

THE ETIOLOGY OF INFECTIOUS DIARRHEA (WINTER SCOURS) IN CATTLE

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To judge from information from various sources there exists in certain dairying sections of this country an epidemic form of diarrhea among cows. Such outbreaks are said to occur during the fall and winter months and for this reason the disease is frequently referred to as winter dysentery. Little is known concerning its etiology, and although both Steffen (1) and Marshall (2) recognize it as an infectious disorder, others hold the view that it is a dietary disease without specific cause. In the main the disease is said to be an afebrile one characterized by the passage of dark brown or blackish brown liquid feces, and because of this fact it is known in certain localities as black scours. The mortality varies; Steffen states that the condition is rarely fatal but Marshall reports 16 deaths among 62 affected cows. All agree that the disease seriously affects the milk yield both during and subsequent to the attack.

The Characterization of the Disease

In all we have had access to 5 herds in which a total of 400 cows were affected.¹ In the main there was relatively little fever although in one small group in which three fatalities occurred two had temperatures of 39.6° and 40.2°C. respectively. The onset appears suddenly; usually a few cows begin to scour, then the diarrhea may spread rapidly throughout the particular group or, as we observed in two

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outbreaks, to all the adults in the herd. As a rule the calves are not appreciably affected.

In general the more severely affected animals are dull, refuse food, and at times exhibit signs of abdominal pain. The feces are fluid, in some cases fetid, usually deep brown to greenish black in color, and often contain blood and mucus. The milk yield is greatly diminished. The diarrhea usually runs a relatively short course from a few hours to 4 or 5 days, when the feces become firmer, the general health improves, and the milk yield begins to increase. In certain instances cows apparently recover but when placed on full diet diarrhea recurs. At times the onset is accompanied by a dry cough and excessive salivation. As a rule the respirations are rapid.

Four cases were autopsied. One was from a small herd where two other cows had died of the disease, two others were slaughtered when acutely ill, and the fourth was a cow which had suffered from an acute attack and after apparent recovery again scoured when heavily fed.

Examination of material from these individuals indicated a catarrhal inflammation of the small intestine (jejunum and ileum) inasmuch as the serosal vessels were injected, the intestinal walls edematous, and the mucosa swollen, wrinkled, and congested. The intestinal content was fluid, usually dark brown in color, and admixed with mucus. The liver in three instances was ochre color, dry in consistency, and readily fractured when bent, and the gall bladder was engorged with dark bile. The heart muscle was dry and brownish red in color. In addition the folds of the abomasum were congested and edematous. The mesenteric lymph glands were enlarged, pale, and juicy.

Histologically well marked changes were observed in the sections of the small intestine where a well defined vascular engorgement was noted. The superficial mucosa was degenerated and overlain with mucus and disintegrated cells. The mucosa was infiltrated with round cells and leucocytes, the vessels of the submucosa engorged, and the connective tissue edematous. Sections of the liver in three of the four cases revealed hydropic degeneration of the parenchymatous cells.

Bacteriological Findings from the Spontaneous Cases

Cultures from liquid feces from acute cases in all outbreaks were made on lactose agar plates containing indicator. From the cows in three herds actively motile Gram-negative rods which failed to ferment lactose were obtained, and at times such organisms made up 90 per cent of the organisms on the plates. From other cases in the same herd they could not be cultivated. Later organisms of this type were correlated with *B. coli mutable* and when fed to healthy calves failed to establish themselves in the intestinal tract.

Blood cultures from acute cases failed to develop suggestive organisms. Media inoculated with bits of organs from the slaughtered animals remained sterile, and inoculations from the intestinal content and mucosa of various portions of the small intestine revealed either *B. coli* or mutable colon bacilli in enormous numbers from all portions of the small intestine.

The only evidence indicating that the disease was an infectious one seemed to lie in the fact that it spread from group to group, but this fact might be explained by some toxic substance in the food or drink. It seemed essential to attempt to reproduce the disease, and our first protocols deal with such experiments.

EXPERIMENTAL

A 4 months old calf (1629) was fed with a small quantity of intestinal content obtained from the jejunum and ileum of a spontaneous infection. The spontaneous case originated in a large dairy herd where diarrhea had been prevalent for 6 weeks. This case was one of the last to appear and after apparent recovery again had diarrhea when heavily fed. The calf developed diarrhea 3 days after feeding, the attack lasting 2 days. Diarrhea was noted again on the 7th and 14th days. The animal was slaughtered 16 days after the feces were administered.

At autopsy a well defined inflammation involving the duodenum, jejunum, and upper portion of the ileum was found. The lesions consisted in a well marked engorgement of the vessels of the serosae. The intestinal walls were thickened. The mucosa was swollen and varied from bright red to pink in color. The content was slimy and tinged with bile. Peyer's patches and solitary follicles were frequently swollen and often overlain with deep red swollen mucosa. There was little abnormal observed in the large intestine. The spleen, liver, and kidneys appeared normal.

Histological examination of fixed and stained material revealed well defined changes in the small intestine; frequently the superficial mucosa was degenerated and often overlain with mucus, leucocytes, and necrotic cells. The capillaries of the mucosa were engorged and leucocytes had invaded the mucosa in large numbers. The secreting layer of the mucous glands had been invaded by leucocytes and aggregates of such cells at times plugged the lumen of the gland. The sub-mucosa was congested and edematous.

The inoculation of lactose agar plates with the contents of various segments of the small intestine revealed large numbers of *B. coli* throughout the jejunum and ileum but no other significant organism.

Bits of the mucosa from the jejunum were washed in several changes of sterile salt solution, ground in a glass grinder similar to that recommended by Hagan (3), and the ground material mixed with sterile salt solution. This suspension was

inoculated into the condensation fluid of tubes of slanted agar to which a few drops of horse blood agar had been added. From the first tube two others in series were inoculated. All tubes were then sealed with sealing wax and after suitable incubation the condensation fluid was examined. Frequently the tubes inoculated directly with the suspension developed rapidly growing organisms, but from the secondary tubes what appeared to be pure cultures of tiny motile vibrios were obtained.

The next calf (1655) was fed liquid feces, mixed with salt solution, from three acute cases of diarrhea. The spontaneous disease had attacked about half the herd up to the time of our visit and within the next week practically every animal developed the disease. The feces which we fed to Calf 1655 contained besides *B. coli*, *B. coli mutable* in considerable numbers. Little of note indicative of enteritis was observed as the result of the artificial infection except that the calf was constipated, the feces clay colored and always contained large masses of clay colored mucus. The animal was slaughtered 12 days after the feces were fed. On autopsy the liver was yellowish brown in color and friable, and on section the color extended throughout. The cut surfaces were granular. The upper and middle portions of the jejunum revealed gross changes similar to those observed in the intestines of Calf 1629. In addition the mucosa of the ileum was swollen and reddened for a distance of 1 m. above the ileocecal valve. Edema and congestion of the leaves of the abomasum were also noted.

Essentially the same histological picture as that encountered in the case of Calf 1629 was noted in the fixed and stained material from Calf 1655. In the latter case the liver revealed distinct changes such as nuclear degeneration of the liver cells and hydropic infiltration of the cytoplasm of such cells, and passive congestion.

Nothing significant developed in the lactose agar plate cultures inoculated with content of the jejunum and ileum. *B. coli* was not found above the middle ileum.

Media inoculated with bits of liver, spleen, and kidney remained sterile. Fragments of the inflamed mucosa of the jejunum were treated in the manner previously described, and when ground, suspended in broth, and inoculated into blood agar, developed after suitable incubation under seal vibrios that resembled those obtained from Calf 1629.

The third calf (1641) when 16 days old was fed feces mixed with salt solution from three acute cases of diarrhea. This material was obtained from another large herd where the disease within the course of 7 days attacked over 160 animals. The scouring was severe in the milch cows but relatively mild in the bulls and young stock. The feces contained no cultivable organism of the colon group other than *B. coli*. Within 24 hours the calf was depressed, and on the 2nd day there was an elevation of temperature (39.5°C.). On the 3rd day the depression was more marked, and the feces were soft, yellow in color, and fetid. The calf's condition improved on the 4th day and it was slaughtered on the 5th day. Autopsy revealed an orange-red liver with rounded borders. There was a well marked patchy inflammation of the mucosa of the small intestine extending

throughout the jejunum and into the anterior half of the ileum. Here the mucosa was reddened, swollen, and frequently overlain with tenacious mucus. The folds of the abomasum were swollen and reddened. The intestinal content was yellowish white in color and contained large quantities of mucus.

Histological examination of sections of the liver revealed well marked cloudy swelling of the parenchymatous cells. The histological changes encountered in sections of the intestine were similar to those found in the two other calves.

B. coli as indicated by lactose agar plate cultures was present in large numbers throughout the ileum and in smaller numbers in the jejunum. Pure cultures of actively motile vibrios developed in the tubes inoculated with the washed mucosa of the upper jejunum but from the middle jejunum and ileum only mixed cultures were obtained.

The experiments indicated that when feces or intestinal content from cases of diarrhea were mixed with salt solution and administered to calves by mouth a definite reaction followed. In two instances a slight temperature reaction accompanied by general depression occurred but in all cases a catarrhal enteritis was found at autopsy. In two instances the feces became soft and fetid and in the other case the feces contained large quantities of mucopurulent material. The administration to calves of feces from spontaneous cases resulted then in a disease of the small intestine accompanied by a mild general reaction. The disease although less severe simulated the malady encountered in the older cows.

In addition from the experimental cases we had succeeded in obtaining cultures of tiny, actively motile vibrios from involved portions of the intestine which might be of etiological significance. It is of interest to note that we had seen organisms of similar morphology in preparations of mucus obtained from the feces of cows with diarrhea and that they had been found in small numbers in mixed cultures made from bits of this mucus.

To establish the relationship of these vibrios to the disease a series of experiments were undertaken. The results are recorded in Table I.

It will be noted that the cultures when mixed with the feed or suspended in salt solution and administered by mouth were capable of producing in most instances recognizable symptoms. Inflammation of the mucosa of the jejunum and anterior ileum was a constant finding. From two of the experimental calves (1669 and 1686) a large number of pure cultures of vibrios were cultivated. Cultures from

TABLE I
The Effect of the Aural Administration of *Vibrios*

| Animal | Age | Fed | Symptoms | Autopsy | Bacteriological findings |
|-----------|---------|--|---|--|--|
| Calf 1669 | 26 days | 2 cultures Calf 1629 1 blood agar Calf 1641 1 leptospira Calf 1655 | Rise of temperature, diarrhea with mucus for 3 days. Killed on 7th day | Cloudy swelling of liver cells; well marked inflammation of lower duodenum, jejunum, and upper half of ileum | Many cultures of vibrios from two portions of jejunum |
| Cow 1683 | 15 mos. | 5, 72 hr. blood agar cultures of vibrios from Calf 1669, 3rd transfer | Rise of temperature, suppression of milk. Passage of bloody mucus containing vibrios, mild diarrhea on 5th day. Killed on 6th day | Inflammation of the mucosa of jejunum and ileum | Vibrios mixed with spore-bearing bacilli. Pure cultures not obtained |
| Calf 1686 | 15 days | 2, 48 hr. blood agar cultures from Calf 1669, 13th transfer | Little general reaction, large quantities of mucopurulent material in feces. Killed on 5th day | Severe inflammation of jejunum and upper half of ileum | Vibrios in large number throughout jejunum and upper ileum |
| Calf 1630 | 3½ mos. | 3 blood agar cultures from Calf 1655 | Diarrhea during first 3 days, rapid recovery. Killed on 8th day | Mild inflammation of jejunum and upper ileum | Vibrios not obtained |

the young cow (1683) also contained vibrios but in every instance mixed with rapidly growing bacilli from which they could not be freed. The other calf (1630) developed a mild diarrhea and at autopsy the lesions although not pronounced could be recognized but the organism could not be cultivated.

The only other organisms which might be regarded as of etiologic significance were the mutable colon bacilli. In certain cases they comprised over 90 per cent of the organisms present in the plate cultures made from feces or intestinal contents but in many cases they were not found. Young broth cultures in amounts of 35 and 40 cc. were administered to calves but as far as we could determine they failed to establish themselves in the intestinal tract. In addition the feces fed to Calf 1641 contained mutable colon bacilli in large numbers but we failed to find them in the fecal cultures or in the intestinal tract at autopsy.

DISCUSSION

Our studies indicate that there exists an infectious disease of cows characterized by severe diarrhea accompanied by marked suppression of milk secretion. In general the same type of symptoms were noted among animals in widely separated herds, and when it was possible to obtain material at autopsy the same lesions were found. Mention has been made of the observations of Steffen and Marshall who characterize the disease with which they dealt as an epizootic dysentery. Our experience indicates, to judge from the natural and experimentally produced disease, that the inflammation has a different distribution from that encountered in human dysentery. The small intestine, particularly the jejunum and upper ileum, seem to be the principal locus.

That the condition is an infectious one there can be little doubt. However, for the purpose of discussion, attention must be given to dietary factors. As a rule the first portion of the ration to be looked at askance is the ensilage. In the first outbreak we encountered the sick received the same ensilage as the older cows in which there was no diarrhea. In another no ensilage had been fed, the animals receiving only the food to which they were accustomed. Two other outbreaks afford strong arguments that the disease is infectious. It is true that

in one of these some of the first cases occurred among cows fed from an old lot of ensilage; nevertheless cows in the same barn fed beet pulp instead of ensilage came down with the disease, and 2 or 3 days later diarrhea appeared among the cows in other barns where there was no question raised as to the quality of the feed. Cows on outlying farms were later affected, where all feed stuffs were of different lots. Evidence in another instance is indicative of the infectious nature of the malady. Here the first cases occurred among the cows in a wing of the main barn, the disease next appearing in the main barn and other large barns connected by runways with it. There had been no change in the character of the feed prior to the outbreak. Direct proof of the infectious nature of the diarrhea is afforded by the findings here recorded when calves were fed suspensions of feces from spontaneous cases. Two of the calves developed diarrhea and the third clinically manifested enteritis by the passage of large quantities of mucus covered feces and mucopurulent material. All three when killed revealed well defined characteristic inflammation of the small intestine. In all three cases vibrios similar in morphology were obtained from the intestinal tract.

In regard to the nature of the etiological agent it may safely be said that the more common types of organisms which would be suspected in such a disease have been ruled out by the methods employed. If organisms of the colon-typhoid group were responsible it is probable that our methods would have demonstrated them, since we readily cultivated *B. coli mutable* from the feces or intestinal tract of certain spontaneous cases. These when fed to calves in amounts as high as 45 cc. of 18 hour broth cultures failed to produce disturbances or, as far as we could tell, to establish themselves in the intestinal tract.

The only organisms encountered which appeared to offer etiologic possibilities were the vibrios isolated from the inflamed intestines of calves which developed enteritis as the result of the ingestion of feces from spontaneous cases. Such organisms had been seen in preparations of mucus obtained from spontaneous cases and in one instance vibrios had been found in mixed cultures made from mucus obtained from the feces. Three calves when fed these cultures developed diarrhea or other evidences of enteritis. A young cow fed a single strain recently isolated from one of the experimental calves developed diar-

rhea with blood stained mucus in the stools, accompanied by almost complete suppression of milk secretion. All four cases had on autopsy an enteritis similar to that encountered in the spontaneous disease and in the calves fed feces from spontaneous cases. From the intestines of two calves fed these cultures vibrios were recovered in pure culture. From the cow vibrios were obtained in mixed culture only, and from the third calf the tubes inoculated with suspension of inflamed intestinal mucosa remained sterile.

SUMMARY

A disease of cows manifested by severe diarrhea has been described. The condition is characterized by the frequent passage of dark brown or black feces, often containing mucus and blood. The principal lesions are catarrhal inflammation of the small intestine and liver degeneration.

By feeding feces from spontaneous cases to calves a similar but milder disease characterized by the same type of enteritis was produced. Vibrios were cultivated from the inflamed intestinal tract of such experimentally induced cases. Pure cultures of the vibrios when fed to other calves, in certain instances, produced diarrhea and a well marked enteritis similar to that observed in both the spontaneous disease and in calves following the feeding of feces from naturally affected cows. Vibrios were recovered from the inflamed small intestine of three out of four animals fed such cultures.

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