels declined slowly throughout the 28-day observation period, but remained significantly higher than in the controls (2 ml of oil, $P < 0.001$; 0.5 ml of oil, $P < 0.05$). Antibody titers in colostral whey from the cows vaccinated with alhydrogel vaccine were also increased (1,140, $P < 0.001$), but by 7 days after calving, antibody was no longer detectable in this group.

The serum antibody titers in 3-day-old calves reflected the colostral antibody titers of their dams. By 28 days of age, the calves born to
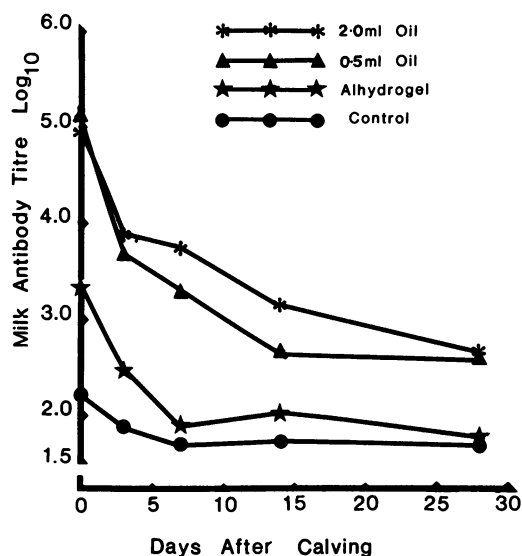

FIG. 1. K99 antibody titers determined by PHA ($\log_{10}$) in colostrum and milk of cows during the first 28 days of lactation.

unvaccinated cows had developed high serum antibody titers to K99 as a result of ETEC infection.

K99 immunological response measured by PHA. The assay of sera and wheys by PHA confirmed the results obtained by ELISA (Fig. 1). In addition, the cow sera collected 28 days after calving were tested, and K99 antibody titers were found to be still significantly raised in all vaccinated cows.

Rotavirus immunological response. The results from the four control cows which had been vaccinated in a previous pregnancy are excluded from these results, and data from seven extra control cows from the same farm not otherwise included in the experiment are incorporated. All cows had prevaccination serum antibody to rotavirus. The serum and milk antibody responses of cows to both doses of oil-adjuvanted vaccine were significant (Table 2).

Cows vaccinated with alhydrogel-adjuvanted vaccine had raised antibody titers in serum and milk, but only colostral antibody titers were significantly higher than in control cows.

Response of calves to ETEC challenge. After ETEC challenge, five of six calves from control cows developed acute enteric disease (disease rating iii or iv) characterized by profuse watery diarrhea, dehydration, and dullness (Table 3). The mean body weight loss of 5.7% and the mean minimum fecal dry matter of 8.4% confirmed the severity of the disease. One calf died 2 days after challenge.

The clinical responses of calves in all three

TABLE 2. Mean rotavirus antibody titers (measured by the neutralization test) in cow serum and whey after K99-rotavirus vaccination

Vaccine	Titer (log_{10}) in cow serum			Titer (log_{10}) in whey at following day after calving:				
	Before vaccination	4 Wk after vaccination	At calving	1	3	7	14	28
None (control)	2.51	2.66	2.36	2.81	2.05	2.02	1.83	1.70
Oil (2 ml)	2.66	3.19	3.46[b]	4.06[a]	3.51[b]	2.86[a]	2.51	2.26[a]
Oil (0.5 ml)	2.81	3.71[a]	3.65[b]	3.96[a]	3.46[b]	2.91[a]	2.41	2.43[a]
Alhydrogel	2.66	3.33	3.29	3.51[b]	2.81	2.36	2.15	2.02

[a] Differs significantly from control value ($P < 0.01$).
[b] Differs significantly from control value ($P < 0.05$).

vaccinated groups were similar. A mild transient diarrhea unaccompanied by systemic disturbance was observed in half of the calves (disease rating ii). All calves gained weight, and fecal dry matter did not fall below 16% in any individual.

Microbiological examination of feces. A serological examination of fecal coliforms was performed for 6 days after ETEC challenge. In control calves, 86% of *E. coli* could be identified as ETEC (Table 4). The proportion of B44 excreted in the feces of calves from vaccinated cows was reduced. In only one calf was there no detectable B44 excretion. Rotavirus was detected in the feces of one control calf with diarrhea and in three clinically normal calves born to vaccinated cows. Cryptosporidial oocysts were not observed in the feces of any calf.

Vaccine toxicity. No toxic effects of K99, used in the vaccines, were detected in mice, and all vaccinated cows remained clinically normal.

DISCUSSION

Calves sucking dams vaccinated with K99 pili in any of the schedules used were protected against the clinical effects of challenge with ETEC, whereas the same challenge produced severe enterotoxic colibacillosis in calves from control cows. In addition, excretion of the challenge strain was significantly reduced in calves from vaccinated cows. Although the oil vaccines produced a much higher immunological response than the alhydrogel vaccine, the degree of protection under these experimental conditions was equally satisfactory.

Most natural ETEC infections occur in calves 1 to 2 days old (2, 10), and increased antibody titers in colostrum alone should be protective in these cases. However, there is experimental evidence that initial rotavirus infection can facilitate ETEC colonization in calves up to 1 week old (17, 20a), and such dual infection of older calves has been reported to occur naturally (10). For this reason, it is desirable to stimulate production of K99 antibody in milk as well as in colostrum, and the oil-adjuvanted vaccine formulation was more effective in this regard than the alhydrogel vaccine. The mean K99 ELISA titers of 7-day milk of cows vaccinated with either dose of the oil-adjuvanted vaccine were higher than those in colostrum of cows which received the alhydrogel-adjuvanted vaccine. As calves of the latter group of cows were resistant to experimental challenge, it is reasonable to suggest that cows vaccinated with oil-based K99 vaccine would confer protection to their calves for at least 7 days and probably longer.

With the methods of vaccine production used with strain B41 (O101:K-:K99) it is likely that antigens other than K99 were present, in particular the O101 somatic antigen and an anionic adhesin (13). However, cross-protection against

TABLE 3. Clinical results of ETEC challenge

Vaccine	No. of calves given the following disease rating:[a]				Wt at 48 h/wt at birth (%)[b]	Minimum fecal dry matter (%)[b,c]
	i	ii	iii	iv		
None (control)	0	1	4	1	94.3 ± 3.6	8.4 ± 2.1
Oil (2 ml)	3	3	0	0	107.5 ± 1.5[d]	28.7 ± 3.2[d]
Oil (0.5 ml)	3	3	0	0	106.7 ± 1.6[d]	24.9 ± 2.9[d]
Alhydrogel	3	3	0	0	103.3 ± 1.5[e]	22.6 ± 1.8[d]

[a] See text for definitions of disease ratings.
[b] Mean ± standard error.
[c] Minimum recorded for each calf over the 4 days after ETEC challenge.
[d] Differs significantly from control ($P < 0.01$).
[e] Differs significantly from control ($P < 0.05$).

TABLE 4. Fecal excretion of ETEC after challenge

Vaccine	Colonies agglutinated (%)[a]
None (control)	86
Oil (2 ml)	49[b]
Oil (0.5 ml)	16[c]
Alhydrogel	32[c]

[a] Mean number of colonies agglutinated by K99 antiserum as a percentage of the total number of colonies tested over the 6 days after challenge.

[b] Differs significantly from control ($P < 0.05$).

[c] Differs significantly from control ($P < 0.01$).

the challenge strain O9:K30:K99 suggests that effective protection was produced in this case by K99 antibodies, even against a strain which may possess colonization properties through K30 (18).

These results confirm those of others who have found that antibody to K99 protects against virulent ETEC challenge (1, 15). However, the incorporation of a rotavirus vaccine greatly increases the value of the immunization regime. No rotavirus challenge was included, but the rotavirus serological response produced in this experiment by the oil-adjuvanted vaccines was consistent with that produced previously, which has been shown to confer substantial protection against rotavirus infection (5, 20, 21). Thus, there was no evidence that the inclusion of K99 interfered with the maternal response to rotavirus vaccination.

An effective bivalent vaccine against ETEC and rotavirus would not prevent all calf diarrhea outbreaks. In particular, disease due to calf coronavirus would continue, as has been found with a previous rotavirus vaccine (22), and cryptosporidiosis would continue to be present. However, the use of such a vaccine could be expected to lead to a useful reduction in morbidity and mortality from diarrhea in young suckled calves and potentially in dairy calves also if the duration of colostrum feeding was prolonged (21).

LITERATURE CITED

1. Acres, S. D., R. E. Isaacson, L. A. Babiuk, and R. A. Kapitany. 1979. Immunization of calves against enterotoxigenic colibacillosis by vaccinating dams with purified K99 antigen and whole cell bacterins. Infect. Immun. 25:121–126.
2. Acres, S. D., G. J. Laing, J. R. Saunders, and O. M. Radostits. 1975. Acute undifferentiated neonatal diarrhea in beef calves. I. Occurrence and distribution of infectious agents. Can. J. Comp. Med. 39:116–132.
3. Acres, S. D., and O. M. Radostits. 1976. The efficacy of a

4. Deans, G. A., Y. C. Ching, R. G. Williams, and L. B. Harden. 1972. Test for Escherichia coli enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. J. Infect. Dis. 125:407–411.
5. Fahey, K. J., D. R. Snodgrass, I. Campbell, A. M. Dawson, and C. Burrells. 1981. IgG₁ antibody in milk protects lambs against rotavirus diarrhea. Vet. Immunol. Immunopathol. 2:27–33.
6. Guinée, P. A. M., J. Veldkamp, and W. H. Jansen. 1977. Improved minca medium for the detection of K99 antigen in calf enterotoxigenic strains of Escherichia coli. Infect. Immun. 15:676–678.
7. Hirata, A. A., and M. W. Brandriss. 1968. Passive hemagglutination procedures for protein and polysaccharide antigens using erythrocytes stabilized by aldehydes. J. Immunol. 100:641–657.
8. de Leeuw, P. W., D. J. Ellens, P. J. Straver, J. A. M. van Balken, A. Moerman, and T. Baanvinger. 1980. Rotavirus infections in calves in dairy herds. Res. Vet. Sci. 29:135–141.
9. de Leeuw, P. W., D. J. Ellens, F. P. Talman, and G. N. Zimmer. 1980. Rotavirus infections in calves: efficacy of oral vaccination in endemically infected herds. Res. Vet. Sci. 29:142–147.
10. Moon, H. W., A. W. McClurkin, R. E. Isaacson, J. Pohlenz, S. M. Skartvedt, K. G. Gillette, and A. L. Baetz. 1978. Pathogenic relationships of rotavirus, Escherichia coli, and other agents in mixed infections of calves. J. Am. Vet. Med. Assoc. 173:577–583.
11. Morin, M., S. Lariviere, and R. Lallier. 1976. Pathological and microbiological observations made on spontaneous cases of acute neonatal calf diarrhea. Can. J. Comp. Med. 40:228–240.
12. Morris, J. A., A. E. Stevens, and W. J. Sojka. 1977. Preliminary characterization of cell-free K99 antigen isolated from Escherichia coli B41. J. Gen. Microbiol. 99:353–357.
13. Morris, J. A., C. J. Thorns, and W. J. Sojka. 1980. Evidence for two adhesive antigens on the K99 reference strain Escherichia coli B41. J. Gen. Microbiol. 118:107–113.
14. Myers, L. L. 1980. Passive protection of calves against experimentally induced and naturally occurring enteric colibacillosis. Am. J. Vet. Res. 41:1952–1956.
15. Nagy, B. 1980. Vaccination of cows with a K99 extract to protect newborn calves against experimental enterotoxic colibacillosis. Infect. Immun. 27:21–24.
16. Nagy, L. K., P. D. Walker, B. S. Bhogal, and T. Mackenzie. 1978. Evaluation of Escherichia coli vaccines against experimental enteric colibacillosis. Res. Vet. Sci. 24:39–45.
17. Runnels, P. L., H. W. Moon, S. C. Whipp, P. J. Matthews, and G. N. Woode. 1980. Interaction of rotavirus and enterotoxigenic Escherichia coli (ETEC) in gnotobiotic calves, p. 343–348. In S. D. Acres, A. J. Forman, and H. Fast (ed.), Proceedings of the Third International Symposium on Neonatal Diarrhea. VIDO, Saskatoon, Canada.
18. Smith, H. W., and M. B. Huggins. 1978. The influence of plasmid determined and other characteristics of enteropathogenic Escherichia coli on their ability to proliferate in the alimentary tracts of piglets, calves, and lambs. J. Med. Microbiol. 11:471–492.
19. Snodgrass, D. R., K. W. Angus, E. W. Gray, and W. A. Keir. 1980. Cryptosporidia associated with rotavirus and an Escherichia coli in an outbreak of calf scour. Vet. Rec. 106:458–459.
20. Snodgrass, D. R., K. J. Fahey, P. W. Wells, I. Campbell, and A. Whitelaw. 1980. Passive immunity in calf rotavirus infections: maternal vaccination increases and prolongs immunoglobulin G₁ antibody secretion in milk. Infect. Immun. 28:344–349.

modified live Reo-like virus vaccine and an Escherichia coli bacterin for prevention of acute undifferentiated neonatal diarrhea of beef calves. Can. Vet. J. 17:197–212.

20a.**Snodgrass, D. R., M. L. Smith, and F. L. Krautil.** 1982. Interaction of rotavirus and enterotoxigenic *Escherichia coli* in conventionally reared dairy calves. Vet. Microbiol. 7:51–60.

21. **Snodgrass, D. R., J. Stewart, J. Taylor, F. L. Krautil, and M. L. Smith.** 1982. Diarrhea in dairy calves reduced by feeding colostrum from cows vaccinated with rotavirus.

Res. Vet. Sci. 32:70–73.

22. **Stair, E. L., M. B. Rhodes, R. G. White, and C. A. Mebus.** 1972. Neonatal calf diarrhea: purification and electron microscopy of a coronavirus-like agent. Am. J. Vet. Res. 33:1147–1156.

23. **Tzipori, S.** 1981. The etiology and diagnosis of calf diarrhea. Vet. Rec. 108:510–514.