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## Calf Diarrhea (Scours): Reproduced with a Virus from a Field Outbreak


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**Calf Diarrhea**

**(Scours): Reproduced  
With a Virus From  
A Field Outbreak**

**Research Bulletin**

**233**

**March 1969**

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## Contents

Acknowledgment .....	1
Introduction .....	2
Materials and Methods.....	2
Bacteriological Examination .....	2
Filtrates .....	3
Experimental Calves .....	3
Route of Inoculation .....	3
Electron Microscopy .....	5
Preparation of Antigen .....	5
Preparation of Conjugates .....	5
Preparation of Material for FA Technique .....	6
Results .....	7
Discussion .....	13
Summary .....	14
References .....	16

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# Calf Diarrhea (Scours): Reproduced with a Virus From a Field Outbreak

By C. A. Mebus, N. R. Underdahl, M. B. Rhodes, M. J. Twiehaus<sup>1</sup>

## Introduction

Special state funds were made available in the spring of 1967 to initiate research of neonatal calf diarrhea. Herds having calf diarrhea were visited and fecal specimens collected.

This fecal material was inoculated orally in experimental calves in an attempt to reproduce diarrhea so that the disease could be studied.

During 1967, nine hysterectomy-derived, colostrum-deprived calves and five caught, colostrum-deprived calves were inoculated. Only one calf developed diarrhea and the disease could not be serially reproduced.

This report is concerned with procedures used and results obtained in 1968 during which time neonatal calf diarrhea has been consistently reproduced.

## Materials and Methods

Calves selected for specimen collection in herds having neonatal diarrhea were either untreated or did not respond to treatment. Calves were examined, temperatures were taken and calves were bled for serum. On several ranches, selected calves were re-bled three to four weeks later.

Fecal material was collected directly into a sterile container. On the ranch, approximately 10 ml. aliquots of fecal material in screw cap tubes were frozen in an alcohol dry ice bath, transported to the laboratory in a dry ice chest, and stored at  $-60^{\circ}$  C. The remainder of the fecal samples was refrigerated with wet ice.

## Bacteriological Examination

Fecal samples routinely were cultured on blood (bovine) agar, tergitol 7 agar,<sup>2</sup> selenite<sup>2</sup> and thioglycollate broths.<sup>2</sup> The selenite broth cultures were transferred to salmonella-shigella (SS) agar. Non-coliforms from SS agar were transferred to Kligler iron agar and the nonlactose fermenters to mediums for "IMVIC" tests.

Fecal cultures were made on experimental calves before inoculation and during the diarrhetic period. At necropsy of calves killed the

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<sup>2</sup> Difco Laboratories, Detroit 1, Michigan.

following tissues were cultured: blood, colon, ileum, jejunum, liver, kidney, spleen, lung and brain.

## Filtrates

Filtrates used for inoculation were prepared by diluting the liquid feces 1-4 with buffered normal saline and centrifuging 15 minutes at 1000 X g. in a refrigerated centrifuge at 4° C. The supernatant was passed through a 5.0, 1.0 and 0.5 micron Seitz<sup>3</sup> filter under 5 lbs. pressure. One ml. was cultured in thioglycollate broth to determine sterility.

## Experimental Calves

Four types of calves were used: dropped, caught, caesarean and hysterectomy.

Dropped calves were born normally and received colostrum unless otherwise noted.

Caught calves were born normally but were not permitted to touch the ground and were transported to the laboratory in a plastic-lined, formaldehyde-gassed box.

Caesarean section calves, following removal from the uterus, were placed immediately in a box that had been fumigated with formaldehyde gas. In the isolation room both the caught and caesarean calves were wiped with sterile towels.

Hysterectomy-derived calves were taken using the hysterectomy hood as described by Sweat (1965).

Calves No. 68-41, 42 and 43 (Table 1) were kept for 30 to 48 hours in the hysterectomy hood in an attempt to maintain the calf bacteria-free during the infection. All other calves were housed in individual isolation rooms. Between calves, floors and walls of the isolation rooms were scrubbed and the rooms fumigated with formaldehyde gas.

Calves were fed twice daily raw, pasteurized or autoclaved milk; quantity of milk offered was equivalent to 8 to 10% of their body weight.

## Route of Inoculation

Duodenal canula: Surgery was performed on the calf in the isolation room. The calf was restrained to the table and the right paracostal area clipped, washed, wiped with 70% ethyl alcohol and tissue infiltrated with a 1:1 dilution of 2.5% procaine hydrochloride with epinephrine 1:10,000<sup>4</sup> and sterile water.

The peritoneal cavity was opened and either the duodenum picked

<sup>3</sup> Republic Seitz Filter Corp., 17 Stone St., Newark 4, N. J.

<sup>4</sup> Locadyne, Norden Laboratories, Inc., Lincoln, Nebraska.



Fig. 1—Calf with a duodenal canula.

up directly or the omentum gently pulled posterior until the greater curvature of the abomasum or duodenum was located. The duodenum just posterior to the pyloric valve was held in the incision and the duodenal visceral peritoneum sutured with braided nylon<sup>5</sup> to the partial peritoneum in the upper  $\frac{2}{3}$  of the anterior edge of the incision. The duodenal visceral and partial peritoneum of the posterior edge were then sutured leaving a  $\frac{1}{2}$  to 1 cm. strip of visceral peritoneum outside the abdominal cavity.

A small incision was made longitudinally in the duodenum and a soft rubber tube ( $1/8'' \times 1/32''$ ) introduced caudally about 10 cm. into the lumen. Two fixation sutures through a larger rubber tube ( $3/16'' \times 3/32''$ ) glued<sup>6</sup> around the canula anchored the canula to the abdominal muscles. The remainder of the peritoneum and the incision were closed (Fig. 1). The infective material was given through the canula.

**Duodenal injection:** The abdominal cavity was opened as for the duodenal canula, the duodenum picked up, and the inoculum introduced into the lumen with a syringe and needle. The incision was closed in a normal manner.

**Oral:** The calves sucked a syringe containing the inoculum.

**Spray:** A fairly small droplet spray was formed using a 5 ml. syringe and 22 gauge needle with the bevel bent up so that the stream of fluid would be deflected to form a fine spray. The bottle with nipple was set by the calf's head and the inoculum discharged upward over

<sup>5</sup> Vetafil, Bengen & Co., Hannover, West Germany.

<sup>6</sup> Hysol Epoxi-Patch Kit, Hysol Corp., Olean, N. Y.

the anterior half of the calf. The calf's head and nose were then rubbed with a gloved hand and the calf permitted to suck the nipple.

Quantity of inoculum: Calves inoculated with fecal material were given 6 to 10 ml. of undiluted untreated feces. Those given filtrate received approximately 25 ml. of a 1-4 dilution of feces; however, a few animals inoculated orally did not suck and some material was lost. Five ml. of filtrate were used in the spray inoculation of calf 68-43. Calf 68-5 received 10 ml. nutrient broth culture of a gram-negative rod and 10 ml. of a gram positive cocci isolated from calf 68-4.

Calf 68-24 was inoculated at the end of the diarrhetic period following filtrate inoculation via the duodenal canula with 10 ml. of a broth culture of *E. coli* isolated two days previously from a field case of calf diarrhea.

Fecal material from experimental calves was collected directly into a jar during the period of diarrhea, cultured as the field cases, and frozen in a dry ice alcohol bath.

## Electron Microscopy

Three hundred ml. of feces from a diarrhetic experimental calf (68-22) were centrifuged at 2930 X g. for 30 minutes in a Spinco<sup>7</sup> ultracentrifuge. The sediment was discarded and the supernatant centrifuged at 105,500 X g. for 20 minutes. The pellets were pooled and the supernatant recentrifuged at 88,000 X g. for 3 hours. Pellets were again pooled.

Each of the pooled pelleted materials was resuspended in 10 ml. of a 0.01 M phosphate buffer, layered over a gradient composed of 5 ml. each of a 40, 30, 20 and 10% sucrose solution and centrifuged at 76,100 X g. for 16 hours. Pellets were resuspended in an equal quantity of phosphotungstic acid, placed on a carbon coated grid and examined with a RCA 3-G electron microscope.

## Preparation of Antigen

Antigen was prepared from the feces of calves 68-42 and 68-43 following the same procedure through sucrose gradient centrifugation as described for the material examined by electron microscopy. The pellet from the sucrose gradient was resuspended in phosphate buffered saline to one-tenth of the original volume of feces and sonified for one minute. The antigen preparation was quick frozen in 4 ml. aliquots and stored at -60° C.

## Preparation of Conjugates

Two domestic white rabbits approximately six months of age, following collection of blood from an ear vein, were each injected intra-

<sup>7</sup> Spinco Division, Beckman Instruments, Inc., Palo Alto, Calif.



muscularly in four sites with 1 ml. of a mixture of equal parts of the above antigen and complete Freund's<sup>8</sup> adjuvant. Four weeks after intramuscular inoculations approximately 30 ml. of blood were collected from an ear vein. Starting one week after bleeding and at weekly intervals for three times, each rabbit was injected intradermally with ½ ml. of antigen. Ten days after the third injection the rabbits were again bled from an ear vein.

Calf 68-41 was bled 24 days following the diarrhetic period and given the above antigen as follows: 24 days, 4 ml. intravenously (i.v.); 38 days, 3 ml. i.v.; 43 days, 3 ml. i.v. and 1 ml. intramuscularly; 51 days, 2 ml. i.v. and ¾ ml. intradermally; and 58 days, ¾ ml. intradermally. At 70 days, the blood was drawn for preparation of a conjugate.

Fluorescein-labeled gamma globulin (FA) was prepared from the postinoculation (PI) bleedings as described by Dunn *et al.* (1966) with the following two changes:

(1) The fluorescein isothiocyanate (FITC),<sup>9</sup> dissolved in 0.5 M sodium carbonate pH 9.0, was added dropwise to the globulin solution to give a final concentration of 25 mg. instead of 20 mg. FITC/gm. protein and 0.05 M sodium carbonate.

(2) The reaction between protein and FITC occurred at 25° C. for 90 minutes instead of overnight in a refrigerator.

## Preparation of Material for FA Technique

Calf 68-48 was inoculated orally with (68-42) filtrate and killed at the onset of diarrhea (16 hours PI). Contents were collected from the upper, middle and lower areas of the small intestine. Intestinal segments 5-7 cm. long from similar areas were ligated, injected with sufficient O.C.T.<sup>10</sup> to slightly dilate the intestine and placed in a -60° C. freezer. Adjacent segments were ligated and filled with 10% buffered formalin.

Pieces cut off the frozen intestinal segments were mounted on a chuck and sectioned approximately 5 μ thick on a refrigerated microtome. Sections were air dried, fixed in acetone and stained with the rabbit and calf origin calf diarrhea, BVD<sup>11</sup> and hog cholera conjugates; and Wright's stain. Formalin fixed tissue was cut with a razor blade, imbedded in paraffin, sectioned at 4 μ and stained with H & E.

Smears of material collected from various levels of the intestine of calves killed, and fecal material from experimental calves (68-22, 24, 29, 30, 31, 42 and 48) and field calves were air dried and fixed in ace-

<sup>8</sup> Difco Laboratories, Detroit 1, Michigan.

<sup>9</sup> Baltimore Biological Laboratories, Baltimore, Md.

<sup>10</sup> Lab-Tek, Westmont, Ill.

<sup>11</sup> Bovine virus diarrhea obtained from National Animal Disease Laboratory, Ames, Ia.

tone. Frozen fecal samples were thawed in a beaker of water at room temperatures, two smears made, and the samples refrozen in an alcohol dry ice bath. One smear was stained with rabbit origin calf diarrhea conjugate and the other smear, from those samples which contained many fluorescing cells, was stained with hog cholera conjugate.

## Results

The cow-calf herd from which the inoculum was obtained has approximately 800 cows and a history of neonatal calf diarrhea each year since 1960.

During the spring of 1967, 275 cows and their 3-6 week old calves were moved from the calving area onto an 800-acre pasture. Eight days later the calves started to have diarrhea and within one week almost all calves in the group were affected. Diarrhea then developed in the younger calves still in the calving area. Material used in this report was collected from these younger animals.

Typically, a calf, normal at night, would be weak, depressed and have watery, yellow feces in the morning. As the interval from the onset of diarrhea increased there were increasing amounts of mucus in the feces, and an occasional animal had green feces containing fairly thick mucus.

Younger calves became dehydrated. Temperatures were usually between 101 and 103.5° F. Calves were treated orally with various antibiotics at the onset of diarrhea and some were given fluids parenterally.

Bacterial examination of feces collected from diarrhetic calves on the ranch revealed a large number of *E. coli*; no *Salmonella* sp. or



Fig. 2. Depressed calf that has a watery, yellow diarrhea.

*Clostridia* sp. were isolated. Other organisms isolated were considered normal. *E. coli* was isolated from many tissues of moribund experimental calves which were killed.

Table 1 lists calves inoculated during the first day of life, the type of calf, route of inoculation, type of material, results and final disposition. All neonatal calves inoculated via the duodenum or orally became sick.

Two to four hours before the onset of diarrhea the calves usually became very depressed, some had mild distention of the abdomen and the majority had saliva hanging from their mouth. Typically, following passage of the meconium, the feces were at first very watery and yellow (Fig. 2). About five hours after onset of diarrhea, as the calf became empty, there was an increase of mucus in the feces.

Fecal material was collected from diarrhetic calves at 30-minute intervals. During the diarrhetic period the calves were extremely depressed—would lie flat with their head and neck extended and shiver. Calves that recovered were generally more alert 12 hours after cessation of diarrhea and within two days were consuming all the milk fed.

Animals reinoculated with filtrate from 12 hours up to 3 weeks following initial diarrhea remained normal. Temperatures prior to and during the diarrhetic period ranged from 101 to 103° F.

All calves listed in Table 1, with the exception of 68-41, 68-42 and 68-43, had at the time of diarrhea large numbers of *E. coli* in their feces. Calf 68-5, a caught calf, had a duodenal canula inserted on the day of birth and was inoculated with cultures of bacteria isolated from 68-4. Diarrhea was not produced.

Calf 68-12, inoculated with field material from a different calf from the same herd as the inoculum for 68-1, developed diarrhea and was very depressed 23 hours postinoculation (PI).

Calf 68-24 developed a watery diarrhea 20 hours after inoculation with 68-22 filtrate. Toward the end of the diarrhetic period a culture of recently isolated *E. coli* was given via the duodenal canula. The feces the first two days following *E. coli* inoculation had a thick cream consistency, and then became thinner so that by the night of the fifth day the feces was watery and yellow. The morning of the sixth day following *E. coli* inoculation the feces were watery and reddish brown. The calf died the morning of the seventh day. Temperatures starting day 0 as the time of *E. coli* inoculation were: 0-102.6, 1-102.0, 2-101.8, 3-103.2, 4-102.6, 5-104.0, 6-102.6.

Calf 68-41, following delivery by hysterectomy, was kept in the hysterectomy hood, inoculated and fed autoclaved milk. This calf developed watery diarrhea 23 hours PI. Fecal material collected at this time and 20 hours PI contained no bacterium that grew on blood agar or in fluid thioglycollate. Calf 68-42 was handled in the same manner. When this calf developed diarrhea 19 hours PI only *B. subtilis* was isolated from the feces.

Table 1. Calves inoculated at 1 day of age.

Calf	How obtained	Route of inoculation	Material	Pas-sage	Results	Final disposition
68-1	C	Canula	Field Feces	0	Diarrhea 15 hours PI	Died day 6 PI
68-2	H	Canula	68-1 Feces	1	Diarrhea 18 hours PI	Killed day 1 PI; temp. 98°
68-3	H	Canula	68-2 Feces	2	Loose stool 24 hrs. PI	Killed day 2 PI; fibrinous peritonitis and suppurative arthritis
68-4	H	Canula	68-3 Feces	3	No defecation	Killed day 3 PI, very depressed
68-5	C	Canula	68-4 Bacteria		Normal feces	Survived
68-7	H	Canula	68-2 Feces	2	Diarrhea 18 hrs. PI	Killed day 2 PI; temp. 99.4°
68-8	C	Canula	68-7 Filtrate	3	Diarrhea 22 hrs. PI	Recovered
68-9	H	Canula	68-7 Feces	3	Diarrhea 21 hrs. PI	Died day 2 PI
68-10	C	Canula	68-8 Filtrate	4	Diarrhea 18 hrs. PI	Killed for tissues
68-11	H	Canula	68-10 Filtrate	5	Diarrhea 21 hrs. PI	Recovered
68-12	H	Canula	Field feces	0	Diarrhea 23 hrs. PI	Killed; very depressed
68-13	H	Canula	68-11 Filtrate	6	No defecation	Killed; very weak
68-16	D	Canula	68-11 Filtrate	6	Diarrhea about 56 hrs. PI	Recovered
68-17	H	Canula	68-11 Filtrate	6	Diarrhea about 36 hrs. PI	Recovered
68-21	H	Injection	68-11 Filtrate	6	Diarrhea about 42 hrs. PI	Recovered
68-22	H	Injection	68-10 Filtrate	5	Diarrhea 32 hrs. PI	Recovered
68-24	D <sup>a</sup>	Canula	68-22 Filtrate	6 <sup>b</sup>	Diarrhea 20 hrs. PI	Died day 7 PI
68-25	H	Oral	68-22 Filtrate	6	Diarrhea 25 hrs. PI	Died day 3 PI
68-36	C	Injection	68-22 Filtrate	6	Diarrhea 19 hrs. PI	Died day 2 PI
68-37	D	Oral	68-22 Filtrate	6	Diarrhea 41 hrs. PI	Recovered
68-38	CS	Oral	68-22 Filtrate	6	Diarrhea 18 hrs. PI	Killed; very depressed, temp. 104.6°
68-39	C	Oral	68-38 Filtrate	7	Diarrhea 20 hrs. PI	Died day 2 PI
68-40	CS	Oral	68-24 Filtrate	7	Diarrhea 20 hrs. PI	Recovered

Table 1. Calves inoculated at 1 day of age (continued).

Calf	How obtained	Route of inoculation	Material	Pas- sage	Results	Final disposition
68-41	H	Oral	68-22 Filtrate	6	Diarrhea 23 hrs. PI <sup>c</sup>	Recovered
68-42	H	Oral	68-22 Filtrate	6	Diarrhea 19 hrs. PI <sup>c</sup>	Recovered
68-43	H	Spray	68-22 Filtrate	6	Diarrhea 25 hrs. PI <sup>c</sup>	Recovered
68-45	C	Oral	68-42 Filtrate	7	Diarrhea 18 hrs. PI	Recovered
68-46	C	Oral	68-22 Filtrate	6	Diarrhea 30 hrs. PI	Died
68-47	C	Oral	68-42 Filtrate	7	Biopsied 13 and 15 hrs. PI	Killed 19 hrs. PI
68-48	C	Oral	68-42 Filtrate	7	Diarrhea 16 hrs. PI	Killed 16 hrs. PI

<sup>a</sup> No colostrum

<sup>b</sup> Inoculated with *E. coli* 28 hours after filtrate

<sup>c</sup> No *E. coli* in feces

C—caught

CS—caesarean

D—dropped

H—hysterectomy

Calves not listed, 68-6, 15, 18, 23, 26, 27, 28, 33, 44, were used in experiments other than those being reported.

Calf 68-43, inoculated with a fine mist of fecal filtrate in the hysterectomy hood, became depressed and would not suck 23 hours PI. Diarrhea started 25 hours following exposure. A few colonies of *B. subtilis* and many colonies of *Neisseria catarrhalis* were cultured from the feces collected during the diarrhetic period and from a rectal swab taken 16 hours following onset of diarrhea.

Rabbit and calf origin calf diarrhea conjugate(s) staining caused bright fluorescence in the epithelial cells of the villi (Fig. 3 and 4) of sections from the upper, middle and lower areas of the small intestine of calf 68-48 and upper area of the small intestine of 68-47.

Fluorescence was not observed when the sections were stained with hog cholera or BVD conjugates or in sections of small intestine from two near term bovine feti, obtained from a local abattoir, stained with rabbit and calf origin calf diarrhea conjugates.

Smears of intestinal contents collected from the above areas of small intestine and feces contained fluorescing cells. These cells when stained with Wright's stain resembled epithelial cells. In sections of formalin fixed segments of intestine taken adjacent to those for frozen sections, the epithelial cells in the lumen appeared to be derived from the ends of the villi.

Fluorescing cells were observed following staining with rabbit origin calf diarrhea conjugate in fecal smears of calves 68-22, 24, 29,

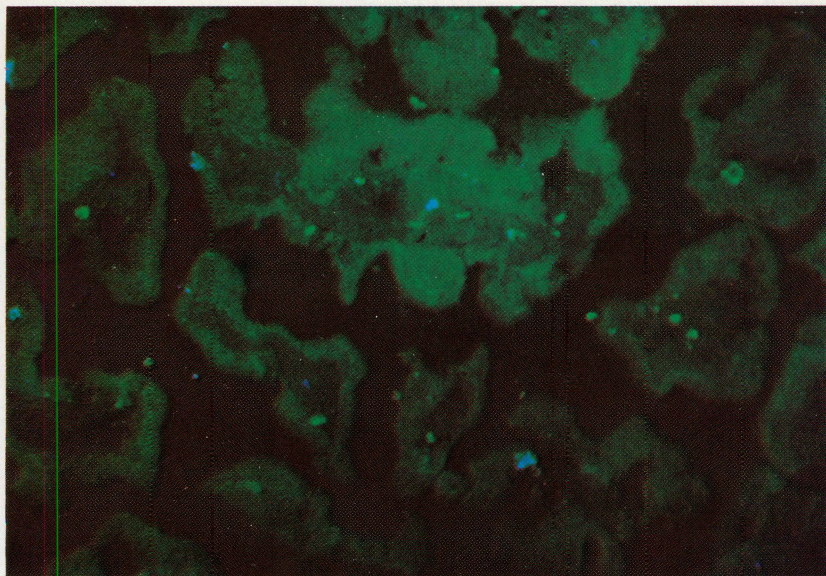


Fig. 3—Section of intestine from a near term bovine fetus, stained with calf scours conjugate. There is no epithelial fluorescence.

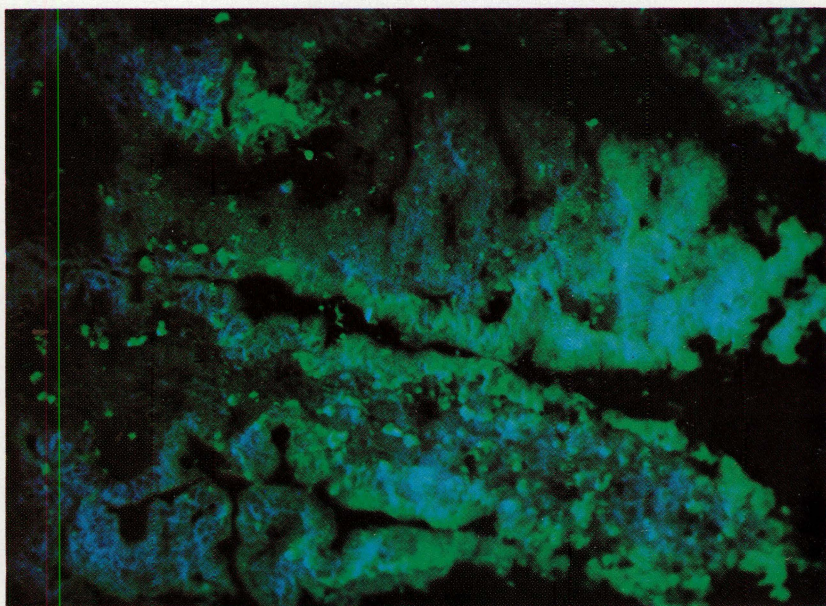


Fig. 4—Section of intestine from a calf (68-48) killed 16 hours postinoculation, stained with calf scours conjugate. The epithelial cells of the villi fluoresce.

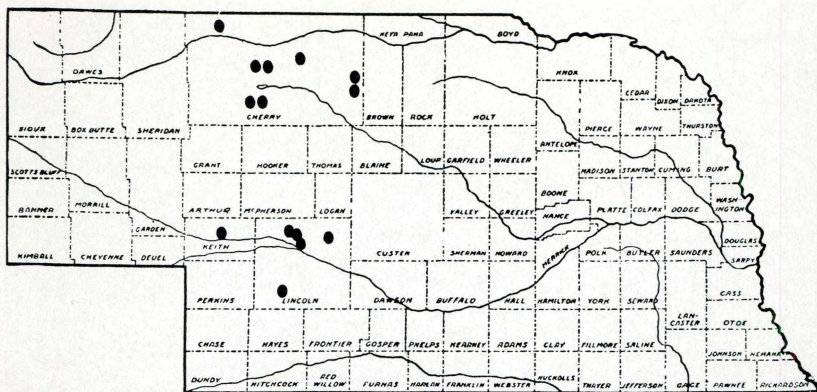


Fig. 5—Locations of herds from which samples of neonatal calf diarrhea contained fluorescing cells.

30, 31, 42, 45 and 28, and in 29 of 51 smears of selected fecal samples from 14 of 15 herds having neonatal diarrhea during the springs of 1967 and 1968. Fluorescence was not observed in smears stained with hog cholera conjugate. Approximate location of the field herds is shown in Fig. 5.

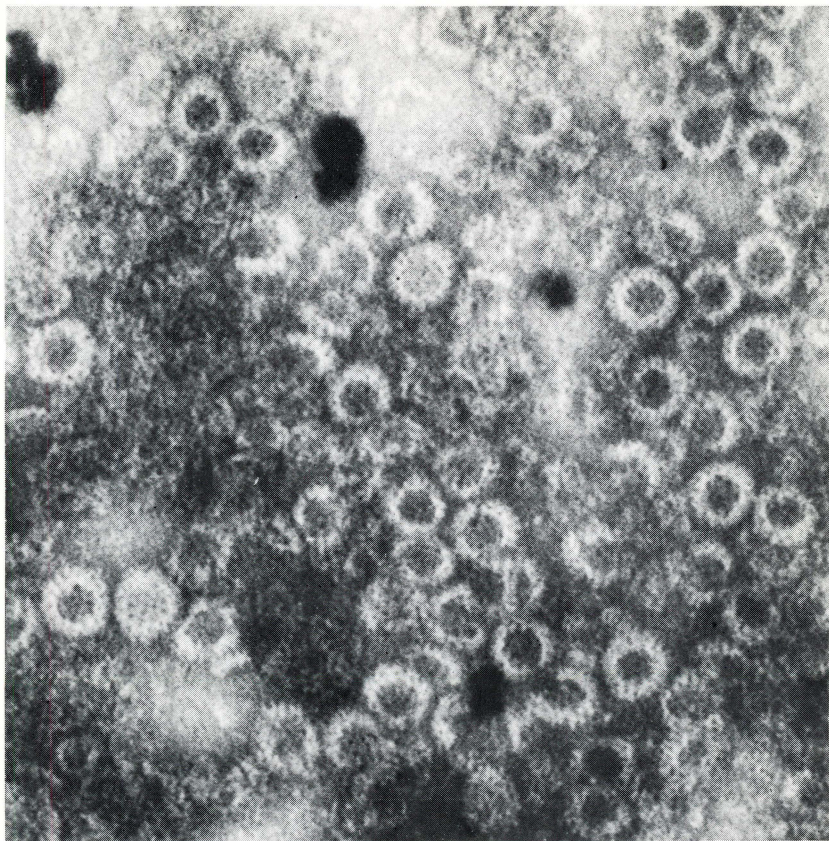
Table 2. Calves used for controls and later inoculation.<sup>a</sup>

Calv	How obtained	Age at surgery	Age at inoculation	Material	Results	Disposition
68-14	H	24 hrs. <sup>b</sup>	6 days	68-11 Filtrate	Brown diarrhea 4 day PI	Recovered
68-19	H	3 days <sup>b</sup>	3 days	68-17 Filtrate	No defecation	Very weak, died 2 day PI
68-20	H	3 days <sup>b</sup>	3 days	68-11 Filtrate	Diarrhea 28 hrs. PI	Died 40 hrs. PI, very weak
68-29	C	18 hrs. <sup>b</sup>	3 days	68-22 Filtrate	Diarrhea about 24 hrs PI	Killed 7 days PI
68-30	C	1 day <sup>b</sup>	5 days	68-22 Filtrate	Diarrhea 4 day old No diarrhea PI	Recovered
68-31	H	5 hrs. <sup>b</sup>		Not inoculated	Diarrhea 3 days	Recovered
68-32	H	1 day <sup>b</sup>	12 days	68-22 Filtrate	Bloody diarrhea 9 days old, diarrhea 6 days PI	Recovered
68-34	C	...		Not inoculated	Diarrhea 8 days old	Recovered
68-35	C	6 days	6 days	68-22 Filtrate	No diarrhea	

<sup>a</sup> These calves were used as controls on experimental surgery and later inoculated with infective material.

<sup>b</sup> Duodenal canula

Calves not listed, 68-15, 16, 17, 18, 21, 22, 23, 24, 25, 26, 27, 28, 33, were used in experiments other than those being reported.



**Fig. 6**—Electron micrograph of viral particles from feces of a diarrhetic calf. The particles have a diameter of approximately 65  $\mu$ . Phosphotungstate negative stain 93,000 X.

Surgery was performed on calves as listed in Table 2 at different ages and the calves were inoculated at varying durations after surgery. None of the calves in which a duodenal canula was placed within 24 hours of birth developed diarrhea until at least three days old. Calves 68-30, 31, 32 and 34 had diarrhea before inoculation. Type of milk fed did not appear to have an effect on severity of diarrhea.

Electron microscopic examination of pelleted material from the feces of a diarrhetic calf revealed many viral particles having a diameter of approximately 65  $\mu$  (Fig. 6).

## Discussion

The inoculation of bacterial cultures via a duodenal canula originally was done in 1967 to bypass the acidity of the abomasum and



flood the upper part of the small intestine with a large number of microorganisms. This was an attempt to reproduce bacterial growth in the upper small intestine as had been described for calf diarrhea (Reisinger 1965).

Calves given these cultures did develop a more loose stool and there was marked reduction in the leucocytic count. Since some effect was observed, field fecal material was inoculated via the canula.

Neonatal calf diarrhea produced experimentally was very similar to the disease observed on the ranch from which the inoculum was obtained. Reproduction of diarrhea with bacteria-free filtrates containing viral particles indicated that a virus was the probable cause of diarrhea but due to the presence of *E. coli* in the diarrhetic material it was necessary to produce diarrhea in calves not contaminated with *E. coli*. This was done in calves 68-41, 68-42 and 68-43.

The spray inoculation of calf 68-43 served two purposes. First, this method of inoculation indicated that a very small quantity of filtrate was required to cause diarrhea; and second, it eliminated the possibility of an endotoxin or other irritant in the filtrate as a cause of diarrhea.

The marked epithelial fluorescence in sections of small intestine collected at the onset of diarrhea and the fluorescing cells in the feces of other experimental calves also substantiated the role of the virus in the experimentally produced neonatal diarrhea. The location of herds from which fluorescing fecal samples were obtained reflects the two areas in which field investigations were made.

Calves in Table 2 on which surgery was performed and/or held for varying periods before diarrhea developed also substantiated the role of the inoculum, and the results reported in Table 1. Diarrhea in calves 68-30, 31, 32 and 34 was believed to have resulted from accidental infection from diarrhetic calves in adjacent rooms. The presence of fluorescing cells in the feces of calves 68-30 and 68-31 support this hypothesis.

On the basis of this research, we believe that the neonatal diarrhea produced in experimental calves was caused by a virus which produced a severe diarrhea for a six to eight hour period. When noninvasive strains of *E. coli* were present, the calf recovered in one to two days; however, when an invasive strain of *E. coli* was present there was an intestinal overgrowth of *E. coli* followed by a septicemia. The calf then developed a temperature of 104 to 105° F. and died in three to five days.

## Summary

Neonatal calf diarrhea typical of that seen in field cases was produced in colostrum-deprived calves by inoculating either feces or bacteria-free filtrates via the duodenum, orally, or by a spray (aerosol).

Three calves, kept free of *E. coli*, developed severe diarrhea following inoculation with a bacteria-free filtrate.

Intense fluorescence in the epithelium of sections of small intestine from two experimental calves was observed following staining with fluorescein-labeled gamma globulin produced with viral antigen prepared from the feces of two experimental diarrhetic *E. coli*-free calves. Fluorescing cells were also present in the feces of experimental and field cases of neonatal calf diarrhea. The etiologic agent of the calf diarrhea reported here is believed to be a virus, observed by electron microscopy, having a diameter of approximately 65 m $\mu$ .

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