INFECTION AND IMMUNITY, Aug. 1981, p. 401-406
0019-9567/81/080401-06$02.00/0

Vol. 33, No. 2

Diarrhea in Lambs: Experimental Infections with Enterotoxigenic *Escherichia coli*, Rotavirus, and *Cryptosporidium* sp.

S. TZIPORI,[1] D. SHERWOOD,[1]* K. W. ANGUS,[1] I. CAMPBELL,[1] AND M. GORDON[2]

Moredun Research Institute, Edinburgh EH17 7JH,[1] and Gastrointestinal Unit, Western General Hospital, Edinburgh,[2] Scotland

Received 25 February 1981/Accepted 5 May 1981

Thirteen gnotobiotic lambs, aged from a few hours to 8 days, were inoculated orally with single infections of enterotoxigenic *Escherichia coli* (ETEC) (four animals), lamb rotavirus (five animals), and *Cryptosporidium* (four animals). Six gnotobiotic and two specific-pathogen-free lambs were co-inoculated with either rotavirus and ETEC (four animals), rotavirus and *Cryptosporidium* (two animals), or ETEC and *Cryptosporidium* (two animals). Lambs 4 days of age and older became only subclinically infected with either rotavirus, ETEC (08:K87:K99 ST^+), or both enteropathogens given simultaneously. Six-day-old lambs inoculated with *Cryptosporidium* became extremely depressed, anorectic, and had intermittent diarrhea. There was no difference in the clinical manifestations, level of disaccharidase activity in the small intestine, or extent of histological damage between lambs inoculated with *Cryptosporidium* alone or together with either of the other two agents. The results indicate that under the conditions of these experiments, lambs become clinically resistant to infection with ETEC, rotavirus, or both agents together, by 4 days after birth, whereas lambs 2 days old or younger were clinically susceptible to infection by these agents. In contrast, they remained clinically susceptible to infection with *Cryptosporidium* up to at least 6 days of age. *Cryptosporidium* infections were not aggravated by coinfection with either ETEC or rotavirus.

Neonatal diarrhea in lambs is considered an important cause of lamb mortality (18). Enteropathogens known to be associated with the disease include enterotoxigenic *Escherichia coli* (ETEC) (2, 22), rotavirus (10, 21), *Cryptosporidium* (3, 23), and possibly astrovirus (20). ETEC are the cause of colibacillosis in many other species and are the subject of many publications (9, 13, 17, 20).

Under field conditions diarrhea in calves (1, 14) tends to be more commonly associated with mixed infections of two or more enteropathogens, but the situation in lambs is unclear and merits investigation. Experimental coinfections with ETEC and rotavirus have been studied in calves (6, 7, 15, 24); the results of these investigations point to the existence of a mechanism of interaction between the two organisms, but the nature and exact site of the interaction remains undefined.

This communication describes clinical and pathological manifestations in gnotobiotic lambs of experimental coinfections involving ETEC, lamb rotavirus, and a calf-derived *Cryptosporidium*.

MATERIALS AND METHODS

Experimental animals. Of 21 Caesarean-derived lambs, 19 were maintained under gnotobiotic conditions in plastic isolators and 2 were maintained under specific-pathogen-free (SPF) conditions. All were fed reconstituted evaporated cows' milk three times daily for the first 3 days and twice daily thereafter. The SPF lambs were kept in an isolated sterile room with no contact with other animals.

Microbiology. (i) ETEC. Strain S13 (08:K87:K99), obtained from H. Williams Smith (Poultry Research Station, Houghton, England), was used in this experiment. The organism produced heat-stable toxin (as tested by the infant mouse assay [5]) but not heat-labile toxin in the Y1 adrenal cell culture test (16). The presence of K99 antigen ($K99^+$) was demonstrated by slide agglutination (8). The inoculum used for lamb infection was 2 ml of Trypticase soy broth (BBL Microbiology Systems) containing 10^8 (lamb no. 2) or 10^{10} viable organisms per ml.

Fecal shedding of strain S13 was demonstrated by testing for $K99^+$ in ten randomly selected colonies from fecal swab cultures plated on sheep blood agar and MacConkey agar plates (8).

(ii) Rotavirus. Fecal filtrate (20% [vol/vol] in water) of the sixth passage of lamb rotavirus in gnotobiotic lambs (21) was used to inoculate lambs, each

receiving 2 ml orally. Rotavirus in the feces of infected lambs was demonstrated by direct electron microscopy (20) and enzyme-linked immunosorbent assay.

(iii) Cryptosporidium. The organism, obtained originally from a calf, was passaged once in newborn suckling SPF rats and twice in SPF lambs (S. Tzipori, K. W. Angus, E. W. Gray, I. Campbell, and F. Allan, Am. J. Vet. Res., in press). A 2-ml homogenate of gut contents (20% [vol/vol] in phosphate-buffered saline) prepared from the second SPF lamb passage was used to inoculate each lamb. The homogenate was examined as described above and found free of enteric viruses and ETEC. Fecal shedding of *Cryptosporidium* oocysts was demonstrated by Giemsa-stained smears.

Inoculation of lambs: single agents. Thirteen lambs up to 8 days of age were inoculated with ETEC (four lambs), rotavirus (five lambs), or *Cryptosporidium* (four lambs) as detailed in Table 1.

Inoculation of lambs with mixed infections. Three groups of lambs aged from 4 to 8 days were inoculated with either ETEC and rotavirus (four lambs), rotavirus and *Cryptosporidium* (two lambs), or ETEC and *Cryptosporidium* (two lambs) (Table 1). As controls, five uninoculated age-matched lambs were used.

TABLE 1. *Response of gnotobiotic and SPF lambs to oral inoculations with ETEC, lamb rotavirus, and Cryptosporidium, singly or in coinfections*

Lamb no.	Age at inoculation (days) with:			Clinical illness[a]	Shedding of organism[a]	Age of lamb at necropsy (days)
	Cryptosporidium	Rotavirus	ETEC			
1[b]	—[c]	—	—	−	−	3–13
2[d]			<1	+	+	<1
3			5	−	+	8
4			5	−	+	10
14			8	−	+	12
9		1		+	+	4
10		2		+	+	5
11		7		−	+	19
15		8		−	+	10
16		8		−	+	12
12	1			+	+	4
13	1			+	+	6
17[e]	6			+	+	8
18	6			+	+	10
5		4	5	−	+	8
6		4	5	−	+	10
7[f]		7	7	−	+	15
8[f]		7	7	−	+	16
19	6		8	+	+	10
20	6		8	+	+	12
21	6	8		+	+	10
22	6	8		+	+	12

[a] Symbols: +, positive; −, not detected.
[b] Consists of five uninoculated control lambs.
[c] —, None.
[d] Lamb no. 2 was infected at 6 and killed at 18 h of age.
[e] Lamb no. 17 died as a result of infection.
[f] Lambs no. 7 and 8 were SPF.

Clinical observations. The lambs were observed daily for clinical signs of illness. When considered necessary some lambs had their milk intake measured. Fecal samples were examined daily for excretion of enteric viruses, ETEC, and *Cryptosporidium* by the methods shown above.

Necropsy. Under terminal anesthesia, representative pieces of intestine were taken from: duodenum, upper and lower jejunum, upper and lower ileum, spiral colon, and cecum. From each sample, portions were taken into 10% formal saline for histology, and duplicate samples were frozen and stored at −20°C for immunofluorescence (IF) and enzymology. Mucosal scrapings were taken from all sites for bacterial counts.

IF. Cryostat sections of lambs infected with ETEC or rotavirus or both were tested with hyperimmune rabbit sera raised against ETEC S13 or rotavirus. Fluorescein-conjugated sheep anti-rabbit antiserum was used as indicator for the indirect IF test.

Enzymology. Small portions of small intestine were analyzed for lactase, maltase, and sucrase activity by the technique described by Dahlqvist (4). Data were analyzed by analysis of variance.

Bacterial counts. Serial, tenfold dilutions of 0.1 g of mucosal scrapings from each intestinal location were plated on MacConkey agar, and viable counts were carried out (12).

RESULTS

Inoculation with a single agent. (i) ETEC. Lamb no. 2, inoculated 6 h after birth with 10^8 organisms, developed profuse diarrhea within 12 h, shed the organism, and was killed 18 h after birth (Table 1). The other three lambs, no. 3, 4, and 14, inoculated with 2×10^{10} organisms when 5 to 8 days old, failed to show any clinical illness although they excreted ETEC in their feces. No decrease in milk intake was observed for lambs no. 3 and 4 (Fig. 1).

Histological examination of the intestines of the above animals revealed pathological changes only in lamb no. 2. The duodenum and upper jejunum of this lamb appeared normal, but throughout the rest of the small intestine, bacteria were seen adhering to the brush borders of villous enterocytes. Pathological changes included hyperemia of mucosal vessels, distention of lacteals, and infiltrates of neutrophils. Small deposits of fibrin-like material were occasionally observed at the base of the mucosa, and limited cytolysis of lymphocytes was seen in the Peyer's patches of the terminal ileum. The colon and cecum were unaffected.

Strain S13-specific fluorescence was seen only in lamb no. 2. Bacteria were seen coating villi from the upper jejunum to the terminal ileum, indicating surface colonization by the ETEC strain throughout the greater part of the small intestine.

(ii) Rotavirus. Five lambs (no. 9–11, 15, 16) were inoculated with rotavirus (Table 1). Lambs

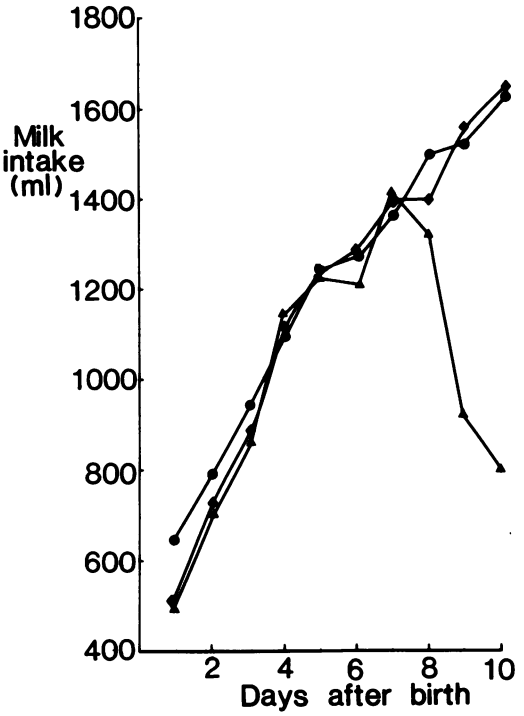

FIG. 1. *Daily milk intake of lambs. Symbols: ●, lambs were inoculated with ETEC only at 5 days (lambs no. 3 and 4), rotavirus only at 8 days (lambs no. 15 and 16), and ETEC at 5 days and rotavirus at 4 days of age (lambs no. 5 and 6); ▲, lambs were inoculated with Cryptosporidium only at 6 days (lambs no. 17 and 18), Cryptosporidium at 6 days, and ETEC at 8 days (lambs no. 19 and 20), and Cryptosporidium at 6 days and rotavirus at 8 days of age (lambs no. 21 and 22); ◆, control (two lambs).*

no. 9 and 10 developed slight depression, anorexia, and diarrhea with virus excretion, whereas lambs no. 11, 15, and 16 developed subclinical infections with rotavirus in their feces. Data were unavailable for lambs no. 9, 10, and 11, but no decrease in milk intake was observed for lambs no. 15 and 16 (Fig. 1).

Lambs no. 9 and 10 had lesions associated with rotavirus infection. The villi in the lower small intestine were stunted, and the lamina propria was infiltrated by mononuclear cells and eosinophils. Lambs no. 11 and 16 had no detectable morphological changes, but the distal intestine of lamb no. 15 contained patches of stunted villi which were, however, clothed by columnar cells. Infiltrates of mononuclear cells were seen in the lamina propria, but the crypts were not elongated. Specific IF studies for rotavirus showed extensive fluorescence in the epithelial cells of lambs no. 9 and 10, extending from the lower jejunum to mid-ileum. Very little specific fluorescence was detected in gut sections obtained from lambs no. 11, 15, and 16.

(iii) Cryptosporidium. Four lambs were inoculated with homogenates containing *Cryptosporidium* oocysts. Two lambs, no. 12 and 13, infected at 1 day of age, developed profuse diarrhea, which soon became intermittent, and lamb no. 13 was moribund when it was killed at 6 days of age. Both lambs had markedly reduced milk intake. Lambs no. 17 and 18, infected when 6 days old, became extremely depressed and passed soft feces, and their milk consumption fell (Fig. 1). Lamb no. 17 died 2 days postinoculation.

Histological examination of the intestines of all four lambs revealed severe stunting and fusion of villi from the jejunum to the terminal ileum; the villi were covered with immature cuboidal cells. The lamina propria contained infiltrates of mononuclear cells. Both small and large intestines were heavily infected with the organism, but the large intestine was only slightly inflamed.

Mixed infections. (i) ETEC and rotavirus. Four lambs (no. 5, 6, 7, and 8) were inoculated with ETEC and rotavirus (Table 1). All four shed both microorganisms in their feces, but none had diarrhea, nor was there any decrease in milk intake (Fig. 1, lambs no. 5 and 6).

No pathological changes were seen in any intestinal site, and IF studies revealed no specific fluorescence against the ETEC, indicating that mucosal colonization had not occurred. However, some fluorescence was observed on the surface of the spiral colon and cecum in older lambs (no. 7 and 8). The extent and distribution of rotavirus-infected cells as detected by IF were similar to those for the single infections in lambs more than 2 days old, viz, patches of small groups of fluorescing cells.

(ii) ETEC and *Cryptosporidium*. Lambs no. 19 and 20 became extremely depressed within 24 h postinoculation with *Cryptosporidium*, and their milk intake was reduced (Fig. 1). ETEC were detected in the feces of these animals, but the bacterial infection did not aggravate the clinical response to *Cryptosporidium*.

Intestinal lesions in these two lambs were much more severe and extensive than in those infected with ETEC and rotavirus. Morphological changes, such as stunting, fusion, and bridging of villi, extended proximally as high as the upper jejunum, and replacement of enterocytes by immature cells was more extensive. Infiltration of the crypts by neutrophils from the lamina propria, with resultant crypt sepsis, was apparent. The large intestines were heavily infected with cryptosporidia. Specific IF tests for ETEC

revealed no mucosal colonization by the organism at any part of the intestine.

(iii) Rotavirus and *Cryptosporidium*. Lambs no. 21 and 22 were inoculated with *Cryptosporidium* at 6 days and rotavirus at 8 days of age, respectively (Table 1). Oocysts were present in the feces at 2 days postinoculation, but rotavirus was not detected in the feces at any time. The lambs were depressed, and their milk consumption dropped 2 days postinoculation with *Cryptosporidium* (Fig. 1).

The histological lesions associated with lambs no. 21 and 22 were similar to those observed for the lambs which has been inoculated with *Cryptosporidium* alone. IF studies for rotavirus revealed very few infected epithelial cells, as in the single infections with rotavirus at the comparable age.

Bacterial counts. Lamb no. 2, inoculated with ETEC, had high bacterial counts at every site of the small and large intestines (10^9 to 10^{10} viable organisms per 0.1 g of mucosal scraping) except the duodenum (10^7). All the other lambs, whether inoculated with ETEC alone or in combination with rotavirus or *Cryptosporidium*, had lower bacterial counts: 10^6 to 10^7 in the lower small intestine and 10^8 in the large intestine.

Enzymology. The lambs were grouped into subclinically affected (group 1), clinically affected (group 2), and controls (group 3) as follows. Group 1 consisted of lamb no. 14 (ETEC only), lambs no. 15 and 16 (rotavirus only), lambs no. 5 and 6 (ETEC and rotavirus). Group 2 consisted of lambs no. 17 and 18 (*Cryptosporidium* only), lambs no. 19 and 20 (ETEC and *Cryptosporidium*), and lambs no. 21 and 22 (rotavirus and *Cryptosporidium*). Group 3 consisted of five uninoculated age-matched controls.

Sites from the duodenum, lower jejunum, and lower ileum were examined for disaccharidase activity, and the mean enzyme level at each site for lactase, maltase, and sucrase was calculated. The lactase results are given in Table 2.

There were no significant differences between or within groups at any site for either maltase or sucrase activity. However, with lactase, significant differences in activity were recorded between groups 1 and 2 ($P < 0.05$) in both the duodenum and lower ileum. In the latter site, a highly significant difference ($P < 0.01$) was obtained for the activities of groups 2 and 3.

TABLE 2. *Lactase activity at different sites along the small intestines of infected and control lambs*

Groups[a]	No. of replicates	Lactase activity (mean ± standard deviation) at: (μmol/min per g of wet wt)		
		Duodenum	Lower jejunum	Lower ileum
1	4	6.69 ± 1.61	8.00 ± 2.03	0.93 ± 0.42
2	5	2.11 ± 0.60[b]	1.15 ± 0.28[c]	0.28 ± 0.15
3	5	4.65 ± 0.49	4.33 ± 0.79	1.85 ± 1.52

[a] See text for composition of groups.
[b] $P < 0.05$ with respect to group 1.
[c] $P < 0.05$ with respect to group 1. $P < 0.01$ with respect to group 3.

DISCUSSION

The results of these experiments show that colostrum-deprived gnotobiotic lambs were susceptible to clinical infection with rotavirus or ETEC when less than 2 days of age, but subclinical infections were produced in lambs more than 4 days of age.

Lambs infected with *Cryptosporidium* all exhibited similar clinical responses, irrespective of whether they were separately challenged with rotavirus or ETEC. Clinical illness, reduced milk intake, reduced lactase activity in the small intestine, and mucosal damage correlated well in *Cryptosporidium*-infected lambs.

Clinical diarrhea and the pathogenesis of diarrhea in newborn lambs caused by ETEC (22) and lamb rotavirus (19) have been described. Subclinical rotavirus infections in lambs inoculated at up to 8 days of age were demonstrated by the shedding of virus in the feces and by IF of gut sections. The ETEC serotype used in this experiment possessed the K99 adherence antigen. However, inoculation of lambs with 10^{10} viable organisms failed to result in colonization of the small intestine of lambs aged 5 days or older, implying a close correlation between adherence, colonization, and clinical diarrhea, on the one hand, and the age of the lamb, on the other. The same organism (08:K87:K99) in combination with another strain (09:K30:K99) has been shown to induce severe diarrhea in three out of seven colostrum-fed young lambs (22), but the authors did not state at what age the lambs were infected.

Coinfection of 4- to 8-day-old lambs with ETEC and lamb rotavirus failed to induce clinical diarrhea, and the use of two SPF lambs did not alter the outcome. Neither evidence of bacterial adherence nor increased viral fluorescence resulted from the dual infection. No statistically significant difference in terms of disaccharidase activity among the control, ETEC-, rotavirus-, or rotavirus-plus-ETEC-infected lambs was observed. In contrast, coinfection of 2-week-old calves with rotavirus and ETEC induced diarrhea in circumstances where infection with a single agent would not (24).

The clinical manifestations, pathogenesis of diarrhea, and age susceptibility of newborn SPF lambs to *Cryptosporidium*, have been described elsewhere (Tzipori et al., in press). At that age *Cryptosporidium* infection induced severe diarrhea, but in lambs 6 days old intermittent diar-

rhea and constipation were observed, indicating that continuous diarrhea is not a consistent clinical manifestation of enterocolitis in older SPF lambs. Terminal examination revealed that 18 h after feeding the stomach still contained large volumes of milk, and the small intestine, which lacked muscular tone and peristaltic action, often contained large amounts of fluid, while the large intestine contained hard and pelleted feces. Thus complete stasis of the gut apparently slowed the rate at which the stomach emptied, thus reducing the milk intake (Tzipori et al., in press).

From previous (Tzipori et al., in press) and the present experiments, it appears that *Cryptosporidium* in lambs is an enteric pathogen which induces a more severe disease in older lambs than does ETEC or rotavirus acting singly or in combination with each other.

Although the work reported here provides information regarding the possible outcome of mixed enteric infections in lambs, the findings do not parallel what happens in calves. Calves are susceptible to rotavirus infection for a longer period than lambs, and dual infection with rotavirus and ETEC caused diarrhea in sucking calves at least 2 weeks of age (24). Further, SPF or colostrum-fed calves older than 1 week appear to be more susceptible to experimental cryptosporidiosis than lambs of similar ages (Tzipori and Sherwood, unpublished data).

The choice of a 2-day interval between inoculations with *Cryptosporidium* (at 6 days) and ETEC or rotavirus (at 8 days) was an attempt to synchronize the incubation periods of the two microorganisms. Other time intervals may have different results. It is significant that the incubation period of *Cryptosporidium* seemed constant at 2 days as oocyst shedding in the feces was noted after this time. It could be argued, for instance, that infection with *Cryptosporidium* before rotavirus may have depleted the small intestine of mature enterocytes for rotavirus infection and replication (11) or, in the case of ETEC, receptor sites on the brush borders may have been blocked by an overwhelming cryptosporidial infection.

Although the *Cryptosporidium* inoculum was not quantitated, the same preparation was used throughout the experiment. Passage of the *Cryptosporidium* inoculum through SPF rats was considered to provide a "biological filtration" of gut bacteria. However, further work is needed to separate the oocysts from feces to obtain a more satisfactory source of infectious material.

ACKNOWLEDGMENTS

We thank I. D. Aitken and R. M. Barlow for their comments and suggestions and the staff of Clinical Studies Department for the derivation and maintenance of gnotobiotic lambs.

D. Sherwood is the recipient of an Agricultural Research Council studentship.

LITERATURE CITED

1. **Acres, S. D., C. J. Laing, J. R. Saunder, and O. M. Radostitis.** 1975. Acute undifferentiated neonatal diarrhoea in beef calves. I. Occurence and distribution of infectious agents. Can. J. Comp. Med. **39**:116–132.
2. **Ansari, M. M., H. W. Renshaw, and N. L. Gates.** 1978. Colibacillosis in neonatal lambs: onset of diarrhoeal disease and isolation and characterization of enterotoxigenic *Escherichia coli* from enteric and septicaemic forms of the disease. Am. J. Vet. Res. **39**:11–14.
3. **Berg, I. E., A. C. Peterson, and T. D. Freeman.** 1978. Ovine cryptosporidiosis. J. Am. Vet. Med. Assoc. **173**: 1586–1587.
4. **Dahlqvist, A.** 1964. Method for assay of intestinal disacharidases. Anal. Biochem. **1**:18–25.
5. **Dean, 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 diarrhoea in children in Honolulu. J. Infect. Dis. **125**:407–411.
6. **Dubourguier, H. C., P. Gouet, O. Mandard, M. Contrepois, and C. Bachelerie.** 1978. Scanning electron microscopy of abomasum and intestine of gnotoxenic calves infected either with rotavirus, coronavirus or enteropathogenic *Escherichia coli* or with rotavirus and *E. coli*. Ann. Rech. Vet. **9**:441–451.
7. **Gouet, P., M. Contrepois, H. C. Dubourguier, Y., Rious, R. Scherrer, J. Laporte, J. F. Vautherst, J. Cohen, and R. L'Haridon.** 1978. The experimental production of diarrhoea in colostrum deprived axenic and gnotoxenic calves with enteropathogenic *Escherichia coli*, rotavirus, coronavirus and in a combined infection of rotavirus and *Escherichia coli*. Ann. Rech. Vet. **9**:433–440.
8. **Guinee, 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**:676–679.
9. **Logan, E. F., G. R. Pearson, and M. S. McNulty.** 1977. Studies on the immunity of the calf to colibacillosis. VII. The experimental reproduction of enteric colibacillosis in colostrum-fed calves. Vet. Rec. **101**:443–446.
10. **McNulty, M. S., J. B. McFerran, D. G. Bryson, E. F. Logan, and W. L. Curran.** 1976. Studies on rotavirus infection and diarrhoea in young calves. Vet. Rec. **99**: 229–230.
11. **Mebus, C. A., M. Kono, N. R. Underdahl, and M. J. Twiehaus.** 1971. Cell culture propagation of neonatal calf diarrhoea (scours) virus. Can. Vet. J. **12**:69–72.
12. **Miles, A. A., and S. S. Misra.** 1938. The estimation of the bactericidal power of blood. J. Hyg. **38**:732–749.
13. **Moon, H. W.** 1974. Pathogenesis of enteric disease caused by *Escherichia coli*. Adv. Vet. Sci. Comp. Med. **18**:179–211.
14. **Morin, M., S. Lariviere, R. Lallier, M. Begin, R. Roy, and R. Etheir.** 1978. Neonatal calf diarrhoea. Pathology and microbiology of spontaneous cases in dairy herds and incidence of the enteropathogens implicated as aetiological agents, p. 347–365. *In* Proceedings of the Second Symposium on Neonatal Diarrhoea. VIDO, Saskatoon, Canada.
15. **Runnels, P. L., H. W. Moon, S. C. Whipp, P. J. Mathews, and G. N. Wood.** 1980. Interaction of rotavirus and enterotoxigenic *Escherichia coli* (ETEC) in gnotobiotic calves, p. 343–360. *In* Proceedings of the Third International Symposium on Neonatal Diarrhoea. VIDO, Saskatoon, Canada.
16. **Sack, D. A., and R. B. Sack.** 1975. Test of enterotoxigenic *Escherichia coli* using Y1 adrenal cells in miniculture. Infect. Immun. **11**:334–336.
17. **Smith, H. W.** 1971. The bacteriology of the alimentary tract of domestic animals suffering from *E. coli* infec-

tion. Ann. N.Y. Acad. Sci. **176**:110–125.
18. **Smith, H. W., and M. B. Huggins.** 1979. Experimental infection of calves, piglets and lambs with mixtures of invasive and enteropathogenic strains of *Escherichia coli*. J. Med. Microbiol. **12**:507–510.
19. **Snodgrass, D. R., K. W. Angus, and E. W. Gray.** 1977. Rotavirus infection in lambs. Pathogenesis and pathology. Arch. Virol. **55**:263–274.
20. **Snodgrass, D. R., and E. W. Gray.** 1977. Detection and transmission of 30 nm virus particles (Astroviruses) in faeces of lambs with diarrhoea. Arch. Virol. **55**:287–291.
21. **Snodgrass, D. R., W. Smith, E. W. Gray, and J. A. Herring.** 1976. A rotavirus in lambs with diarrhoea. Res. Vet. Sci. **20**:113–114.
22. **Sojka, W. J., J. A. Morris, and C. Wray.** 1978. Enteric colibacillosis in lambs with special reference to passive protection of lambs against experimental infection by colostral transfer of antibodies from ewes vaccinated with K99, p. 52–63. *In* Symposium of the Twelfth International Congress of Microbiology, Munich.
23. **Tzipori, S., K. W. Angus, I. Campbell, and L. Clerihew.** 1981. Diarrhea due to *Cryptosporidium* infection in artificially reared lambs. J. Clin. Microbiol. **14**: 100–105.
24. **Tzipori, S. R., J. T. Makin, M. L. Smith, and F. L. Krautil.** 1981. Clinical manifestation of diarrhea in calves infected with rotavirus and enterotoxigenic *Escherichia coli*. J. Clin. Microbiol. **13**:1011–1016.