Clinical and Pathological Observations on the Experimental Passage of Swine Dysentery

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ABSTRACT

The length of incubation for 36 eight and 12 week old swine in eight experimental passages averaged 11 days and ranged from five to 24 days. The duration of diarrhea for 24 of these swine averaged 6.4 days and ranged from two to 19 days. The consistent macroscopic lesion was a colitis and, subsequently, a typhlitis. In the swine euthanized on the first day of diarrhea, the colitis was most intense in the coils near the apex of the colon and, frequently, these swine had a hyperemia of the fundus of the stomach. The amount of visible blood in the colon varied. Organisms identified microscopically and ultrastructurally as spirochetes were observed commonly in the feces and the mucosal glands of the colon of swine with a diarrhea, but not in the adjacent mesenteric lymph nodes. These spirochetes which were the most numerous on the first day of diarrhea, could not be isolated and propagated in vitro. Swine which recovered naturally or were medicated at the height of a diarrhea, developed a resistance to swine dysentery. Colon from infected swine remained infectious when stored at -77°C for nine months but not when stored at -16°C. Feces from infected swine were not infectious after lyophilization and storage at -12°C.

RÉSUMÉ

Au cours de huit passages expérimentaux effectués chez 36 porcs âgés de huit et 12 semaines, la période d'incubation de la dysenterie varia de cinq à 24 jours et dura en moyenne 11 jours. Chez 24 de ces sujets, la diarrhée persista en moyenne 6.4 jours, allant cependant de deux à 19 jours. La lésion macroscopique constante se traduisit par une colite, suivie de typhlite. Chez les porcs sacrifiés dès le début de la diarrhée, la colite s'avéra particulièrement intense dans les replis avoisinant l'apex du côlon et la muqueuse de la grande courbure de l'estomac manifesta souvent de l'hyperémie. La quantité de sang décelable dans le côlon s'avéra irrégulière. On observa souvent dans les fèces et les glandes de la muqueuse du côlon des porcs diarrhéiques, mais non dans les ganglions mésentériques correspondants, des organismes que la microscopie et l'ultra-structure permirent d'identifier comme spirochètes. On ne put isoler et cultiver in vitro ces organismes que l'on observa en plus grand nombre le premier jour de la diarrhée. Les porcs qui guérirent naturellement ou que l'on traita au plus fort de la diarrhée devinrent résistants à la dysenterie. Le côlon des porcs infectés conserva sa virulence pendant neuf mois lorsqu'on le congela à -77°C, mais non à -16°C. Les fèces de ces sujets perdirent leur virulence à la suite de la lyophylisation et de l'entreposage à -12°C.

INTRODUCTION

Many of the clinical observations on swine dysentery have been made on infected herds and not on individual swine (10). The length of the incubation period has been known to vary considerably. Some have questioned whether swine develop an enduring immunity to the disease (13). Previously, *Vibrio coli* was considered to be the cause of swine dysentery (1-4, 8, 10). More recently, the principal etiological agent is thought to be a spirochete, *Treponema hyodysenteriae* (5-7, 14, 15). The

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TABLE I. Length of Incubation and Diarrhea in Swine Infected with Swine Dysentery and Used for the First Eight Passages of the Disease

Age of Swine, Weeks	Length of Incubation			Length of Diarrhea		
	No Swine Observed	Average Days	Range Days	No Swine Observed	Average Days	Range Days
8	12	10.4	5-24	17	6.8	3-19
12	12	13.8	5-23	7	4	2-5
Both age groups combined	36	11	5-24	24	6.4	2-19

consistent method of inducing swine dysentery in susceptible swine is by the oral administration of either feces or diced colon from swine that have developed clinical signs of the disease.

This study is a report on: 1) the latitude in susceptibility, clinical course, and lesions induced with 16 passages of swine dysentery; 2) efforts to isolate and propagate spirochetes *in vitro;* 3)) the development of immunity to the disease; and 4) the preservation of infectivity in infected colon.

MATERIALS AND METHODS

Swine

A total of 69 specific-pathogen-free (SPF) swine of Yorkshire-cross breeding were used for this study. Of these swine, 37 eight and 12 week old were used for the first eight passages of swine dysentery in the following order: 24 were permitted either to die or recover from the disease; three were euthanized on the first day of diarrhea, three were euthanized after two days of diarrhea, two were euthanized after four days of diarrhea, one never developed diarrhea, and four were used in the experiment on the inducement of immunity. Six eight week old and six 12 week old SPF swine which were euthanized on the first day of diarrhea were used for the last eight passages. An additional 20 eight week old SPF swine were used for experiment II on the inducement of resistance, controls on the inducement of resistance, and testing the infectivity of frozen inoculum. Most of the euthanized swine were used to provide infective inoculum for drug studies. All swine were born and raised at the Veterinary Research Farm, University of Missouri and they never had been given antibiotics in their feed before the time of exposure. The experimental swine were fed a ration containing 16% protein and 80% corn.

Inoculum

Each pig was exposed by administering, orally, approximately 30 gm of fresh diced colon from swine infected with swine dysentery.[1]

Criteria for Evaluating Infection

Criteria used to evaluate the presence or absence of infection were: 1) attitude and appetite, 2) duration and type of diarrhea such as bloody or blood-free, 3) observation of spirochetes in feces, 4) death, and 5) lesions. To be designated as infected, all swine had to have a diarrhea with spirochetes in the feces. Swine were observed four times a day.

Fecal Staining

Smears of feces from each pig prior to developing diarrhea and during the period of having a diarrhea were stained with victoria blue 4-R[2] in an attempt to identify spirochetes (11).

Necropsy

All swine were necropsied and portions of the colon, cecum, posterior ileum, fundus of the stomach, and the mesenteric lymph nodes of the colon were fixed in 10% buffered formalin.

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[1]Inoculum was originally recovered from an epizootic of swine dysentery in Missouri.

[2]Hellige, Garden City, N.Y.

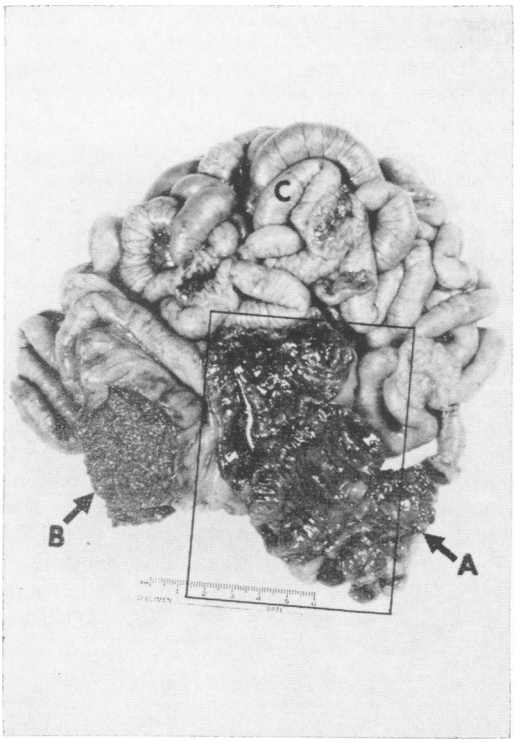

Fig. 1. Intestines from an eight week old pig on the first day of diarrhea which was hemorrhagic. The incubation period was five days. Notice the muco-hemorrhagic appearance (A) of the middle of the colon and the normal appearing cecum (B). Small intestine (C).

Bacteriological Isolation

Procedures and media previously described were used in an attempt to isolate and propagate *T. hyodysenteriae* from mucosal scrapings of the colon from swine on the first day of diarrhea (7). The same procedures were used for isolating *Vibrio* spp. Fecal swabs from swine with diarrhea were streaked on brilliant green agar in an attempt to isolate *Salmonella* spp during the period when the swine had a diarrhea.

Histopathological and Ultrastructural Techniques

Formalin-fixed tissues were dehydrated,[3] cleared,[3] infiltrated and embedded with paraffin.[4] Sections were cut 6 μ thick and stained with hematoxylin and eosin and victoria blue 4-R (12). Using standard techniques, thin sections were cut from the colon of infected swine on the first day of diarrhea and viewed with an electron microscope (12).

Evaluation of Immunity

In experiment I, one naturally infected and four experimentally infected swine were subsequently exposed three times to infective inoculum at intervals of four weeks. With each subsequent exposure, one previously unexposed pig was exposed. The last three experimentally infected swine (C,D,E) were medicated with streptomycin sulfate two days after the development of their initial bloody diarrhea to prevent possible death. In experiment II, the three of nine swine which survived infection were reexposed twice again at six week intervals.

Preservation of Inoculum

Colon and contents from infected swine were stored at -77°C and -16°C for nine

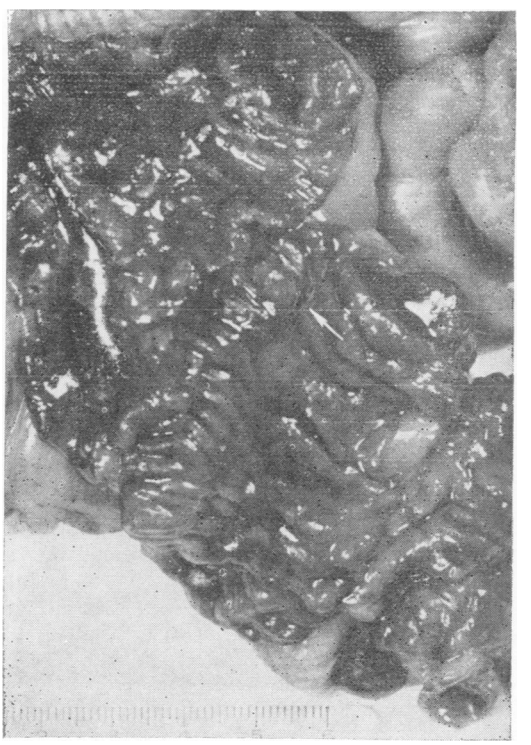

Fig. 2. Magnification of the colon (rectangle) in Fig. 1. Notice the strains of fibrin (arrow).

[3]Dehydrant and Clearing Agent, Technicon Co., Chauncey, N.Y.

[4]Paraplast, Aloe Scientific, St. Louis, Mo.

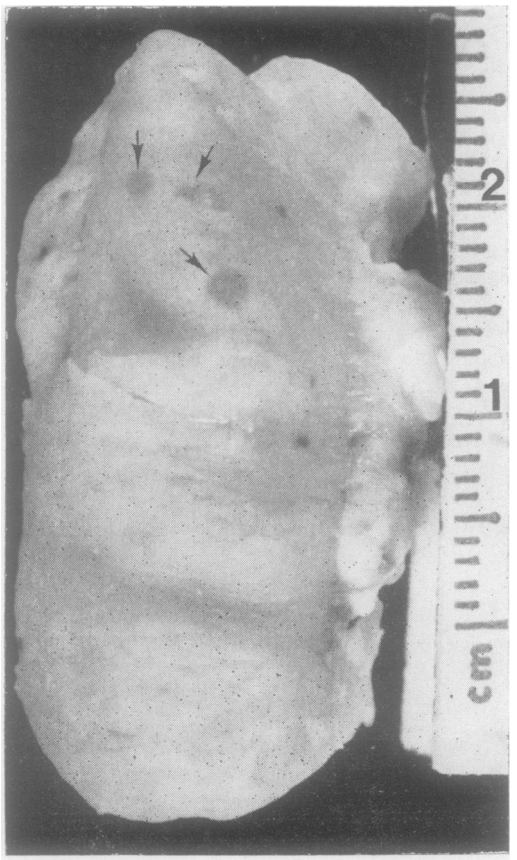

Fig. 3. Enlarged submucosal glands which appeared as opaque spots (arrows) on the serosal surface of the colon from a pig euthanized on the second day of diarrhea.

months. Bloody feces from infected swine were lyophilized and stored at -12°C for nine months.

RESULTS

Susceptibility

Of the 37 swine exposed in the first eight passages, 36 developed either a bloody or a blood-free diarrhea characteristic for swine dysentery. Of the 24 swine in the above group in which the disease was allowed to run its course, 18 died. Of the six swine which recovered, five developed a bloody diarrhea and one had a blood-free diarrhea. No difference in susceptibility was seen between the eight week and 12 week old swine, as the six recovered swine were equally divided between the two age groups. The pig which did not develop a diarrhea after the first exposure was resistant to a subsequent exposure. Of the 12 swine exposed in the last eight passages, all developed either a bloody or blood-free diarrhea.

Clinical Signs

The mean for the incubation period for the swine used in the first eight passages was 10.4 days (ranged from five to 24 days) for the eight week old swine and 13.8 days (ranged from five to 23 days) for the 12 week old swine (Table I). Combining both age groups, the mean for the incubation period was 11 days. The mean for the duration of diarrhea in the eight week old and 12 week old swine was 6.8 days (ranged from three to 19 days) and four days (ranged from two to five days), respectively.

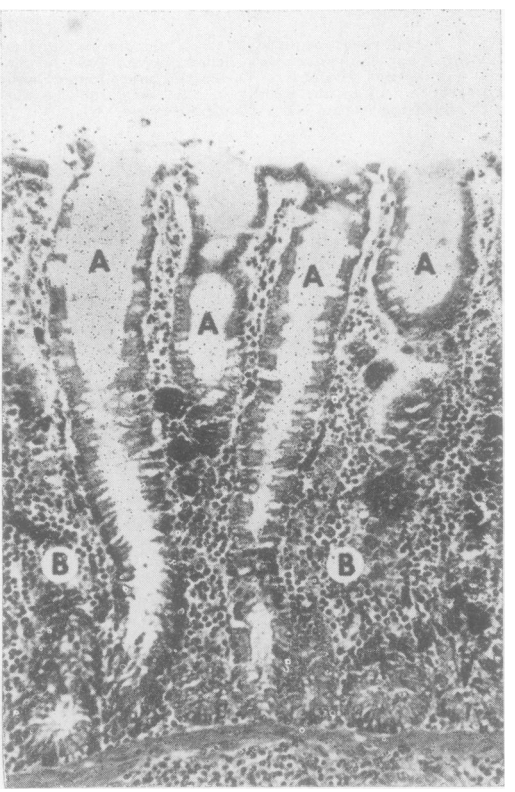

Fig. 4. Dilated mucous glands (A) in the colon of an infected pig on the second day of bloody diarrhea. Mucosa is infiltrated with numerous erythrocytes and some lymphocytes (B). H & E 95X.

The diarrhea in all swine was preceded by a soft stool which lasted from six to ten hours. During the first and second day of the diarrhea, there was a copious amount of mucus. In some swine, the first mucus was relatively free of blood; whereas, in other swine it was mixed with blood. In some swine, the diarrheic state remained blood-free throughout the course of the disease. The amount and duration with which mucus and blood were prominent in the diarrhea varied between swine and with the length of the diarrhea.

After two or three days of diarrhea, all of the exposed swine were gaunt. The feed consumption and stamina during the period of diarrhea varied among swine. The swine with a blood-free diarrhea frequently ate; whereas, most of the swine with a bloody diarrhea did not. With some swine, anorexia was intermittent. Some of those swine with a blood-free diarrhea lived for several weeks and became progressively weaker until they could hardly stand; yet these swine would occasionally nibble feed.

Microscopic Examination of Feces

Spirochetes similar to those previously reported were observed in stained fecal smears obtained from all swine with a diarrhea (5, 6, 7). Spirochetes were most numerous in the feces at the beginning of the diarrhea and in the feces containing visible blood. Erythrocytes were observed microscopically in the feces and colon sections of swine with macroscopic blood-free diarrhea.

Isolation of Spirochetes

The spirochetes in the colon on the first day of diarrhea could not be isolated and propagated *in vitro* using the techniques previously described (7). Organisms identified as *Vibrio* spp were isolated in four of five attempts at culturing scrapings of the infected colon.

Macroscopic Lesions

In swine euthanized on the first day of the diarrhea, the colitis was most intense in the several centrifugal and centripetal coils near the apex of the colon (Figs. 1 and 2). In all swine euthanized on day 2, the inflammation involved the entire colon but not the cecum. All of these swine had moderate amounts of feed in their stomach and many had a hyperemia of the fundus. In all of these swine, the mesenteric lymph nodes draining the colon were congested.

The colitis was more intense and had extended into the cecum in the swine euthanized on the fourth day of diarrhea. The amount of blood in the colon varied. All of the swine which died from swine dysentery had a colitis, typhlitis, and a hyperemia of the fundus of the stomach. Those swine that died having a bloody diarrhea, also had congestion in the mesenteric lymph nodes.

As the disease progressed, many swine developed a diphtheritic membrane in the colon. Strands of fibrin were often visible over the serosa of the colon. Frequently, the submucosal glands of the colon were enlarged and visible through the serosa as opaque spots (Fig. 3). No visible lesions were observed in the rectum or ileum of any infected swine.

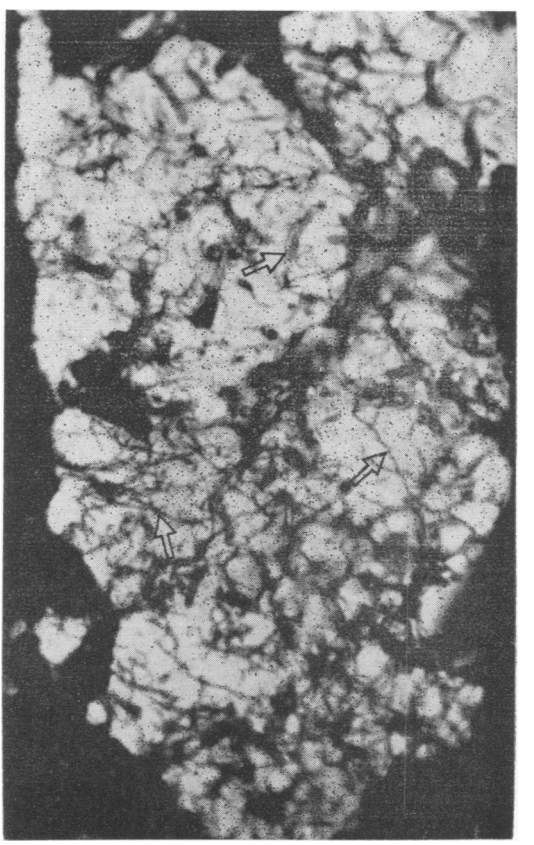

Fig. 5. Spirochetes (arrow) in the dilated mucous glands of the colon on the second day of a bloody diarrhea. Slide stained with victoria blue 4-R. 1,700 X.

TABLE II. Development of Resistance to Swine Dysentery After Repeated Exposure with Infected Colon

Swine	Age Wks	First Exposure: Length of Incubation, Days	First Exposure: Length of Diarrhea, Days	First Exposure: Type of Diarrhea	Treatment and Recovery	Second Exposure: Length of Incubation, Days	Second Exposure: Length of Diarrhea, Days	Second Exposure: Type of Diarrhea	Third Exposure: Length of Incubation, Days	Third Exposure: Length of Diarrhea, Days	Third Exposure: Type of Diarrhea	Fourth Exposure: Type of Diarrhea
Experiment I												
Pig A[a]........	Unknown	Unknown	4	BD[b]	Not treated, recovered	7	2	BF[b]	9	1	BF	No diarrhea developed
" B..............	8	7	2	BD	Not treated, recovered	6	1	BF	7	1	BF	" " "
" C..............	8	14	2	BD	Treated[c]	9	2	BF	No diarrhea developed			" " "
" D..............	12	24	2	BD	Treated[c]	7	1	BF	9	1	BF	" " "
" E..............	12	19	2	BD	Treated[c]	8	1	BF	6	1	BF	" " "
Experiment II												
9..............	8	5-24	2-20	BF & BD	No treatment, 3 survived	No diarrhea developed			No diarrhea developed			Not exposed

[a]Pig came from the farm where the initial infected inoculum was obtained
[b]BD = Bloody diarrhea; BF = Blood-free diarrhea
[c]Injected intramuscularly with 300 gm of streptomycin sulfate

Microscopic Lesions

On the first day of diarrhea, the mucous glands of the colon were dilated (Fig. 4). Spirochetes were consistently observed in these glands in sections stained with victoria blue 4-R (Fig. 5). As the disease progressed, the goblet cells of the mucous glands became depleted of mucus.

The initial lesion in the colon was a copious discharge of mucus. This was followed by an exudation of plasma and erythrocytes which varied greatly. Necrosis was minimal and involved only the superficial mucosa. Often, a pseudomembrane consisting of necrotic tissue and fibrin adhered to the mucosal surface. Erythrocytes were observed outside of the vessels in the lamina propria in swine with either a bloody or blood-free diarrhea; however, generally, there was not a marked infiltration of neutrophils or lymphocytes. Plasma cells were rarely seen.

The mesenteric lymph nodes draining the colon were enlarged, edematous and often contained large amounts of blood in the peripheral sinuses. Spirochetes were never observed in microscopic sections of the lymph nodes stained with victoria blue 4-R.

Microscopically, there was a dilation of the blood vessels in the mucosa of the gastric fundus in the swine with visible hyperemia of this region; however, erythrocytes were never observed outside of these vessels.

No microscopic lesions were observed in the colon, cecum or stomach in the exposed swine which previously had developed either a bloody or blood-free diarrhea, but either had recovered naturally or were treated while having a diarrhea.

Electron Microscopic Appearance of Spirochetes

Numerous organisms with a protoplasmic cylinder and surrounded by a thin envelope containing 12 to 18 axial fibrils were observed in thin sections of the mucous glands of the colon from swine euthanized on the first day of diarrhea when viewed with an electron microscope. These organisms were approximately 0.3 μ in diameter and 9 μ long. These features were characteristic of the spirochetes previously reported as the possible etiological agent for swine dysentery (5, 6, 7). Migration of the spirochetes into the tissues had been observed but not fully evaluated.

Development of Resistance

None of the five swine, which developed a bloody diarrhea after the first exposure to infected colon, had macroscopic blood in their stool after the second and third exposures (Table II). In contrast, the diarrhea, after the second exposure, was blood-free and transitory. One pig was resistant to the third exposure and none developed a diarrhea after the fourth exposure. The three swine in experiment II which survived the initial exposure to swine dysentery, were resistant to the second and third exposures.

Preservation of Inoculum

Colon and contents from infected swine, which were stored at -77°C for nine months, were proven to be infective in two groups of swine. However, the colon and contents from infected swine, which were stored at -16°C for nine months, and the bloody feces from infected swine which were lyophilized and stored at -12°C, were not infective for susceptible swine.

DISCUSSION

The clinical signs of the infected swine, which varied between swine and passages, were the length of incubation, duration of diarrhea, type of diarrhea, and the attitude and appetite. The clinical sign consistently observed was a diarrhea except for one of 60 exposed swine which never developed a diarrhea. No explanation can be presented for this natural resistance, although it has been suspected from field observations.

The macroscopic lesions that were variable, was the amount of blood in the colon and feces. The consistent macroscopic lesions in those swine which died of the disease was a colitis and a typhlitis. The consistent microscopic lesion was a muco-hemorrhagic colitis with spirochetes in the dilated mucous glands. Frequently, a hyperemia of the fundus of the stomach was observed.

The variation observed in the length of the incubation period in this study may partially explain why an epizootic of swine dysentery has the characteristic of lingering in a herd for many months. One reason for the variation in the length of incubation may be several factors which could alter the concentration and pathogenicity of the causative agent before it reaches the colon, such as the juices of the stomach and intestine.

It is possible that the severity of the disease could be decreased by the presence of certain secondary bacteria. These organisms may expedite the development of a pseudomembrane which aids in decreasing the massive exudation.

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