

VIBRIONIC ENTERITIS IN CALVES

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In a previous paper (1) we called attention to a disease of adult cows characterized by profuse diarrhea from which by indirect means we obtained vibrios and these when fed to healthy calves produced a well defined enteritis. It seemed likely that organisms of this class might be responsible for certain forms of enteritis in calves, especially inasmuch as various types of intestinal disease of obscure etiology are known to exist. That vibrios can be the cause of a more or less chronic intestinal disease is brought out in the present paper.

The Disease as Encountered among Calves

During the latter months of 1929 and 1930 many of the older calves in a large dairy herd were affected with a complication of diseases. Through the kindness of Dr. Theobald Smith who studied the respiratory aspect of the problem, we obtained several gastrointestinal tracts of calves suffering with pneumonia but later many cases were encountered where the cause of death or general debility was directly correlated with intestinal disease.

As a rule calves over 2 weeks old were affected. The unthrifty appearance of such animals attracted attention. The tail and buttocks were often covered with feces and the abdomen distended. The feces were soft or gummy in consistency and varied from clay colored to dark brown in color. They frequently contained blood and mucus and when suspended in water were noted to contain irregular, firm, sticky masses, and these when crushed were found on microscopic examination to contain large numbers of leucocytes often embedded in mucus. As a rule the disease as we have encountered it is more or less chronic but a few cases dying after a short illness have been seen. During the later portion of 1929 and the early months of 1930 only a few sporadic cases appeared but with the onset of colder weather a well marked outbreak occurred, and the disease has been

largely responsible for the slaughter or death of many calves. We have tabulated the more important pathological and bacteriological findings of a number of cases.

TABLE I
The Distribution of Intestinal Lesions and Bacteriological Findings in the Spontaneous Disease

No. of calf	Age	Distribution of lesions	Bacteriological findings	
			Vibrios	The distribution of <i>B. coli</i> as determined by lactose agar plates
	<i>days</i>			
1652	18	Inflammation of small intestine	Pure culture from lower jejunum and upper ileum	
1682	57	Patchy inflammation of jejunum and upper ileum	Mixed culture of vibrios from jejunum	<i>B. coli</i> throughout jejunum and ileum
1699	50	Inflammation most marked in middle jejunum and upper ileum	Vibrios in pure culture from middle jejunum and upper ileum	<i>B. coli</i> and streptococci from middle jejunum and ileum
1700	28	Lower half of jejunum and upper half of ileum	Vibrios in pure culture from the middle jejunum	<i>B. coli</i> not found in jejunum
1728	18	Inflammation throughout jejunum. Severe inflammation in ileum extending close to ileocecal valve	Pure culture from middle jejunum. Vibrios mixed with <i>B. coli</i> in ileum	Middle jejunum sterile. <i>B. coli</i> present in middle ileum
1731	68	Inflammation throughout small intestine	Vibrios not obtained from intestine. All tubes inoculated with bits of liver developed vibrios	<i>B. coli</i> throughout ileum
1745	51	Patchy inflammation of jejunum. Severe inflammation throughout ileum	Vibrios in pure culture from lower ileum	<i>B. coli</i> as far as the middle ileum
1748	56	Inflammation of the abomasum and small intestine	Pure cultures of vibrios from duodenum and mixed from stomach and remainder of small intestine	<i>B. coli</i> in liver and small intestine below the middle jejunum

From Table I it can be said that most of our material was obtained from calves between 18 and 68 days old, but in all probability the ani-

mals are infected relatively early in life. Mention has been made of the unthrifty appearance of the calves frequently accompanied by chronic diarrhea. Autopsy reveals well marked intestinal lesions.

The folds of the gastric mucosa (abomasum) are often reddened and swollen. The small intestine, from the duodenum to the ileum, reveals well marked changes. The vessels of the serosae are injected and the intestinal walls appreciably thickened. The mucosa is reddened, the folds enlarged, and the solitary follicles swollen and overlain with reddened mucosa. The content is rich in mucus, desquamated epithelial cells, and leucocytes. The liver when involved is yellowish brown in color and fractures easily. The gall bladder is usually well filled with normal appearing bile. The mesenteric lymph glands are pale and enlarged. Vibrios are at times readily demonstrated in properly prepared films from involved portions of the intestine. In other cases they are not readily found. Histologically the intestinal lesions consist of superficial degeneration of the mucosa, engorgement of the vessels of the mucosa, and infiltration of the cores of the villi and mucous layer with round cells and leucocytes. Leucocytic infiltrations into the lumen of the mucous glands are not uncommon, and edema of the lymphoid structure of the solitary follicles with leucocytic infiltration, congestion, and edema of the submucosa is usually found. At times hyperplasia of the endothelial elements of the submucosa has been encountered. The liver cells may be degenerated and infiltrated with fat.

Since most of our cases were regarded as of some duration, it seemed desirable to endeavor to ascertain the nature of the acute disease as it occurred spontaneously. With this in view young vigorous calves were mingled with those presenting chronic symptoms and later slaughtered at an abattoir. The more important details are given in Experiment 1.

Experiment 1.—Three calves ranging in age from 6 to 8 days were housed with a 2 months old calf which presented symptoms of chronic enteritis. Vibrios had been obtained from the inflamed intestines of older calves kept in the same pen. Two younger calves from the same source as those exposed to natural infection were slaughtered at the time the other three were exposed. Their intestinal tracts appeared normal and vibrios were not found. The calves were under observation for periods of 14, 16, and 21 days respectively.

Calf 1707 had fever and diarrhea 10 days after exposure and the feces contained blood. Diarrhea persisted until the 14th day when the calf was slaughtered. Characteristic severe inflammation of the upper and middle jejunum and the lower portion of the ileum was found. Many apparently pure cultures of vibrios were obtained from the washed and ground mucosa from inflamed regions. In addition pure cultures of vibrios were also obtained from the liver. *B. coli* was

present in the content in large numbers throughout the ileum and in the lower jejunum; above this region it was present in smaller numbers.

10 days after exposure the feces of Calf 1709 were soft and contained blood. The calf had fever. It was slaughtered 21 days after the experiment started. A moderately severe enteritis which began in the upper portion of the jejunum and extended practically throughout this region of the bowel was encountered. A more severe inflammation was observed in the lower portion of the ileum. Vibrios were obtained from the upper and middle jejunum but below this point the cultures were overgrown with the ordinary intestinal forms. Cultures from the liver failed to show vibrios but all contained anaerobes. Lactose plate cultures revealed *B. coli* in large numbers throughout the jejunum and ileum.

Calf 1710 revealed symptoms of gastric disorder and scoured on one occasion. When slaughtered on the 14th day it revealed a mild patchy congestion of the mucosa of the upper jejunum and ileum. Vibrios were not found in the cultures from the intestinal tract and liver. *B. coli* was present in the content in large numbers throughout the small intestine.

The experiment indicates that infection takes place relatively early in life and that in the main the disease encountered in the herd is a relatively chronic one, but the location and general type of reaction encountered in the acute disease is identical with that manifested in cases of longer standing. Furthermore the negative results obtained by the examination of two calves originating from the same source and born about the same time as the calves used in the experiment strengthen the argument that infection is acquired from the spontaneous infection in the older calves and that the disease may be maintained by the introduction of susceptible individuals.

Inasmuch as the indications pointed to the vibrios as the etiological agent it seemed desirable to test their pathogenicity on calves and the following experiment covers this phase of the problem.

Experiment 2.—Calf 1714 when 16 days old was fed three 48 hour blood agar cultures of vibrios obtained from the middle jejunum of Calf 1707. The cultures had been under cultivation for 3 months and during this period had been transferred eleven times. Symptoms indicative of enteritis, other than a slight general depression 2 days subsequent to feeding the culture, were not noted. When the calf was slaughtered 7 days after artificial infection a mild gastrointestinal catarrh was found. The principal involvement was in the upper and middle jejunum and irregularly scattered areas in the ileum where the mucosa was swollen and congested. Vibrios were obtained from the upper jejunum but the primary cultures from the middle jejunum and ileum were overgrown with *B. coli* and vibrios were not obtained from these regions. The liver was sterile.

On the whole the reaction was mild. The intestinal process seemed to be regressing but the organism was recovered. It seemed possible that the culture might have lost some of its virulence on cultivation, so that it was decided to test the pathogenicity of a freshly obtained strain and for this purpose the vibrios obtained from Calf 1714 were chosen.

Calf 1721 when 18 days old was fed six 72 hour blood agar cultures of vibrios obtained from the jejunum of Calf 1714. The cultures were of the third transfer and the strain had been under cultivation for 3 weeks. There was an increase in bodily temperature for the first 2 days following artificial infection and during this time the feces became dark, soft, and fetid. These were the only clinical manifestations noted except that the feces throughout the observation always contained large quantities of thick yellow mucus. In certain instances this material made up about half the stool. Microscopically it was composed of thick mucus loaded with leucocytes and epithelial cells. On the 5th day the feces became much softer and the animal was slaughtered.

In contrast to the ill defined clinical manifestations there was a severe inflammation of the small intestine which began at the pylorus and extended throughout the duodenum, jejunum, and the anterior half of the ileum. The intestinal walls were thickened, the vessels of the serosae injected, and the mucosa swollen, wrinkled, and bright red in color. The content was slimy and below the middle jejunum bile-stained. The liver appeared normal.

Many cultures of vibrios were obtained from the washed mucosa of the upper and middle jejunum and from the middle ileum but below this point the tubes were overgrown with *B. coli*. The tubes inoculated with bits of liver remained sterile. Lactose agar plate cultures revealed small numbers of *B. coli* in the content of the middle ileum and large numbers below this point. No *B. coli* and relatively few other colonies developed on the plates filmed with content from the duodenum and jejunum.

Both calves fed cultures of the vibrios manifested relatively slight clinical manifestations, but the organisms were nevertheless recovered from inflamed portions of the bowel. The inflammatory process in both instances resembled that found in the spontaneous disease and those cases which contracted the natural infection under experimental conditions.

DISCUSSION

A disease affecting calves from 18 to 68 days old has been described. In general it may be said that the clinical picture, other than a general condition of malnutrition and unthriftiness often accompanied by attacks of diarrhea and an irregular, moderate increase in bodily temperature, is confusing. Our data indicate that infection may take place relatively early, but the animals manifest at first only slight clinical manifestations, the symptomatology becoming more pro-

nounced as the disease becomes more chronic. That such animals may become a ready prey to other infections must be recognized. Our first cases seemed to be in general of this class and it was assumed that the intestinal disease had been superimposed on other conditions. The experiment in which young vigorous calves were exposed to older calves regarded as chronic cases of enteritis indicated that such infections may be acquired relatively early and may for a time be overlooked. A further argument that the disease is an independent one, and as such is not dependent on a preexisting one, is that calves may actually die as the result of such infection. Under certain conditions when the malady becomes chronic the clinical effects become so pronounced that slaughter is advisable. In one large herd many calves between the ages of 1 and 2 months have recently been slaughtered because of this disease.

The distribution of the lesions is characteristic. The upper half of the jejunum seems to be the most frequent locus, but inflammation of the mucosa of the abomasum is not uncommon and inflamed patches often of considerable size are frequently encountered in the upper, middle, and lower ileum. The process is one of congestion, loss of superficial mucosa, infiltration of the mucosa with leucocytes and round cells, and edema and congestion of the submucosa. The exudate overlying the inflamed mucosa and appearing in the feces is mucopurulent.

The etiology seems clear; the vibrios which were cultivated from the more chronic spontaneous disease, and from the young calves contracting the infection in a natural manner, all appeared alike in morphology and were all actively motile. In addition they all possessed certain well defined nutritive requirements. At times they could be readily demonstrated in films of the inflamed mucosa and were frequently found in many cultures from various portions of the involved intestine. However, in cases of long standing they are not easily obtained, since *B. coli* and other intestinal forms thrive readily in the content and serve to suppress the growth of the delicate vibrios in the test tube. In three instances they were cultivated from the liver. Experimental proof exists that vibrios that have been under cultivation for considerable periods still possess the property of passing from the mouth through the stomach and localizing in the small intestine and there inciting a process resembling that found in the spontaneous infection.

SUMMARY

An intestinal disorder of calves has been described. The clinical manifestations are usually observed in calves 2 or more weeks old. Our experiments indicate that infection may take place relatively early in life and may for a time produce only a mild reaction, but as the disease becomes more chronic the clinical manifestations become more pronounced. The anterior portion of the jejunum is the primary locus of infection but in more chronic cases practically the whole small intestine may be involved. Vibrios were cultivated from the inflamed intestinal mucosa in both the acute and more chronic spontaneous cases. Vibrios were also obtained from the acutely involved intestine of young calves experimentally exposed to natural infection. On three occasions similar vibrios were found in cultures from the liver. When a single strain of vibrios which had been under cultivation for 3 months was fed to a young calf subclinical infection was produced and the organism recovered. This strain after three passages on media when fed to a calf produced a severe inflammation of the jejunum and ileum and from these areas the organism was recovered.

REFERENCE

1. Jones, F. S., and Little, R. B., *J. Exp. Med.*, 1931, **53**, 835.