Pathological and Microbiological Observations Made on Spontaneous Cases of Acute Neonatal Calf Diarrhea

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ABSTRACT

The purpose of this report is to describe clinical signs, gross and microscopic lesions, bacteriological and immunofluorescence observations made on spontaneous cases of acute neonatal calf diarrhea (NCD) in dairy and beef herds. The following diagnostic tools were used: 1) direct smears of intestinal content, 2) Escherichia coli counts, 3) aerobic bacterial cultures of the small intestine and other organs (The O serogroup and the enterotoxigenicity of the E. coli isolated was determined), 4) detection of the two Nebraska NCD viruses (reo-like and corona-like) by the fluorescent antibody technique and 5) histological examination on different segments of the digestive tract.

The following etiological diagnoses were suggested after post mortem examination of 55 cases of NDC (34 were submitted alive): reo-like virus only (1), reo-like virus + E. coli (4), reo-like virus + cryptosporidium (2), reo- + corona-like viruses (5), reo- + corona-like viruses + cryptosporidium (3), reo- + corona-like viruses + infectious bovine rhinotracheitis virus (1), coronavirus-like agent only (2), coronavirus-like agent + mycotic abomasitis (1), coronavirus-like agent + crytosporidium (1), E. coli only (6), cryptosporidium only (5), mycotic abomasitis (3) , mycotic rumenitis + reticulitis (1) and undetermined (20). Most of the calves in the last group were submitted dead.

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RÉSUMÉ

Cet article décrit les signes cliniques, les lésions macroscopiques et histologiques, ainsi que les résultats d'études bactériologiques et d'immunofluorescence relatifs à des cas spontanés de diarrhée néo-natale aiguë qui se produisirent dans des élevages de bovins laitiers et de boucherie. Les auteurs utilisèrent les méthodes de diagnostic suivantes: 1) l'examen de frottis du contenu intestinal; 2) le comptage des Escherichia coli; 3) la culture aérobie du contenu de l'intestin grêle et d'autres organes, ainsi que la vérification du groupe O et de l'entérotoxigénicité des souches d'E. coli isolées; 4) la recherche, à l'aide de l'immunofluorescence, des deux virus (réo et corona) de la diarrhée néo-natale isolés au Nebraska; 5) l'examen histologique de tous les segments de l'intestin grêle et du côlon.

L'application de ces méthodes à l'étude de 55 cas de diarrhée néo-natale chez autant de veaux, dont 34 arrivèrent encore vivants à la salle de nécropsies, permit aux auteurs de formuler les diagnostics suivants: un cas d'infection à réovirus; quatre cas d'infection mixte: réovirus et E. coli; deux cas d'infection mixte: réovirus et Cryptosporidium; cinq cas d'infection mixte: virus réo et corona; trois cas d'infection mixte: virus réo et corona et Cryptosporidium; un cas d'infection mixte: virus réo et corona et virus de la rhino-trachéite infectieuse bovine; deux cas d'infection par le virus corona; un cas d'infection mixte: virus corona et inflammation fongique de la caillette; un cas d'infection mixte: virus corona et Cryptosporidium; six cas d'infection à E. coli; cinq cas d'infection à Cryptosporidium; trois cas d'infection fongique de la caillette; un cas d'infection fongique du réseau et du rumen; 20

cas d'étiologie indéterminée, dont la plupart correspondaient à des veaux reçus morts.

INTRODUCTION

Acute neonatal calf diarrhea (NCD) is responsible for major economic losses in many dairy and beef herds (23, 31). Morbidity rate of 100% with a mortality rate reaching almost 100% are often encountered. Several different infectious agents have been implicated either alone or in combination (1, 20, 34). *Escherichia coli* (19), *Salmonella* (25), *Providencia stuartii* (34) and chlamydial agents (5, 29, 32) are bacteria suggested as etiological agents of the disease. Several viruses such as the Nebraska NCD viruses (reo-like and corona-like) (8, 13, 14, 15, 16, 17), bovine viral diarrhea virus (9), infectious bovine rhinotracheitis virus (2), adenoviruses (3, 11, 12), parvoviruses (33) and enteroviruses (32) can cause NCD. Recently, coccidia of the genus *Cryptosporidium* were reported in the intestinal tract of a calf with neonatal diarrhea (18). Overfeeding, overpopulation, cold temperature, bad hygiene, artificial feeding and colostrum deprivation are all predisposing factors which can be important in the complex etiology of the disease.

The purpose of this report is to describe clinical signs, gross and microscopic lesions, bacteriological and immunofluorescence observations made on spontaneous cases of acute NCD in dairy and beef herds. It was felt that such a study should be helpful for a better understanding of the disease and could provide a tool to improve diagnosis.

MATERIALS AND METHODS

ANIMALS

Fifty-five calves were submitted for necropsy in their first two weeks of age because of a severe NCD problem. Thirty-four of these calves were submitted alive and the others were dead for a variable period of time.

HISTOPATHOLOGICAL EXAMINATION

Calves submitted alive were electrocuted and exsanguinated. Sections of the duodenum, upper, middle and lower jejunum, ileum and colon were collected for histological examination. They were fixed in 10% neutral buffered formalin and processed for paraffin tissue sections according to conventional methods. Sections 6.0μm thick were stained with hematoxylin, phloxin and safran (HPS) (10) and MacCallum-Goodpasture. In some cases, 3.0μm thick sections of segments of intestine were prepared and stained with a Giemsa or Macchiavello. Histological examination was also performed on the oesophagus, rumen, reticulum, omasum and abomasum of a certain number of the calves particularly when gross lesions were observed in these organs.

FLUORESCENT ANTIBODY TECHNIQUE

Intestinal specimens obtained from the upper, middle and lower jejunum and from the colon were rapidly frozen at —40°C for immunofluorescence studies. Using commercial conjugates[1] the fluorescent antibody tissue section technique (FAT) which was described previously was used for the detection of the reo-like virus and the coronavirus-like agent (20).

BACTERIOLOGY

Portions of the upper, middle and lower jejunum, liver, spleen and kidneys were submitted for bacterial isolation. The specimens were streaked onto blood agar and MacConkey agar plates which were then incubated aerobically at 37°C for 24 hours. Two or three isolated *E. coli* colonies from the upper jejunum of some of the calves were picked up for serological and enterotoxigenicity determinations. Those isolates were maintained on trypticase soy agar at room temperature.

The O serogroup of the isolates was identified using rabbit OK antisera against *E. coli* strains belonging to the following serogroups: O8, O9, O26, O55, O78 and O101. The tube agglutination test

[1]Reovirus conjugate and coronavirus conjugate. Norden Laboratories, Lincoln, Nebraska 68501.

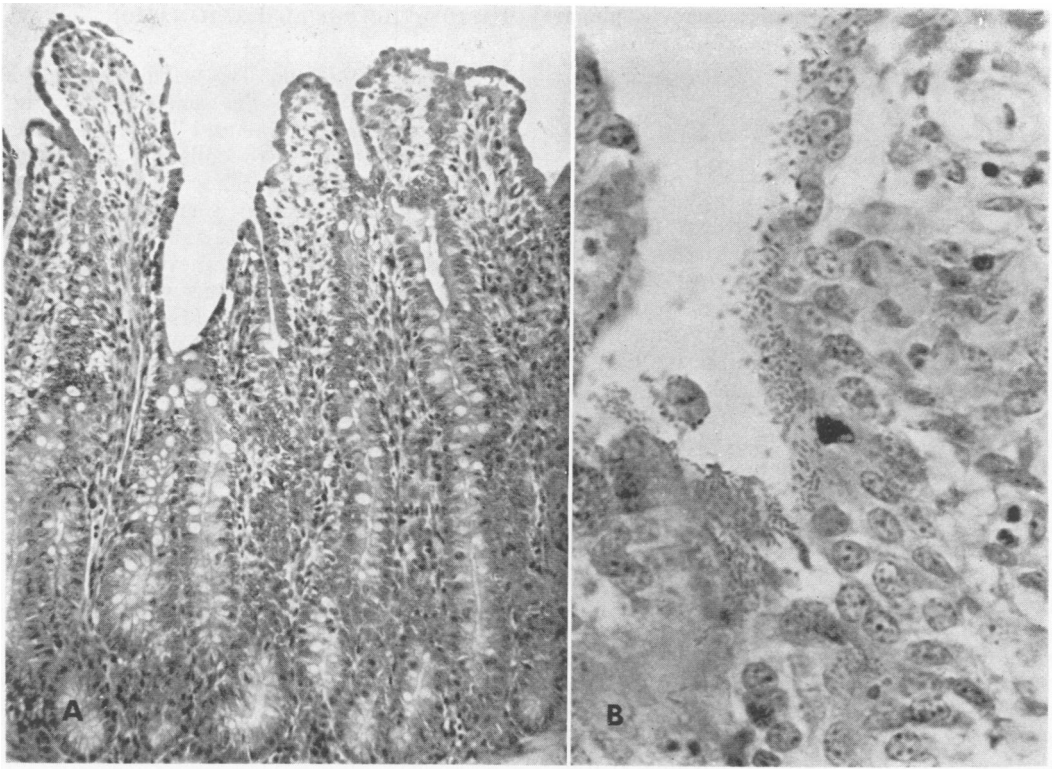

Fig. 1. Section from the middle jejunum of a four day old calf from which reo-like virus antigen was demonstrated by the FAT. a. The villi are shorter than normal and covered by cuboidal epithelial cells. HPS. x110. b. Gram stained section from the same area. Large numbers of gram-negative bacilli are present on the surface of low columnar or cuboidal epithelial cells lining a villous. x440.

as described by Sojka (30) was used for O group determination. Using the slide agglutination test with an absorbed K99 antiserum some isolates were tested for the presence of the K99 antigen.

Enterotoxigenicity of the *E. coli* strains isolated from the jejunum of the diarrheic calves was determined in colostrum-fed Holstein calves. The calves six to seven days of age were deprived of feed 24 hours prior to surgery. Calves were initially tranquilized with acepromazine maleate (5 mg per animal) and general anesthesia was induced by the intravenous administration of 5 to 8 gm of sodium pentobarbital. Infusion of a 2% carbocaine solution was done along the *linea alba* before the incision through the abdominal wall. Sixty 10 cm loops were prepared starting at a point on the small intestine three meters from the pylorus. Test loops were injected with 1 ml of overnight cultures in brain heart infusion[2]. Starting from the most anterior segment, for every five loops prepared one was injected with a nonenterotoxigenic negative control *E. coli* and for every ten loops one was injected with an enterotoxigenic positive control *E. coli*. Eighteen hours after surgery the calves were sacrificed and the test section of small intestine was removed intact. Positive loops were identified by comparing with the nearest positive and negative control loops.

The bacterial population of the small intestine was estimated by an examination of Gram stained direct smears from the intestinal content of upper, middle and lower jejunum. Viable *E. coli* counts were performed from a portion of the small intestine located 60 cm from the pylorus and from another one at 90 cm from the ileocecal valve of a certain number of calves. Between 0.2 gm and 1.0 gm of intestinal content was collected from each specimen and was suspended in sterile Ringer's solution at a dilution of 1:10. After agitation, serial dilutions were conducted and the number of coliforms was estimated by

[2]Gibco, Grand Island Co., New York.

plating five 20μl drops of each dilution onto MacConkey agar.

RESULTS

Clinical Signs and Gross Lesions

The calves submitted for necropsy were in their first two weeks of life and had a watery and yellowish diarrhea which was sometimes tinged with blood. These calves had diarrhea for not more than three days when they were received and most of them had signs of dehydration of variable severity. Most of the calves submitted alive had not been treated.

At necropsy, the following gross lesions were generally observed 1) dehydration and 2) the whole intestinal tract distended by a yellow watery content which often contained gas and poorly digested milk or only the large intestine distended by fluid. The intestinal mucosa was usually normal on gross examination. Whatever the etiology was the clinical signs and gross lesions were almost always the same.

Histopathological, Immunofluorescence and Bacteriological Studies

The summary of the final etiological diagnosis is presented in Table I.

TABLE I. Etiological Diagnosis Suggested After Post Mortem Examinations of 55 Cases[a] of Spontaneous Neonatal Calf Diarrhea

Etiological Diagnosis	Number of Cases
Reo-like virus only	1
Reo-like virus + *E. coli*	4
Reo-like virus + Cryptosporidium	2
Reo- + Corona-like viruses	5
Reo- + Corona-like viruses + Cryptosporidium	3
Reo- + Corona-like viruses + IBR virus	1
Coronavirus-like agent only	2
Coronavirus-like agent + Mycotic abomasitis	1
Coronavirus-like agent + Cryptosporidium	1
E. coli only	6
Cryptosporidium only	5
Mycotic Abomasitis	3
Mycotic Rumenitis + Reticulitis	1
Undetermined[b]	20

[a]34 calves were submitted alive
[b]19 out of the 20 calves in this group were submitted dead

Reo-like virus — A three day old calf was submitted alive for necropsy because of a mild diarrhea of one day duration. Using the FAT, reo-like virus antigen was demonstrated in the cytoplasm of several absorptive cells in the lower jejunum. Coronavirus-like antigen was not demonstrated and the direct smears revealed a small number of mainly gram-positive bacteria at all levels of the jejunum. Several villi in the middle and lower jejunum and in the ileum were moderately shorter than normal and covered by low columnar or cuboidal epithelial cells. There was hypercellularity of their lamina propria. The duodenum, upper jejunum and colon were histologically normal.

Reo-like virus + E. coli — Reo-like virus antigen was detected by FAT in the cytoplasm of absorptive cells in the jejunum of four calves, two to seven days of age, which were submitted alive from herds with a severe NCD problem. Direct smears revealed an abundant gram-negative bacterial flora in the middle and lower jejunum. *E.*

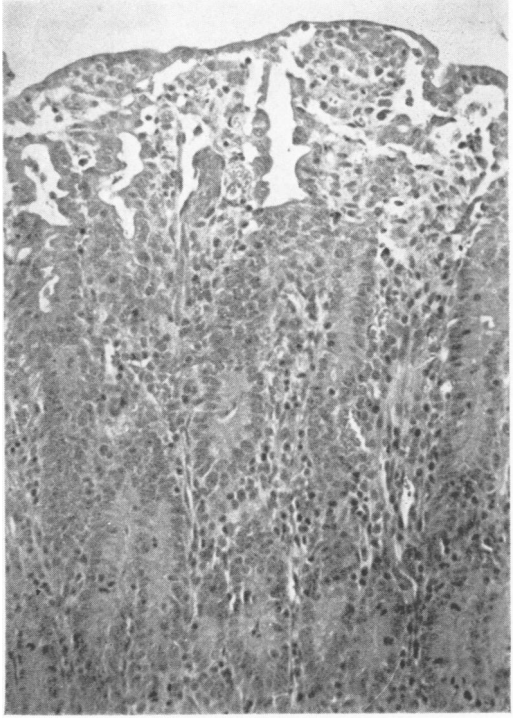

Fig. 2. Section from the lower jejunum of a seven day old calf from which both Nebraska NCD viruses (reo-like and corona-like) were demonstrated by the FAT. Notice the severe villous atrophy and replacement of columnar absorptive cells by cuboidal epithelial cells. HPS. x175.

TABLE II. Analysis of the *Escherichia coli* Population of the Small Intestine of Diarrheic Calves

Final Etiological Diagnosis	Calf no	E. coli × 10^5/g of Intestinal Content: Upper jejunum	Lower jejunum	O Group	K99 Ag	Ent[a]
Reo + *E. coli*	5	>90,000	>90,000	U[b]	+	+
Reo + Cryptosporidium	6	620	35,000	U	–	–
Reo + Corona	11	ND[d]	ND	U	–	–
	12	ND	ND	U	–	–
Reo + Corona + Cryptosporidium	15	100	98	U	–	–
Reo + Corona + IBR	16	13	430	O8	–	–
Corona	17	1	12	U	–	–
Corona + Mycotic abomasitis	19	30	140	C8	–	–
Corona + Cryptosporidium	20[c]	14	>10,000	U	–	–
E. coli	21	ND	ND	U	–	+
	22	ND	ND	U	–	–
	23	62	46,000	U	–	–
	24	1,100	>90,000	U	–	–
	25	>90,000	24,000	U	–	–
	26	1,400	1,000	U	–	+
Cryptosporidium	30	ND	ND	U	–	–
	31	ND	ND	U	–	–
Undetermined	38[c]	ND	ND	U	–	–
	39[c]	ND	ND	U	–	–
	40[c]	ND	ND	U	–	–
	44[c]	ND	ND	U	+	+
	47	ND	ND	U	–	–
	48[c]	17,000	63,000	U	–	–
	49[c]	2,300	56,000	U	–	–
	50[c]	2,400	2,600	C8	–	–
	51[c]	3,700	ND	U	–	–
	52[c]	4,000	4,500	U	–	–
	53[c]	20	6	U	–	–
	54[c]	<1	ND	U	–	–

[a]The enterotoxigenicity was determined by the gut loop technique in the calf
[b]U: untypable by the O antisera· O8, O9, O26, O55, O78 and O101
[c]Calves were received dead
[d]ND: not determined

coli counts performed on the intestinal tract content from the upper and lower jejunum of one of these calves were in the high range (Table II) and the *E. coli* isolate was enterotoxigenic and possessed the K99 antigen. Large numbers of *E. coli* were also obtained from the liver, spleen and kidneys of the four calves. Histological findings in the intestinal mucosa of the four calves were essentially similar: 1) several villi were blunt, shorter than normal and the epithelial cells covering their upper part or tip were undergoing degeneration and desquamation (Fig. 1), 2) the upper part or tip of other villi was covered by cuboidal or simple squamous epithelial cells and in both denuded and nondenuded villi there was hypercellularity of the lamina propria and 3) large numbers of gram-negative bacilli were aggregated on the surface of the denuded and nondenuded villi and in the intestinal lumen (Fig. 1). These lesions were usually more severe in the middle and lower jejunum and ileum. The duodenum and upper jejunum were usually spared or the lesions were milder than those observed in the lower parts of the small intestine. Significant histological lesions were not demonstrated in the colon of these calves.

Reo-like virus + Cryptosporidium — Reo-like virus antigen was detected by the FAT in the jejunum of two (three and five day old) calves submitted alive for necropsy with acute neonatal diarrhea. Direct smears revealed a poor bacterial flora at all levels of the jejunum from which only a small number of a nonenterotoxigenic *E. coli* was isolated from one of the calves (Table II). Histological examination revealed normal villi in the duodenum and upper jejunum. In the middle and lower jejunum and in the ileum, histological lesions similar to those demonstrated in the only calf with a reo-like virus infection alone were observed except for the fact that these two calves had a more severe denudation of the villi. In the microvillous border of absorptive cells lining the upper half of villi in the middle, lower jejunum and ileum, there were organisms having the morphological characteristics of coccidia of the genus *Cryptosporidium* (6, 7, 18, 24, 26).

Reo-like virus + Coronavirus-like agent — By the FAT, the two Nebraska NCD viruses (reo-like and corona-like) were detected in the cytoplasm of absorptive cells in the small intestine of five calves, four to 14 days of age, submitted alive for necropsy with acute neonatal diarrhea. Reo-like virus antigen was not detected in the absorptive and crypt cells of the colon but coronavirus-like antigen was found. Direct smears revealed a poor bacterial flora at all levels of the jejunum and no evidence of bacteremia was found. The *E. coli* isolated from two of the calves were nonenterotoxigenic and could not by typed (Table II). Histological lesions observed in the intestinal tract of the five calves were essentially similar: 1) the duodenum was usually spared, 2) the upper jejunum of one calf was normal whereas in the four others several villi were moderately shorter than normal, blunt and covered by cuboidal epithelial cells with a poorly developed brush border. The tip of some villi was denuded. In the middle jejunum lesions were similar to those observed in the upper jejunum. In the lower jejunum and ileum of the five calves most of the villi were very atrophic and lined by cuboidal or simple squamous epithelial cells (Fig. 2). The upper part or tip of a variable number of villi was denuded and in some of the calves large numbers of desquamated cells could be seen in the intestinal lumen. In the colon there were focal areas of absorptive cell degeneration and desquamation with denudation and their replacement by cuboidal cells (Fig. 3). A variable number of crypts were lined by cuboidal cells and their lumen was dilated and filled with desquamated dead cells (Fig. 3).

Reo-like virus + Coronavirus-like agent + Cryptosporidium — In three cases of acute NCD (calves five to 14 days of age), reo-like virus and the coronavirus-like antigens were demonstrated by the FAT. Histological lesions in the jejunum and colon of these calves submitted alive for necropsy were similar to those described

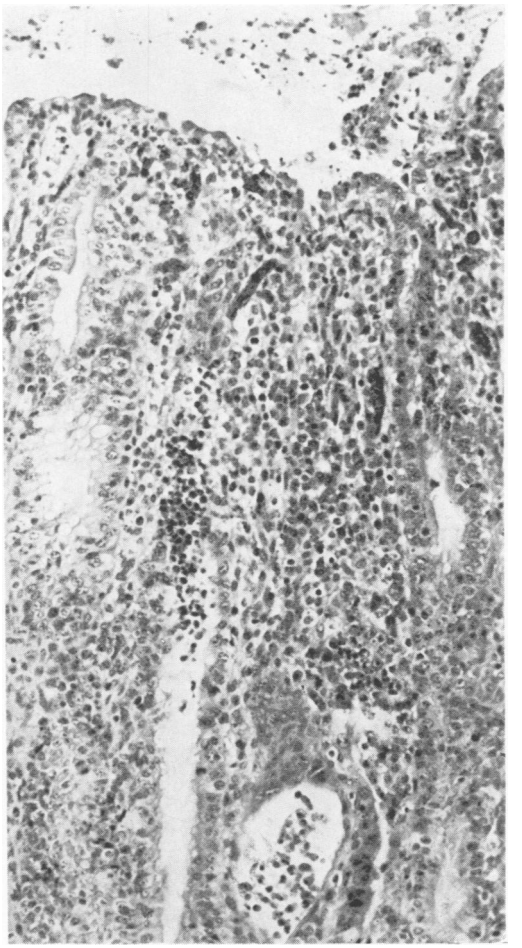

Fig. 3. Section from the colon of a seven day old calf from which coronavirus-like antigen was demonstrated by the FAT. Notice foci of absorptive cells degeneration and desquamation with denudation of the lamina propria. In other areas the absorptive cells are cuboidal rather than columnar. There is an increased number of mononuclear cells in the lamina propria and notice the presence of cryptitis. HPS. x140.

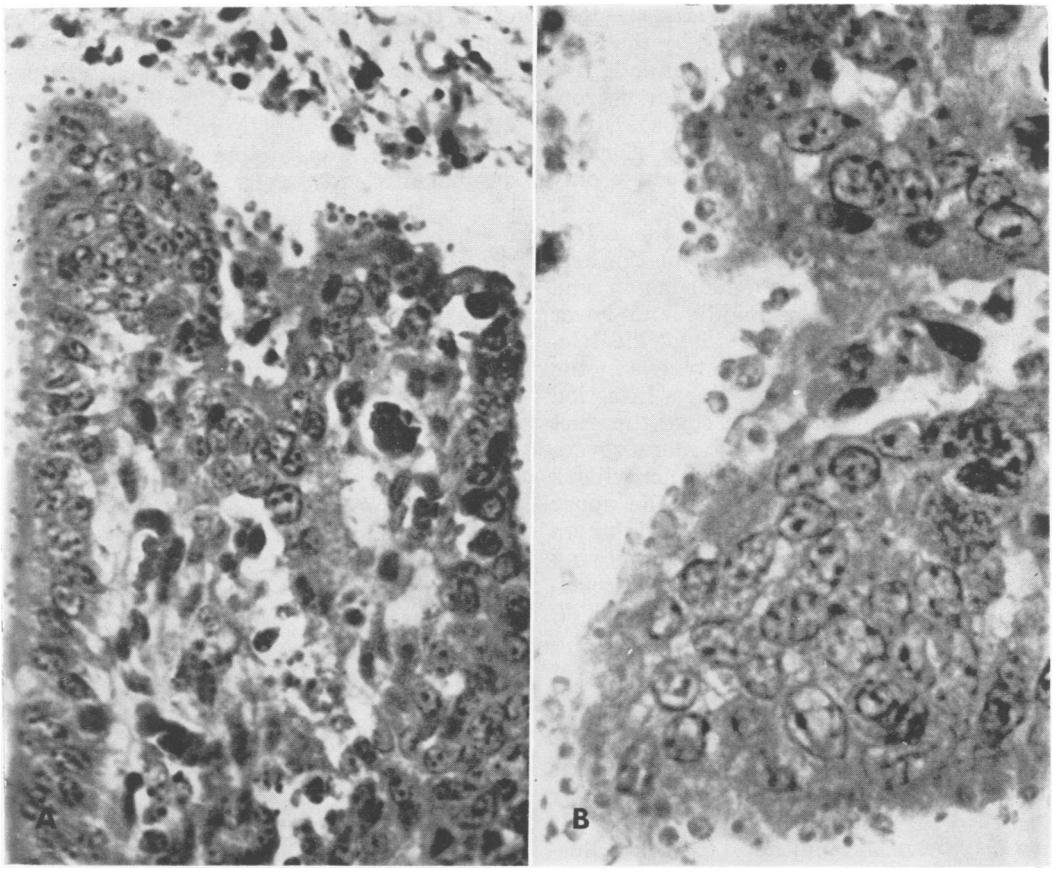

Fig. 4. Section from the lower jejunum of a 14 day old calf from which both Nebraska NCD viruses (reo-like and corona-like) were demonstrated by the FAT. a. There is a severe villous atrophy and replacement of columnar absorptive cells by cuboidal epithelial cells. Notice the large numbers of organisms with the morphological characteristics of coccidia of the genus Cryptosporidium **in the brush border of several absorptive cells. HPS. x430. b. Same area. Notice the large numbers and various stages of cryptosporidia on the surface of degenerating absorptive cells. HPS. x925.**

in the previous five calves infected with the two viruses. In the lower jejunum and ileum of the three calves large numbers of organisms with the morphological characteristics of coccidia of the genus *Cryptosporidium* were observed on the surface of the degenerating or cuboidal cells covering the atrophic villi (Fig. 4). Small numbers of a nonenterotoxigenic *E. coli* were found in the small intestine (Table II) of one of these calves.

Reo-like virus + Coronavirus-like agent + Infectious bovine rhinotracheitis virus —A one week old calf was submitted alive for necropsy because of a severe, profuse and yellowish diarrhea. There were small amounts of mucus and blood in the diarrheic feces. Morbidity and mortality among the calves in the herd was 100%. This problem had started following the introduction in the herd of five cows. In the following weeks all the cows in the herd had suffered the respiratory form of IBR. The calving period began at this time and all the calves were lost with the clinical signs described above. In the calf submitted there was a severe fibrinonecrotic laryngitis and tracheitis with bronchopneumonia. In the mucosa of the cranial half of the oesophagus there were white, slightly raised linear foci approximately 2-4 mm in length and in the ventral portions of the rumen and reticulum there were masses of grayish material attached to the mucosa. Histological examination of the oesophagus, rumen and reticulum revealed lesions similar to those described for IBR infection in calves (2). IBR virus was isolated from the lung and jejunum of this animal on calf testis cell cultures. The Nebraska NCD viruses (reo-like and corona-

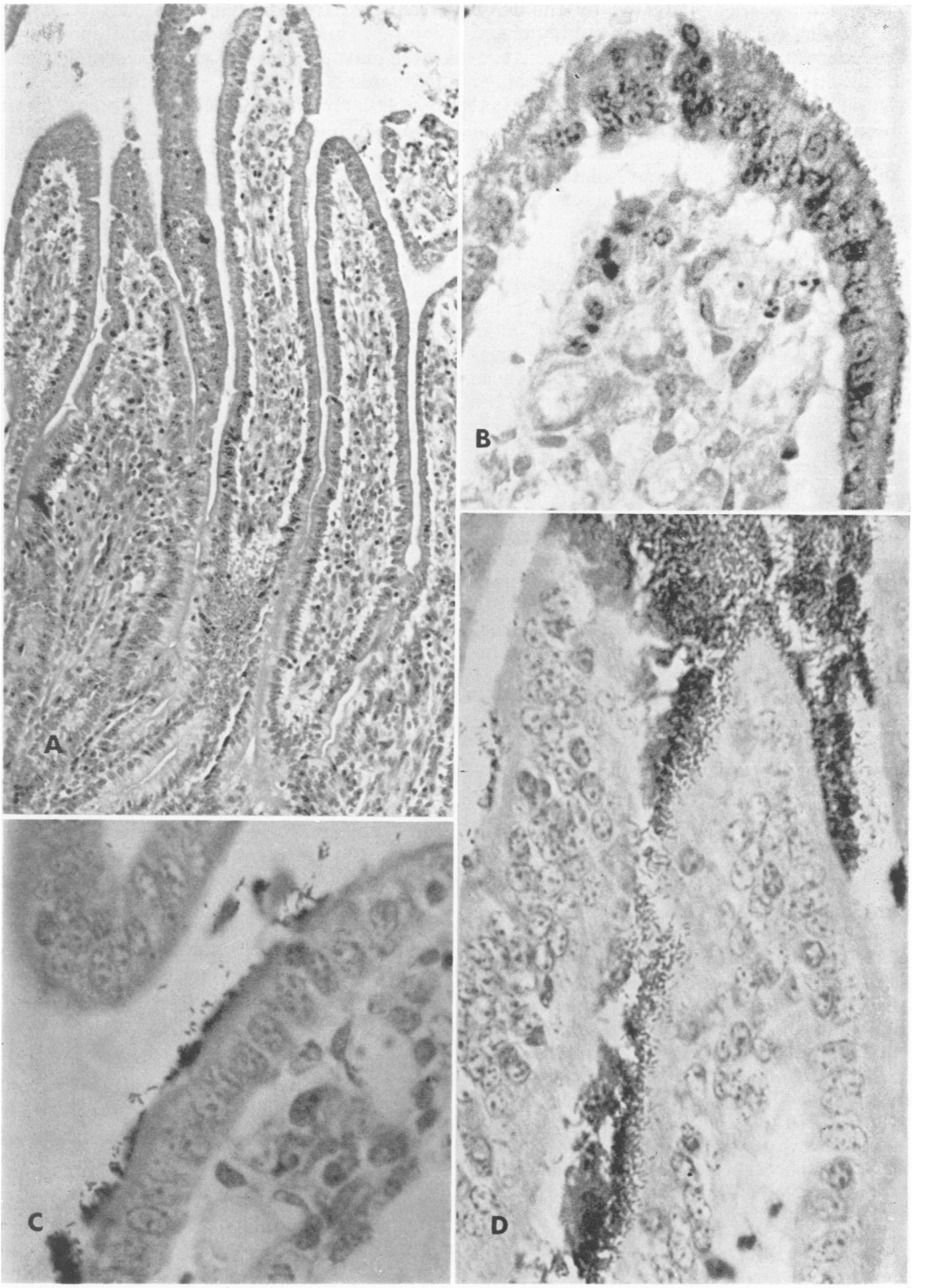

Fig. 5. Section from the middle jejunum of a five day old calf. The Nebraska NCD viruses were not demonstrated and a loop dilating E. coli was isolated a. Villi appear normal in length. HPS. x104. b. Higher magnification of one of the villi shown in the previous figure. Large numbers of gram-negative bacilli are present on the surface of degenerating and low columnar absorptive cells. HPS. x700. c and d. Large numbers of gram-negative bacilli are present on the surface of the absorptive cells and in the lumen. Gram. x700.

like) were also demonstrated by the FAT and histological lesions in the jejunum and colon were similar to those described previously in the calves with this combined infection. Direct smears from intestinal content of the upper and lower jejunum revealed a poor bacterial flora and only a small number of *E. coli* was isolated (Table II). This *E. coli* belongs to serogroup O8 and was not enterotoxinogenic.

Coronavirus-like agent — Only the coronavirus-like agent was demonstrated by the FAT in the small intestine and colon of two calves (five and ten days of age) with severe NCD. Histological and bacteriological findings were very similar to those described in the calves infected with the two Nebraska NCD viruses.

E. coli — In six cases of acute NCD (calves two to seven days of age) submitted alive for necropsy, searches for the Nebraska NCD viruses were unsuccessful. *E. coli* counts performed on intestinal content from the upper and lower jejunum of four of these calves were in the high range for three of them (Table II). Direct smears usually revealed an abundant gram-negative bacterial flora at the different levels of the jejunum. None of the *E. coli* strains isolated could be O grouped nor possessed the K99 antigen. Two of the six strains showed evidence of enterotoxin production (Table II). A moderate number of *E. coli* were isolated from the liver, kidneys and spleen of one calf, whereas in all the others only a few were obtained. In the jejunum of these calves, villi were normal in length or slightly shorter than normal and they were covered by absorptive cells either normal in appearance or undergoing slight degeneration (Fig. 5). In some areas the absorptive cells were subcolumnar or cuboidal in shape and denudation was uncommon. Large numbers of gram-negative bacilli were present on the surface of the absorptive cells and in the intestinal lumen (Fig. 6). Only rarely were they seen in the apical cytoplasm of the absorptive cells and these bacteria were usually more numerous in the middle and lower parts of the small intestine.

Cryptosporidium — Five calves (five to 15 days of age) were submitted alive from three different herds with a severe NCD problem. Searches for the Nebraska NCD viruses gave negative results. Direct smears revealed small numbers of mostly gram-positive bacteria at all levels of the jejunum and the *E. coli* found in two of these calves were untypable and nonenterotoxigenic (Table II). The intestinal villi particularly those in the middle and lower jejunum and in the ileum were slightly shorter than normal and covered by normal or low columnar absorptive cells (Fig. 6). Denudation was uncommon and there was hypercellularity of the lamina propria. In the microvillous border of the absorptive cells, particularly those covering the upper half of the villi, there were numerous organisms which were identified as coccidia belonging to the genus *Cryptosporidium* (6, 7, 18, 24, 26). The same organisms were also observed with lesser frequency in the crypts of Lieberkühn and lesions of cryptitis were present. It was impossible to demonstrate oocytes in the fecal samples obtained from two of these calves.

Mycotic abomasitis, rumenitis and reticulitis — In three calves with NCD problems numerous erosive and ulcerative lesions were observed in the abomasal mucosa. Histological lesions were similar in all cases and consisted of focal areas of necrosis and hemorrhages in the mucosa with occlusion of several mucosal and submucosal vessels by thrombi (Fig. 7). Periodic acid schiff stain revealed large irregular branching nonseptate hyphae either free in the necrotic areas of the mucosa or in the thrombi (Fig. 7). Search for inclusion bodies characteristic for infectious bovine rhinotracheitis or adenovirus infections gave negative results. In the rumen and reticulum of one calf, raised areas of necrosis covered by a yellowish cheesy material were observed. Histological lesions were similar to those described in the abomasum of the previous calves.

Cases of undetermined etiology — Most of the calves in this group were submitted dead and post mortem changes in their intestinal tract were variable in severity. In the jejunum of a calf submitted alive several crypts of Lieberkühn were distended and their lumen contained neutrophils and desquamated cells. The villi were normal. A special stain (Macchiavello) for demonstration of chlamydial elementary bodies gave negative results. Bovine viral diarrhea virus was not searched for. Out of the 12 *E. coli* strains isolated only one was enterotoxigenic and this strain was

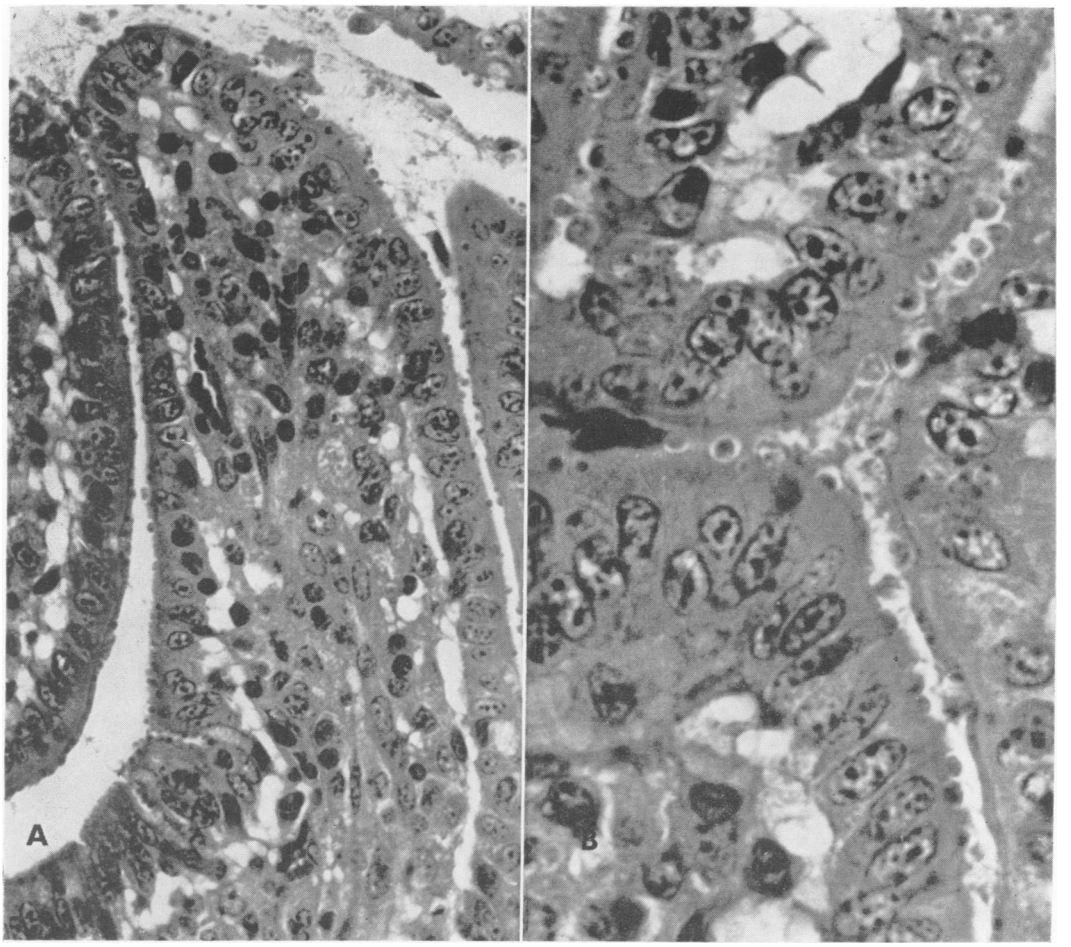

Fig. 6. Section from the lower jejunum of a five day old calf. The Nebraska NCD viruses were not demonstrated. a. Villi are normal in length and notice the large numbers of organisms with the morphological characteristics of coccidia of the genus Cryptosporidium in the brush border of several absorptive cells. HPS. x240. b. Large numbers and various stages of cryptosporidia are present in the brush border of absorptive cells on adjacent villi. HPS. x1100.

shown to possess the K99 antigen (Table II).

DISCUSSION

In the study reported herein, all the calves in which a reo-like virus infection was demonstrated by the FAT had histological lesions in their jejunum similar to those already described in experimentally infected animals (16) but had an intact colon. In four out of these five calves there was evidence for a concomitant *E. coli* infection as shown by the aggregation of large numbers of gram-negative bacilli on the intestinal mucosa. Unfortunately, only one case was submitted for the characterization of the *E. coli* population. In this case an enterotoxigenic strain possessing the K99 antigen was present in very high numbers (Table II). These calves were the only virus infected ones showing evidence of *E. coli* invasion into the internal organs. In these four calves the intestinal lesions were more severe than those observed in the calf in which only a reo-like virus infection had been detected and this would tend to support the suggestion of Mebus (14) that a bacterial overgrowth may delay restoration of the jejunal absorptive cells destroyed by the reo-like virus or may even cause further injury to the epithelium.

In the present study, the calves in which the Nebraska coronavirus-like agent was demonstrated either alone or in combination with the reo-like virus all had similar

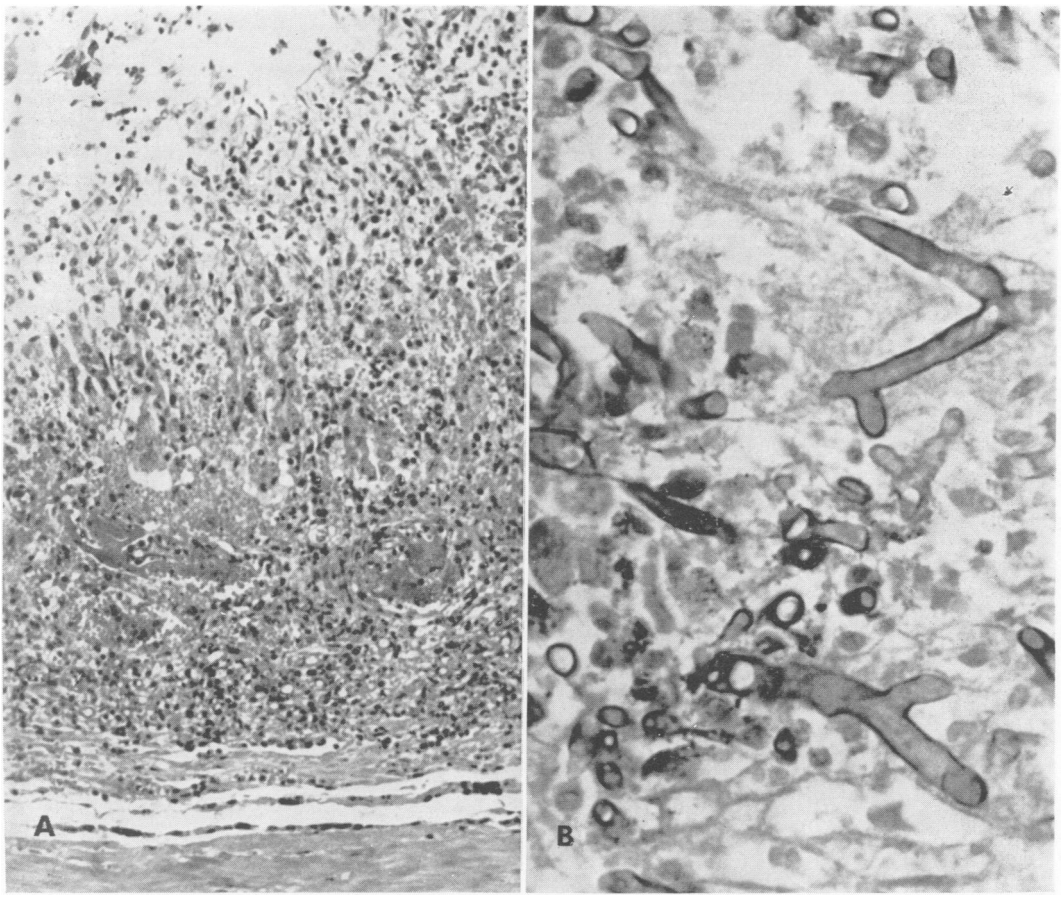

Fig. 7. Section from the abomasum of a ten day old calf. a. Mycotic abomasitis. The mucosa is necrotic and hemorrhagic and there are vessels occluded by thrombi. HPS. x175. b. Mycotic abomasitis. Notice the branching nonseptate hyphae. PAS. x700.

histological lesions in their jejunum and colon. These lesions were similar to those described in calves experimentally infected with the virus (17) and tended to be more severe in the middle and lower jejunum and in the ileum whereas lesions in the colon were variable in severity from one calf to another. The histological findings in the small intestine of these calves support the suggestion of Mebus (14) that lesions caused by the coronavirus-like agent are more severe and persistent than those caused by the reo-like virus alone. *E. coli* infection did not seem to be involved and the bacterial flora of the small intestine of these calves was reduced as suggested by the direct smears. We feel that the extensive damages to the digestive surface of the small intestine and the absorptive surface of the colon combined with an alteration of the intestinal bacterial flora could explain the severe diarrhea and dehydration suffered by these calves. Contrary to what is reported in the literature (8, 14), infection with the coronavirus-like agent alone or in combination with the reo-like virus was observed in calves less than one week old and among these calves morbidity and mortality was very high whereas in older calves, mortality was lower. It is also interesting to notice that the reo-like virus agent was demonstrated in calves up to two weeks of age.

On the basis of the aggregation and increased numbers of gram-negative bacilli in the jejunum and the absence of other known enteropathogenic microorganisms, a diagnosis of *E. coli* infection alone was suggested in six out of the 55 NCD cases studied. Bacteremia was evident in only one of these calves and only two of the four *E. coli* strains tested were enterotoxigenic. In the jejunum of the six calves, villi were normal in length or slightly shorter

than normal and they were covered by absorptive cells either normal in appearance or undergoing slight degeneration. These calves had been submitted alive and the intestinal specimens were well preserved. The degenerative changes observed could be related to the large numbers of gram-negative bacilli on the surface of the absorptive cells or could have been caused by another undetected infectious agent. The lesions were much less severe than those detected in the intestinal tract of calves with a combined reo-like virus-*E. coli* infection. In an attempt to find other criteria for the recognition of diarrhea caused by *E. coli*, enumeration of coliforms in the small intestine was done. Since most of the workers recognized that proliferation of *E. coli* into the small intestine is a feature of enteric colibacillosis (19), in this study we were considering the presence of small numbers of *E. coli* into the jejunum as an evidence that the diarrhea was not caused by *E. coli*. From the literature it appears that not more than 10^5 x *E. coli* per gram of content is found in the upper small intestine of healthy calves of not more than two weeks of age (4, 28). In this study, all the *E. coli* counts were over 10^5 x *E. coli* per gram, however, all those which were not more than 10^7 x *E. coli* per gram were considered to be in the normal range. In the establishment of the final diagnosis the *E. coli* enumeration was never used as the sole criterion and was never considered if the calf was dead upon arrival as it is known that *E. coli* counts increase rapidly after death (28). As the presence of K99 antigen on the surface of *E. coli* cells seems associated with the enteropathogenicity of *E. coli* (22) we looked for it in all the *E. coli* isolated. Only two strains were shown to possess that antigen and they were both enterotoxigenic. The low incidence of *E. coli* infection alone observed in this study with absence of bacteremia or septicemia in most of the cases was also reported by Smith (27). Septicemic colibacillosis in calves is principally a disease of immunoglobulin deficient animals (19, 27) and the low incidence of this form of the disease in our area could be explained by the fact that most of the farmers are aware of the importance of very early colostrum feeding and disinfection of the umbilicus.

In five cases of acute NCD, organisms with the morphological characteristics of coccidia of the genus *Cryptosporidium* were observed in the microvillous border of absorptive cells particularly in the lower jejunum and ileum. Only mild to moderate lesions of villous atrophy and blunting with hypercellularity of the lamina propria were present. Histological examination was the most useful tool to demonstrate the organisms in the intestine and it was impossible to demonstrate oocytes in the fecal samples obtained from two of the infected calves. In these five cases, the participation of other infectious agents was not demonstrated and treatments with sulfonamides were helpful to control the disease in the affected herds. The morphology of the organisms and the histological changes observed in the calves of our study were similar to cryptosporidiosis described in calves (18), guinea pigs (6), turkeys (26) and Rhesus monkeys (7). We have not observed prominent lesions in the colon similar to those described by Meuten *et al* (18) and the histological lesions in the jejunum were not as severe as those described by Panciera *et al* (24) in an older calf which had suffered a chronic diarrhea. Recently, we have observed the same organisms in the lower jejunum of two pigs which also had transmissible gastroenteritis. A combination of cryptosporidial organisms with the two Nebraska NCD viruses was demonstrated in three calves. In these animals, lesions in the lower jejunum and ileum were severe and similar to those observed with the mixed viral infection alone. We feel that the demonstration of cryptosporidial organisms either alone or in combination with other infectious agents in the intestinal tract of calves with acute neonatal diarrhea is an interesting finding and further studies should be undertaken for a final proof of their pathogenicity.

In several of the NCD cases reported in this paper infectious agents were thought to be involved either alone or more often in combination and usually there was a fair correlation between the bacteriological, immunofluorescence and histopathological studies. We feel that the combination of the laboratory methods used in this study could be used routinely for the detection of the etiological agents responsible for NCD and the submission of live animals which have not been sick for more than one or two days is essential. In this study, the intestinal tract of most of the calves submitted dead was unsuitable for histopathological and immunofluorescence

studies and the results from the bacteriological studies were difficult to interpret. We feel that histological examination of the intestinal tract of these calves is very important for the following reasons: 1) one can appreciate better the meaning of the results obtained from the other laboratory procedures used and 2) it is possible to make observations which can be very useful to understand better the etiology and pathogenesis of a NCD case.

This study shows the complexity of recognizing the etiological agents of NCD and we do not eliminate the possibility that other agents were involved in the cases described herein. We also recognized that most of the NCD cases reported in this study were severe and impossible to control by conventional therapy and they might not reflect the majority of NCD cases which can be handled by the practitioner and are rarely submitted alive for necropsy. We feel that the laboratory approach described in this paper can be useful to establish etiological diagnosis on a good number of NCD cases and can contribute to a better understanding of this complex disease.

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