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Characterization of Treponema hyodysenteriae isolated from outbreaks of swine dysentery

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by

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INTRODUCTION

Swine dysentery has existed as a disease entity since 1921 when the first descriptions were reported. It is a debilitating disease characterized clinically by diarrhea with mucus and blood. Postmortem observations reveal lesions consisting of catarrhal to mucohemorrhagic enteritis confined to the large intestine. Pigs are usually affected at 7-14 weeks of age and frequently experience more than 1 attack of the disease. Morbidity and mortality rates can be as high as 90% and 25%, respectively.

The economic significance of this disease was emphasized by a report of Livestock Conservation, Incorporated. They estimated losses to the pork industry as a result of swine dysentery at $34,000,000. Costs are incurred due to death loss, feed costs through decreased rate of gain of surviving animals, and medication and veterinary care.

The etiology of swine dysentery remained undetermined until 1971. Prior to this time Vibrio coli had been implicated as the etiologic agent. However, conflicting reports from research investigators around the world demonstrated the questionable involvement of V. coli in swine dysentery.

In December of 1971 and in January of 1972, two independent groups of investigators demonstrated the reproduction of swine dysentery with pure cultures of an anaerobic spirochete (Type-1 spirochete, large spirochete, Treponema

hyodysenteriae). Both groups of investigators noted the presence of Type-1 spirochetes or T. hyodysenteriae in the colonic lesions of pigs affected with swine dysentery and reisolated the organisms from diseased animals to confirm Koch's postulates. Subsequent reports from investigators in 7 different laboratories have also demonstrated the production of swine dysentery with pure cultures of T. hyodysenteriae-like organisms. These results indicate the primary role of T. hyodysenteriae in the etiology of swine dysentery.

All of the above investigators cultured this anaerobic organism on blood agar media incubated in GasPak jars containing anaerobic atmospheres. Attempts by several investigators to grow T. hyodysenteriae in liquid media failed; however, a few characteristics of T. hyodysenteriae were determined in blood agar slant cultures with differential media overlays under deoxygenated CO_2.

The present studies were undertaken to: 1) support and confirm the primary etiologic role of isolates of T. hyodysenteriae-like organisms from geographically separated outbreaks of swine dysentery, 2) improve procedures for the isolation of the organism from intestinal material, 3) develop a liquid medium suitable for the more complete characterization and separation of pathogenic and nonpathogenic isolates of T. hyodysenteriae-like organisms.

REVIEW OF LITERATURE

Swine Dysentery--the Disease

Definition

Swine dysentery is a mucohemorrahagic diarrhea of swine. The disease was first described in 1921 in Indiana by Whiting, Doyle, and Spray, and has since been reported by workers in most swine rearing areas throughout the world (Sorensen, 1970). There are several synonyms for this disease including bloody dysentery, bloody scours, black scours, vibrionic dysentery, and hemorrhagic enteritis. The disease has been reported in most breeds of pigs and is not known to be transmissible to other species. Swine dysentery affects primarily the weanling-age pig with many outbreaks occurring in animals at 7-14 weeks of age (Lussier, 1962; Harris and Glock, 1971).

Economic significance of swine dysentery is related to swine management practices. In recent years, the pork industry has gradually experienced an increase in feeder pig production (Paul, 1972). These pigs are born and weaned (at 4-6 weeks) on one farm, then sold and shipped to other farms where they are fed to market weight and sold to slaughter. This selling and shipping process results in the comingling of animals from different herds (with various disease backgrounds) and subsequent stress of those animals. This

practice, coupled with the confinement rearing of swine, permits an environment suitable for the maintenance of the causative factors of many infectious diseases, including swine dysentery.

In the United States, swine dysentery is estimated by Livestock Conservation Commission (LCI) to cause an annual loss of $34,000,000 to the pork industry (Rosse, 1972). The disease can reach a mortality rate of 30% in weanling pigs. Substantial losses are incurred due to poor feed conversion by animals surviving the disease. The LCI report (Rosse, 1972) states this loss as the requirement of 100 pounds of additional feed to produce 100 pounds of pork as compared to control animals. Much evidence to support this reasoning has been accumulated in studies on certain therapeutic agents. For example, 8-10 week old pigs infected by exposure to colonic material from pigs acutely affected with dysentery show a 3-fold increase in feed conversion and a 2-fold decrease in rate of gain as compared to uninfected control animals (Harris et al., 1972b).

Although this infectious enteric disease of swine is quarantinable in many states, the incidence of swine dysentery among swine in the United States is difficult to determine. Quarantine procedures usually represent a greater loss to the producer and as a result treatment is often begun, without knowledge of the local veterinarian or

state regulatory officials, to avoid quarantine. In Iowa in 1969, 159 herds were quarantined (Harris and Glock, 1971); and there are currently 100 herds under quarantine (National Hog Farmer, 1974). In March, 1971, Wisconsin started a quarantine program which is believed to have been effective in the reduction of cases of dysentery in that state. A quarantined herd may not be sold except to slaughter and removal of the quarantine requires complete depopulation (National Hog Farmer, 1974).

Clinical characteristics

The clinical characteristics of swine dysentery were described in great detail by the early workers in the disease (Whiting et al., 1921; Whiting, 1928). More recent descriptions are very similar and indicate that swine dysentery does exist as a distinct disease entity (Deas, 1960; Lussier, 1962; Alexander and Taylor, 1969; Harris and Glock, 1970; Simmons, 1972).

Alexander and Taylor (1969), on the basis of the clinical signs and history, designated outbreaks of swine dysentery as either typical or atypical. In a study of 249 field cases of swine dysentery Lussier (1962) classified outbreaks as peracute, acute, subacute or chronic. It is convenient to combine these two systems.

Typical acute swine dysentery outbreaks begin gradually affecting one or two pigs in a group. The first clinical

signs may be insidious, particularly in large groups, and include loss of appetite and passage of soft feces. Early in the disease the fecal composition will be normal, but soon after the appearance of diarrhea, streaks of blood and mucus are seen. The quantity of blood and mucus will vary but usually both increase in relation to the amount of digested and undigested food. In this acute stage of the disease the appearance of the feces is dark red to chocolate and of semiliquid to watery consistency. The perineum is usually wet and stained with fecal material. A febrile response may accompany swine dysentery, however, this is not always the case. The pigs become depressed and dehydrated, lose weight, and may continue to show loss of appetite. A particularly striking characteristic of the disease is the "tucked up" or gaunt appearance of these animals which probably results from abdominal pain. If pigs suffering from dysentery are housed in a confined area a particular foul odor is noted and easily recognized by animal caretakers.

The incubation period is usually 1-2 weeks and the course of the disease may vary from 3-4 days to 3-4 weeks. Pigs that recover from the acute state of the disease may remain normal, depending upon treatment and management procedures. The typical acute clinical signs of dysentery may occur in recovered pigs. Often this first exacerbation

is more severe than the original attack. Another possible outcome, frequently seen after ineffective treatment or no treatment, is progression from acute to chronic swine dysentery. In these cases the characteristic signs include diarrhea of grayish color containing pieces of necrotic mucous membranes, but no blood.

Atypical swine dysentery is noted in studies of individual outbreaks and includes the peracute, rapidly fatal form of the disease. Lussier (1962) reports these as cases where pigs died without showing any symptoms. Sorensen (1970) states this may be the first indication of the beginning of a swine dysentery outbreak.

Swine dysentery is also occasionally observed in animals outside the 7-14 week range. Alexander and Taylor (1969) state that sows may be severely affected soon after the finishing stock develop the disease. Clinical signs in adult stock are similar to those of weanling pigs. If treatment and management are such that the disease is maintained in the herd during farrowing, the new-born piglets may also contract swine dysentery. Whiting (1924) reported a 2-5% mortality rate for sows with a 50-60% mortality rate for their piglets (Whiting et al., 1921). Swine dysentery in nursing piglets is characterized by soft to semiliquid feces often containing mucus but no blood (Harris and Glock, 1973).

Swine dysentery is a communicable disease. If control measures are not taken early in the outbreak and management is poor, the morbidity rates reach 90%. Spread of the disease through a herd is via the ingestion of feed and water contaminated with feces (Whiting, 1924). Terpstra et al., (1968) reported transmission of the disease from swine dysentery affected pigs to susceptible pigs by caretakers who did not change clothing or footwear between isolation units.

Pathological characteristics

Hematological changes Hematologic changes due to acute swine dysentery have been studied by Glock (1971) in three trials involving 24 pigs. The animals were infected with colon contents and mucosal scrapings from field cases of swine dysentery. He observed hemoconcentration (increased packed cell volume, increased erythrocyte count and increased total plasma protein) due to fluid loss in affected animals. An increase in erythrocyte sedimentation rates and fibrinogen levels indicated inflammation, as did the observation of a marked left shift in circulating neutrophils. The total leukocyte count did not consistently increase.

The most consistent hematologic changes in pigs affected with dysentery were in electrolyte balance. Serum sodium and chloride declined throughout the course of the

disease while serum bicarbonate levels decreased and serum potassium levels increased just before death. Nearly normal blood pH values indicated that the acidosis resulting from depletion of bicarbonate was compensated for by respiratory alkalosis until just prior to death.

This work confirms an earlier summarization of hematologic changes in pigs affected with swine dysentery from the Minnesota Veterinary Diagnostic Laboratory. In this summary Sorensen (1970) reported hemoconcentration and acidosis. He also noted a mild anemia probably due to the intestinal hemorrhage. These studies indicate the cause of death in swine dysentery to be dehydration and acidosis.

Macroscopic lesions The lesions of swine dysentery are typically limited to the gastrointestinal tract, specifically the cecum, colon and rectum (Whiting et al., 1921; Whiting, 1924, 1928). Whiting (1928) reported hemorrhage and hyperemia in the fundic stomach. Numerous other workers (Lussier, 1962; Davis, 1961; Warner, 1965; Alexander and Taylor, 1969; Harris and Glock, 1971, 1972) have also noted this lesion but Warner (1965) and Harris and Glock (1971, 1972) considered it nonspecific.

Lussier (1962) found the small intestine to be normal in most cases, however, increased mucoid exudate and mild hyperemia were occasionally seen throughout the length of the small intestine. This lesion was seen commonly in the

terminal ileum in sharp contrast to the normal intestine above it. Doyle (1958) cites this feature as particularly characteristic of dysentery.

Gross observations of the large intestine at post mortem include an inflamed serosal surface frequently spotted with pale raised nodules (Harris and Glock, 1971, 1972). The intestinal wall and the mesentery appear edematous. If the dysentery is more chronic the serosal surface may appear dry and granular (Lussier, 1962).

The contents of the colon are of soft and watery consistency and include mucus, fibrin, blood, and feces in varying proportions. Catarrhal, hemorrhagic and necrotic inflammation of the mucosa are encountered in cases of swine dysentery depending on the severity and duration of the infection. Lussier (1962) has described three types of inflammation: 1) catarrhal inflammation is characterized by increased quantities of mucus and swelling of the intestinal wall with irregular congestion and petechial hemorrhages; 2) hemorrhagic inflammation was not frequently encountered. Either catarrhal or hemorrhagic inflammation can show a rough eroded mucous membrane covered with necrotic material; and 3) necrotic inflammation begins as focal necrosis with diphtheritic accumulation. As the disease progresses, these areas coalesce to form a necrotic membrane which is usually sloughed off leaving a granular hemorrhagic

surface. Erosion of this type does not penetrate deeply.

Microscopic lesions Microscopic examination of the lesions of dysentery show an exudate on the mucosal surface consisting of mucus, fibrin, red blood cells, lymphoid cells and bacteria (Whiting et al., 1921). The area beneath the intestinal epithelium is edematous and there may be considerable quantities of extravasated blood as well as congested or thrombosed blood vessels. The epithelium may be destroyed and pushed away from the mucosa. Crypt epithelium shows increased numbers of goblet cells and the crypts are filled with mucus and may be distended. Numerous bacteria of a variety of morphotypes are observed within the crypts and on the mucosal surface. These will be discussed in later sections. The raised nodules on the serosal surface are dilated submucosal glands containing mucus, cell debris, bacteria, and inflammatory cells (Harris and Glock, 1971, 1972).

Microscopic examination of stained tissue sections of later stages of the disease may show complete exhaustion of the goblet cells, then a lack of epithelial covering and zones of necrosis. Eventually the necrosis will cover most of the luminal surface, but usually deeper tissues remained unaffected.

Leucocytic infiltration of varying intensity may be seen in the mucosa. The primary cell type is lymphocytic

and occasionally large numbers of polymorphonuclear leukocytes are observed.

Occasionally only a portion of the colon may appear to be involved by macroscopic or microscopic observation. Warner (1965) speculated that the apex of the spiral colon was affected initially and then the lesion progressed throughout the large intestine. In most cases both cecum and colon are involved and rectum may be involved to a less severe extent.

Swine Dysentery--the Etiology

Etiology--1971 to present

The primary role of a specific disease agent in swine dysentery was finally elucidated in 1971 after 50 years of study on the disease. The agent is an anaerobic spirochete and was first isolated from a field case of swine dysentery in a study by Taylor and Alexander (1971). This organism (Type-1 spirochete, isolate A-1) was inoculated in pure culture into 7 minimal disease (MD) pigs. Five developed signs and lesions of dysentery after an incubation period of 8-11 days.

In a simultaneous study Harris et al. (1972a; Glock and Harris, 1972) inoculated specific pathogen free (SPF) pigs with a morphologically similar anaerobic spirochete (large spirochete, strain B78). Two of 4 pigs inoculated with pure

cultures of the large spirochete developed the signs and lesions of dysentery. In the same study, 12 of 15 pigs which were inoculated with the large spirochete in combination with Vibrio coli and vibrio-like organisms developed signs and lesions of swine dysentery. The incubation period was 6-14 days. The large or Type-1 spirochetes were reisolated from animals that developed the disease in both studies.

The organism was isolated on fresh or prereduced blood agar plates by both groups of investigators using a millipore filtration procedure (Taylor and Alexander, 1971; Harris et al., 1972a, d). The plates were incubated anaerobically at 37° C for 2-5 days. Evidence of growth of the organism appeared as clear hemolysis of the blood agar with little or no surface growth. Animals were inoculated orally with blood agar plate cultures prepared by transferring agar from the hemolytic areas to fresh plates. Neither group was able to propagate the organism in a broth medium.

Characteristics of the organism were determined by growth on bovine blood agar slants with broth overlays incubated under deoxygenated CO_2 by Harris et al. (1972a). It was a gram negative, motile spirochete, 0.32-0.38 μm in diameter and 6.0-8.5 μm long, with 7-9 axial fibrils. The organism would only grow in an anaerobic atmosphere, was

catalase and oxidase negative, and produced small amounts of acetic acid from media containing glucose. The organism was named Treponema hyodysenteriae.

Taylor and Alexander (1971) reported the presence of a morphologically similar spirochete (isolate 4/71) in uninoculated controls and in the preinoculation samples. They were able to isolate this spirochete and to differentiate it from the spirochete in the inoculum on the basis of antibiotic sensitivity pattern and hemolysis. In contrast, Harris et al. (1972a) reported the uninoculated animals in their study to be free of T. hyodysenteriae.

Several groups of investigators from laboratories around the world have since reproduced swine dysentery in susceptible animals by oral inoculation of pure cultures of Treponema hyodysenteriae-like organisms (Akkermans and Pomper, 1973; Olander et al., 1973; Olujic, et al., 1973; Hamdy and Glenn, 1974; Brandenburg, 1974; Taylor, 1974; Sofrenovic and Olujic, 1974; Hudson et al., 1974; Smibert, R. M., Virginia Polytechnic Institute and State University, personal communication, 1972).

To date three studies concerning the inoculation of pigs with cultures of Treponema hyodysenteriae have not resulted in swine dysentery. Two of these were studies involving germ-free pigs and will be discussed later. The third was a study by Hudson et al. (1974), which showed that

T. hyodysenteriae lost virulence for pigs after 80 in vitro passages. The same strain at 12 in vitro passages produced signs and lesions of swine dysentery in 10 of 13 orally inoculated pigs.

Etiology--1921-1971

Swine dysentery as a disease entity was very much in existence from 1921 to 1971 as veterinarians and producers observed its spread throughout the United States and much of the world during this period of time (Sorensen, 1970). Research in the area of swine dysentery involved many aspects of the disease but concentrated on etiology and treatment. The early reports (Whiting et al., 1921; Whiting, 1924, 1928) emphasized the lack of involvement of hog cholera virus, Bacillus suispestifer (Salmonella choleraesuis), Bacillus coli (Escherichia coli), Bacillus necrophorum (Fusobacterium necrophorum), and amoebae. Another possible agent observed in "enormous numbers" in the feces of pigs affected with the disease was a spirochete. Mixed cultures of spirochetes and other bacteria grown in meat-piece serum water were fed to two pigs. Both pigs remained healthy for 12 days (Whiting et al., 1921).

In 1928 Whiting reported the consistent occurrence of a particular comma-shaped microorganism in the feces of animals with dysentery, and the absence of organisms with

similar morphology in feces of normal swine. This organism was not isolated and inoculated in pure culture into susceptible animals until 1944, when Doyle reported the production of diarrhea in 6 of 8 pigs orally inoculated with blood agar plate cultures containing the organism. Doyle states, however, that the diarrhea was less severe than in typical cases of swine dysentery. This work was confirmed by James and Doyle in 1947 when 50 of 60 animals developed the disease after inoculation with pure cultures of the organism protected with gastric mucin. The organism was characterized and named Vibrio coli (Doyle, 1948) and thereafter the disease was frequently referred to as vibrionic dysentery.

In reviewing the literature on swine dysentery through the ensuing years one is faced with both contradiction (Boley et al., 1951; Davis, 1961; Deas, 1960; Maninger, 1960; Andress et al., 1968; Andress and Barnum, 1968; Kashiwazaki et al., 1971; Sofrenovic et al., 1973; Terpstra et al., 1968; Hughes et al., 1972) and support (Roberts, 1956a, b; Truszczynski, 1957; von Ballmoos, 1950) of the above work. While many accepted V. coli as the cause of swine dysentery and used its presence as a guideline in the diagnosis of the disease, confusion arose when several workers reported the frequent occurrence of V. coli in healthy pigs (Olujic et al., 1972; Deas, 1960; von Ballmoos, 1950; Andress and

Barnum, 1968; Terpstra et al., 1968; Harris and Glock, 1971, 1972). The confusion has been increased due to the reported presence of two biochemically distinct types of V. coli (Doyle, 1944; Deas, 1960). One was seen in both healthy and dysentery affected pigs while the other was only seen in the diseased pigs.

An enlightening study of the etiology of the disease was presented by Warner (1965). He produced the disease using embryonated chicken eggs which had been inoculated with filtered dysenteric colonic material and incubated for 18 hours. The 0.8 μm millipore filtrate contained vibrio, Spirocheta spp. and other bacteria. The egg fluids after incubation contained increased numbers of vibrio, bacilli and cocci, but the spirocheta were not seen. Vibrio coli in pure culture (isolated on blood agar plates) did not produce disease in 36 inoculated animals. In an attempt to eliminate the supposed loss of virulence on artificial media, Warner passaged a 0.45 μm millipore filtrate in embryonated chicken eggs. Acute swine dysentery was produced in 8 of 12 animals inoculated with this material. Warner did not observe bacteria other than V. coli in direct smears and was not able to isolate any other bacteria from the filtrate or egg fluids by his methods. He concluded that the organism was in pure culture in the 0.45 μ filtrate and that previous isolates had lost virulence by even a few passages

on artificial media. Warner therefore supported the conclusions of James and Doyle that V. coli was the primary etiological agent of swine dysentery.

Current status of Vibrio coli

The exact status of V. coli in the etiology of swine dysentery has not been determined. The MD and SPF pigs used by Taylor and Alexander (1971) and Harris et al. (1972a) harbored Vibrio spp. prior to inoculation. Deas (1960) suggested V. coli was present as a normal commensal in nearly all pigs.

Hamdy and Glenn (1974) stated that the disease produced by feeding susceptible pigs both V. coli and T. hyodysenteriae was more severe than the disease produced by T. hyodysenteriae alone. They did not report presence or absence of V. coli in control animals or preinoculation rectal swab samples. Sofrenovic and Olujic (1974) reported a shorter incubation period for development of swine dysentery in animals inoculated with both V. coli and T. hyodysenteriae as compared to those inoculated with T. hyodysenteriae alone or in combination with Escherichia coli or Clostridium perfringens. Harris et al. (1972a; Glock and Harris, 1972) did not make a conclusion on either the severity or incubation period of the disease produced by T. hyodysenteriae in combination with V. coli. Their

data indicated 50% of the pigs inoculated with T. hyodysenteriae alone and 80% of the pigs inoculated with T. hyodysenteriae in combination with V. coli developed the disease.

In an attempt to determine the role of V. coli in swine dysentery, Taylor (1972) inoculated hysterectomy-derived, colostrum-deprived (HDCD) isolation-reared pigs with Type-1 spirochetes. He stated the pigs were free of Vibrio spp. and Type-1 spirochetes by phase microscopic and cultural techniques, however they did develop a colonic flora from exposure to the environment. Two of the three inoculated pigs developed mucoid diarrhea with blood and the early lesions of swine dysentery at 13 and 14 days post-inoculation.

Germ-free (GF) and SPF pigs were inoculated with pure cultures of T. hyodysenteriae alone and in combination with V. coli by Harris et al. (1972c). The SPF pigs receiving either inoculum developed signs and lesions of swine dysentery while neither produced signs or lesions of dysentery in the GF pigs. The organisms became established in the colons of the GF animals as they were reisolated at the conclusion of the study. The authors suggested the normal flora contributed to the production of swine dysentery.

Brandenburg (1974) has also inoculated GF pigs with T. hyodysenteriae alone and in combination with V. coli or

Peptostreptococcus spp. These inocula did not produce the disease in GF pigs while 4 of 4 conventional pigs developed signs and lesions of dysentery.

These results indicate a synergistic mechanism of infection for this disease. The intestinal microbiota may provide either the necessary additional agents or the specific biochemical conditions which enable T. hyodysenteriae to produce the dysentery response.

Association of spirochetes with enteric disease in swine

The determination of the primary involvement of a spiral-shaped organism, T. hyodysenteriae, as the causative agent of swine dysentery was the culmination of a hypothesis developed by several workers in enteric diseases of swine.

As early as 1894, Smith reported the presence of spiral-shaped organisms in ulcers of the colon in swine. The production of hog cholera with cultures of an anaerobic spirochete, Spirocheta suis (frequently found in the blood and colonic ulcers of cases of hog cholera), led King and co-workers, Drake, Baeslack, and Hoffman (1913, 1915) to believe it was the primary etiologic agent of the disease.

Whiting, Doyle, and Spray (1921) made a limited attempt to define the involvement of spirochetes seen in the feces, colonic mucosa, and colonic crypts of pigs affected

with swine dysentery. However, the oral inoculation of susceptible pigs with mixed cultures containing spirochetes did not produce the disease.

In a review of the enteritidies of swine, Gray (1939) characterized the condition called spirochetosis as a hemorrhagic diarrhea with mucus, which was diagnosed by the demonstration of spirochetes by microscopic examination. He also described swine dysentery which was diagnosed clinically as diarrhea with blood, and characterized by lesions confined to the large intestine. Gray reported that the exact cause of swine dysentery was unknown. His recommendation for treatment of spirochetosis was arsenic and he reported that it was specific for that condition.

A Russian worker, L'vov (cited by Warner, 1965, and Taylor, 1972), considered the etiology of swine dysentery to be of spirochetal origin. He did not report the characteristics of the organism, and the syndrome which he described was different from swine dysentery in that the lesions were not confined to the large intestine and seemed to be more systemic in distribution.

Spirochetes were associated with swine dysentery from the treatment aspect by Carpenter and Larson (1952). Feces from pigs affected with swine dysentery contained spirochetes and vibrio similar to that described by Doyle (1944). Feces from animals which were apparently cured by treatment with

arsenicals still contained vibrio but the spirochetes were completely eliminated.

In 1956 Roberts (1956b) noted the presence of spirochetes among many other types of bacteria at the openings of the crypts in colonic tissue from pigs affected with swine dysentery.

Warner (1965) also described the presence of spiral-shaped organisms in the feces, colonic mucosa, and filtrate material which he used in his study of swine dysentery. He referred to these organisms as Spirocheta spp. and Borrelia hyos but did not describe enough characteristics of the organism for classification. He concluded the spirochetes were opportunists in the lesions of swine dysentery.

In 1968, a period of intense study on the relationship of spirochetes to swine dysentery was initiated as a result of the report by Terpstra et al. (1968). These workers precipitated gamma globulin from a pool of serum collected from convalescent cases of swine dysentery. The gamma globulin was conjugated with fluorescein isothiocynate and used in a direct fluorescent antibody test (FAT) on intestinal contents and feces from pigs acutely affected with swine dysentery. The organism which almost always fluoresced, was a spirochete of two or three turns with pointed ends. Terpstra was not able to cultivate these

organisms in either pure or mixed culture. Infrequently, spirochetes which were of similar morphology and gave positive fluorescence were observed in normal animals.

In 1969 Tesouro reported the direct microscopic observation of spirochetes in intestinal contents of dysentery affected pigs, and described the characteristic serpentine motility and size (7-12 μm x 0.25-0.30 μm). Smears stained with aniline dyes suggested classification of the organism as a Borrelia. In electron microscopic observations the organisms lacked flagella and possessed axial filaments, facts which supported its inclusion in the family Treponemataceae. Tesouro was not able to culture the organism.

The observation of morphologically similar spirochetes in the feces and colonic mucosa of pigs affected with swine dysentery has been reported by several workers (Roberts and Simmons, 1970; Espinasse and Redon, 1970; Todd, Hunter, and Clark, 1970; Eriksen and Andersen, 1970; Taylor, 1970, Taylor and Blakemore, 1971; Harris and Glock, 1971; Blakemore and Taylor, 1970, Hunter and Ross, 1972; Krizanova et al., 1972; Simmons, 1972; Ritchie and Brown, 1971).

Taylor (1970) reported observation of 6 different morphological types of spirochetes by phase contrast microscopic examination of feces from swine dysentery-affected and normal animals. Type-a (Type-1) was seen only in pigs

affected with dysentery. The other types were seen in both dysentery-affected and normal animals. Harris and Glock (1971) reported observation of two types of spirochetes from pigs affected with swine dysentery. The first was a Treponema-like organism (later referred to as small spirochetes), the second was Borrelia-like (later referred to as large spirochetes) based on a classification scheme by Listgarten and Socransky (1965). The Borrelia-like organism was not observed in healthy pigs. The isolation and cultivation of both of these spirochetes will be discussed later.

Spirochetes (Type-a, Type-1, Borrelia-like, large spirochetes, or Treponema hyodysenteriae) were more clearly demonstrated as the possible agent of swine dysentery in electron microscopic examination of thin sections of colonic lesions of pigs experimentally affected with swine dysentery by Blakemore and Taylor (1970), and Taylor and Blakemore (1971). They reported the presence of several types of bacteria which could not be identified along with an organism showing the structural characteristics of a spirochete adjacent to the colonic mucosa and in the crypts. This spirochete measured 0.30 to 0.37 μm in diameter with an electron-dense central core (0.25-0.30 μm) surrounded by a group of 13 axial fibrils and was enclosed in a double membrane. These spirochetes were also present between and within

degenerative cells of the colonic mucosa.

Glock (1971, 1974) expanded work along this line in a sequential study of the development of the disease. He examined the large intestines of pigs orally inoculated with mucosal scrapings of infected pigs by phase, light, and electron microscopy. Small spirochetes, large spirochetes, and vibrio were demonstrated by phase microscopy of affected animals. Vibrio and small spirochetes were also observed in normal animals. The large spirochete, morphologically similar to T. hyodysenteriae, was first observed in the colonic mucosa by phase microscopy 2 days after inoculation. Signs and macroscopic lesions of the disease were noted 3-6 days after inoculation. Histological lesions progressed from catarrhal to mucofibrinous to fibrinonecrotic enteritis. Numerous large spirochetes were observed at the luminal surface of infected animals prior to development of clinical signs and gross lesions of the disease. As the disease progressed large spirochetes were observed to be massed in the crypts; the microvilli of epithelial cells adjacent to the spirochetes were shortened and irregular. Spirochetes were observed free within the cytoplasm of viable but damaged epithelial cells.

Spirochetes with morphology and dimensions similar to T. hyodysenteriae have recently been observed by Kennedy et al. (1973) in scanning electron microscopic preparations

from the colons of pigs orally inoculated with the colonic mucosa of naturally occurring cases of swine dysentery. The spirochetes were numerous in the colons of animals at 5 and 8 days postinoculation, however, they were not seen in animals at 3 days postinoculation or in the normal control pig.

Swine Dysentery--Diagnosis and Prevention

Diagnosis

Recent additions to our knowledge of the etiology of swine dysentery should improve diagnostic methods and preventive measures for the disease. Recent reviews concerning diagnosis emphasize the following: history and clinical signs of the outbreak; postmortem examination of acutely affected untreated animals; and the observation of numerous T. hyodysenteriae-like organisms from the colonic lesions (Harris, 1974; Glock, 1973).

Detection of spirochetes in feces or colonic lesions of swine dysentery suspects can be accomplished best by phase contrast or darkfield microscopic examination of wet-mount preparations. The advantage to the live preparation is observation of characteristic serpentine motility. Since normal swine may harbor spirochetes of similar morphology it is important to observe T. hyodysenteriae-like organisms in large numbers comprising a major portion of the

flora. Fixed smears may be stained with crystal violet, dilute carbol fuchsin (Glock, 1971), or Victoria Blue-4-R (Olson, 1973) stains. Treponema hyodysenteriae may be demonstrated in sections of the colonic mucosa by Warthin-Starry, Goodpasture (Glock, 1971), and Victoria Blue 4-R stains (Olson, 1973). Examination of rectal swabs from live animals by the above methods may be used in diagnosis, however, mucosal scrapings are more reliable. With any of these methods care should be taken not to be confused by the small spirochete which is smaller in diameter and length and more tightly coiled than T. hyodysenteriae (see later sections) and may be seen in relatively large numbers in normal swine.

Workers in Holland (Terpstra et al., 1968; Akkermans and Pomper, 1973) have developed a fluorescent antibody test (FAT) which has been used in confirmation of the diagnosis of swine dysentery. Convalescent swine sera (from pigs acutely affected with swine dysentery) was absorbed against Vibrio coli and then used in an indirect FAT on fecal or colonic smears. They concluded the FAT was a valuable diagnostic procedure when the contents or feces were of abnormal consistency and when pigs submitted for examination had not been treated.

Lee and Olson (1973) reported use of an indirect FAT using convalescent swine sera on impression smears from the

colons of pigs affected with swine dysentery. They used this method with dilutions of sera to determine circulating antibody titer to spirochetes.

DeGeeter and Harris (1974) used an indirect FAT confirmation of the presence of T. hyodysenteriae-like spirochetes observed in rectal swab material from pigs affected with swine dysentery.

Saunders and Hunter (1974) have also developed a similar indirect fluorescent-antibody staining technique (FAST) for the diagnosis of swine dysentery. The antisera was prepared by hyperimmunization of rabbits with pure cultures of killed T. hyodysenteriae in Freund's adjuvant. This eliminated necessity of absorption with V. coli.

None of these groups reported absorption of the antisera with small spirochetes or nonpathogenic T. hyodysenteriae-like isolates form normal swine.

A serum agglutination test for the diagnosis of swine dysentery was developed by Hunter and Saunders (1973). They tested swine serum samples from farms with and without a history of swine dysentery. The samples from either type of farm could be divided into high and low level responses at either 100% or 50% agglutination. The high level sera from dysentery farms were significantly higher than the high level sera from dysentery-free farms. But the low level sera from either farm gave nearly identical agglutina-

tion responses. The authors did not make note of how recently the pigs had been exposed to swine dysentery and did not sample herds with active outbreaks of swine dysentery. They suggested the test may be useful for detection of the disease on a herd basis rather than on individual pigs.

There are several recent reviews on swine dysentery which cover adequately the subject of differential diagnosis (Harris and Glock, 1973; Glock, 1973; Harris, 1974). The basis for many of these diagnostic procedures is the isolation or demonstration of the causative agent of other enteric diseases and lack of T. hyodysenteriae.

Akkermans and Pomper (1973) examined the feces of pigs suffering enteritidies other than swine dysentery. The FAT for "spirochete-like" organisms was negative for 169 cases which had been diagnosed as other types of enteritis.

Prevention

There are no products currently available for the successful immunization of pigs against swine dysentery. The clinical observations of large groups of animals and the progression of the disease through a particular herd have led many to believe that no active immunity is conferred as a result of the disease. Harris and Glock (1973) have collected individual data on 4 pigs affected with swine dysentery. All 4 developed clinical signs of swine

dysentery by day 5 of the experiment. One pig died between day 5 and day 10, and 2 of the remaining pigs continued to show sporadic loose and watery feces with mucus. The fourth pig recovered and remained clinically normal through the 60 day observation period.

Olson (1974) conducted a study on the individual clinical signs of pigs inoculated with diced colon of dysentery affected pigs. Thirty-six of 37 exposed animals developed the disease. In a group of 24 the disease was allowed to run its course, and 18 died. Of the 6 remaining, recovered pigs, 5 developed bloody diarrhea. The sixth pig remained clinically normal. The one pig which resisted the original exposure also resisted a second challenge.

Taylor (1972) also reported that some pigs resist challenge with colonic scrapings from pigs affected with dysentery. He concluded that this natural resistance may be very dose dependent, based on the observation by Alexander and Taylor (1969) that the cyclic nature of the disease seemed to be related to the length of time required for multiplication of the infectious agent to the individual pig minimal infective dose.

Olson's (1974) study also examined the development of resistance to swine dysentery after repeated exposure with diced infective colon at 6 week intervals. Fourteen pigs were used in 2 experiments and all developed signs of

dysentery after the first exposure. Six of these pigs died, 5 recovered without treatment, and 3 were treated with streptomycin sulfate and recovered. The 8 recovered pigs were challenged three more times with diced colon and 5 of 8 developed blood-free diarrhea after the second dose, 4 of 8 developed blood-free diarrhea after the third dose, and none of 8 developed diarrhea after the fourth dose.

Hudson et al. (1974) orally inoculated 8 pigs with isolate A-1 (Type-1 spirochetes) after 80 in vitro passages. The pigs were observed for 30 days and none developed signs of swine dysentery. At 30 days this group along with a previously uninoculated group were orally inoculated with isolate A-1 (Type-1 spirochetes) after 12 in vitro passages. Ten of 13 previously uninoculated pigs and all 8 of the immunized pigs developed signs and lesions of swine dysentery.

In 1971 Genho et al. reported "some immunity" to swine dysentery from the intramuscular injection of globulins harvested by ammonium sulfate precipitation from sera of previously infected animals. No data or results were presented in the abstract.

Therapy

In 1969 Kunesh stated that treatment of swine dysentery would not be efficient until the disease was reproduced with

pure cultures. The logical therapeutic agent could then be used after *in vitro* antibiotic sensitivities of the agent or agents were determined. Several compounds have been evaluated *in vivo* in naturally occurring swine dysentery or in pigs inoculated with colonic material from pigs acutely affected with swine dysentery. These compounds so evaluated are currently in use: arsenicals (Smith *et al.*, 1967; Olson and Rodabaugh, 1973; Boley *et al.*, 1951); carbadox (Wilson and Roe, 1971; Kornegay *et al.*, 1968; Davis *et al.*, 1968); streptomycin (Doyle, 1954; Salisbury *et al.*, 1951); bacitracin (Boley *et al.*, 1951); neomycin (Kunesh, 1969); tylosin (Doornenbal, 1965); and sulfonamides (Boley *et al.*, 1951). Other compounds which have been reported to be efficacious in the treatment of swine dysentery include: dimetridazole (Griffin, 1972; Bech and Hyldgard-Jensen, 1972; Cottereau, 1971; Fox, 1974); ronidazole (Taylor, 1974; Sutherland *et al.*, 1974; Olson and Rodabaugh, 1974); ipronidazole (Messersmith *et al.*, 1973); gentamicin (Harris *et al.*, 1972b); lincomycin-spectinomycin (Hamdy, 1974; DeGeeter and Harris, 1974); virginiamycin (Miller *et al.*, 1972); and mix-9 (Pujic and Campa, 1973). These have not yet been approved for use in swine in the United States by the Federal Drug Administration.

Since the etiology of swine dysentery has been more clearly defined, several drug studies have noted the

association of Treponema hyodysenteriae-like organisms with the disease. Success of the treatment in these studies included the remission of clinical signs and the disappearance of T. hyodysenteriae-like organisms from the feces (Griffin, 1972; Harris et al., 1972b; Messersmith et al., 1973; DeGeeter and Harris, 1974; Miller et al., 1972; Hamdy, 1974; Olson and Rodabaugh, 1973, 1974; Sutherland et al., 1974; Taylor, 1974; Espinasse and Redon, 1970).

Two studies of the efficacy of a chemotherapeutic agent (ronidazole) have been conducted in pigs affected with swine dysentery produced by T. hyodysenteriae in pure culture (Taylor, 1974; Harris, D. L., Iowa State University, unpublished data, 1973.)

The in vitro sensitivities of pure cultures of T. hyodysenteriae have been determined for a limited number of compounds (Taylor, 1972; Harris and Glock, 1973; Messersmith et al., 1973; Sutherland et al., 1974; DeGeeter and Harris, 1974).

Spirochaetales

Classification

The name "spirochete" (Greek: "coiled hair") was first given in 1838 to a large, free-living, flexible organism found in fresh and salt water (Spirocheta plicatilis) by Ehrenberg (Breed et al., 1957). Spiral-shaped organisms

have since been named spirillum, spirocheta, spironema, spiroschaudinni and vibrio. Observations of the rod structure of Vibrio and Spirillum and of their propulsion by flagella have separated these organisms from the five genera of helically coiled organisms in the order Spirochaetales, family Spirochaetaceae (Smibert, 1973a, b).

For a time spirochetes were considered by some to be protozoan. As late as 1959, van Thiel considered spirochetes to be more closely related to protozoa for several reasons: 1) spirochetes often had an intermediate host; 2) diseases caused by spirochetes were characterized by reoccurrence and relapse; and 3) these diseases were often successfully treated with arsenicals and mercurials.

Breed et al. (1957) described spirochetes as slender flexuous organisms which were 6-500 μm long, spiral-shaped; some possessed axial fibrils, but not flagella for locomotion, which was rapid whirling or spinning. The organisms were best observed by dark-field microscopy but could also be stained with aniline dyes, Giemsa, and silver nitrate. Spirochetes which were 30-500 μm long with definite protoplasmic structure, were classified in Family I--Spirochaetaceae, including the genera Spirochaeta, Saprospira and Cristispira. Family II--Treponemataceae were 4-16 μm long with no obvious protoplasmic structures. The genera within this family were Borrelia, Treponema and Leptospira.

These were separated by affinity for aniline dyes (Borrelia stain easily, Treponema and Leptospira stain poorly with aniline dyes) and relationships to oxygen (Treponema were anaerobic; Leptospira were aerobic).

Eight species of Treponema were described:

T. pallidum[1]	the cause of syphilis in man.
T. pertenue[1]	the cause of yaws in man.
T. calligyrum	isolated from smegma, nonpathogenic.
T. genitalis	isolated from male and female genital tract, nonpathogenic.
T. microdentium	isolated from normal mouth.
T. mucosum	isolated from pyorrhea.
T. carateum[1]	the cause of pinta in man.
T. cuniculi[1]	the cause of rabbit spirochetosis.

Breed et al. (1957) did not discuss the Reiter treponeme (T. phagedenis btp reiterii) or T. refringens (Nichols) strains of avirulent T. pallidum which were being used in nutritional and antigenic studies at the time (Wallace and Harris, 1967).

Listgarten and Socransky (1965) utilized electron microscopic techniques in their examination of three groups of spirochetes. They proposed a system of classification of

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[1]Not cultivatable in vitro.

spirochetes based on the morphologic characteristics of the organisms. Spirochete morphology is covered in detail later in this discussion. The groups were:

Small spirochetes--protoplasmic cylinder diameter of 100-250 mμ, with 2-4-2 or 1-2-1 arrangement of axial fibrils.

Intermediate spirochetes--protoplasmic cylinder diameter of 200-500 mμ, with 3-6-3 to 20-40-20 arrangement of axial fibrils.

Large spirochetes--protoplasmic cylinder diameter 500 mμ and larger with 12-24-12 to more than 20-40-20 arrangement of axial fibrils.

These authors noted variability in size and number of fibrils of organisms in the log phase of growth and recommended observation of only resting cells. They hoped the classification scheme would apply to the species of Treponema which had not been cultivated in vitro.

In 1969 Socransky et al. reported the biochemical differentiation of 3 human oral small spirochetes which had been isolated and cultivated in vitro. Basic differences in serum requirement, glucose and lactate utilization and fermentation, indol and ammonia production, and gas chromatographic analysis of end products resulted in suggested classification of the 3 as separate species of Treponema even though all 3 were of similar morphology.

Smibert (1973a, 1974) (forthcoming 8th edition of Bergey's Manual of Determinative Bacteriology) describes members of the order Spirochaetales as slender, flexuous, unicellular, helically coiled, motile organisms, 5-500 μm long, 0.1 to 3.0 μm wide with 1 or more complete turns in the helix. Bizarre forms such as bullae (swollen parts of the cell envelope), round bodies (coccoid forms, spheres, coiled up cells), and granules (broken cells) occur especially in old cultures. The order has just one family--Spirochaetaceae, with five genera:

Spirochaeta--free living in nature, oxygen tolerant anaerobes and facultative anaerobes.

Borrelia--cause of relapsing fever in man; tick and louse borne; oxygen tolerant anaerobes.

Treponema--some pathogenic species; found in intestine, oral cavity, and genital tract of man and animals; strict and oxygen-tolerant anaerobes.

Cristispira--found in intestinal tract of molluscs; largest with most numerous axial fibrils; not cultivated.

Leptospira--saprophytes and pathogens, obligately aerobic, thin, tightly coiled with hooked ends.

The treponemes are 5-20 μm long and 0.09 to 0.5 μm wide with 1 or more axial fibrils. Those that have been cultured require serum or volatile fatty acids, are catalase

and oxidase negative, and have a fermentative metabolism. The type species is Treponema pallidum which has not been cultivated in vitro. This fact has made classification of the treponemes difficult. The genus is separated into 1) noncultivatable pathogenic treponemes which are propagated in laboratory animals:

T. pallidum--cause of syphilis in man;

T. pertenue--cause of yaws;

T. carateum--cause of pinta;

T. paraluis-cuniculi--cause of rabbit syphilis;

Endemic syphilis treponeme--nonvenereal syphilis;

Treponema FB--causes a natural disease in primates;

and 2) cultivatable treponemes:

T. phagedenis--isolated from phagedenic ulcer on human genitalia, also from anal and genital area of normal chimpanzees, nonpathogenic biotypes Reiter and Kazan;

T. macrodentium--isolated from gingival crevice of man, nonpathogenic;

T. refringens--normal flora of human genitalia, biotypes refringens and calligyrum;

T. denticola--oral cavity of man and chimpanzees; nonpathogenic, biotypes denticola and comondonii;

T. orale--ginginal crevice of man;

T. scoliodontum--oral cavity of man;

T. vincentii--oral cavity of man;

T. hyodysenteriae--associated with lesions of swine dysentery (Smibert, 1973a,b, 1974).

The following table from Smibert (1973b) lists characteristics of treponemes and provides a method of speciation of the above cultivatable organisms (see Table A1 in the Appendix). The organisms were grown in peptone yeast basal media prepared by prereduced anaerobically sterilized (PRAS) methods and supplemented with rabbit serum, cocarboxylase, and diacetyl tartaric acid ester of tallow monoglycerides (TEM-4T) in an atmosphere of deoxygenated N_2 or CO_2.

Structure

Knowledge regarding spirochetal morphology has been greatly advanced by the use of the electron microscope and by recent contributions which have enabled better cultivation of several more organisms in this family.

The helical structure of spirochetes includes at least 1 turn and there may be several; the frequency and amplitude of the helices also vary. Spirochetes have three main structures: 1) the outer envelope which is a trilaminar membrane covering, 2) the protoplasmic cylinder, and 3) the axial fibrils (Pillot and Ryter, 1965).

Table 1. Characteristics of treponemes[a] (Smibert, 1973a)

Species	Glucose	Lactose	Fructose	Sucrose	Mannitol	Indol	H_2S	1%Glycine, gr.	Lactate Used	Esculin Hydrolysis	Cell Diameter, μ
T. phagedenis biotype Reiter	+	+	+	-	+	+	w	+	-	-	0.25-0.35
T. phagedenis biotype Kazan	+	+	+	-	+	+	w	+	-	+	0.25-0.35
T. refringens biotype refringens	-	-	-	-	-	+	+	-	-	+	0.25-0.35
T. refringens biotype calligyrum	-	-	-	-	-	+	+	+	-	+	0.25-0.35
T. denticola biotype denticola	-	-	-	-	-	+	+	-	-	+	0.15-0.25
T. denticola biotype comondonii	-	-	-	-	-	-	+	v	-	+	0.15-0.25
T. oralis	-	-	-	-	-	+	+	ND	+	ND	0.15-0.25
T. scoliodontum	-	-	-	-	-	-	-	-	-	-	0.10-0.15
T. macrodentium	+	-	+	+	-	-	+	ND	-	-	0.15-0.25
T. vincentii	-	-	-	-	-	+	+	-	-	-	0.25-0.35
T. hyodysenteriae[b]											0.35-0.45

[a]Code: + = positive reaction or weak acid formation without gas; - = negative reaction or no acid formation; v = variable results (some strains +, some -); w = weak reaction; ND = no data available.

[b]Associated with swine dysentery.

The fibrils are filaments located between the protoplasmic cylinder and the outer envelope. They are inserted by subterminal attachment discs in equal numbers at each end of the protoplasmic cylinder and extend toward the opposite end of the cell, overlapping in the center. This results in nomenclature such as 2-4-2 regarding the axial fibril arrangement. The unattached ends of the fibrils may even extend beyond the ends of the cell but they are still enclosed in the outer envelope. The fibrils are approximately 150-200 A in diameter (Listgarten and Socransky, 1964) and are primarily protein with trace amounts of hexose (Bharier and Rittenberg, 1971; Joseph and Canale-Parola, 1972). Sykes and Miller (1973) have reported that axial fibrils of T. pallidum from infected rabbit testes were hollow while axial fibrils of T. denticola and T. reiteri were solid. The 12-15 fibrils of the Leptospira are encased in a sheath which has been called an axostyle (Bharier and Rittenberg, 1971). Bharier and Rittenberg (1971) and Joseph and Canale-Parola (1972) have noted the similarity of the axial fibrils to bacterial flagella.

Theoretically, the fibrils have been supposed to function in locomotion of the organisms, but conclusive evidence to that end has not been reported. When the fibrils were removed from spirochetes, the organisms were nonmotile and retained spiral morphology. However, the process used to

strip the outer envelope and fibrils was harsh and the authors (Joseph and Canale-Parola, 1972) could not directly report that the fibrils were responsible for locomotion. Their studies did show that the helical shape of spirochetes was due to the peptidoglycan layer. Sprirochetal motility consists of rapid rotation about the long axis of the helix, flexion, and forward or backward motion along a corkscrew or serpentine path (Smibert, 1973a). Jahn and Landaman (1965) have studied spirochetal locomotion and considered it an active bending process which travels in a helical wave along the body.

The ring forms, spherical shapes, and round bodies of spirochetes appear to develop with age, and in media with less than optimal nutrients. Wilcox and Guthe (1966) cite studies by Rose and Morton in which 5-day cultures of treponemes contained 2% ring forms and 11-day cultures contain 5% ring forms. The viability of these bizarre forms is uncertain; however, Bryant (1952) reported the successful subculture of a bovine rumen treponeme which contained predominately round bodies.

The recognition of the distinct morphologies of two intestinal spirochetes of swine was important in the studies on the etiology of swine dysentery. Small spirochetes were described as 4-7 μm in length and 0.24-0.30 μm in diameter

with 1-2-1 or 2-4-2 pattern of axial fibrils. These treponemes were tightly coiled and moved in a corkscrew path. They were seen in the luminal contents of the colons and feces of both healthy and dysenteric pigs (Harris et al., 1972d). Treponema hyodysenteriae (synonym large spirochete) was 6-8.5 μm long and 0.32-0.38 μm wide with 7-9 axial fibrils at each end. In contrast to the small spirochetes, T. hyodysenteriae moved in a serpentine path with looser, less frequent coils (Harris et al., 1972d). Pure cultures of T. hyodysenteriae have repeatedly produced signs and lesions of swine dysentery (see earlier sections in this review).

Nutrition

The metabolic and morphologic characteristics of cultivatable treponemes have been studied in a variety of cell-free media. Frequently the initial isolation and first subpassage have been achieved with great difficulty. Often, treponemes do not subpassage in the primary isolation medium. Of primary consideration in this regard are the atmospheric conditions to which the organisms are exposed, the oxidation-reduction potential, and essential nutritive value of the medium.

An anaerobic atmosphere can be provided by several methods (see later section in this review). Treponemes

were shown (Steinman et al., 1954), to require CO_2 and for that reason atmospheres using mixtures of H_2 and CO_2 are frequently used in cultivation. The CO_2 requirement can also be fulfilled by addition of bicarbonate to the medium in which case, N_2 provides a satisfactory anaerobic atmosphere (Hardy and Munro, 1966; Smibert and Claterbaugh, 1972; Bryant, 1952).

The oxidation-reduction potential of media used for the cultivation of treponemes is usually lowered by reducing agents such as thioglycollic acid, sodium sulfite or cysteine hydrochloride. Such media can be used immediately after preparation or stored under an anaerobic environment until used.

The basal medium used in cultivation of treponemes is usually protein rich. Beef or pork heart infusions or peptone solutions are commonly used. Yeast extract is often added as a source of vitamins and cofactors. Steinman et al. (1954) have shown the requirement of some treponemes for coenzyme A, sodium pyruvate, and citrovorum factor. These growth factors are present in yeast extract. Smibert and Claterbaugh (1972) have chemically defined a medium for a treponeme (PR-7) which was isolated from the intestine of swine. This organism required most of the amino acids, some short chain fatty acids, CO_2, heme, glucose, nicotinamide, folic acid, pyridoxal, thiamine, riboflavin,

pantothenate, choline, α-lipoic acid and biotin. A number of the cultivatable treponemes have been shown to require (or be greatly stimulated by) cocarboxylase (Smibert, 1971, 1973b).

All of the cultivatable treponemes require supplementation of basal media with animal tissue or fluids (serum, ascitic fluid, rumen fluid) or bacterial filtrates as a source of fatty acids. Nouguchi grew spirochetes in sterile water supplemented with pieces of sterile tissue (liver, heart, kidney) and serum (cited in Wilcox and Guthe, 1966). Since that early work, several people have successfully replaced the serum with various fatty acids. Oyama et al., (1953) applied the principle Davis and Dubos (1947) had reported with tubercle bacillus to treponemes. The serum in a medium which supported growth of T. reiteri could be replaced by crystalline albumin which was in turn replaced by lipid-free albumin and oleic acid. Media supplemented with lipid-free albumin alone would not support growth of the organism. The function of albumin was to carry and detoxify the essential lipid. This capacity was saturated at protein: oleate ratios greater than 1:4. At ratios greater than 1:4, growth of the organism was not obtained because of the toxicity of free oleic acid.

Power and Pelczar (1959) also demonstrated replacement

of serum by palmitic, stearic, and oleic acids for maximum growth of the Reiter treponeme. All three were present in TEM-4T and growth was 2 to 3 times better than with serum supplemented media.

Socransky and Hubersak (1967) cultured T. dentium in media containing ascitic fluid or rabbit serum and spermine and isobutyric acid. In this medium the serum was successfully replaced by α-2 globulin.

A medium for culture of the Noguchi strain of T. pallidum was devised by Rajkovic (1967). It included TEM-4T, cholesterol, and Tween 80. He was able to passage the organisms 25 times in this medium.

More recently Johnson et al. (1970) and Johnson and Eggebraten (1971) demonstrated the unaltered incorporation of medium lipid components into the treponeme cell. They reported the requirement of Treponema pallidum Kazan 5 and Reiter for a pair of fatty acids. One was saturated and at least 14 carbons in length; the other was unsaturated and 15 carbons long.

Several species of Treponema require rumen fluid (Smibert, 1971, 1973b). Bryant (1952) isolated a rumen fluid-requiring spirochete ("Borrelia spp.") from the bovine rumen. Wegner and Foster (1960) worked with a similar "Borrelia" and demonstrated the successful replacement of rumen fluid in media by n-valerate and isovalerate. The

rumen fluid for the PR-7 spirochete from the intestine of swine was successfully replaced by short chain fatty acids n-valerate, isobutyrate, acetate, and pyruvate (Smibert and Claterbaugh, 1972).

Hardy et al. (1963, 1964) observed the satelliting of colonies of oral spirochetes around colonies of an anaerobic diphtheroid. Culture filtrates from this anaerobic diphtheroid supported growth of the spirochetes in broth with or without additional supplementation of serum. The culture filtrate was further separated by a variety of techniques and was successfully replaced by isobutyrate in medium for growth of the spirochetes. Socransky et al. (1964) successfully replaced serum with isobutyric acid and cocarboxylase in medium for growth of T. microdentium. The isobutyric requirement could also be fulfilled by several polyamines.

The concentration of serum and ascitic fluid used in most of the above experiments was 10-20%, however, Hardy and Munro (1966) reported 10%to be inhibitory. They obtained maximum growth with 0.5-0.75% serum. Oyama et al. (1953) reported the minimum effective concentration of sodium oleate was 0.0002%.

Smibert and Claterbaugh (1972) suggested that treponemes cannot synthesize de novo the fatty acids which are used as precursors for long chain fatty acids and eventually

cellular phospholipids. Some of the treponemes appear to be able to synthesize long chain fatty acids from short chain fatty acid precursors and others cannot.

Isolation

Since treponemes are most frequently encountered in areas with high bacterial populations, methods for their isolation have been complicated and exacting. These methods usually rely upon the small size and motility of treponemes. Also important in the isolation procedures are the essential nutritive factors of the medium and the anaerobic atmosphere.

Isolation has been successfully reported by use of centrifugation (Ecker and Wed, 1931; Thomas et al., 1972); dilution (Kast and Kolmer, 1940), electrophoresis (Schmale et al., 1970), DEAE cellulose columns (Ginger and Katz, 1970), and passage of the material containing treponemes through various filters (Wichelhausen and Wichelhausen, 1942; Chandler and Clark, 1970; Taylor, 1972; Harris et al., 1972d; Harris, 1974).

The ability of treponemes to migrate through various filters has been used by several (Wichelhausen and Wichelhausen, 1942; Loesche and Socransky, 1962; Smibert, 1971, 1973b) to isolate the organism. Suspensions containing the organisms are applied to the surface of the filter which

rests on an agar medium in a petri dish. The plates are incubated anaerobically and treponeme growth appears as a subsurface haze in the agar beneath the filter and contaminating growth. Smibert (1973b) has recommended the additional inoculation of anaerobic broth media with the original specimen. This is incubated at 25° C and inoculated to subsequent membrane filters if the first is grossly contaminated. Rosebury and Foley (1941) inoculated suspensions containing treponemes into wells cut in the solid agar medium; the spirochetes migrated out into the agar leaving the contaminants behind. Variations of these spirochete "well-plates" have been used by Hampp (1943), Hanson and Cannefax (1964), Hanson (1970), and Smibert (1973b). The contaminating bacterial growth and tissue debris are believed to provide essential nutrients for growth of spirochetes by these methods.

Unique filtration methods were used by Harris et al. (1972a, d; Harris 1974) in the isolation of T. hyodysenteriae. Suspensions of dysenteric colonic mucosa in phosphate buffered saline were allowed to settle and the supernate was filtered through a series of cellulose acetate filters of decreasing pore sizes. This method facilitated the passage of the spirochetes while gradually eliminating contaminants. Filtrates were then inoculated to fresh bovine blood agar plates and incubated anaerobically.

Hanson (1970) reported the use of polymyxin B sulphate and crystal violet in the isolation of treponemes from several animals, to inhibit contaminating bacteria growth. Smibert (1973b) reported use of polymixin B, nalidixic acid, cycloserine, furacin and sulfonamides to reduce or completely inhibit contaminating bacterial growth.

Wichelhausen and Wichelhausen (1942) cultivated human oral treponemes by intramuscular injection of guinea pigs. The suspensions which they injected contained several types of contaminating organisms but usually the abscesses which developed contained primarily spirochetes. This method for increasing the titer of spirochetes and the inoculum has not been commonly accepted.

While these methods assure the purity of treponemes from contaminating bacteria, they do not necessarily assure the homogeneity of the isolate. Cloning methods which depend upon the growth of a single, well-isolated colony have been difficult to use in isolation of treponemes. Bryant (1952) obtained colonies of rumen treponemes in PRAS rumen fluid medium, which was inoculated while molten, under a stream of deoxygenated gas with diluted suspensions containing the treponemes. The tubes were spun while cooling to form a thin layer of solidified agar on the sides of the tube. Socransky et al. (1959) succeeded in obtaining surface colonies of T. microdentium on veal heart infusion blood agar

plates when inoculation and incubation were carried out in the anaerobic atmosphere of a glove bag. Rosebury and Reynolds (1964) reported similar results in isolating oral treponemes from human gingival crevices.

Smibert (1973b) routinely isolates treponemes using the migration-through-filter technique. These isolates are further purified by streaking roll tubes or spirochete well plates and isolated colonies are then picked to broth for characterization.

Hardy et al. (1963) reported the successful cultivation of treponemes as surface colonies, was due to the use of minimal concentrations of agar in liquid media.

Treponeme colonial growth is diffuse with an undefined edge which frequently spreads as concentric circles (Hanson and Cannefax, 1965; Smibert, 1973b; Harris et al., 1972d). Frequently the growth is more subsurface than surface. Colonies may appear in 48 hours (Bryant, 1952; Smibert, 1973b) but may take as long as 6-10 days (Hardy et al., 1963). They may not reach maximum size for 3-4 weeks (Hardy et al., 1963).

Characterization

The most comprehensive system for characterization and speciation of cultivatable treponemes has been compiled recently by Smibert (1971, 1973b). The characteristics (for

121 isolates) which are most reliable for speciation (10 groups; species and biotype) are cell width, fermentation of mannitol and sucrose, esculin hydrolysis, 1% glycine tolerance, indol production, conversion of lactate to propionate, and short chain fatty acid products from glucose. Other characteristics determined by Smibert include fermentation of several sugars; hydrolysis of starch and hippurate; gelatin, meat, and milk digestion; nitrate and neutral red reduction; 1% bile tolerance; gas, ammonia, acetylemethylcarbinol, hemolysin, lipase, lecithinase, catalase, and hydrogen sulfide production; conversion of threonine to propionate, and pyruvate utilization (see Table A1 in the Appendix).

The characteristics of a few treponemes have been reported by several others (Bryant, 1952; Hespell and Canale-Parola, 1971; Harris et al., 1972a,d; Harris and Kinyon, 1974; Smibert, 1971, Smibert and Claterbaugh, 1972; Socransky et al., 1969). Of special interest to this author are reports by Gates (1923), Wichelhausen and Wichelhausen (1942) and Hardy et al. (1963) which described the hemolysis of blood agar media by treponemes.

Smibert (1973b) and Socransky et al. (1969) did not detect gas production by the treponemes they studied. Bryant (1952) reported production of CO_2 from glucose by Borrelia isolated from the bovine rumen. Krichevsky and

Hampp (1966) reported the production of CO_2 by 37 treponeme isolates from the human and canine oral cavity. Four of the 37 strains produced equal amounts of H_2 and CO_2. Hespell and Canale-Parola (1971) detected CO_2 production by T. denticola from the fermentation of amino acids and glucose.

A rumen spirochete, strain $B_2 5$, has been shown to hydrogenate linoleate to octadec-trans-11-eonate (Sachan and Davis, 1969). This reaction was stimulated by methyl and benzyl viologen which indicates the involvement of an electron carrier in the hydrogenation reaction (Yokoyama and Davis, 1971).

Treponemes grown in liquid or semi-liquid media reach maximum concentration in 2-4 days (Bryant, 1952; Hardy et al., 1964; Oyama et al., 1953; Power and Pelczar, 1959; Smibert, 1973b; Smibert and Claterbaugh, 1972). Generation times for cultivatable treponemes range from 4-20 hours (Hardy, 1973).

Treponeme--host relationships

Macfie (1916), Parr (1923) and Hanson (1970) have reported the presence of spirochetes in the intestinal tract of normal mammals (dog, cat, rat, cattle, sheep, goat, pig, monkey, chimpanzee, mouse, guinea pig, gerbil, fox, racoon, opposum, and bat). Hanson (1970)

has cultivated many of these treponemes.

Savage et al. (1971), Davis and Savage (1972), Davis et al. (1973), and Savage (1972) have demonstrated the association of spirochete organisms with the colonic epithelium of SPF mice. The spirochetes establish as part of the colonic flora of 3-5 day old mice, before the association of fusiform organisms, and are considered as part of the autochthonous flora of these mice.

Davis et al. (1972) observed two types of spirochetes packed in the crypts of the ceca of rats. Occasionally these spirochetes were also seen attached to the surface epithelium or in the lamina propria either extracellularly or within phagocytes. Leach et al. (1973) also reported the presence of dense populations of spirochetes in the crypts of the ceca of rats and dogs but very few spirochetes were observed in the cecal contents of rats and dogs. Diarrhea in rats was stimulated by oral administration of magnesium sulfate. As a result, spirochetes were dislodged from the crypts as evidenced by increased numbers of spirochetes in cecal contents and feces.

Bryant (1952) reported that anaerobic spirochetes represent 4% of the total microbial flora of the rumen.

Treponema phagedenis biotypes Kazan and Reiter have been isolated from the anal and genital regions of monkey and man (Smibert, 1971).

Two morphologically distinct types of treponemes have been observed and isolated from the intestinal tract of normal swine. The smallest has been referred to as pig feces strains (Smibert, 1971), Type-2 spirochetes (Taylor, 1972), small spirochetes (Harris et al., 1972d), PR-7 strain (Smibert and Claterbaugh, 1972) and PN-5 strain (Saheb and Berthiaume, 1973; Saheb and Richer-Massicotte, 1972). These organisms have not been associated with colonic epithelium of pigs but do appear in the colonic contents as early as 2 weeks of age. The second morphological type, T. hyodysenteriae-like, has been observed most frequently in swine dysentery affected animals (see earlier section), however, T. hyodysenteriae-like spirochetes have also been isolated from normal healthy swine (Taylor, 1972). This isolate differed from the morphologically similar organisms present in swine dysentery affected pigs, in antibiotic susceptibility and hemolytic pattern on blood agar media.

Treponemes have been associated with pathological conditions in the intestines of primates, dogs, and swine. Harlan and Lee (1967) noted spirochetes attached to epithelial cells in lesions of "intestinal spirochetosis" in man. Lee et al. (1971) could not correlate the presence of spirochetes in rectal biopsies or appendices with a disease entity; and Takeuchi et al. (1971) has reported 12% of normal

monkeys and 0.9% of normal human beings have spirochetes attached to colonic epithelial cells.

A syndrome in dogs characterized clinically by diarrhea and pathologically by mucodesquamative colitis, has been referred to as "canine spirochetosis" and several laboratories have reported the presence of large numbers of spirochetes in the diarrheic stools of dogs (Jungherr, 1937; Craige, 1948; Pindak et al., 1965; Zymet, 1969; Mortensen, 1970; Goudswaard and Cornelisse, 1973; van Ulsen and Lambers, 1973).

Goudswaard and Cornelisse (1973) and van Ulsen and Lambers (1973) have applied the FAT (developed for T. hyodysenteriae in swine by Terpstra et al. (1968) and Akkermans and Pomper (1973)) to feces of dogs with diarrhea. Spirochete organisms fluoresced and they concluded that the organism was identical to T. hyodysenteriae. However, the specificity of this test for T. hyodysenteriae has not been determined.

The association of treponemes with swine dysentery has been discussed. Spirochetes have also been associated with the experimental reproduction of Trichuris suis infection in swine (Beer and Rutter, 1972). The clinical and pathological manifestations of this infection are similar to swine dysentery and in this report the authors noted the presence of numerous spirochetes (7.8-9.5 μm x 0.30-0.35 μm)

in lesions of the colons of infected animals. Spirochetes of similar size and morphology were not observed in control animals. This spirochete differed from T. hyodysenteriae in that it had only 5-7 axial fibrils.

Porcine ulcerative spirochetosis is a type of skin lesion in swine also known as necrotic ulcer or ulcerative granuloma. Types of the disease include foot-rot, necrotic jaws, arthritis, and scirrhous cord. The lesions have been noted to contain spirochetes and fusiforms. Osborne and Ensor (1955) have reproduced foot-rot only when material from these lesions, which were injected into the laminae of susceptible pigs, contained motile spirochetes. Osborne did not isolate the spirochete. Blandford et al. (1972) and Harcourt (1973) have reported ulcerative lesions on the outer ear of swine. Two of 40 affected pigs which they studied contained spirochetes but these organisms were not isolated.

Kujumgiev et al. (1971) have reported the isolation of T. suispraeputiale from preaputial diverticulum of swine.

Anaerobic Bacteriology

General

Anaerobic bacteria have been recognized since the time of Leeuwenhouk. However, the techniques for achieving anaerobiosis did not make great advances until the 1950's.

The methods used in anaerobic culture of bacteria have sought to reduce the oxygen level of the medium and atmosphere by physical or chemical means. Physical reduction methods included boiling of the medium prior to inoculation, to drive off the oxygen. Also used were a variety of containers which could be evacuated with vacuum pumps or flushed with inert gases. The chemical reduction of media was accomplished by the addition of reducing substances such as glucose, sodium sulfite, cysteine, sodium thioglycollate and glutathione. Pieces of cooked sterile tissue (containing glutathione) were the reducing agents of cooked meat medium. Small balls of cotton or metallic iron were also used as reducing agents. Reducing agents (pyrogallol and phosphorous) were used by many to remove oxygen from the chamber. Several combinations of these methods provide suitable anaerobiosis for many organisms.

An anaerobic jar which depended upon removal of oxygen in the presence of hydrogen and a heated palladium catalyst was developed by McIntosh and Fildes in 1916 (cited in

Sonnenwirth, 1972). There were many modifications of this jar, including the Brewer jar. The use of a shredded palladium catalyst which was active at room temperature by Stokes (1958) and the gas-generating package of Brewer and Allgeier (1966) were advances which made anaerobic culture methods practical in small laboratories.

Large anaerobic chambers (glove boxes) provide an oxygen-free enclosed space. The glove boxes can be equipped with an air lock to introduce or remove specimens and media; several pairs of gloves; and incubators (Aranki et al., 1969; Leach et al., 1971). These glove boxes have made it possible to isolate and identify anaerobic bacteria without ever exposing the specimen or isolates to oxygen.

In 1950, Hungate developed a technique for working with very strict anaerobes from the bovine rumen. His methods made it possible to produce, tube, sterilize, and inoculate both solid and liquid media in the absence of oxygen. The methods lower the oxidation-reduction potential of the media and prevent formation of organic peroxides. Hungate's methods were modified by Moore (1966) (Virginia Polytechnic Institute (VPI) method) and this technique has made it possible to isolate and identify strict anaerobes using prereduced anaerobically sterilized media at "bench top" conditions with sublethal exposure to oxygen (Holdeman and Moore, 1973).

Treponeme-related

Treponemes range in their sensitivity to oxygen. Loesche (1969) considered T. macrodentium, T. denticola, and T. orale to be strict anaerobes as they were sensitive to levels of oxygen greater than 0.5%. Treponema macrodentium would not grow in atmospheres containing even 0.3% oxygen.

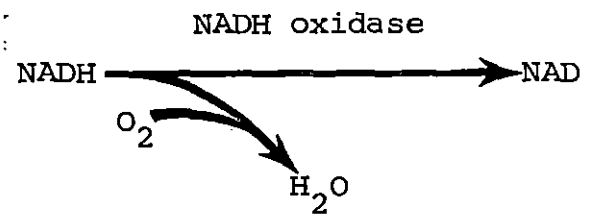
Lack of treponeme growth in aerobic environments has been noted by several others (Bryant, 1952; Hanson and Cannefax, 1964; Harris et al., 1972a, d; Saheb and Richer-Massicotte, 1972; Socransky et al., 1969; Smibert, 1971, 1973a, b).

The anaerobic collection of specimens to be cultured for anaerobic organisms is essential to their recovery (Holdeman and Moore, 1973). Socransky et al. (1959) and Rosebury and Reynolds (1964), emphasized the importance of inoculating as well as incubating in anaerobic conditions in order to achieve cultivation of treponemes as surface colonies.

Treponema hyodysenteriae is an oxygen-tolerant anaerobe. It grows well on blood agar medium which does not contain reducing agents but has been freshly prepared and incubated under H_2 and CO_2. Isolation procedures and cultures of T. hyodysenteriae on blood agar plates are handled in "bench top" atmospheres without loss of viability (Harris, 1974).

Taylor (1972) could not isolate Type-1 spirochetes using prereduced media.

The basis of oxygen-sensitivity by anaerobes has not been thoroughly investigated. O'Brien and Morris (1971) found that oxygen increased the activity of NADH oxidase in cultures of Clostridium acetobutylicum. They speculated that anaerobes tolerate a small amount of oxygen by this mechanism:



If the concentration of oxygen becomes too high the supply of NADH required for other biosynthetic functions is exhausted and multiplication is halted.

Kelly (1971) reported the possible consumption of oxygen in Borrelia hermsi (cause of relapsing fever in man) as the organism would not grow in tubes filled completely to the top with medium.

Kawata (1967) has reported presence of a_2 and b cytochromes in the Reiter treponeme. Recently, Cox and Barber (1974) have demonstrated oxygen uptake by virulent Treponema pallidum (extracted from rabbit testicle). This uptake was

sensitive to cyanide, azide, chorpromazine, and amytal. These facts suggested presence of a cytochrome oxidase mediated electron transport system possibly coupled to oxidative phosphorylation.

MATERIALS AND METHODS

Isolates

Isolates of Treponema hyodysenteriae-like organisms (ThLO) were collected from dysentery and nondysentery origins as described in Table 2. Isolates A-1, 4/71, N-1, N-2, 300/8, 1037 and strain B78 (the type species of T. hyodysenteriae) were obtained in pure culture from other investigators. The original isolates and the isolates of ThLO from pigs affected with pure culture induced swine dysentery (reisolates) were isolated from the colons by the following procedure:

A. Preparation of tissue for isolation:

1. Approximately 4-6 inches of spiral colon (isolate B205 was isolated from the rectum) from the affected animal was obtained and stored in a styrofoam chest with ice, or in a mechanical refrigerator at 4^{o} C until isolation procedures were carried out. This was usually within 24-48 hours on pure culture affected pigs; however, specimens which had been mailed were occasionally not received and isolated from until 4-9 days after necropsy.

2. The colon was opened longitudinally with sterile scissors.

3. The colonic mucosa was removed with a sterile microscope slide and ground to smooth consistency with sterile mortar and pestle.

Table 2. Origin of isolates of Treponema hyodysenteriae-like organisms

Isolate No	Origin			Referring Veterinarian	Lesion Descr Colon[c]	No of ThLO/Field Phase Exam[d]	Additional Observations[e]
		[a]	[b]				
B78	IA	SD	PC	D.L.Harris ISU, Ames	NA	NA	NA
B137	IA	SD	FS	Gipple Crawfordsville	Hm,F	+	Trichuris suis present in colon
B138	IA	SD	FS	Gonnerman Pochahontas	F,N	+	Ascarids present in intestine
B140	MN	SD	FS	H.H.Kanning Fairmont	C,F	1-2	-
B143	IA	SD	EX	R.D.Glock ISU, Ames	N,Hm	10-15	-

[a] State or country where isolate originated. Symbols: SD = swine dysentery outbreak; PWD = postweaning diarrhea in a pig; CE = catarrhal enteritis in a puppy; N = normal pig.

[b] Symbols: EX = isolate was obtained from pigs experimentally infected with crude SD inoculum taken from the original natural outbreak; PC = isolate was obtained in pure culture; FS = isolate was obtained from infected pigs in the natural outbreak.

[c] Symbols: NA = not applicable; Hm = hemorrhage; F = fibrin; N = necrosis; C = catarrah; HyM = hyperemic mucosa; G = gas; Fl = fluid.

[d] Symbols: NA = not applicable; + = ThLO present but not quantitated.

[e] Symbols: NA = not applicable; - = no other gross lesions.

Table 2 (Continued)

Isolate No	Origin		Referring Veterinarian	Lesion Descr Colon[c]	No of ThLO/Field Phase Exam[d]	Additional Observations[e]
	[a]	[b]				
B153	IN	SD FS	H.J.Olander West Lafayette Purdue U.	C,Hm	4-6	-
B163	MN	SD EX	H.H.Kanning Fairmont	M,Hm	2	-
B164	NB	SD FS	D.L.Harris ISU, Ames	M,F	2-3	-
B169	CAN	SD EX	D.A.Barnum U. of Guelph Guelph, Ontario	M,Hm	<1	-
B170	NB	SD FS	C.M.Hibbs U. of NB North Platte	M,Hm	4-6	-
B171	KS	SD EX	G.A.Kennedy KSU, Manhattan	M,Hm	1-2	-
B173	CAN	SD FS	D.A.Barnum U. of Guelph Guelph, Ontario	M,Hm,C	2-4	-
B175	NB	SD FS	C.M.Hibbs U. of NB North Platte	M,Hm,C	5-15	-

Table 2 (Continued)

Isolate No	Origin			Referring Veterinarian	Lesion Descr Colon[c]	No of ThLO/Field Phase Exam[d]	Additional Observations[e]
		[a]	[b]				
B179	IN	SD	EX	A.H.Hamdy Kalamazoo, MI	M,Hm	12	-
B204	IA	SD	FS	R.E.Messersmith Nutley, NJ	M,Hm	+	Mycoplasma pneumonia in lungs
B205	CO	SD	FS	D.J.Moeller Englewood	HyM,M	+	-
B206	KS	SD	FS	G.A.Kennedy KSU, Manhattan	M,C,Hm	3-4	-
B211	IL	SD	FS	D.C.Hoefling U. of IL, Peoria	M,C,Hm	10-15	-
B223	CO	SD	FS	D.J.Moeller Englewood	M,Hm	12-15	-
B224	IL	SD	FS	D.C.Hoefling U. of IL, Peoria	M,Hm	4-6	-
B228	IA	SD	EX	R.Glock ISU, Ames	M,C,Hm	10	-
B230	IL	SD	FS	D.C.Hoefling U. of IL, Peoria	Hm	4-6	-
B231	FL	SD	FS	H.J.Smith Roopville, GA	H,M,C	8-10	-

Table 2 (Continued)

Isolate No	Origin [a]		[b]	Referring Veterinarian	Lesion Descr Colon[c]	No of ThLO/Field Phase Exam[d]	Additional Observations[e]
B234	MO	SD	EX	L.D.Olson U. of MO, Columbia	M,HyS	2-5	-
B254	NC	SD	FS	H.J.Smith Roopville, GA	M,Hm	15-20	-
B256	IA	PWD	EX[f]	D.L.Harris ISU, Ames	G,Fl	10	10^7 CFU *E. coli*/ml in jejunum
B259	SD	SD	FS	D.C.Pankratz Freeman	M,Hm	4-5	-
Puppy	IA	CE	FS[g]	R.W.Carithers ISU, Ames	C,Hm	+	Coccidia in intestine
A-1	GB	SD	PC	D.J.Taylor Cambridge U. England	NA	NS	NA
4/71	GB	N	PC	D.J.Taylor Cambridge U. England	NA	NA	NA

[f] Isolate from experimental control pig with postweaning diarrhea.

[g] Isolate from natural outbreak of catarrhal enteritis in puppies.

Table 2 (Continued)

Isolate No	Origin [a]		[b]	Referring Veterinarian	Lesion Descr Colon[c]	No of ThLO/Field Phase Exam[d]	Additional Observations[e]
N-1	JAP	SD	PC	S.Oda Niigata U. Niigata City	NA	NA	NA
N-2	JAP	SD	PC	S.Oda Niigate U. Niigate City	NA	NA	NA
300/8	NETH	SD	PC	J.W.Akkermans Central Vet Inst Rotterdam	NA	NA	NA
1037	NETH	SD	PC	J.W.Akkermans Central Vet Inst Rotterdam	NA	NA	NA

4. Approximately 2 g of ground mucosa were suspended in 18 ml of sterile phosphate buffered saline, 0.01 M, pH 7.2-7.4 (PBS--see page 79). The suspension was vigorously mixed for 1 minute.

B. Isolation by Titration:

1. The suspension from A was serially diluted ten-fold in PBS to 10^{-8}. Using a calibrated platinum loop, 0.01 ml of each dilution was streaked on one-quarter of a blood agar plate (see page 73).

2. Blood agar plates were also streaked with approximately 0.05 ml from the 10^{-2} and 10^{-3} dilutions for isolation.

C. Isolation by Filtration:

1. The suspension from A was centrifuged at 4° C for 10 min at 55 X G to remove particulate matter.

2. The supernate was passed via syringe through Swinney holder containing cellulose acetate membrane filters[1] of decreasing pore sizes: prefilter, 8 μm, 5 μm, 1.2 μm, 0.8 μm, 0.65 μm and 0.45 μm. Only the 0.65 μm and 0.45 μm filters were sterilized before use.

3. Blood agar plates were streaked for isolation with 0.05 ml of the 0.8 μm, 0.65 μm, and 0.45 μm filtrates. The 0.45 μm filtrate was also lawn streaked in 0.01 ml, 0.1

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[1]Millipore Corporation, Bedford, Massachusetts.

ml and 0.5 ml amounts on blood agar plates.

All plates were incubated at 37° C in H_2:CO_2 anaerobic atmospheres. Plates were examined for growth of ThLO every 2 days and held for as long as 18 days. Growth was evidenced by zones of hemloysis in the blood agar. Pieces of agar from the hemolytic areas were transferred to fresh blood agar media or to tubes of liquid media on the tip of 1 ml plastic pipettes. If only a small zone of hemolysis was available to transfer, it was streaked in a very small area on the new plate to prevent dilution of ThLO.

Pure cultures of isolates were obtained by subculturing 2-4 times on solid media. At each passage a portion of the material was simultaneously inoculated into thioglycollate[1] broth to detect the presence of contaminating bacteria--especially Vibrio spp.

Strict adherence to accepted cloning methods was not possible until the development of liquid media. Even then the use of agar to broth to agar cloning methods was tedious because of the necessity of transferring agar, and the mechanical unlikelihood of successfully transferring agar from a single zone to a tube of broth. Therefore all isolates were cloned by a modified procedure: always transferring from the last part of a streak, or when possible from the last dilution

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[1]Difco Laboratories, Detroit, Michigan.

of the titration. Isolates in these studies were used at the lowest possible *in vitro* passage (5-15 passages) except for the following: B78, greater than 24 passages; A-1 and 4/71, 15-25 passages; N-1 and N-2, 34-40 passages; and 300/8 and 1037, unknown. Isolates 300/8 and 1037 were passaged *in vitro* 5-10 times during the present studies.

Isolates of ThLO were harvested from blood agar plate cultures with PBS and preserved by freezing at -80° C. Suspensions were made by adding 2 ml of sterile PBS to the surface of a 4 day culture. The surface was then streaked with bacteriological loop to remove surface growth and small pieces of agar. The material was pipetted into sterile tubes under deoxygenated CO_2, and stoppered. The stoppered tubes were frozen immediately. Cultures of ThLO in liquid media were also preserved by freezing at -80° C in sterile stoppered tubes under deoxygenated CO_2. In addition, the suspensions harvested with PBS from blood agar slant cultures and cultures in liquid medium were lyophilized. Twenty-four tubes of suspensions harvested from blood agar slant material were frozen at -80° C and 8 vials of the same material were lyophilized. A frozen culture (from -80° C) was checked monthly and a lyophylized culture was checked every 3 months over a 2 year period for viability by thawing or hydrating the preserved sample and inoculating blood agar plates which were incubated anaerobically and observed for growth.

Culture Media

Solid

Blood agar plates used in isolation and purification, propagation, and colony count determinations of ThLO were prepared from two different basal media containing 5% bovine blood. In initial studies, ThLO were cultured on tryptose with yeast extract agar medium containing: 3 g beef extract,[1] 5 g NaCl, 10 g tryptose,[1] 10 g proteose-peptone No. 3,[1] 5 g yeast extract,[1] 18 g agar,[1] and 1000 ml distilled H_2O. In latter studies the medium used was trypticase soy agar (TSA)[2] (40 g in 1000 ml distilled H_2O).

Both of the above basal media were sterilized 15 min at 121° C, and cooled to 48° C before the addition of 5% bovine blood. The blood was aseptically collected in sodium citrate (1 g/100 ml of blood) procured from the same Holstein cow[3] (No. 30), and used within 1 month of collection. Twice in the past 2 years blood collected from cow No. 30 has not supported growth of ThLO, and an attempt to use bovine blood from another cow resulted in no growth of ThLO.

Blood from different animal species was also collected

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[1]Difco Laboratories, Detroit, Michigan.

[2]BBL, Division of Bioquest, Cockeysville, Maryland.

[3]Iowa State University, Veterinary Clinical Hospital.

in sodium citrate from clinically normal animals. The human blood was O positive, and was collected from a laboratory technician in this department. Plasma of blood from all species of animals used in these experiments were negative for treponemal antibody by the rapid plasma reagin (RPR)[1] test (Portnoy et al., 1962).

Blood agar plates were prepared fresh the day of use. In some studies 24-48 hour-old plates were used if they had been held prereduced in GasPak jars[2] with H_2:CO_2 (Brewer and Allgiers, 1966), generator envelopes[2] for the interim. All plates within an isolation study or a colony count determination were of the same age to insure consistency.

Isolates B137 through B203 were isolated on tryptose with yeast extract blood agar plates. Isolates B204 through B263 were isolated on TSA blood agar plates. The inocula for transmission experiments SD No. 58 through 72 were grown on tryptose with yeast extract blood agar plates while inocula for SD No. 80 through 109 were grown on TSA blood agar plates.

Liquid

Thioglycollate broth was prepared according to manufacturer's directions, dispensed in 7 ml amounts in screw

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[1]Hynson, Westcott and Dunning, Inc., Baltimore, Maryland.

[2]BBL, Division of Bioquest, Cockeysville, Maryland.

capped tubes, and autoclaved. Immediately before use, the tubes were boiled 5-10 min to drive off accumulated O_2.

Peptone yeast[1] (PY), peptone yeast with glucose[2] (PYG) and E broth were prepared and dispensed in 7 ml amounts in rubber stoppered anaerobe tubes[3] by prereduced anaerobically sterilized (PRAS) methods as described by Holdeman and Moore (1973). Peptone yeast serum (PYS) and peptone yeast rumen fluid (PYRF) were prepared and dispensed in 7 ml amounts in stoppered anaerobe tubes by PRAS methods as described by Smibert (1971).

Aerobically prepared trypticase soy broth without dextrose (TSB)[4] was rehydrated according to manufacturers directions in a 300 ml amount in a 1000 ml Erlenmeyer flask, the pH was adjusted to 6.85, the medium was brought to a boil, and boiled for 10 min. The medium was cooled at room temperature for 15 min and then pipetted in 7 ml amounts to stoppered anaerobe tubes. Prereduced anaerobically sterilized TSB was rehydrated according to manufacturer's directions in 400 ml amounts in 500 ml boiling flasks, and 0.4

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[1]Difco Laboratories, Detroit, Michigan.

[2]J. T. Baker Chemical Co., Phillipsburg, New Jersey.

[3]Bellco Glass, Inc., Vineland, New Jersey.

[4]BBL, Division of Bioquest, Cockeysville, Maryland.

ml/100 ml medium of resazurin[1] solution (11 mg/44 ml distilled H_2O) was added. The medium was brought to a boil and boiled for 10 min. The reazurin would not reduce to yellow regardless of length of boiling time. After boiling, a stream of deoxygenated CO_2 was bubbled through the medium which was held in an ice bath. When cool, cysteine hydrochloride[2] 0.05 g/100 ml medium was added and pH was adjusted to 6.4. The gas flow was changed to deoxygenated N_2, and the medium was tubed in 7 ml amounts in stoppered anaerobe tubes under deoxygenated N_2. For detection of the gaseous products of ThLO, TSB was prepared with cysteine hydrochloride and resazurin as above, but was bubbled under deoxygenated N_2 only.

All media in stoppered anaerobe tubes were autoclaved in presses[3] for 15 min at 121° C. The pH after autoclaving was 6.6 to 7.2 for all media. Supplements were added immediately before inoculation.

Supplements for liquid media Colonic mucosa from normal and swine dysentery affected pigs was ground with mortar and pestle and diluted in PBS. The resulting suspensions were filtered through 0.22 μm cellulose acetate

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[1]Matheson, Coleman and Bell, Norwood, Ohio.

[2]Sigma Chemical Co., St. Louis, Missouri.

[3]Bellco Glass, Inc., Vineland, New Jersey.

filters. These colonic filtrate preparations were added to liquid and solid media prior to autoclaving.

Both E and PYRF media contained 30% (V/V) rumen fluid[1] (Holdeman and Moore, 1973), which was added prior to autoclaving.

Normal rabbit sera[2] (NRS) from pooled sources (negative for treponemal antibodies by the RPR test were prepared as described by Smibert (1973b) and added to autoclaved media immediately prior to inoculation in 10% concentrations (V/V). Cocarboxylase[3] and TEM-4T[4] were also prepared as described by Smibert (1973b) and added to autoclaved media immediately before inoculation and added in 0.005 mg/ml and 0.16 mg/ml final concentrations, respectively. Fetal calf serum (FCS)[5] was obtained sterile in 500 ml amounts. This was dispensed by means of a sterile automatic syringe to sterile screw capped tubes and added to media in 10% (V/V) final concentration. Supplements other than TEM-4T were stored at -20° C until used.

Media containing rumen fluid were also supplemented with

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[1]Kindly supplied by Dr. M. J. Allison, NADC, Ames, Iowa.

[2]Pel-Freeze Biologicals, Inc., Rogers, Arkansas.

[3]Sigma Chemical Co., St. Louis, Missouri.

[4]Witco Chemical Corp., New York, New York.

[5]Grand Island Biological Co., Grand Island, New York.

FCS, NRS, or NRS with TEM-4T and cocarboxylase as were the other basal media.

Routine culture media systems were PRAS-TSB with 10% (V/V) FCS inoculated with 7% inoculum (V/V) under deoxygenated H_2:CO_2 or CO_2 alone; or aerobically prepared TSB with 10% FCS (V/V) with 7% inoculum (V/V) under deoxygenated H_2:CO_2.

Experiments for the determination of requirements of minimum FCS and minimum inoculum were conducted as block titrations, simultaneously, using the following pattern for either isolate B204 or B234 under H_2:CO_2 or CO_2 alone:

	FCS							
Inoculum	20%	15%	10%	5%	1%	0.5%	0.1%	0%
10%								
7%								
4%								
1%								

An experiment for the determination of the growth curve of ThLO was conducted. Thirty-six tubes of PRAS-TSB were supplemented with 10% FCS and inoculated (7% V/V) with a 3 day growth of isolate B204 at 8th passage under H_2:CO_2. Three tubes were randomly chosen, vigorously mixed, observed for growth, opened, examined by phase microscopy, and colony counts were determined every 6 hours for 72 hours. The colony counts were performed by plating each dilution on 2

blood agar plates. The dilutions were done in duplicate from each of the three tubes. Generation time was calculated according to the formula from Frobisher (1969).

Colony count determinations were made on the 2nd and 4th days of incubation of cultures, by removing 0.5-1.0 ml of culture from tubes under a stream of deoxygenated H_2:CO_2 or CO_2. The culture was diluted 100-fold in PBS and then serially diluted 10-fold in PBS to 10^{-6} and streaked on blood agar media which was incubated in H_2:CO_2 anaerobic atmospheres. Colony count determinations were performed on the day a particular culture was transferred, to calculate the number of CFU/ml in the subpassage at the zero hour.

The diluent used in the studies was phosphate buffered saline (PBS 0.01 M) and was prepared by combining 85.7 ml of a 0.1 M stock solution of Na_2HPO_4 with 14.3 ml of a 0.1 M stock solution of KH_2PO_4 and 900 ml of 0.85% NaCl. The solution was thoroughly mixed and the pH before and after autoclaving was 7.2-7.4.

Anaerobiosis

Anaerobic atmospheres for plate media were provided in vented GasPak jars with cold palladium catalyst. The jars were evacuated with a pump and flushed with H_2 3 times, evacuated a 4th time and filled to 10 lbs vacuum with H_2. Carbon dioxide was added to 5 lbs vacuum and the resulting

mixture was 80% H_2 and 20% CO_2. This was the H_2:CO_2 anaerobic atmosphere used for all GasPak jars in all studies of this work, except for the demonstration of H_2 stimulation, where GasPak H_2:CO_2 generator envelopes and CO_2 alone were used in GasPak jars with cold palladium catalyst. Methylene blue indicators were colorless in all 3 atmospheres.

GasPak jars were opened every 2 days and the plates examined for colonies. After opening, jars were always evacuated within 2-4 hours. Freshly inoculated blood agar plates were similarly evacuated as soon as possible to prevent excess exposure to O_2.

Anaerobic atmospheres for liquid media were provided by incubating foil-covered or screw-capped tubes in GasPak jars with H_2:CO_2 anaerobic atmospheres or by inoculating media in special rubber stoppered anaerobe tubes under a stream of deoxygenated H_2:CO_2, CO_2, or N_2. Deoxygenation was accomplished by passing the gases through a glass column of copper filings heated to 350° C. The mixture of H_2 and CO_2 in these studies was 50:50, the gases were combined by use of a gas proportioner[1] after each had been deoxygenated.

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[1]Curtin-Matheson Scientific, Inc., Houston, Texas.

Animals

The Yorkshire, Hampshire and Crossbred swine used in these studies were obtained from the specific pathogen-free (SPF) herd maintained at the Veterinary Medical Research Institute at Iowa State University. This herd has been free of swine dysentery for 23 years. The SPF Duroc swine were obtained from Sam Kennedy, Clear Lake, Iowa, whose herd has been free of swine dysentery for 6 years. Both swine herds use antibiotic-free feed. The Beagle puppies were obtained from Marshall Research Animal Incorporated, Northrose, New York. The pigs were not vaccinated. The puppies received live distemper and hepatitis vaccine but were not immunized for leptospirosis.

The majority of pigs and all puppies were housed in previously fumigated 4 ft x 4 ft isolation cages, with forced filtered air systems and slatted floors draining into buckets of Roccal[1] disinfectant. The fumigant was 5 g potassium permanganate mixed with 10 ml 40% formalin and was allowed to react 24 hours. The cage exhaust fans were run for 48 hours prior to the introduction of pigs or puppies.

Strict isolation was maintained between the 10 cages. Feed and water could be added without opening the door. Caretakers wore latex gloves and clean coveralls when it was

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[1]Winthrop Laboratories, Division of Sterling Drug Inc., New York, New York.

necessary to handle the animals (i.e.; collection of rectal swabs). The gloves were scrubbed in Roccal disinfectant between cages.

Pigs inoculated with isolates B231 and B234 in SD No. 100, and the 6-week-old pigs in Ex SD 93 were housed in concrete isolation pens (4 under 1 roof). Maintenance of strict isolation in this system was more difficult as the walls which separated pens were only 4 feet high.

Room temperature for the first week of a given experiment utilizing 2-week-old pigs was 80-90° F. After the first week, the temperature was adjusted to 70-80° F.

Two-week-old pigs were abruptly weaned when put on experiment and were fed a pig starter ration of 18% protein until the third week of the experiment. At this time the pigs were fed a pig grower ration of 16% protein.

The 6-week-old pigs were maintained in the VMRI herd until the experiment was initiated and then were given the pig grower ration.

The 6-week-old Beagles were fed Gaines Meal.[1] All pigs and puppies were brought into the isolation cages at least 2 days prior to the start of an experiment. Feed was withheld for 18-24 hours prior to inoculation. Animals were inoculated once a day for 2 days. Part of the inoculum was

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[1]General Foods Corp., White Plains, New York.

given with feed on both days and animals were put back on feed ad libidum on Day 3 of the experiment. Water was available ad libidum throughout the experiment.

Blood agar plate cultures were used as inocula for all pathogenicity determinations except isolates B140 and B204 in SD No. 81 which were grown in aerobically prepared TSB with FCS to determine the virulence of ThLO in a liquid medium.

Blood agar plate cultures were inoculated from either blood agar plate cultures or broth cultures. Agar to agar transfers were made with 0.1 ml of chopped agar and 0.3 ml PBS which were lawn streaked over the surface of the plate. Broth to plate transfers were made by lawn streaking 0.3 ml of broth over the agar. Plates were incubated 3-4 days in H_2:CO_2 anaerobic atmospheres. Agar was removed from petri plates and placed in plastic bags by use of sterile microscope slides. The material was mashed by hand-kneading the bag, and approximately 1 g was removed for a colony count determination. The 2-week-old pigs received 4-8 mashed blood agar plates per pig per day (80-160 g). Twenty-to-forty g of this material was given directly via syringe to the 2-week-old pigs. The remainder of the inoculum was mixed with feed and left for the pigs to eat. Broth cultures were given at the same dosage. The 6-week-old pigs and puppies were not inoculated individually, however, they

were observed to eat the entire inoculum. The older animals received 10 plates per animal per day mixed with feed.

Crude inoculum was prepared by suspending the ground colonic mucosa from pigs acutely affected with swine dysentery in PBS (1:1). This was administered orally in 20 ml amounts via syringe to pigs which had been starved for 18-24 hours.

Animals were observed daily for fecal consistency (normal, soft, loose, or watery); fecal composition (normal, mucus, blood, or mucus and blood); and general condition (normal, depressed, gaunt, or moribund). Presence or absence of ThLO, Vibrio spp., small spirochetes in the feces was determined once preinoculation, and every 2 days postinoculation by means of rectal swab samples. Rectal swab specimens were collected on sterile, dry, cotton-tipped applicators which were immersed in 0.5 ml of PBS and held at 4° C until observation by phase microscopy was performed.

Pigs to be necropsied were stunned by electrocution, and exsanguinated. Postmortem observations of stomach, small intestine, colon, cecum and rectum as well as other body organs were recorded. Portions of the colon and any abnormal body organs were fixed in 10% buffered formalin for observation by light microscopy. These fixed tissues were embedded in paraffin, sectioned (6 μm), and stained with hematoxylin and eosin (Glock, 1971).

Eight to 10 inches of the spiral colon was also placed in a sterile plastic bag. Portions of this were: 1) used to reisolate ThLO; 2) frozen at -80° C; 3) used in an attempt to isolate Salmonella spp.; and 4) used for phase microscopic examination of the mucosa for presence of ThLO, vibrio and small spirochetes.

Salmonella spp. were isolated by streaking contents from preinoculation rectal swabs or aseptically opened colons on Tergitol-7[1] medium. The rectal swab or approximately 1 g of colonic tissue was added to tubes containing 20 ml of Tetrathionate broth.[1] After 18-24 hours incubation, approximately 0.05 ml of tetrathionate broth was streaked to brilliant green agar with sulfadiazine[2] (BG). Lactose negative colonies on either T_7 or BG were inoculated to differential media and identified as outlined in Oetjen and Harris (1973). All media were incubated at 37° C. Suspects which were biochemically compatible with Salmonella spp. and which were agglutinated in the presence of salmonella polyvalent antisera[1] were serotyped at the National Animal Disease Center.[3]

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[1]Difco Laboratories, Detroit, Michigan.

[2]BBL, Division of Bioquest, Cockeysville, Maryland.

[3]Dr. B. Blackburn, NADC, Ames, Iowa.

A phase microscope (Leitz, with Heine condenser)[1] magnification 630x, was used in determining: 1) the presence or absence of ThLO in rectal swab material and colonic scrapings; and 2) the relative amount of growth of isolates of ThLO in various liquid media. Rectal swab material to be observed was prepared by placing a small drop of PBS buffer containing feces on the slide. For pure culture examination, a small drop of culture (from loop or pipette) was placed on the slide. Mucosal scrapings were prepared by gently scraping away feces and mucus; a sharp instrument (edge of slide or broken applicator stick) was used to make a deep mucosal scraping. This tissue was then mixed with a small drop of PBS on the slide. All preparations were coverslipped before examination.

Characterization

Differential media

Reactions of ThLO in differential media were considered valid if: 1) during the 12-15 day incubation period cultures developed the characteristic shimmer of growth, or if at the end of the incubation period some shimmer of growth was obvious; 2) 0.05 ml of each differential culture would grow on

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[1]Ernst Leitz, Wetzlar, West Germany.

TSA with blood; and 3) ThLO were observed in the culture by phase microscopic examination.

Most differential media were prepared according to the same techniques used for PRAS-TSB and tubed in 7 ml amounts. Exceptions are explained below, when necessary. Differential media are designated in the following notation, the basal medium-substrate (concentration of substrate). Autoclave times for differential media are as outlined in Holdeman and Moore (1973).

All these media were supplemented with 10% FCS (except PRAS-TSB-esculin, 1%) and inoculated (7% V/V) under deoxygenated atmospheres. All were incubated at 37° C in a slanted rack for 12-15 days, except 1) esculin (see page 88) and 2) media to determine ability of ThLO to grow at 25° C, 30° C and 42°C.

Differential media inoculated for the determination of acid production from substrates included PRAS-TSB without additional substrate and PRAS-TSB with the following (1g/100 ml of medium, 1%): amygdalin,[1] arabinose,[2] cellobiose,[1] erythritol,[3] fructose,[4] glucose,[4], glycogen,[3] inositol,[5]

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[1]Sigma Chemical Co., St. Louis, Missouri.
[2]Difco Laboratories, Detroit, Michigan.
[3]Nutritional Biochemical Corp., Cleveland, Ohio.
[4]J. T. Baker Chemical Co., Phillipsburg, New Jersey.
[5]Matheson, Coleman and Bell, Norwood, Ohio.

lactose,[1] maltose,[2] mannitol,[3] mannose,[4] melezitose,[4] melibiose,[4] rhamnose,[4] raffinose,[2] ribose,[5], salicin,[2] sorbitol,[6] starch,[7] sucrose,[1] trehalose,[2],xylose,[2] adonitol,[2] dulcitol,[2] glycerol,[1] galactose,[8] inulin,[8] sorbose,[5] pectin,[4] tartaric acid,[3] dextrin,[4] mucin,[2] galacturonic acid,[5] gluconic acid,[4] and glucuronic acid.[4]

After incubation the pH of the media (with and without substrate) was determined by the placement of a single probe pH electrode[9] into the culture tube.

Acid production from 1% esculin[4] in PRAS-TSB was measured as above, however, the medium was inoculated with 10% inoculum and 0.5% FCS and was compared after 12-15 days incubation to a control PRAS-TSB with 10% inoculum and 0.5% FCS. This was done in order to obtain accurate information regarding esculin hydrolysis by ThLO. Esculin in

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[1]J. T. Baker Chemical Co., Phillipsburg, New Jersey.

[2]Difco Laboratories, Detroit, Michigan.

[3]Mallinkrodt Chemical Works, St. Louis, Missouri.

[4]Sigma Chemical Co., St. Louis, Missouri.

[5]Nutritional Biochemical Corp., Cleveland, Ohio.

[6]Matheson, Coleman and Bell, Norwood, Ohio.

[7]Fischer Scientific Co., Pittsburgh, Pennsylvania.

[8]Pfanstiehl Laboratories, Inc., Waukegan, Illinois.

[9]Coleman Instruments, Maywood, Illinois.

PRAS-TSB-esculin was completely hydrolyzed after 2 days incubation when uninoculated tubes were supplemented with 10% FCS. Esculin hydrolysis was detected by use of ferric ammonium citrate (Holdeman and Moore, 1973). After 8 days incubation esculin in PRAS-TSB-esculin was slightly hydrolyzed when uninoculated tubes were supplemented with 0.5% FCS.

Since 4 days of incubation in PRAS-TSB-esculin were required for growth of ThLO, the following method was devised. One ml of a culture of ThLO in PRAS-TSB-esculin with 0.5% FCS under CO_2 was aseptically removed from the culture tube after 4, 6, and 8 days incubation. The culture and uninoculated, incubated, PRAS-TSB-esculin and 0.5% FCS were serially diluted 2-fold in PBS; 0.5 ml of reagent was mixed with each tube. The uninoculated control was hydrolyzed at the 1:0 dilution after 8 days incubation but not at 1:2 or 1:4, while esculin hydrolysis positive cultures of ThLO hydrolyzed esculin in all dilutions through 1:8.

To detect hippurate hydrolysis, 1% sodium hippurate[1] was added to PRAS-TSB. An uninoculated control with 10% FCS was set up with each inoculated PRAS-TSB-hippurate culture. After the incubation period, 1 ml amounts of the uninoculated control medium were titrated with 0.1, 0.2, 0.3,

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[1]Difco Laboratories, Detroit, Michigan.

0.4, 0.5, 0.6, or 0.7 ml sodium hippurate reagent (ferric chloride). The minimum amount of reagent (usually between 0.5 ml and 0.6 ml) which resulted in a clear uninoculated control was added to the culture. Presence of a brown precipitate was recorded as hydrolysis.

The ability of ThLO to liquefy gelatin[1] was determined in medium prepared as described by Holdeman and Moore (1973), with the exception that TSB was used as the basal medium.

Chopped meat medium was prepared from horse meat as described in Holdeman and Moore (1973) with the exception that the basal medium was TSB. After the incubation period the culture was observed for digestion and blackening of the meat. Production of indol was tested from both chopped meat and SIM medium[2] as outlined in Holdeman and Moore (1973).

SIM medium was prepared as in Holdeman and Moore (1973), with the exception that cysteine hydrochloride and resazurin were not added. The medium was also examined for the presence of H_2S (Holdeman and Moore, 1973).

Nitrate[2] was prepared as described in Holdeman and Moore (1973). Reduction of nitrate to nitrite was determined as described in Holdeman and Moore (1973).

Catalase production was determined (as described by

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[1]Difco Laboratories, Detroit, Michigan.

[2]BBL, Division of Bioquest, Cockeysville, Maryland.

Holdeman and Moore, 1973) on TSA slants containing 10% FCS, which had been inoculated by streaking the slant with a loop of ThLO.

The ability of ThLO to metabolize glucose to acetylmethcarbinol was assayed (as described by Holdeman and Moore, 1973) from cultures grown in TSB-glucose (1%) prepared with cysteine, without resazurin, and supplemented with 10% FCS. This culture was also used to assay for production of ammonia (described by Holdeman and Moore, 1973).

The production of gas by ThLO was determined in TSA aerotolerance (described by Holdeman and Moore, 1973) deep agar tubes containing 10% FCS which were inoculated while molten at 48° C. The tubes were incubated with foil covers which also tested for the ability of ThLO to grow in the presence of O_2. An alternative method for the determination of production of gas was inoculation of tubes of TSB with cysteine hydrochloride and resazurin prepared under N_2 only, which contained Durham tubes. When Durham tubes were added to medium prepared under both CO_2 and N_2, bubbles of gas were observed in the FCS supplemented uninoculated controls.

The ability of ThLO to reduce neutral red[1] was tested by

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[1]J. T. Baker Chemical Co., Phillipsburg, New Jersey.

addition of 0.1 mg/100 ml (final concentration) neutral red to PRAS-TSB without cysteine or resazurin. The medium was a faint pink after preparation and remained pink after supplementation until slight growth of ThLO was observed.

The production of lecithinase and lipase (Holdeman and Moore, 1973) by ThLO was determined on TSA containing 10% FCS and 20% egg yolk which was inoculated and incubated in anaerobic H_2:CO_2 atmospheres of GasPak jars for 6 days.

Presence of oxidase was determined on 6 day growth of ThLO on TSA with blood by addition of fresh 1% N.N-dimethyl-para-phenylene diamine monohydrochloride.[1]

Urease production was tested in urea[2] medium prepared as described by Holdeman and Moore (1973). The production of urease is observed by increase in pH compared to control.

The ability of ThLO to grow in 2% bile[2] (Holdeman and Moore, 1973) in PRAS-TSB with glucose, was determined.

The ability of ThLO to grow in 1% glycine[3] (Holdeman and Moore, 1973) in PRAS-TSB with glucose, was determined.

The ability of ThLO to grow in PRAS-TSB-NaCl (3.0) or (6.5) was determined.

The ability of ThLO to grow in PRAS-TSB at pH 9.6 or 6.0

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[1]Matheson, Coleman and Bell, Norwood, Ohio.

[2]Difco Laboratories, Detroit, Michigan.

[3]Sigma Chemical Co., St. Louis, Missouri.

was determined.

The ability of ThLO to grow in the presence of 1.0 μM iodoacetic[1] acid was determined in PRAS-TSB with glucose.

Lactate,[1] pyruvate,[1] tartaric acid, galacturonic acid, gluconic acid and glucuronic acid were prepared by addition of the substrate 1g/100ml PRAS-TSB; however, these media were prepared from the acid moeity of the compound and required additional buffering. This was provided by salt solution (Holdeman and Moore, 1973). This approach was taken because of the results of preliminary data which indicated growth in PRAS-TSB-lactate was possible only if the medium included buffer. Lactate and pyruvate were assayed for utilization by gas chromatography (see page 94). Galacturonic, gluconic, and glucuronic media were assayed for acid production.

Threonine[2] (1%) was prepared 1g/100 ml PRAS-TSB; assay for conversion of threonine to propionate was by gas chromatography.

All differential media (uninoculated) were supplemented with FCS, under a stream of deoxygenated CO_2 or N_2, incubated 12 days, and analyzed on 4 different occasions. The pH of uninoculated, supplemented media under CO_2 was 6.2 ± .2. The

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[1]Sigma Chemical Co., St. Louis, Missouri.

[2]Nutritional Biochemical Corp., Cleveland, Ohio.

pH of uninoculated, supplemented media under N_2 was $6.9 \pm .3$.

Quality controls were run on several of the special test procedures and carbohydrate substrate fermentations of these differential media. Anaerobic bacteria were inoculated into the PRAS-TSB differential media to determine the reliability of the substrates.

Gas chromatography

Cultures of ThLO in PRAS-TSB; PRAS-TSB-glucose, fructose or maltose; PRAS-TSB-lactate, pyruvate and threonine were analyzed for alcohols and volatile and nonvolatile short chain fatty acid products as described by Holdeman and Moore (1973) by use of a Varian Aerograph[1] thermal conductivity gas chromatograph.

Uninoculated control media PRAS-TSB, PRAS-TSB-glucose were also analyzed and found to contain acetic and lactic acid. Peak height of uninoculated control media was subtracted from peak height of cultures in PRAS-TSB and PRAS-TSB-glucose. These corrected values were then compared. Cultures which produced higher peak heights of a particular acid in PRAS-TSB with glucose, fructose, or maltose than in PRAS-TSB were considered to produce that acid.

Peak heights of lactic and pyruvic acid in cultures of

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[1]Varian Aerograph, Walnut Creek, California.

ThLO in PRAS-TSB-lactate or pyruvate were compared to uninoculated control media. Disappearance of lactate and pyruvate were recorded as utilization of that fatty acid by cultures of ThLO.

Conversion of threonine to propionic acid was determined by comparison of propionic acid peaks from the culture in PRAS-TSB-threonine to those in PRAS-TSB.

Gaseous products of ThLO were analyzed by gas chromatography by injecting atmospheres above cultures of ThLO into a Beckman[1] GC-2A gas chromatograph and a Loenco[2] respiration and blood gas analyzer. The Beckman instrument was operated at column temperature of 40° C, detector current of 150 mA, with a 6 foot long, 1/4 inch diameter stainless steel column packed with silica gel and carrier gas of argon. It detected H_2 and N_2. The Loenco instrument (Model AD 2000) was operated at column temperature of 53° C, detector current 150 mA, with helium as the carrier gas. This analyzer had 2 columns with a CO_2 stripper between them; and detector at the end of each column. The first column was 3 feet long, 1/4 inch diameter aluminum, packed with 80-100 mesh Porapak Q, and detected a composite peak of all gases but CO_2, and a CO_2 peak. The second column was 12 feet long, 1/4 inch diameter

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[1]Beckman Instruments, Inc., Fullerton, California.

[2]Envirotech Corp., Mountain View, California.

aluminum, packed with 30-60 mesh molecular sieve, and it separated O_2, N_2 and CO.

A tuberculin syringe was used to obtain and inject the samples. It was rinsed with H_2 prior to insertion through the stopper of culture tubes; rinsed 5 times with the atmosphere above cultures and withdrawn containing 800 µl of gas. Three hundred µl samples were injected after the needle had been flushed with the excess atmosphere in the syringe.

Peaks were identified according to retention time as compared to standards of pure and mixed gas standards injected each day. The gas proportioner was used to prepare H_2:CO_2 standards in the following ratios: 100:0; 80:20; 50:50; 20:80; 0:100. The peaks obtained from each standard were plotted as peak height versus proportion of individual gases in the mixture. A linear relationship was obtained. Approximate percentages of H_2 and CO_2 produced by ThLO were read from the graph.

Atmospheres above uninoculated control PRAS-TSB supplemented with FCS contained no H_2 and small quantities of CO_2. Very small quantities of CO_2 were also present in atmospheres above FCS supplemented, uninoculated TSB prepared with cysteine hydrochloride and resazurin and N_2 only.

Electron microscopy

Electron microscopic observations of negative stains of isolates of ThLO were performed on a Hitachi Hu-11A electron microscope at 50 Kv.

Photomicrographs were contact printed for measurement of diameter and length and to count axial fibrils. Sizes were calculated by dividing the size on contact prints by magnification. Student's "t" (Steel and Torrie, 1960) was used to statistically analyze the data.

Cultures for electron microscopic observation were either PBS harvests from blood agar plate cultures or aerobically prepared TSB or PRAS-TSB with FCS. The cultures were centrifuged for 30 min at 70,000 X G to pellet ThLO. These pellets were suspended in distilled H_2O and mixed in the following ratio with 2.5% phosphotungstic acid[1] (PTA) (neutralized with KOH) and 2% bovine serum albumin (Cohn Fraction V),[2] 5:5:1. This mixture was incubated at room temperature for 15 min and then was transferred to a glass nebulizer. The nebulizer was held 2-5 cm in front of a Formvar[3] coated 100 mesh copper grid and sprayed 2-3 times. Grids were examined immediately.

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[1]Allied Chemical and Dye Corp., New York, New York.

[2]Sigma Chemical Co., St. Louis, Missouri.

[3]Vaughn, Inc., Memphis, Tennessee.

RESULTS

Thirty-four isolates of Treponema hyodysenteriae-like organisms (ThLO) were collected as presented in Table 2. Thirty-one of the isolates were cultured from colons of pigs affected with swine dysentery and are referred to as dysentery isolates. Three isolates (Puppy, B256, and 4/71) are referred to as nondysentery isolates as they were not isolated from cases of SD. Strain B78 and isolates A-1, 4/71, N-1, N-2, 300/8, and 1037 were isolated by other investigators and were obtained in pure culture for these studies. For ease in tabulation, data concerning strain B78 (the type species of T. hyodysenteriae) has been grouped with the dysentery "isolates".

Twenty-seven isolates of ThLO were isolated by either titration or filtration methods from intestinal material for these studies (Table 3). Titration methods were successful in obtaining zones of hemolysis free of contaminating organisms in 13 of 21 attempts. Filtration methods resulted in zones of hemolysis free of contaminating organisms in all 28 attempts.

The isolates were grown in a variety of cell-free media for the following studies: 1) transmission, 2) growth, and 3) characterization. Table 4 lists each isolate and the studies in which they were included.

Table 3. Isolation of Treponema hyodysenteriae-like organisms from field specimens on bovine blood agar plates in H_2:CO_2 anaerobic atmospheres

Isolate No	Titration[ab]						Filtration[cb]				
									Volume of 0.45 μm[d]		
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	0.8 μm[e]	0.65 μm[e]	0.01 ml	0.1 ml	0.5 ml
B137	ND	ND	ND	ND	ND	ND	ND	+	ND	+	ND
B138	ND	ND	ND	ND	ND	ND	ND	+	ND	+	ND
B140	OG	+	+	-	-	-	ND	+	+	+	ND
B143	OG	OG	-	-	-	-	ND	+	ND	+	ND
B153	ND	ND	ND	ND	ND	ND	+	+	ND	ND	ND
B163	OG	OG	±	+	+	-	+	+	+	+	+
B164	ND	ND	ND	ND	ND	ND	+	+	+	+	+
B169	OG	+	+	-	-	-	+	+	+	ND	ND
B170	OG	OG	-	-	-	-	-	-	+	+	+
B171	OG	OG	-	-	-	-	-	-	-	+	-

[a]Serial dilutions of dysenteric colonic mucosal scrapings in PBS, plated on bovine blood agar plates and incubated in H_2:CO_2 atmospheres for 6 days.

[b]Symbols: ND = not done; + = zones of hemolysis containing ThLO were observed; ± = small zones of hemolysis containing ThLO were observed; - = no zones of ThLO hemolysis were observed; +OG = ThLO hemolysis was overgrown by contaminants; OG = presence of contaminating bacteria but not ThLO hemolysis.

[c]Filtration of dilute suspensions of dysenteric colonic mucosal scrapings through cellulose acetate filters of various sizes.

[d]Volumes from 0.45 μm filtrates of 0.01 ml to 0.5 ml were lawn streaked.

[e]Less than 0.1 ml of filtrate streaked for isolation.

Table 3 (Continued)

Isolate No	Titration[ab]						Filtration[cb]				
									Volume of 0.45 μm[d]		
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	0.8 μm[e]	0.65 μm[e]	0.01 ml	0.1 ml	0.5 ml
B173	OG	-	-	-	-	-	-	-	+	+	+
B175	OG	-	-	-	-	-	+	+	+	+	±
B179	ND	ND	ND	ND	ND	ND	ND	+	ND	+	ND
B204	OG	+	+	+	-	-	+OG	+	+	+	+
B205	OG	OG	OG	+	+	-	+	+	-	-	-
B206	OG	OG	+	+	+	-	+	+	±	+	OG
B211	OG	OG	OG	-	-	-	OG	OG	+	+	OG
B223	OG	OG	+	+	+	-	+	+	+	OG	OG
B224	OG	OG	OG	+	-	-	+	+	+	OG	OG
B228	OG	OG	+	+	+	-	+	+	+	+	+
B230	+OG	+OG	+OG	+	+	-	+	+	+	+	+
B231	ND	OG	OG	+	+	-	ND	+	ND	ND	ND
B234	OG	OG	-	-	-	-	OG	OG	-	+	+
B254	OG	OG	+	+	+	-	+	+	+	-	-
B256	OG	OG	-	-	-	-	OG	+	-	-	-
B259	+OG	+OG	+OG	+	+	-	+	+	-	+	+
Puppy	OG	OG	OG	ND	ND	ND	ND	+	+	ND	ND

Table 4. Studies conducted on 34 isolates of Treponema hyodysenteriae-like organisms of dysentery and nondysentery origin

Isolate No	Transmission[a]	Electron Microscopy[b]	Differential Characterization[c]	Gas Analysis[d]
		Dysentery Isolates		
78	+	+	+	+
137	+	+	-	-
138	+	-	-	-
140	+	+	+	-
143	-	-	-	-
153	+	+	+	+
163	+	+	+	+
164	+	-	-	-
169	+	+	+	-
170	-	-	-	-
171	+	+	+	-
173	+	+	+	-
175	+	-	-	-
179	-	+	+	-
204	+	+	+	+
205	+	-	-	-
206	+	-	-	-
211	+	-	-	-
223	+	-	-	-

[a] Transmission experiments to determine pathogenicity.

[b] Electron microscopic observation to determine morphology and size.

[c] Inoculated to differential media to determine characteristics.

[d] Analysis of atmosphere above cultures to determine gaseous products.

Table 4 (Continued)

Isolate No	Transmission[a]	Electron Microscopy[b]	Differential Characterization[c]	Gas Analysis[d]
224	+	-	-	-
228	+	-	-	-
230	+	-	-	-
231	+	-	-	-
234	+	+	+	-
254	+	+	+	-
259	+	+	+	-
A-1	+	+	+	+
N-1	-	+	+	-
N-2	-	+	+	-
300/8	-	+	+	-
1037	-	+	+	-
(Total 31)	24	18	17	5
		Nondysentery Isolates		
B256	+	+	+	+
Puppy	+	+	+	+
4/71	+	+	+	+
(Total 3)	3	3	3	3

Transmission

Pathogenicity--dysentery isolates

Swine dysentery was produced in 47 of 63 pigs which were orally inoculated with pure cultures of dysentery isolates of ThLO (Table 5). The pigs were inoculated and housed as pairs. Each pair received pure cultures of a single dysentery isolate (24 isolates, 31 separate inoculations). Twenty-two of the 24 dysentery isolates produced SD in at least 1 of 2 inoculated pigs. Isolates B140, B204, B231, and B234 produced SD in 7 of 8, 5 of 6, 2 of 4, and 3 of 4 inoculated pigs respectively.

Clinical signs of SD were first observed in pigs at an average of 8 days postinoculation (DPI) (range 3-25 DPI). The first signs usually included watery diarrhea of normal composition and depression; these progressed rapidly to diarrhea with mucus and blood, and gauntness. Of the 47 pigs that developed SD after oral inoculation with pure cultures, 17 became moribund. At nearly the same time (8 DPI, range 3-25 DPI), large numbers of ThLO were present in the feces. The first pig of an inoculated pair to reach this stage of the disease was killed and necropsy was performed (Table 5).

Lesions of SD were noted in all pigs suspected of having the disease. These lesions were usually limited to the large

Table 5. Reproduction of swine dysentery with pure cultures of dysentery isolates of Treponema hyodysenteriae-like organisms

Inoculum						SD Expt No[d]	Breed[e]
Isolate No	Origin	Passage	Culture[b]	Titer Day 1[c]	Titer Day 2[c]		
B78	IA	25	TBAS	10^6	NA	46	H
B137	IA	9	BAP-1	10^7	NA	58	Y
B138	IA	9	BAP-1	10^4	NA	58	Y

[a]Macroscopic lesion descriptions; symbols: HyS = hyperemic serosal surface; M = mucus; Hm = hemorrhage; NGL = no gross lesions; HyM = hyperemic mucosal surface; C = catarrah; F = fibrin; N = necrosis; Sn = serosal nodules; PeHm = petechial hemorrhage; EW = edematous wall and mesentery; Ps = pseudomembrane; ND = not done; + = cecum involved; - = cecum not involved.

[b]Inoculum was grown in culture medium: TBAS = tryptose blood agar slant; BAP-1 = tryptose with yeast extract blood agar; BAP-2 = TSA blood agar; Aer-TSB = aerobically prepared TSB with FCS.

[c]Number of ThLO (CFU/ml inoculum). Symbols: NA = not applicable as pigs were inoculated on Day 1 only; ND = not done.

[d]Transmission experiments on several ThLO isolates were conducted and controlled at the same time within a single SD experiment no (see Table 7 for controls).

[e]Symbols: H = Hampshire; Y = Yorkshire; D = Duroc; X = Crossbred.

[f]DPI of first observation of semiliquid feces.

[g]DPI of first phase microscopic observation of ThLO in rectal swab material.

[h]Numerator = number with clinical signs of SD; denominator = number inoculated.

[i]Numerator = number which became moribund; denominator = number inoculated.

Pig No	Clinical Signs of SD[f]	Observation of ThLO[g]	Postmortem Observation[a]		Totals	
			Colon	Cecum	SD[h]	Moribund[i]
5B	3	8	HyS,M	+	2/2	1/2
5G	5	8	HyS,M	+		
L	7	7	M,Hm	-	1/2	0/2
S	-	-	NGL	-		
II	6	7	HyS,HyM	-	1/1	0/1
ND	ND	ND	ND	ND		

Table 5 (Continued)

Inoculum						SD	
Isolate No	Origin	Passage	Culture[b]	Titer Day 1[c]	Titer Day 2[c]	Expt No[d]	Breed[e]
B140	MN	4	BAP-1	10^7	NA	58	Y
B140	MN	7,9	BAP-1	10^6	10^8	68	D
B140	MN	7,9	BAP-1	10^6	10^8	68	D
B140	MN	9	Aer-TSB	10^6	10^7	80	D
B153	IN	8	BAP-2	10^6	10^6	80	D
B163	NM	6	BAP-2	10^6	ND	100	D
B164	NB	7	BAP-2	10^7	ND	100	D
B169	CAN	7	BAP-1	10^5	ND	69	Y
B171	KS	8	BAP-2	10^7	10^6	80	D
B171	KS	8	BAP-2	10^7	10^5	101	D
B173	CAN	9	BAP-2	10^6	10^7	81	Y
B175	NB	7,8	BAP-1	10^6	ND	72	H
B204	IA	7	BAP-2	10^7	10^6	80	D
B204	IA	7,8	Aer-TSB	10^5	10^6	80	D

[j] Pigs were observed for 30 DPI; some recovered and some were subsequently rechallenged--therefore postmortem findings may differ from the expected, considering clinical signs.

Pig No	Clinical Signs of SD[f]	Observation of ThLO[g]	Postmortem Observation[a]		Totals	
			Colon	Cecum	SD[h]	Moribund[i]
I-0	9	11	C,HyM	-	2/2	1/2
I-2	8	9	C,HyM	-		
0025	5	4	F,N,Hm,M	+	2/2	1/2
3081	4	3	HyM,M,Hm	-		
0047	8	7	C,HyM	+	2/2	1/2
0066	9	7	F,Sn	-		
4533	-	-	ND	ND	1/2	0/2
4504	7,25	25	ND	ND		
1006	11	9	PtHm,HyM	+	2/2	0/2
5502	7	7	F,M,HyM,EW	-		
4-11	5	6	F,M,HyM,EW	-	2/2	1/2
4-12	9	9	ND	ND		
9-33	3	6	HyM,Sn	-	2/2	0/2
9-34	7	-		ND		
1	18	18	M,Hm	-	1/2	0/2
0	-	-	M,Hm,F[j]	+		
7504	-	-	NGL	ND	0/2	0/2
7502	-	-	NGL	ND		
7-7	-	-	ND	ND	0/2	0/2
7-15	-	-	ND	ND		
7851	15	15	HyM,M	+	2/2	0/2
7855	11	11	HyM,M,G	+		
711M	7	9	C,HyM	-	2/2	0/2
711F	7	9	NGL[j]	ND		
6502	-	-	NGL	ND	1/2	0/2
5530	6	6	HyM,M,H	+		
6505	19	19	NGL[j]	ND	2/2	0/2
1001	8	6	HyM,F,N,M	-		

Table 5 (Continued)

Inoculum							
Isolate No	Origin	Passage	Culture[b]	Titer Day 1[c]	Titer Day 2[c]	SD Expt No[d]	Breed[e]
B204	IA	6,7	BAP-2	10^8	10^8	93	X
B205	CO	6	BAP-2	10^7	10^6	84	D
B206	KS	4	BAP-2	10^6	10^6	84	D
B211	IL	8,9	BAP-2	10^7	ND	100	D
B223	CO	5	BAP-2	10^6	ND	100	D
B224	IL	8	BAP-2	10^7	ND	91	D
B228	IA	6	BAP-2	10^7	ND	100	D
B230	IL	5	BAP-2	10^8	ND	91	D
B231	FL	8	BAP-2	10^7	ND	91	D
B231	FL	8	BAP-2	10^7	ND	100	D
B234	MO	6	BAP-2	10^6	ND	91	D
B234	MO	8	BAP-2	10^6	10^6	101	D
B254	NC	6	BAP-2	10^6	ND	109	D
B259	SD	9	BAP-2	10^6	ND	109	D
A-1	GB	5	BAP-2	10^8	10^8	101	D

[k]Pig had semiliquid feces with mucus 1 day only.

Pig No	Clinical Signs of SD[f]	Observation of ThLO[g]	Postmortem Observation[a]		Totals	
			Colon	Cecum	SD[h]	Moribund[i]
2286	8	8	HyM,M	+	2/2	1/2
2283	8	8	Ps,C,Hm,M	6		
0068	3	4	HyS,C,F	+	1/2	0/2
0048	-	6	M,Hm[j]	+		
0028	-	-	M,Hm[j]	+	1/2	0/2
3029	8	8	C,F,N,HyM	±		
12-24	5	6	C,HyM,M	-	2/2	1/2
12-25	11	12	HyM,M,C	-		
11-40	7	8	C,Hm,EW	-	2/2	1/2
11-41	5	6	C,Hm,M	-		
6-46	6	6	C,Hm	+	2/2	2/2
6-31	7	7	C,Hm	+		
10-18	7	6	HyM,Ps,C	-	2/2	2/2
10-19	5	6	HyM,M			
7-32	7	6	M,Hm	-	1/2	1/2
7-39	16[k]		ND	ND		
8-37	7	6	NGL[j]	ND	1/2	1/2
8-49	-	-	ND	ND		
8-4	7	7	M,HyM	+	1/2	0/2
8-5	-	-	ND	ND		
9-33	13[k]	-	ND	ND	1/2	0/2
9-41	7	6	HyM,M,EW	+		
3-5	5	6	ND[j]	ND	2/2	1/2
3-3	6	6	HyM,Hm,C,N	+		
8-8	5	5	HyM,C,M,F	-	2/2	1/2
8-17	10	9	TW,F,M,Sn	+		
3-9	-	-	NGL	ND	0/2	0/2
3-36	-	-	NGL	ND		
1-36	6	5	HyM,M	+	2/2	1/2
1-37	5	4	HyM,C	∓		
				Grand Totals	47/63	17/63

intestine. The serosal surface of the colon was frequently hyperemic and occasionally serosal nodules were present. The mesentery and colonic tissue often appeared edematous. Gross lesions of the mucosal surface of the colon varied from mucosal hyperemia with mucus, to catarrhal inflammation, pseudomembranous accumulation, and necrosis (Table 5, Figures 1, 2, 3). Microscopically, the crypts appeared dilated and goblet cells were increased in number. The mucosal surface sometimes completely lacked epithelial covering. In such cases the surface was covered with an adherent layer of mucus, fibrin, red and white blood cells, and bacteria. The vessels of the lamina propria and submucosa appeared congested and frequently areas of hemorrhage were found. Leukocytic infiltration of the tissues was noted in varying degrees, as was pavementing of leukocytes in the vessels of the submucosa.

No other gross lesions were observed in these pigs except an isolate A-1 inoculated pig (pig no. 1-36 in SD 101) where lesions resembling mycoplasma pneumonia were present in the cardiac lobes of the lung; and an isolate B234 inoculated pig (pig no. 33 in SD 101) which had spleenomegaly and excess fluid in the peritoneal cavity. The two pigs which were inoculated with isolate B173 (SD 81) developed lesions of swine dysentery in the colon and cecum; and large numbers of ThLO were present in the mucosa. However, the small

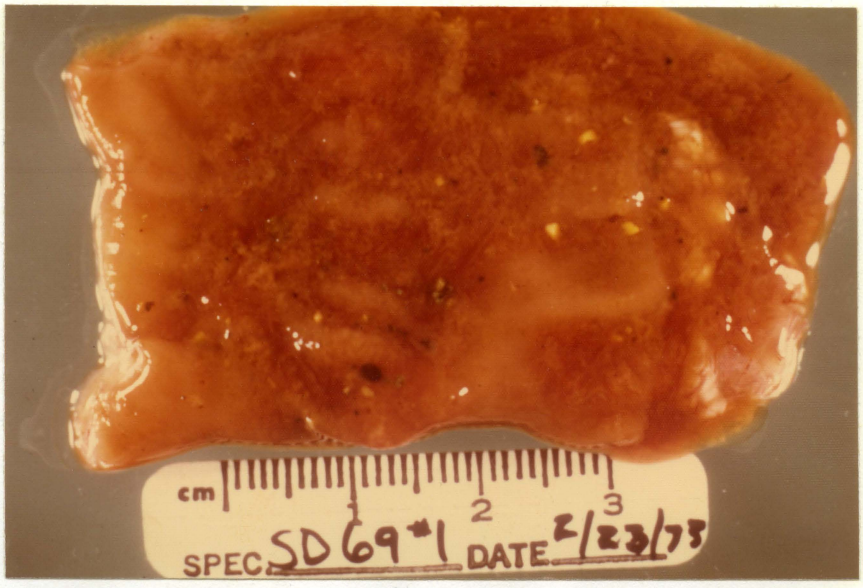

Figure 1. Mucosal surface of spiral colon of 2-week-old pig inoculated with isolate B169. The surface is covered with mucofibrinous exudate and focal areas of hemorrhage are present

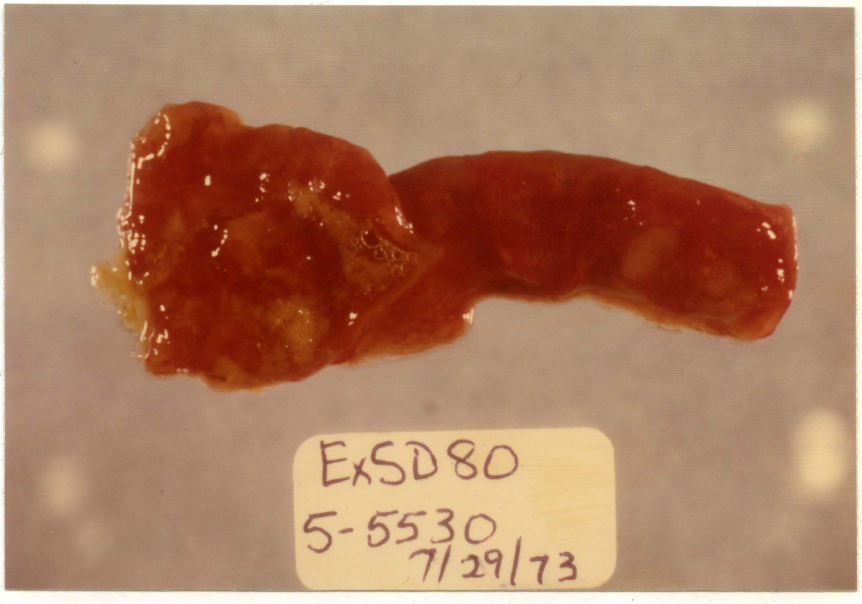

Figure 2. Mucosal and serosal surface of 2-week-old pig inoculated with isolate B204. The serosal and mucosal surfaces are hyperemic, and the mucosal surface is covered with catarrhal exudate

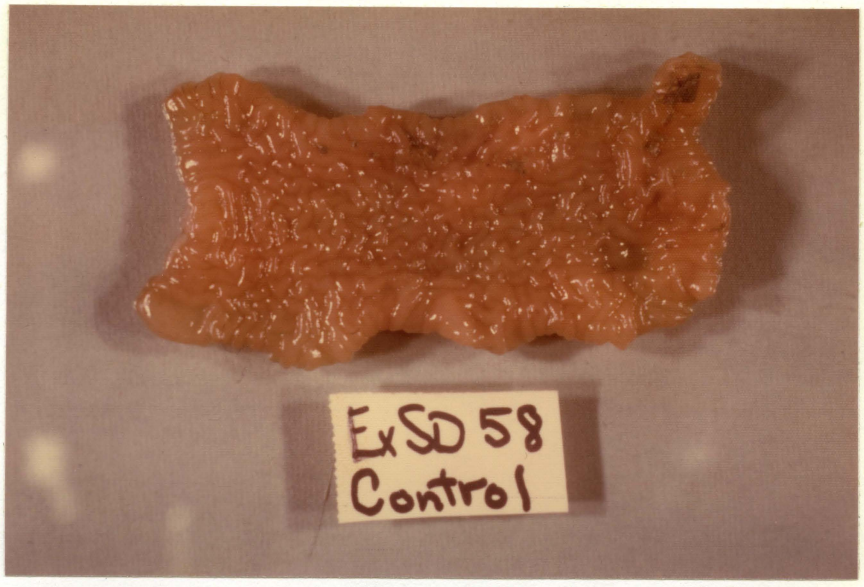

Figure 3. Mucosal surface of spiral colon of uninoculated control pig

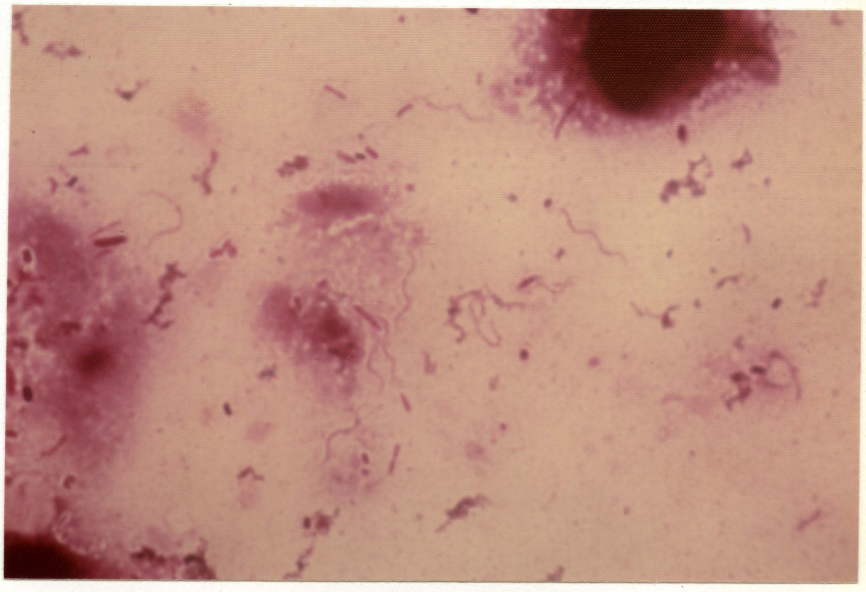

Figure 4. Gram's stain (with carbol fuchsin substituted for Safranin in counterstain) of deep mucosal scraping from colon of pig (No. 5530, SD No. 80) affected with SD produced by isolate B204. x1600

intestine was distended with bile colored, watery fluid and gas. Escherichia coli was present in fluids from the jejunum at 10^7 CFU/ml.

Phase microscopic observations of scrapings from the colonic mucosa of all necropsied pigs affected with SD, revealed the presence of large numbers of ThLO (2-4/field (f) to 20-30/f) (Figure 4). Treponema hyodysenteriae-like organisms were reisolated from 24 of the 31 pairs inoculated, by both titration and filtration methods (Table 6). The average number of CFU of ThLO per gram of colonic mucosa was 8×10^7 and ranged from 10^5 to 10^9. In contrast to the original isolation data (Table 3), titration methods proved as successful as filtration methods in obtaining zones of hemolysis free of contaminating organisms for subculture.

Two dysentery isolates did not produce SD in susceptible pigs. B171 was inoculated into 4 pigs (SD 80 and SD 101) and B259 was inoculated into 2 pigs (SD 109). None of these pigs developed signs or lesions of SD and ThLO were never present in the feces.

Dysentery isolate B204 (6th and 7th passage) was grown on TSA with blood and orally inoculated at 10^8 CFU/ml into 2 6-week-old Beagle puppies (SD 93). The puppies remained normal throughout a 30 day observation period. Feces from these puppies never contained ThLO organisms.

Table 6. Isolation of Treponema hyodysenteriae-like organisms from acute cases of swine dysentery induced with pure cultures of dysentery isolates

Isolate No[e]	SD Expt No	Pig No	Inoc Iso No[f]	Titration[ab] 10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
B115	46	5G	B78	ND	ND	ND	ND	ND
B150	58	III-L	B137	OG	OG	+	+	+
B147	58	II	B138	+OG	+	-	-	-
B146	58	I-2	B140	+OG	+	-	-	-
B149	58	I-0	B140	+OG	+OG	+	+	+
B182	68	0066	B140	OG	OG	OG	OG	-
B183	68	0025	B140	OG	OG	OG	-	-
B207	80	5502	B153	OG	OG	OG	+OG	+
B250	100	4-11	B163	+OG	+OG	+OG	+	-
B249	100	9033	B164	OG	+OG	+OG	+	+

[a]Serial dilutions of dysenteric colonic mucosal scrapings in PBS, plated on bovine blood agar plates and incubated in H_2:CO_2 atmospheres for 6 days.

[b]Symbols: ND = Not done; + = zones of hemolysis containing ThLO were observed; + = small zone of hemolysis containing ThLO were observed; - = no zones of ThLO hemolysis were observed; +OG = ThLO hemolysis was overgrown by contaminants; OG = presence of contaminating bacteria but not ThLO hemolysis.

[c]Filtration of dilute suspensions of dysenteric colonic mucosal scrapings through cellulose acetate filters of various sizes.

[d]Volumes of 0.45 μm filtrates from 0.01 ml to 0.5 ml were streaked.

[e]Isolate number given to ThLO isolated from pure culture induced SD.

[f]Original dysentery isolate number which was inoculated into pigs and caused SD.

[g]Less than 0.1 ml of filtrate streaked for isolation.

Titration[ab]		Filtration[cb]				
				Volume of 0.45 μm[d]		
10^{-9}	10^{-10}	0.8 μm[g]	0.65μm[g]	0.01 ml	0.1 ml	0.5 ml
ND	ND	ND	+	ND	+	ND
+	ND	ND	+	ND	+	+
–	ND	ND	+	+	+	+
–	ND	+	+	+	+	ND
–	–	ND	+	+	+	ND
–	–	+	+	+	+	+
–	–	OG	+OG	+	+	+
–	ND	+	+	+	+	+OG
–	–	+	+	±	+	+
–	ND	+	+	+	+	+

Table 6 (Continued)

Isolate No[e]	SD Expt No	Pig No	Inoc Iso No[f]	Titration[ab] 10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
B174	69	1	B169	OG	OG	+	+	–
B209	81	7855	B173	OG	OG	OG	OG	–
B185	72	8-B1	B175	OG	OG	OG	+	+
B208	80	5530	B204	OG	OG	+	+	+
B210	80	1001	B204	OG	OG	+	+	+
B245	93	2283	B204	+OG	+OG	+	+	+
B226	84	0068	B205	+OG	+OG	+	+	+
B229	84	3029	B206	+OG	+OG	+	+	+
B252	100	12-24	B211	OG	OG	OG	OG	+
B251	100	11-40	B223	+OG	+OG	+	+	+
B238	91	6-31	B224	OG	OG	+	+	+
B239	91	6-46	B224	OG	OG	OG	+OG	+
B248	100	10-18	B228	+OG	+OG	+	+	+
B241	91	7-32	B230	OG	OG	OG	–	–
B253	100	8-4	B231	OG	OG	OG	+	+
B257	101	3-3	B234	OG	OG	OG	–	–
B263	109	8-8	B254	+	+	+	+	+
B255	101	1-36	A-1	OG	OG	+OG	+OG	+

[h]Zone of ThLO hemolysis appeared only after 6 days incubation.

Titration[ab]		Filtration[cb]				
				Volume of 0.45 μm[d]		
10^{-9}	10^{-10}	0.8 μm[g]	0.65 μm[g]	0.01 ml	0.1 ml	0.5 ml
ND	ND	ND	+	+	+	ND
–	–	+OG	+OG	–	–	–
+	–	OG	+	–	–	–
–	–	+	+	+	+	+OG
–	–	+	+	+	+	+
–	–	ND	ND	ND	ND	ND
–	ND	+	+	+	+	+OG
ND	ND	+	+	+	+OG	+OG
+	–	OG	+	+	+	+OG
–	–	+	+	ND	+	+
+	–	+	+	+	+	+
+	–	+OG	ND	+	+	+
–	–	+	+	+	+	+
–	ND	OG	+	–	+	+
–	–	+	+	+	+	+
–	–	+	+	+[h]	+	+
ND	ND	+	+	–	+	+
+	ND	OG	OG	+	+	+

Pathogenicity--nondysentery isolates

One ThLO isolate from a healthy pig (4/71); a ThLO isolate from a pig with postweaning colibacillosis (B256); and a ThLO isolate from a puppy with catarrhal enteritis (Puppy) were grown on TSA with blood and orally inoculated into pigs. In addition, the Puppy isolate was orally inoculated into puppies (see Table 7). None of these 10 pigs or 2 puppies developed signs or lesions typical of SD. Occasionally, ThLO were seen in the feces of Duroc pigs inoculated with B256, and in the feces of Beagles inoculated with Puppy isolate. The pigs which were inoculated with 4/71 experienced transient diarrhea of normal composition. The 6-week-old pigs which received Puppy developed clinical signs of gastrointestinal dysfunction including vomition and watery diarrhea with pieces of pseudomembranous catarrh. Postmortem examination of both of these pigs revealed necrotic ileitis. The lesions were in the terminal ileum and colon, and consisted of heavy diphtheritic accumulations which formed a cast of the intestine. These lesions were not considered a result of the inoculum, since pigs from the same source housed in either strict isolation or in the original herd also occasionally developed necrotic ileitis; however, ThLO (B244) was isolated from one of these pigs.

Table 7. Determination of pathogenicity of nondysentery isolates for pigs and puppies

Inoculum					Animals			Observations	
Isolate No	Origin	Passage	Titer Day 1[a]	Titer Day 2[a]	SD Expt No[b]	Breed[c]	Animal No	Clinical Signs[d]	Phase Observations of ThLO
B256	IA	9	10^7	10^7	109	D	1-1	N	occ susp[e]
							1-3	N	occ susp
B256	IA	9	10^7	10^7	109	Y Y	6-S	N	-
							6-L	N	-

[a]Number of ThLO (CFU/ml of inoculum).

[b]Transmission experiments on several dysentery and nondysentery isolates were conducted and controlled at the same time within a single SD experiment number.

[c]Symbols: D = Duroc; Y = Yorkshire; H = Hampshire; X = Crossbred; B = Beagle.

[d]Symbols: N = normal; V = vomission; W = watery; M = mucus; SL = semiliquid.

[e]Occasional observation of ThLO in feces.

Table 7 (Continued)

Inoculum					Animals			Observations	
Isolate No	Origin	Passage	Titer Day 1[a]	Titer Day 2[a]	SD Expt No[b]	Breed[c]	Animal No	Clinical Signs[d]	Phase Observations of ThLO
Puppy	IA	8	10^8	10^8	72	H	9-B	N	-
							9-G	N	-
Puppy	IA	8	10^8	10^9	93	X	81	V,W,M[f]	occ susp
							82	V,W,M[f]	occ susp
Puppy	IA	8	10^8	10^9	93	B	81	N	occ susp
							78	N	occ susp
4/71	GB	5	10^8	10^7	101	D	6	SL[g]	
							13	SL[g]	

[f]Lesions of necrotic ileitis at postmortem.

[g]Transient diarrhea lasting 1-2 days.

Subsequent observations of the second pig of an inoculated pair

When possible the second pig of each inoculated pair was observed for the 30-day period. In several inoculations the second pig of the pair also developed severe SD and was killed and necropsied. The second pigs which had been inoculated with isolates B234 (SD 101), B231 (SD 91), and B163 (SD 100) developed SD from 5-10 DPI, recovered 14-20 DPI, and remained normal. When necropsied, these pigs did not have gross lesions of SD and ThLO were not present in the colonic mucosa.

One pig inoculated with isolate B204 (pig no. 6505 in SD 80) developed SD at 19 DPI, and continued to show signs of the disease and shed ThLO for the remainder of the 30 day observation period. At that time he was treated with 0.006% Ronidazole (Merck) in the water. The pig appeared to be recovering by 24 hours and was normal when killed and necropsied at 48 hours. There were no gross lesions of SD in the colon or cecum, and ThLO were not present in the colonic mucosa.

Both pigs inoculated with isolate B175 (SD 72) developed clinical signs of SD at 7 DPI, and ThLO were present in the feces at 9 DPI. The first pig was necropsied in the acute stage and the second pig (711 F) was observed until 45 DPI. During this time, the pig recovered and exacerbated

(with clinical signs of SD and large numbers of ThLO in the feces) 4 times in 4-10 day cycles. When killed and necropsied the pig was clinically normal and there were neither gross lesions of SD or ThLO in the colonic mucosa.

The second pigs in the pairs inoculated with isolates B169, B205, and B206 did not develop SD in the 30 day observation period. At the end of the observation period these pigs were challenged with crude SD inoculum. Pigs previously challenged with isolates B169 and B205 developed the disease 6 and 19 days later. The pig which had previously been challenged with isolate B206, remained normal through 21 DPI with crude SD inoculum, and then developed diarrhea without blood or mucus. No ThLO were detected in the feces.

Both pigs which had received Puppy isolate in SD 72 remained clinically normal through 30 days observation. They were then hyperimmunized orally for 5 consecutive days, with 20th passage Puppy isolate grown on tryptose with yeast blood agar. After 5 days rest, these pigs and a previously uninoculated control, were challenged with crude swine dysentery inoculum. All 3 developed signs and lesions of SD and large numbers of ThLO were present in the colonic mucosa.

Controls

Control animals (Table 8) were inoculated with freshly prepared blood agar. These pigs infrequently developed signs of postweaning colibacillosis. This diarrhea was usually of a transitory nature, often just after introduction to the isolation cage. Treponema hyodysenteriae-like organisms were seen in 2 control pigs which had been affected with postweaning diarrhea for 4-7 days. One of 2 attempts to isolate these organisms was successful (B256). Lesions included gas and watery feces in the small intestine and colon. Fluids from the jejunum contained 10^6-10^7 CFU/ml of Escherichia coli.

All other swine controls remained clinically normal and ThLO were not observed in feces or in the colonic mucosa. Attempts to isolate ThLO from control pigs were negative.

The Beagle controls were also clinically normal, however, they occasionally shed small numbers of ThLO. These organisms were not isolated from rectal swab material in 2 titration attempts.

Other microfauna

Vibrio-like organisms and small spirochetes were observed in low numbers (1/3f to 3/f) in rectal swab samples of all pigs in these studies, although not always from each rectal swab sample. There were high numbers of Vibrio-like

Table 8. Uninoculated control animals used in transmission experiments with dysentery and nondysentery isolates of Treponema hyodysenteriae-like organisms

SD Expt No[a]	No of Control Animals	Breed[b]	No of Animals with Diarrhea[c]	Phase Observation of ThLO[d]
46	2	H, Y	0/2	0/2
58	1	Y	0/1	0/2
68	4	D	1/4	0/2
80	2	D	0/2	0/2
81	2	Y	0/2	0/2
84	2	D	0/2	0/2
91	2	D	2/2	0/2
93	2	B	0/2	2/2
93	1	X	0/1	0/2
101	5	D	3/5	2/5
109	2	D	0/2	0/2

[a]Number of SD experiment (see Tables 4 and 7 for inoculated animals).

[b]Symbols: H = Hampshire, Y = Yorkshire; D = Duroc; X = Crossbred; B = Beagle.

[c]Numerator = number of animals with diarrhea; denominator = number of control animals.

[d]Numerator = number of animals with ThLO in phase microscopic examination of rectal swab material; denominator = number of control animals.

organisms (8-10/f) present in inoculated pigs when large numbers of ThLO were also present, as the pig was showing clinical signs of SD. Vibrio spp. were frequently isolated but were not identified.

Salmonella spp. were not isolated from any pigs in these studies. Two of the Beagles (uninoculated controls) in SD 93 harbored Salmonella schwarzengrund.

Balantidium coli was observed from rectal swab material in 1 pig inoculated with isolate B254 (pig no. 8-8 in SD 109) and in 1 pig inoculated with isolate B205 (pig no. 0068 in SD 84).

Growth

Development of a liquid medium

These studies on ThLO isolates began when the organism was cultivatable only on solid blood agar media (slants or plates). The basal medium in the preparation was tryptose with yeast extract, which was supplemented with 5% citrated bovine blood. For a time, efforts at obtaining growth in liquid media utilized anaerobic media known to cultivate other treponemes (PY,PYG, E, and thioglycollate; supplemented with either bovine blood, bovine plasma, rabbit serum, TEM-4T, or filtered colonic mucosal preparations). However, these supplemented liquid media did not support growth of ThLO in GasPak jars with H_2:CO_2 GasPak generator

envelope, or under deoxygenated N_2 or CO_2.

An important step in the development of a liquid medium was a study comparing various anaerobic atmospheres provided in GasPak jars with palladium catalyst. Treponema hyodysenteriae-like organisms were stimulated by H_2 (Figure 5).

Growth of ThLO in a semisolid medium was obtained in experimentation subsequent to the demonstration of H_2 stimulation of the organism. The organism grew in tryptose broth with yeast extract, and 0.45% agar, when the medium was prepared by aerobic methods, supplemented with bovine blood, and inoculated under H_2:CO_2. It would also grow in the same basal medium prepared by PRAS methods if the medium had been oxidized prior to supplementation with bovine blood and was inoculated under H_2:CO_2. Without prior oxidation of PRAS tryptose broth with yeast extract, poor or no growth was obtained (see Figure 6).

The next step which advanced the development of a liquid medium suitable for growth of ThLO was use of trypticase soy agar (TSA) as the basal medium for isolation, purification, and colony counts of the organisms. The TSA (supplemented with bovine blood) promoted better growth of ThLO than tryptose with yeast extract (supplemented with bovine blood) in isolation of the organisms from colons of pigs affected with swine dysentery (Table 9) and in colony

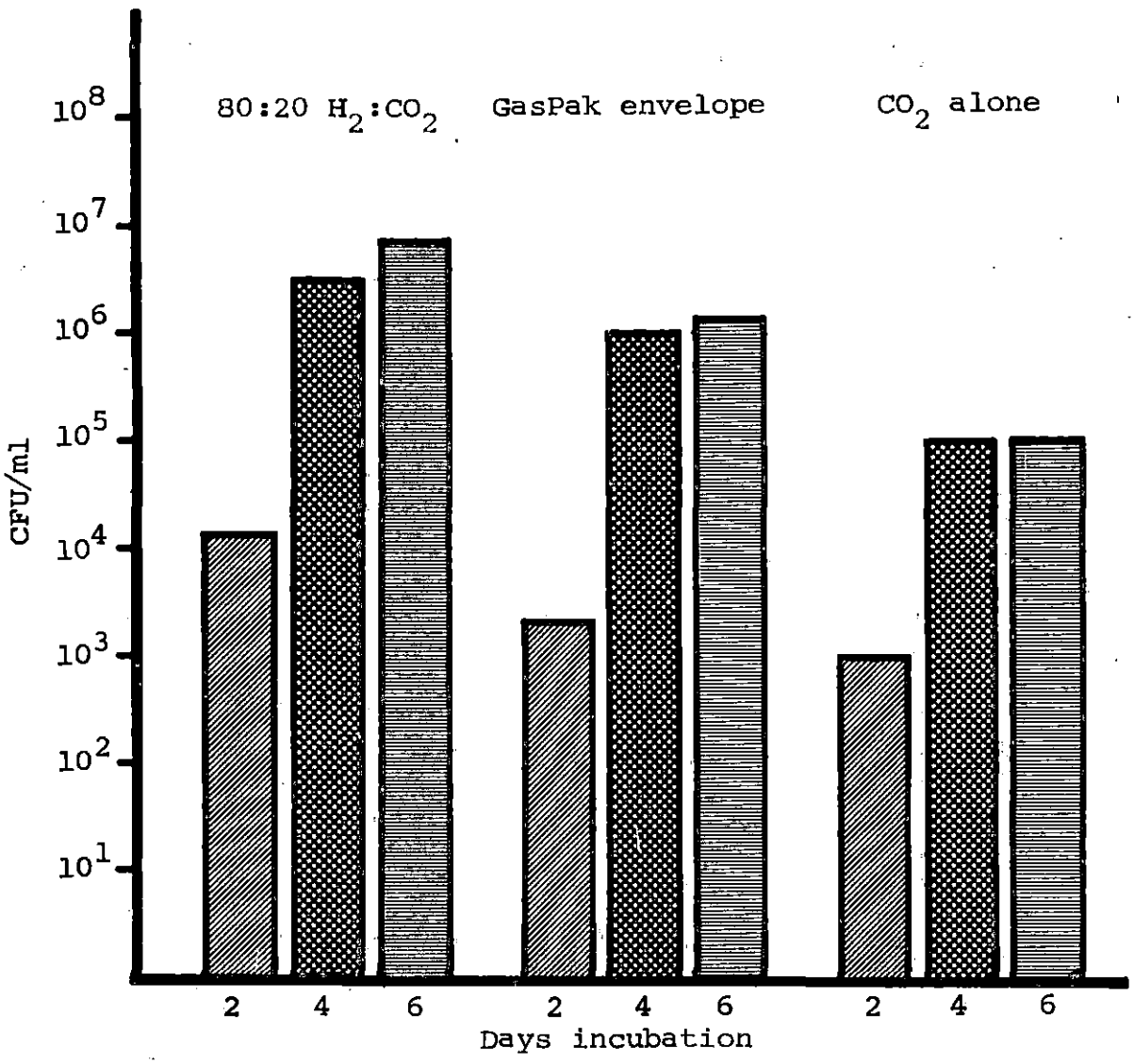

Figure 5. A comparison of three concentrations of H_2 in anaerobic atmospheres of GasPak jars with cold palladium catalyst for the growth of Treponema hyodysenteriae (strain B78, serially diluted in PBS, plated on tryptose with yeast extract agar and blood and incubated; jars opened every 2 days and examined for growth)

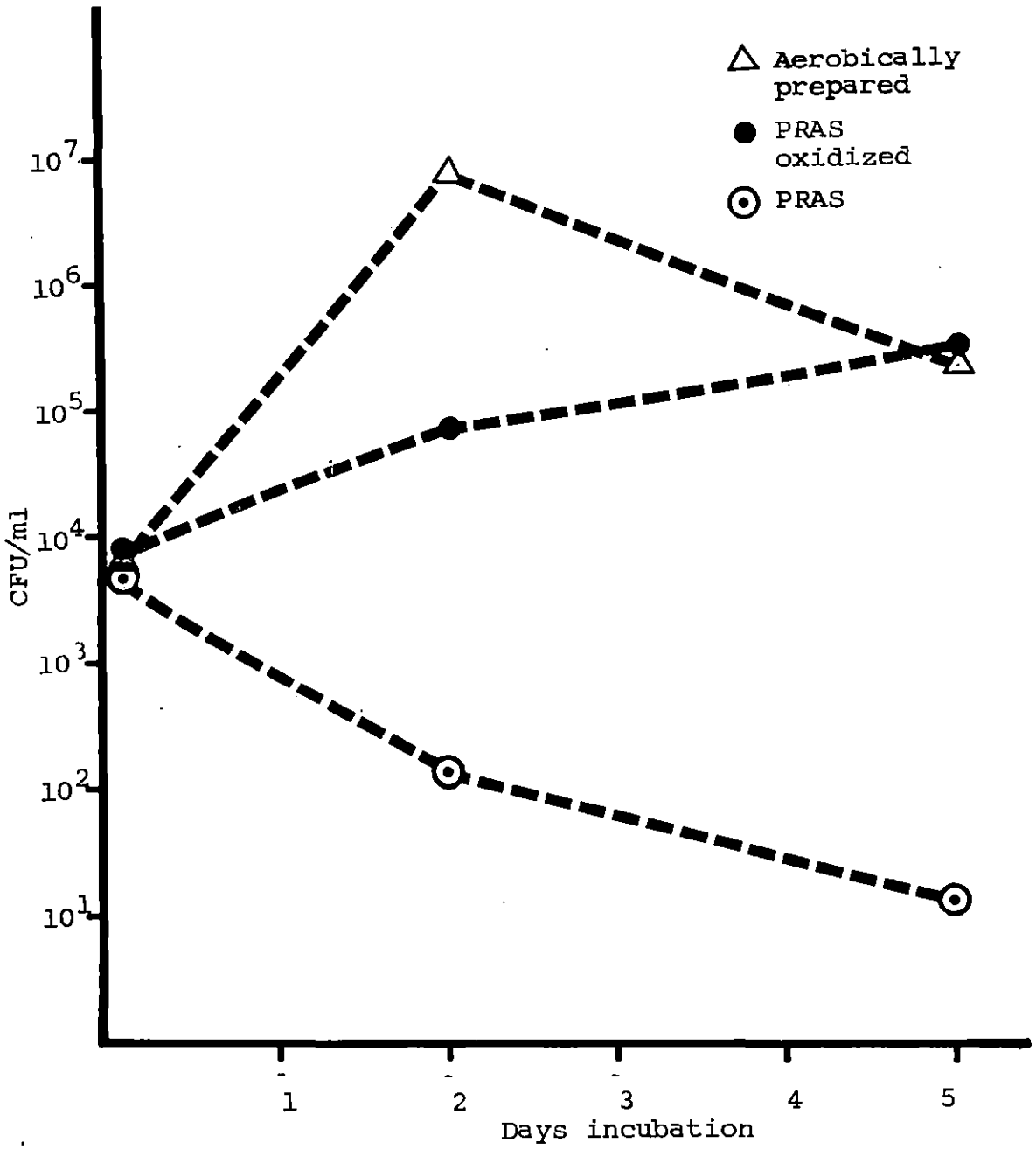

Figure 6. A comparison of growth of Treponema hyodysenteriae (strain B78) in PRAS, PRAS-oxidized, and aerobically prepared semisolid tryptose with yeast extract supplemented with 5% bovine blood

Table 9. A comparison of two blood agar media for the isolation of *Treponema hyodysenteriae*-like organisms from three separate outbreaks of swine dysentery

Isolate No	B204				B205						B206			
	TA Y[a]		TSA[b]		TA Y			TSA			TA Y		TSA	
	2da[c]	4da	2da	4da	2da	4da	6da	2da	4da	6da	2da	4da	2da	4da
	Titration[d]													
10^{-4}	OG[e]	OG	+OG	+OG	-C	-C	-C	-C	-C	-C	OG	OG	OG	OG
10^{-5}	-C	+C	+C	+C	-C	-C	-C	-C	-C	-C	OG	OG	+OG	+OG
10^{-6}	-	-	-	20z	-C	-C	-C	-C	-C	-C	-C	-C	+C	+C
10^{-7}	-	-	-	3z	-	-	-	-	-	15z	-	-	-	12z
10^{-8}	-	-	-	-	-	-	-	-	-	1z	-	-	-	4z

[a] Tryptose agar with yeast extract supplemented with 5% bovine blood.

[b] Trypticase soy agar supplemented with 5% bovine blood.

[c] Plates were incubated in H_2:CO_2 anaerobic atmospheres; GasPak jars were opened every 2 days and examined for zones of ThLO hemolysis.

[d] Serial dilutions of dysenteric colonic mucosal scrapings in BPS, plated on blood agar plates and incubated in H_2:CO_2 atmospheres for 6 days.

[e] Symbols: OG = presence of contaminating bacteria but not ThLO hemolysis; +OG = ThLO hemolysis was overgrown by contaminants; -C = area contained 3-100 colonies of contaminating bacteria but no zones of ThLO hemolysis; +C = area contained 3-100 colonies of contaminating bacteria and numerous zones of ThLO hemolysis; Z = zones of hemolysis = colonies of ThLO; +P = zones of ThLO hemolysis free of contaminating bacteria; - = no growth; ND = not done.

Table 9 (Continued)

Isolate No	B204				B205						B206			
	TA Y[a]		TSA[b]		TA Y			TSA			TA Y		TSA	
	2da[c]	4da	2da	4da	2da	4da	6da	2da	4da	6da	2da	4da	2da	4da
							Titration[d]							
10^{-9}	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-10}	-	-	-	-	ND	ND	ND	ND	ND	ND	-	-	-	-
							Filtration[f]							
0.8 μm streak	+OG	+OG	+OG	+P	OG	OG	OG	OG	OG	+P	ND	ND	+P	+P
0.65 μm streak	OG	+OG	+OG	+P	-	-	-	OG	P	+P	ND	ND	+P	+P
0.45 μm streak	-	+P	+P	+P	-	-	-	-	-	-	ND	ND	P	+P
0.45 μm x 0.01 ml	ND	ND	ND	ND	-	-	-	-	-	-	ND	ND	-	+P
0.45 μm x 0.1 ml	+OG	+OG	+OG	+OG	-	-	-	-	+P	+P	ND	ND	+P	+P
0.45 μm x 0.5 ml	+OG	+OG	+OG	+OG	-	-	-	-	-	-	ND	ND	+P	+P

[f]Filtration of dilute suspensions of dysenteric colonic mucosal scrapings through cellulose acetate filters of various sizes.

Table 10. A comparison of two blood agar media for growth of Treponema nyodysenteriae-like organisms

Isolate No[a]	Tryptose Agar with Yeast Extract Bovine Blood	Trypticase Soy Agar Bovine Blood
B169	$< 10^2$[b]	$< 10^2$
B203	$< 10^2$	7×10^7
B179	9×10^4	9×10^6
B78	4×10^2	8×10^6
B78	2×10^2	9×10^3
B140	$< 10^2$	2×10^6

[a]Cultivated in various types of broth; diluted in PBS, plated on the above fresh media, and incubated in H_2:CO_2 atmosphere for 8 days.

[b]Average number of ThLO (CFU/ml) from duplicate platings on the above media.

counts of pure cultures of the organisms (Table 10).

With the apparent advantages provided by H_2 atmospheres and trypticase soy nutrients, a liquid medium supplemented with 10% FCS was developed. Trypticase soy broth (TSB) prepared by either aerobic or PRAS methods, supplemented with 10% fecal calf serum (FCS), and incubated at 37°C under an anaerobic atmosphere supported growth of ThLO. Prereduced anaerobically sterilized TSB (PRAS-TSB) with 10% FCS supported growth of ThLO when inoculated under either

deoxygenated H_2:CO_2 or deoxygenated CO_2 alone. Aerobically prepared TSB with 10% FCS supported growth of the organism only when inoculated under the H_2:CO_2 atmosphere (Table 11).

The selection of a basal medium for *in vitro* characterization of ThLO isolates was based upon a series of experiments which were conducted on strain B78 and isolates B204 and B234. The basal media under study were: TSB (PRAS and aerobic), E, PY, PYRF, and PYS. These were supplemented with either FCS (various concentrations), 10% NRS, 10% NRS with TEM-4T and cocarboxylase, or nothing at all. The medium was then inoculated under either deoxygenated CO_2 or deoxygenated H_2:CO_2 and incubated at 37° C in a slanted rack.

Comparative experimentation of various combinations of the isolates, media, supplements, and atmospheres revealed that strain B78 would grow in almost everything. By contrast, isolates B204 and B234 were at low passage and would not grow or survive subculture in combinations other than TSB supplemented with FCS. In such systems when no growth was obtained beyond the first or second passage, colony counts of the cultures during 1-2 weeks of incubation showed a steadily decreasing number of ThLO (CFU/ml) over time. Frequently, when the systems were identical except for the CO_2 or H_2:CO_2 atmosphere; those cultures under H_2:CO_2 grew to higher titer and successfully passaged to the second and third subculture. Colony counts of subsequent passages of

Table 11. The amount of growth[a] of isolates of Treponema hyodysenteriae in liquid media

Isolate No	Passage No	Days Incubated	Serial Passage Number[b] 1	2	3	4	5	6
PRAS-TSB with FCS and H_2:CO_2								
B78	50	0	$7x10^4$[c]	$7x10^4$	$1x10^5$	$4x10^6$	$1x10^4$	$6x10^5$
		2	$4x10^6$	$9x10^4$	$6x10^7$	$1x10^7$	$3x10^7$	$9x10^6$
		4	$9x10^5$	$2x10^6$	$5x10^7$	$2x10^5$	$9x10^6$	$4x10^7$
B204	6	0	$1x10^6$	$3x10^6$	$2x10^5$	$2x10^6$	$1x10^5$	$7x10^6$
		2	$5x10^4$	$5x10^6$	$9x10^6$	$1x10^5$	$7x10^6$	$3x10^7$
		4	$4x10^7$	$8x10^6$	$2x10^7$	$2x10^6$	$1x10^8$	$1x10^7$
		6	ND[d]	$3x10^6$	ND	ND	ND	ND
B234	5	0	$5x10^6$	$7x10^3$	$7x10^4$	$3x10^4$	$1x10^5$	$7x10^5$
		2	$1x10^4$	$1x10^5$	$9x10^4$	$2x10^6$	$1x10^5$	$4x10^5$
		4	$2x10^4$	$9x10^5$	$4x10^5$	$3x10^6$	$1x10^7$	$4x10^6$
		6	$1x10^5$	ND	ND	ND	ND	$1x10^7$

[a]Serially diluted in PBS; plated on TSA with bovine blood; incubated 6-8 days in GasPak jar with H_2:CO_2 and cold palladium catalyst.

[b]Growth in broth from each passage was transferred (7% V/V) to an uninoculated tube (next passage) on the last day that colony count was performed.

[c]Number of CFU/ml.

[d]Not determined.

Table 11 (Continued)

Isolate No	Passage No	Days Incubated	Serial Passage Number[b] 1	2	3	4	5	6
			PRAS-TSB with FCS and CO_2					
B78	58	0	$1x10^5$	$3x10^4$	$1x10^5$	$6x10^5$	$7x10^6$	$2x10^6$
		2	$6x10^7$	$1x10^5$	$3x10^7$	$9x10^5$	$1x10^7$	$9x10^5$
		4	$4x10^5$	$2x10^6$	$9x10^6$	$1x10^8$	$2x10^7$	$1x10^8$
B204	6	0	$1x10^6$	$5x10^4$	$7x10^3$	$7x10^4$	$5x10^5$	$5x10^5$
		2	$8x10^4$	ND	-[e]	$1x10^4$	$9x10^5$	$5x10^4$
		4	$7x10^5$	$1x10^5$	$1x10^6$	$7x10^6$	$8x10^6$	$2x10^5$
		6	ND	ND	ND	ND	ND	$3x10^7$
B234	5	0	$5x10^6$	$2x10^3$	$7x10^5$	$7x10^4$	$4x10^4$	$1x10^3$
		2	$1x10^4$	$1x10^6$	$7x10^2$	$4x10^5$	$1x10^5$	$9x10^3$
		4	$3x10^4$	$1x10^7$	$9x10^4$	$6x10^5$	$1x10^4$	$9x10^5$
		6	ND	ND	$1x10^6$	ND	ND	ND

[e] - = negative; less than 10^2 CFU/ml.

Table 11 (Continued)

Isolate No	Passage No	Days Incubated	Serial Passage Number[b] 1	2	3	4	5	6
Aerobically Prepared TSB with FCS and H_2:CO_2								
B78	47	0	$7x10^4$	$6x10^5$	$2x10^6$	$2x10^6$	$7x10^4$	$2x10^6$
		2	$8x10^6$	$1x10^7$	$7x10^6$	$1x10^8$	$1x10^7$	$9x10^6$
		4	$9x10^6$	$2x10^7$	$2x10^7$	$9x10^5$	$2x10^7$	$1x10^8$
B204	6	0	$1x10^6$	$7x10^4$	$6x10^5$	$3x10^6$	$3x10^5$	$2x10^4$
		2	$1x10^7$	$1x10^5$	$2x10^6$	$3x10^7$	$1x10^6$	$2x10^7$
		4	$1x10^6$	$9x10^6$	$4x10^7$	$4x10^6$	$1x10^8$	$9x10^6$
		6	ND	$8x10^6$	ND	ND	ND	ND
B234	5	0	$5x10^6$	$7x10^3$	$1x10^4$	$6x10^5$	$2x10^5$	$7x10^5$
		2	$1x10^4$	$1x10^4$	$3x10^7$	$9x10^6$	$9x10^6$	$1x10^8$
		4	$2x10^4$	$1x10^7$	$9x10^6$	$3x10^6$	$1x10^7$	$9x10^7$
		6	$1x10^5$	$3x10^5$	ND	ND	ND	ND

these cultures often revealed maintenance and death.

These comparisons were repeated 3 times with isolate B204 and 2 times with isolate B234. The inoculum for one replicate of each isolate was pieces of agar from zones of hemolysis of cultures on TSA with blood. Both isolates grew from this inoculum in PRAS-TSB and aerobically prepared TSB, when supplemented with FCS and inoculated under H_2:CO_2. Both isolates also grew when inoculated into PRAS-TSB supplemented with FCS, under CO_2. Isolate B204 grew from blood agar plate inoculum in E broth with FCS under H_2:CO_2, but this growth did not survive subpassage. Media and supplements other than TSB (PRAS or aerobically prepared) with FCS were rejected as unsuitable for use in the in vitro characterization of isolates of ThLO for the following reasons: 1) the increased length of incubation (11 to 16 days) required to reach titers of sufficient concentration to successfully passage; 2) the inability of ThLO to consistently reach titers greater than 10^5 CFU/ml; 3) the cloudiness of uninoculated control medium in PY, PYRF, and PYS supplemented with NRS or NRS with TEM-4T and cocarboxylase; and 4) the inability of cultures from blood agar plates to initiate growth in PY, PYRF, or PYS.

Growth of ThLO in TSB with FCS is evidenced by a shimmery appearance of the medium when shaken and viewed by transmitted light after 24-48 hours of incubation. After

72 hours of incubation the medium is more turbid (see Figure 7). This growth usually represents an increase of 10 to 1,000 colony forming units (CFU) per milliter of broth when compared to the original concentration of the organism. Maximum growth is usually 10^7 organisms/ml and occasionally cultures reach 10^8 CFU/ml.

All 34 isolates of ThLO have been propagated in PRAS-TSB with FCS and H_2:CO_2. There was not a requirement for adaptation to this system since abundant growth of these strains occurred as early as the second in vitro passage.

Studies to determine the minimum necessary concentrations of inoculum and FCS in PRAS-TSB were performed. Isolate B234 would not passage in PRAS-TSB which had not been supplemented with FCS, under either H_2:CO_2 or CO_2. Isolate B204 passaged 6 times in PRAS-TSB without FCS under H_2:CO_2 but did not passage under CO_2 alone. Transfer interval for isolate B204 in FCS-free PRAS-TSB, under H_2:CO_2, was 8 days with 10% inoculum and 9-14 days with 7%, 4%, or 1% inoculum. The maximum growth in these cultures was never above 1×10^6 CFU/ml.

Both isolates B204 and B234 grew and passaged easily with as little as 1% inoculum and 0.5% FCS in PRAS-TSB under H_2:CO_2. The transfer interval was at 3-4 days and growth was first observed from 24-48 hours. Maximum growth was 10^7-10^8 CFU/ml. Isolate B204 in PRAS-TSB with 0.1% FCS

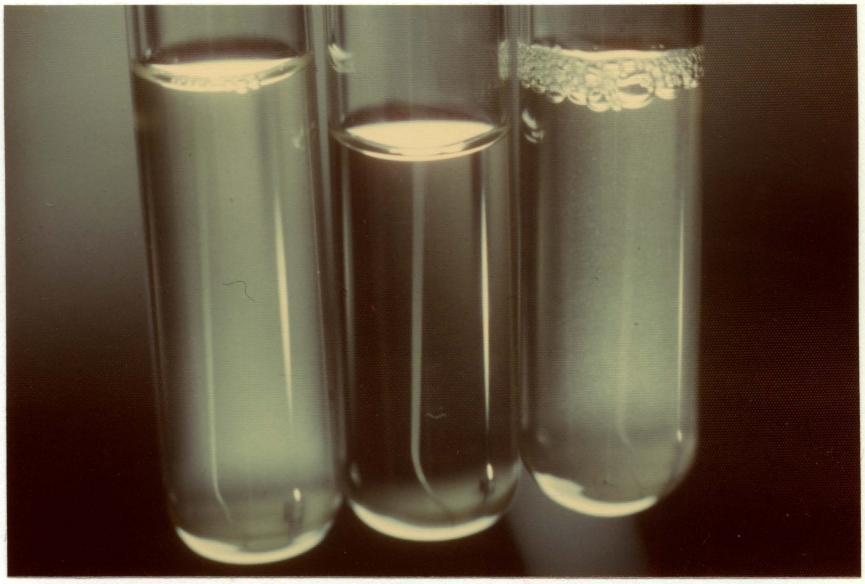

Figure 7. Characteristic appearance of growth of ThLO in aerobically prepared TSB with FCS under H_2:CO_2 Left tube inoculated with B204; center tube - uninoculated media control; right tube inoculated with isolate B169. Growth of isolate B169 frequently appears granular

under CO_2 would not grow, even with the 10% inoculum. Isolate B234 was not inoculated into PRAS-TSB with 0.1% FCS under CO_2.

Concentrations of FCS as high as 20% were not inhibitory for either isolate under either gas, however, the passage interval for both isolates in medium with 15% or 20% FCS under either gas was occasionally delayed 24 hours.

Maximum growth of most ThLO cultured in PRAS-TSB with 10% FCS under H_2:CO_2 was usually obtained by 4 days. Colony counts beyond that point frequently remained at 10^4-10^5 CFU/ml, and passage of 10 and 12 day cultures was frequently successful. Figure 8 is a growth curve for B204 in PRAS-TSB with FCS under H_2:CO_2. Generation time was calculated to be 6 hours.

Growth of ThLO on TSA with blood from 6 animal species

Strain B78, and isolates B204, B140, B256, Puppy, A-1, and 4/71 were inoculated to fresh TSA plates supplemented with 5% citrated bovine, horse, sheep, porcine, rabbit, or human blood and incubated in H_2:CO_2 anaerobic atmospheres. All 7 isolates grew on bovine, horse, sheep, porcine, and rabbit blood in TSA after 2 days incubation, and the amount of growth was increased after 4 days incubation. Trypticase soy agar supplemented with human blood (0 positive) supported growth of isolates B140 and Puppy after 4 and 6 days incubation. The other isolates did not grow at all or grew

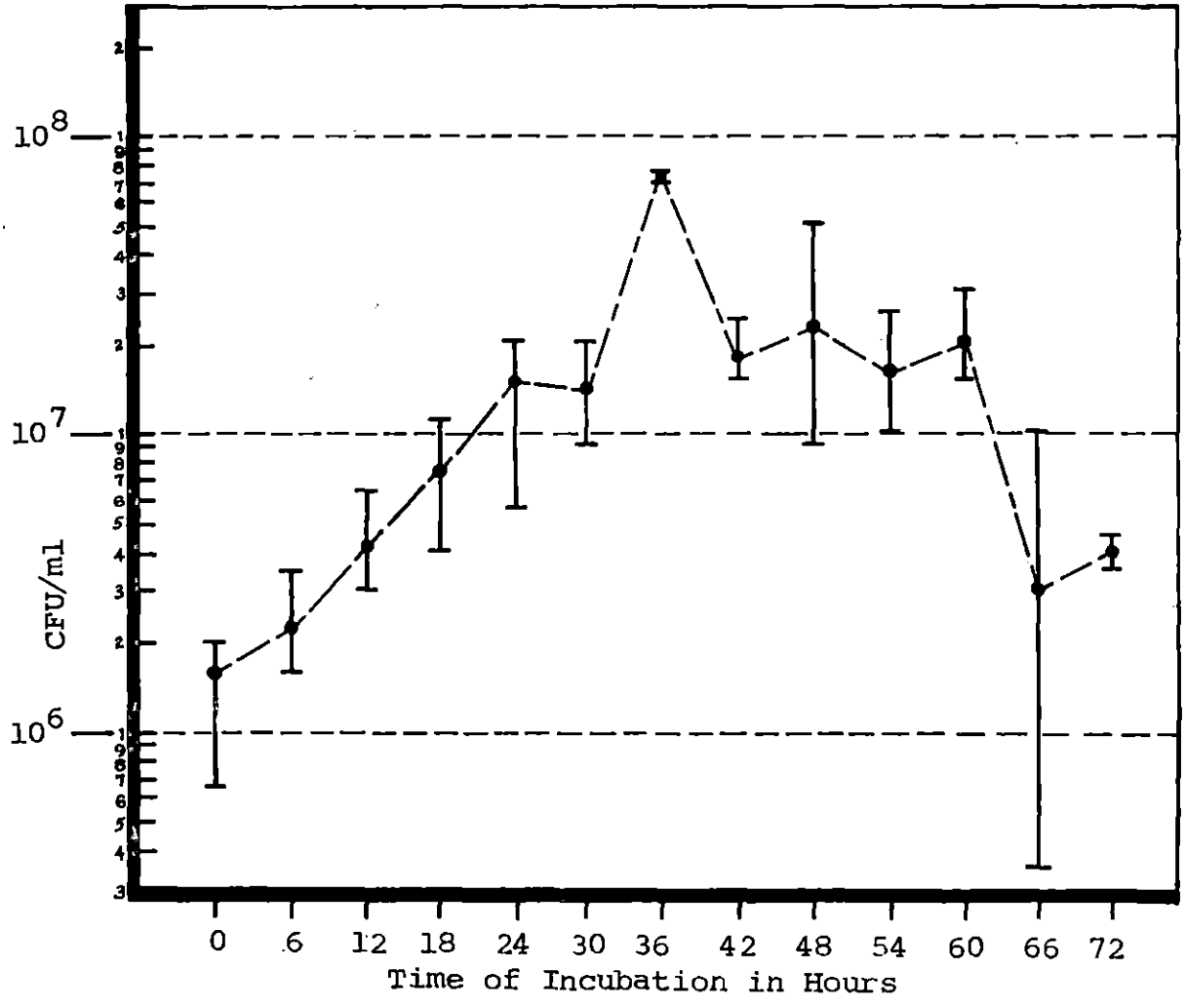

Figure 8. A growth curve for Treponema hyodysenteriae-like organism (isolate B204)

sparingly after 6 days incubation on TSA with human blood. Isolates B204, Puppy, and 4/71 were successfully subpassaged 3 times on all 6 types of blood agar at 4 or 6 day intervals.

Colony counts of 3 isolates in broth cultures on the 6 different bloods show quantitatively the variability of the source of blood for supporting the growth of ThLO (see Table 12). Propagation of strain B78 and isolates B140 and B204 produced a double zone of hemolysis on TSA with sheep blood (Figure 9).

Preservation

Treponema hyodysenteriae (strain B78) was viable after 24 months of storage at -80° C and for 24 months after lyophilization.

Characterization

Morphology

Treponema hyodysenteriae-like organisms were of an "S" helical morphology by light or phase microscopic observations (Figure 10). The organisms usually possessed 2 or 3 turns, however, long forms with 7 or 8 turns were also seen. "Round-bodies" were observed from old cultures that had been grown either on blood agar or in liquid media. Measurements of ThLO in electron photomicrographs (Figures 11-14) averaged 327 nm in diameter, 8.5 μm in length with 8-9 axial fibrils (Table A3, Appendix). "Student's t"

Table 12. A comparison of trypticase soy agar supplemented with blood from six different species for the growth of three isolates of Treponema hyodysenteriae-like organisms

Blood[b]	B78[a]			B140			B204		
	2 da[c]	4 da	6 da	2 da	4 da	6 da	2 da	4 da	6 da
Bovine	3×10^5[d]	4×10^6	9×10^6	TN x 10^3[e]	6×10^5	4×10^6	5×10^6	7×10^6	8×10^6
Horse	5×10^6	1×10^7	1×10^7	7×10^5	6×10^6	7×10^6	6×10^6	7×10^6	2×10^7
Sheep	8×10^6	8×10^6	9×10^6	1×10^6	1×10^7	3×10^7	2×10^6	5×10^6	6×10^6
Porcine	9×10^6	9×10^6	9×10^6	3×10^5	4×10^6	5×10^6	1×10^7	1×10^7	1×10^7
Rabbit	5×10^6	1×10^7	1×10^7	4×10^6	9×10^6	Completely hemolyzed	4×10^6	2×10^7	2×10^7
Human	TN x 10^2	TN x 10^2	TN x 10^2	4×10^4	1×10^5	2 c 10^5	TN x 10^2	TN x 10^2	TN x 10^2

[a] Isolates were cultured in PRAS TSB with FCS under CO_2 for 2 days, then serially diluted in PBS, plated on blood agar plates and incubated in H_2:CO_2 anaerobic atomspheres.

[b] Blood was collected aseptically in sodium citrate and used within 2 weeks.

[c] GasPak jars were opened every 2 days and plates were examined for zones of ThLO hemolysis.

[d] Average number of ThLO (CFU/ml) from duplicate dilutions and platings on each of the above media.

[e] TN = too numerous to count individual zones.

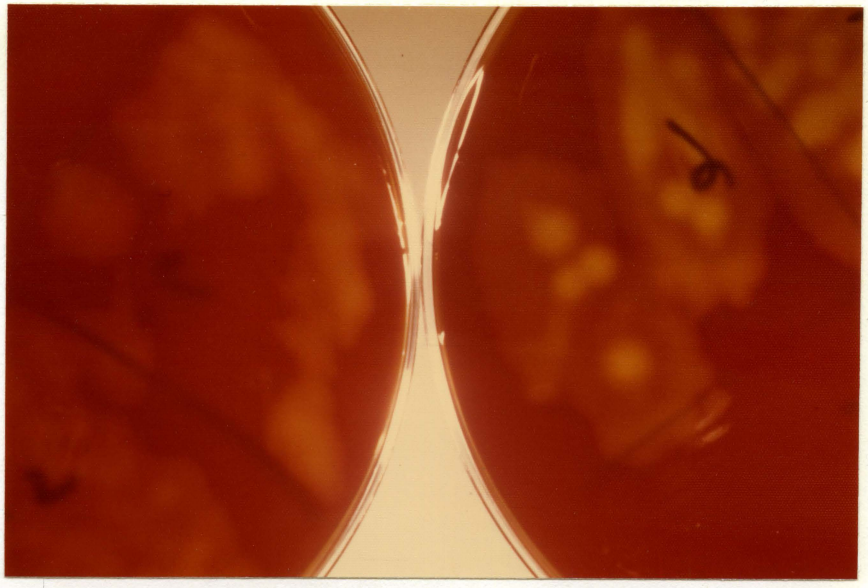

Figure 9. Comparison of growth of ThLO (isolate B204) on TSA with either bovine blood (left) or with sheep blood (right). Note the more complete hemolysis of sheep red cells in the center of zones

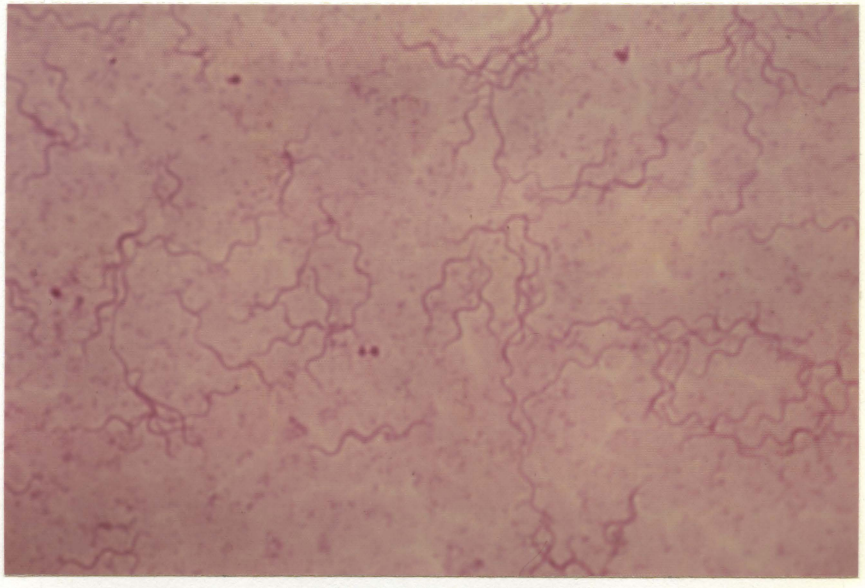

Figure 10. Carbol fuchsin stain of ThLO (isolate B204). x1600

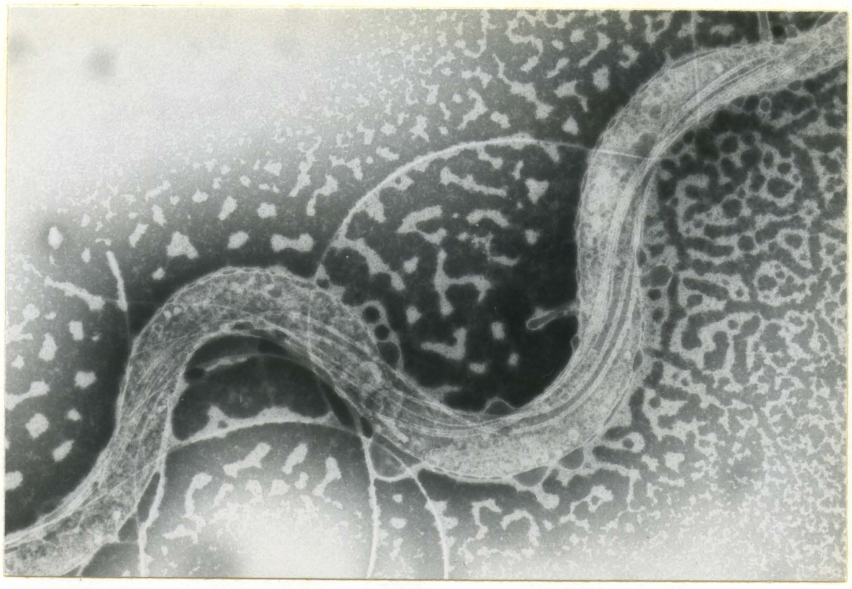

Figure 11. Electron photomicrograph of isolate B140 (negatively stained with PTA). x13,125

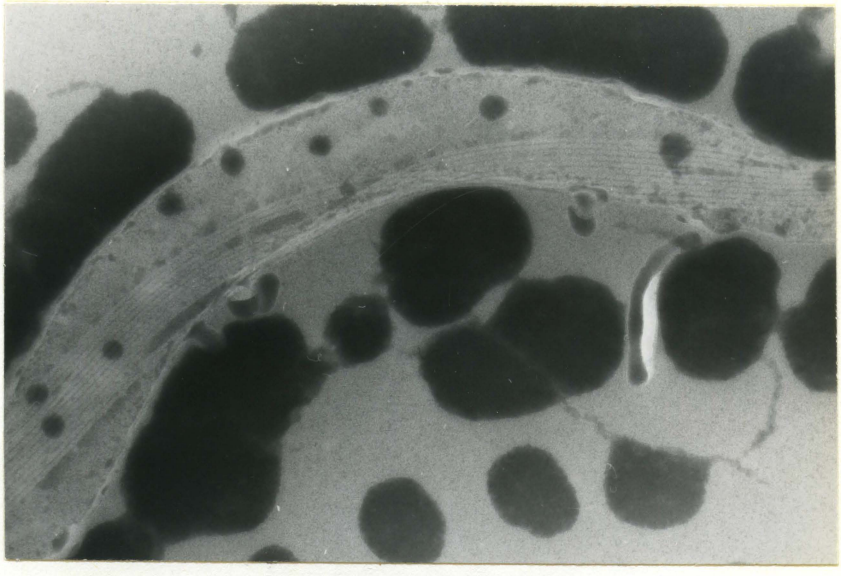

Figure 12. Electron photomicrograph of isolate B140 (negatively stained with PTA). x46,200

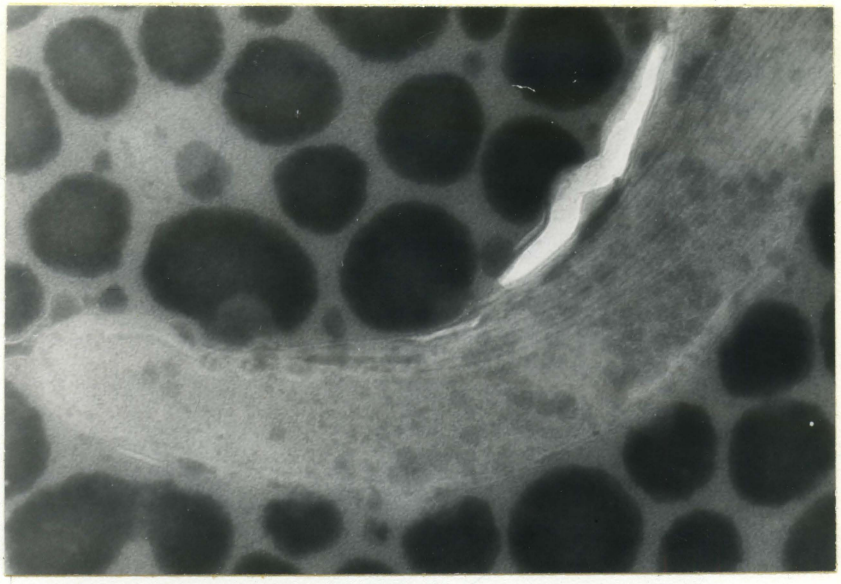

Figure 13. Electron photomicrograph of isolate B153 (negatively stained with PTA). Note insertion discs. x64,800

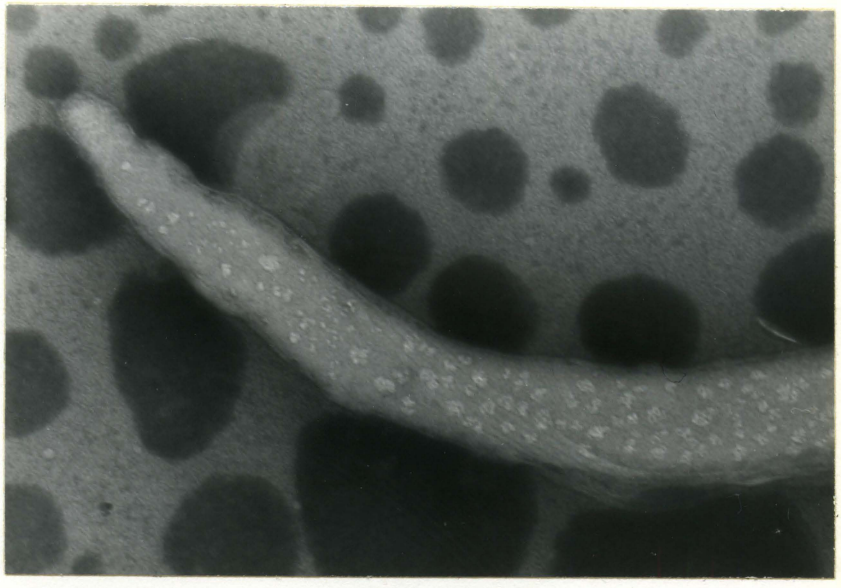

Figure 14. Electron photomicrograph of Puppy isolate (negatively stained with PTA). The isolate appears to be thinner than other isolates but statistical comparisons did not detect a difference. x64,800

analysis of these data did not show a statistical difference between isolates and the mean.

The characteristic motility of all these isolates was "snake-like", "whip-like", or "lashing", however, isolate B256 was unusual in its motility. The isolate was noticeably different because of a "bending-from-the-middle, touch-your-toes" type of movement. And, this isolate was always motile in comparison with the other isolates which were not usually so active. Figure 15 illustrates a culture of isolate B256 in which an organism is curved in a shape similar to that seen in the observations by phase microscopy.

Differential media

Tables A3, A4, and A5 in the Appendix list the reactions in differential media of all isolates. None of the isolates of ThLO fermented any of the following carbohydrates when grown under CO_2, H_2:CO_2, or N_2: amygdalin, arabinose, erythritol, esculin, glycogen, inositol, mannitol, mannose, melezitose, melibiose, raffinose, rhamnose, sorbitol, starch, sucrose, xylose, adonitol, dulcitol, glycerol, inulin, tartaric acid, dextrin, galacturonic acid, gluconic acid, or glucuronic acid. None of the isolates produced the following: catalase, lecithinase, lipase, ammonia, acetylmethylcarbinol, oxidase, or urease. One isolate (Puppy) produced H_2S in 1

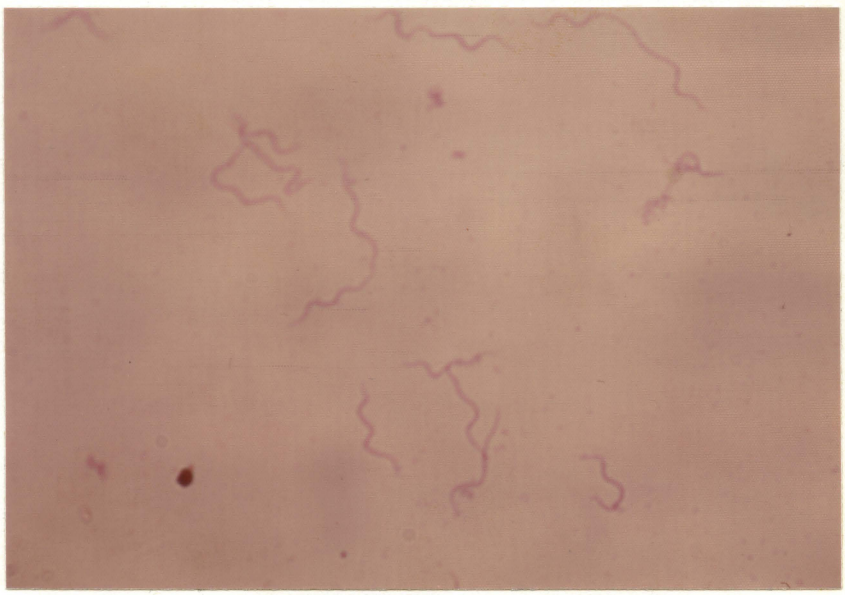

Figure 15. Carbol fuchsin stain of ThLO (isolate B256). Note the organism in lower right corner with "U" shape morphology. x1600

of 3 determinations; no other isolates produced H_2S. The isolates did not liquefy gelatin, reduce nitrates, utilize lactate, blacken mucin, and did not digest or blacken meat. Temperatures of 25° C and 30° C did not usually support growth of ThLO and the organisms would not grow in medium at pH of 9.6; medium with 1% glycine; or medium with 30% or 6.5% salt. None of the isolates grew in aerobic conditions.

All ThLO hydrolyzed esculin; grew in the presence of bile and iodoacetic acid; reduced neutral red; produced gas; and grew at 37° C and 42° C, regardless of the gaseous atmosphere under which they were inoculated. All isolates hemolyzed blood agar media used in primary isolation and subsequent cultivation. This hemolysis is not as complete as the complete hemolysis of the B-hemolytic streptococci, no is it green hemolysis. It is similar to the outer incomplete zone of hemolysis seen with cultures of Clostridium perfringens and Staphylococcus aureus (Figures 16-17). The 3 nondysentery isolates appeared less hemolytic than the dysentery isolates (see Figure 18). Young cultures of the dysentery isolates did not usually have surface colonies in the zones of hemolysis, however, nondysentery isolates produce large amounts of surface growth (see Figure 19).

The concept of weaker hemolysis of nondysentery isolates was further illustrated by observations on growth of

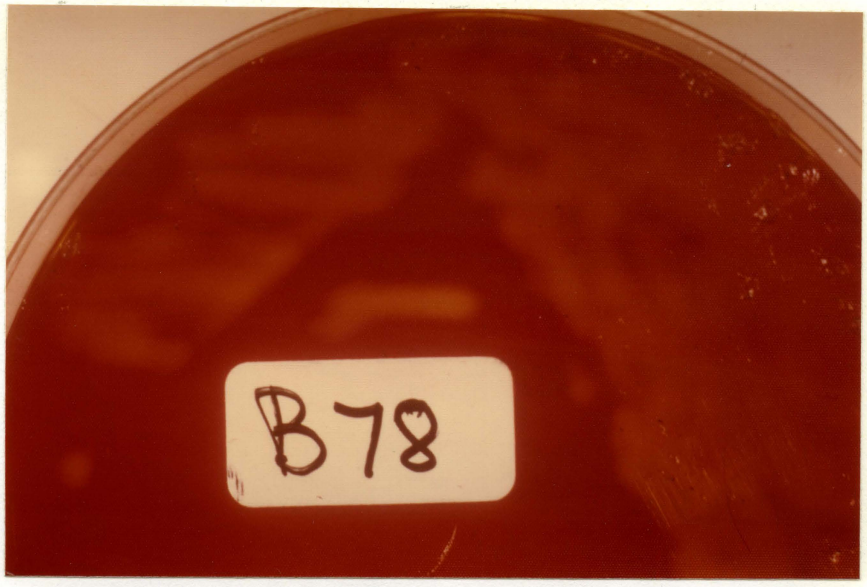

Figure 16. Illustration of type of hemolysis produced by dysentery isolate B78 on TSA with bovine blood

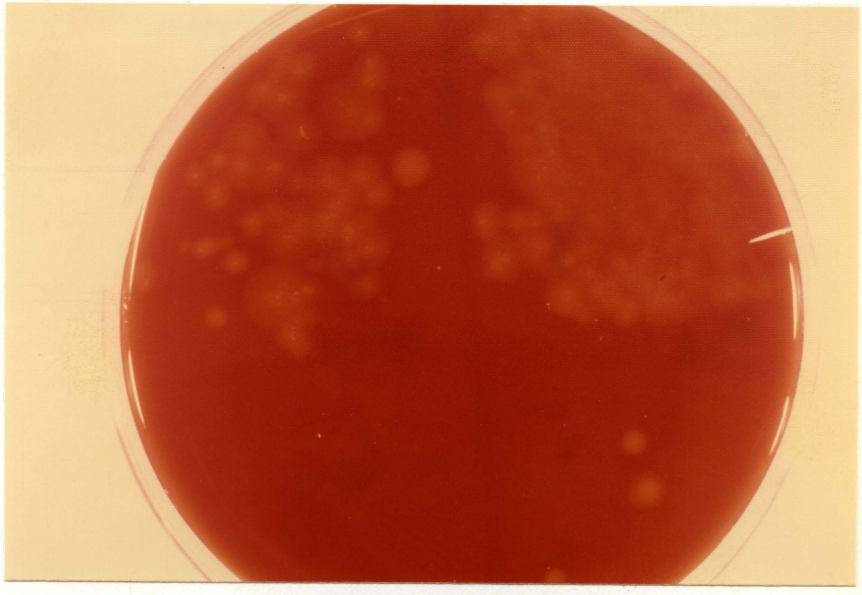

Figure 17. Demonstration of colony count determination of broth culture of isolate B204 on TSA with bovine blood. Note the lack of distinct colonies in the hemolytic zones

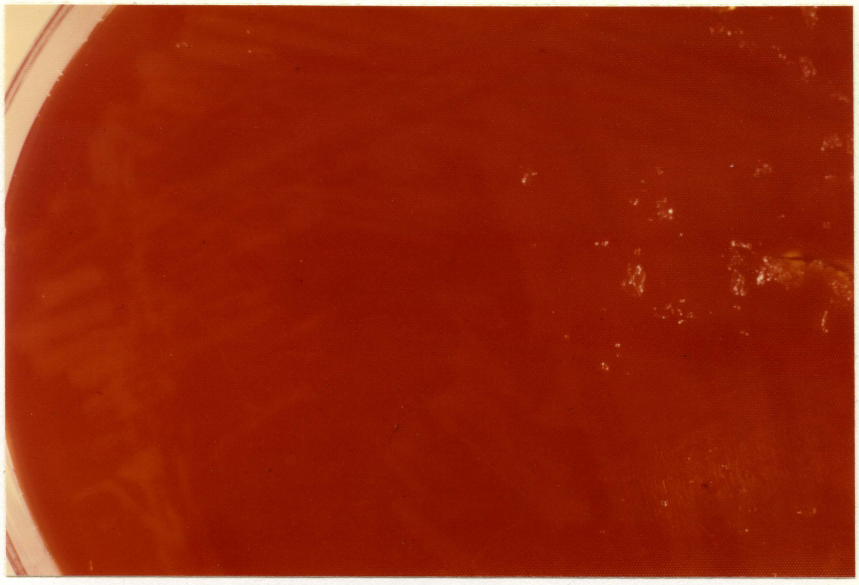

Figure 18. Illustration of the type of hemolysis produced by a nondysentery isolate (B256) of ThLO on TSA with bovine blood. All nondysentery isolates caused this type of hemolysis

Figure 19. Illustration of surface growth of ThLO (isolate B256) often produced by cultures of nondysentery isolates on TSA with bovine blood

dysentery (B204 and B78) and nondysentery isolates (B256) in liquid PRAS-TSB medium containing 5% bovine blood, under CO_2, inoculated with agar from blood agar plates containing the organisms. Growth of isolates B204 and B78 resulted in partial hemolysis of the red blood cells after 24 hours incubation and complete hemolysis after 48 hours incubation. Cultures of isolate B256 were hemolyzed after 4 days incubation and completely hemolyzed after 5 days of incubation. Turbidity of the medium above the settled red cells was observed in cultures of isolate B256 after 3 days incubation.

The variable characteristics of the isolates in differential media inoculated under CO_2 are presented in Table 13, and the variable characteristics for 2 isolates (B78 and B204) inoculated under N_2 are presented in Table 14. Differential media inoculated under H_2:CO_2 yielded fermentation characteristics exactly like that of media inoculated under CO_2 except for the positive fermentation of lactose by isolates B78 and B204 in H_2:CO_2 atmospheres.

Minor amounts of acetic acid and n-butyric acid were the end products of fermentation of carbohydrate media by all isolates of ThLO. Occasionally pyruvic acid and isovaleric acid were also produced from carbohydrate fermentation, however, these were very small (less than 5 mm) peaks.

Table 13. Variable characteristics of isolates of Treponema hyodysenteriae-like organisms[a]

Differential Test	Isolate: Dysentery Origin																Nondysentery Origin		
	B78	B140	B153	B163	B169	B171	B173	B179	B204	B234	B254	B259	N-1	A-1	300/8	1037	B256	Puppy	4/71
Hemolysis	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	wk	wk	wk
Cellobiose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Fructose	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	+	+
Glucose	+	-	+	+	-	-	-	-	+	-	+	+	+	-	-	+	ND	-	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Maltose	+	-	+	+	+	-	-	-	+	+	+	+	-	+	-	-	+	-	-
Indol	+	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	-	-	-
Hippurate	±	-	±	-	-	±	-	-	-	±	±	-	-	-	-	-	-	-	+
Pyruvate	+	+	+	+	+	-	ND	-	+	±	+	±	+	+	+	+	+	-	+

[a]Symbols: β = beta hemolysis; wk = weak beta hemolysis; - = does not possess characteristic; + = possesses characteristic (pH > 0.25 units lower than control); ± = weak; ND = not done.

Table 14. Comparison of the production of acid Treponema hyodysenteriae-like organisms grown in media with carbohydrate substrate inoculated under N_2 and CO_2[a]

Differential Test	Isolate No and Atmosphere			
	B78, N_2	B78, CO_2	B204, N_2	B204, CO_2
Fructose	+ 1/1	- 3/3	++ 1/1	+ 1/2
Glucose	+ 1/1	+ 2/3	++ 1/1	+ 1/2
Lactose	+ 1/1	- 1/1	+ 1/1	- 2/2
Maltose	++ 1/1	+ 1/1	++ 1/1	+ 1/1
Ribose	- 1/1	- 1/1	+ 1/1	- 1/1
Salicin	ND	- 1/1	+ 1/1	- 1/1
Trehalose	- 1/1	ND	++ 1/1	- 1/1
Galactose	- 1/1	- 1/1	++ 1/1	- 1/1
Sorbose	- 1/1	- 1/1	+ 1/1	- 1/1

[a] Symbols: + = production of acid from substrate, >0.25 pH units lower than control; ++ = production of acid from substrate, 0.5-1.1 pH units lower than control; - = did not produce acid from substrate; ND = not done. Numerator = number of attempts which gave characteristic; denominator = number of attempts.

All isolates of T. hyodysenteriae-like organisms produced moderate quantities of gas (Figures 20 and 21). The isolates produced both H_2 and CO_2 as determined by gas chromatographic analysis of the atmospheres above cultures of dysentery and nondysentery isolates (Table 15).

Table 16 illustrates the effect of age of culture, substrate, and atmosphere on the production of H_2 by 6 isolates of ThLO.

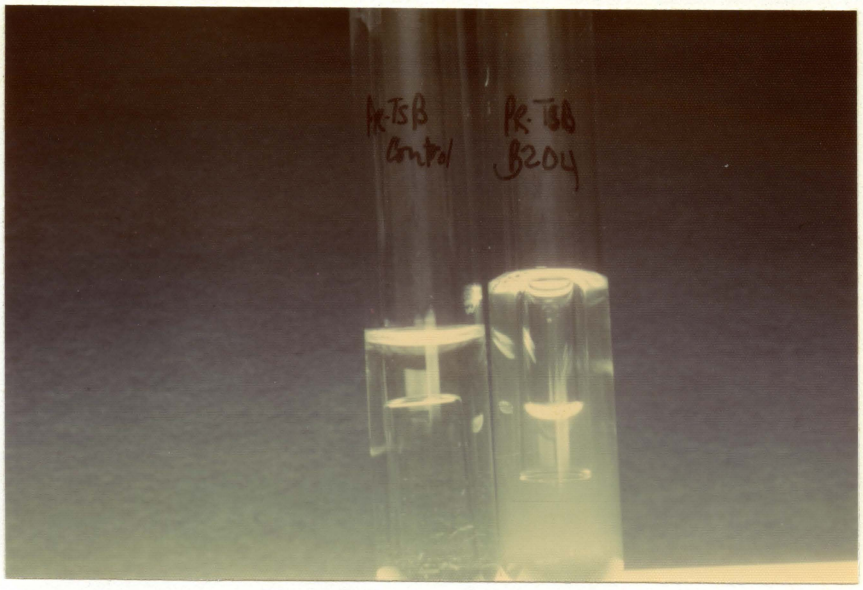

Figure 20. Illustration of the amount of gas produced by isolates of ThLO (B204, right tube) in Durham tubes in TSB with FCS, cysteine hydrochloride, and resazurin prepared and inoculated under N_2 only. Left tube is uninoculated control

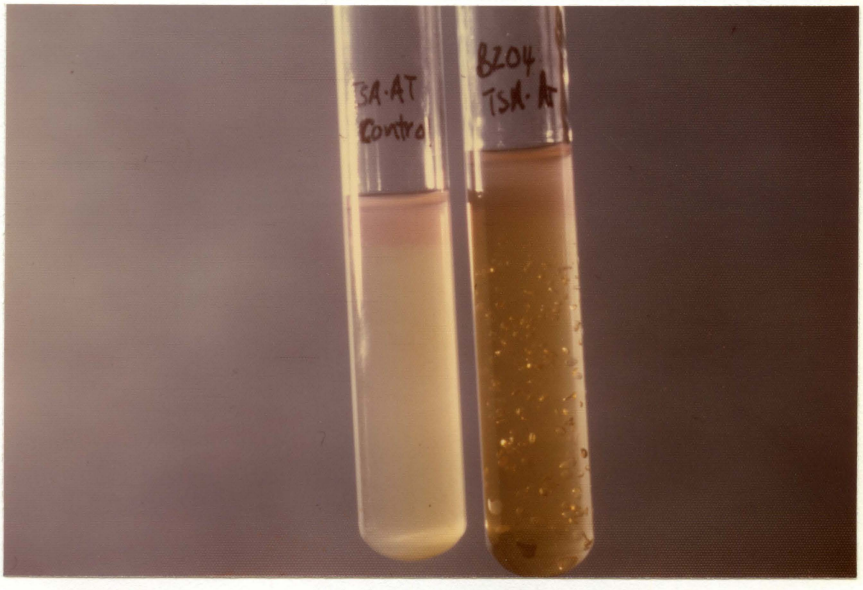

Figure 21. Illustration of the amount of gas produced by isolates of ThLO (B204, right tube) in TSA aerotolerance deep agar containing 10% FCS. Left tube is uninoculated control

Table 15. Production of H_2 and CO_2 by dysentery and non-dysentery isolates of Treponema hyodysenteriae-like organisms

Isolate No	Origin[a]	Medium[b]	Percentage Gas Produced[c]	
			H_2	CO_2
B78	SD	TSB-N_2	16%	6%
B153	SD	PRAS-TSB	8%	ND
B163	SD	PRAS-TSB	25%	ND
B204	SD	TSB-N_2	32%	1%
A-1	SD	TSB-N_2	16%	8%
Puppy	NSD	TSB-N_2	20%	22%
B256	NSD	TSB-N_2	13%	18%
4/71	NSD	PRAS-TSB	10%	26%
Uninoculated Control		TSB-N_2	-	1%
Uninoculated Control		PRAS-TSB	-	4%

[a]Symbols: SD = dysentery origin; NSD = nondysentery origin.

[b]TSB-N_2 = TSB prepared with PRAS methods, cysteine hydrochloride and resazurin, but bubbled with N_2 only; both were supplemented with 10% FCS and inoculated under N_2.

[c]Symbols: ND = not done; - = no gas produced; % = as compared to standard.

Table 16. Effect of age of culture, medium substrates and opening[a] on production of H_2 by isolates of Treponema hyodysenteria-like organisms[b]

Isolate No	Media	Atmosphere	Age	H_2 Peak Height in mm
B78	PRAS-TSB	CO_2	3 day	3400
B78	PRAS-TSB	N_2	3 day	3300
B78	PRAS-TSB-Gluc	N_2	3 day	6800
B78	PRAS-TSB	CO_2	3 day	1350
B78	PRAS-TSB	CO_2	10 day	3500
B78	PRAS-TSB	CO_2	6 day opened at 4 day	0
B204	PRAS-TSB	CO_2	4 day	3150
B204	PRAS-TSB-Gluc	CO_2	4 day	3900
B204	AMC	CO_2	4 day	4400

[a] Culture was opened for sampling under stream of deoxygenated CO_2.

[b] Uninoculated controls (PRAS-TSB, PRAS-TSB with glucose and AMC and PRAS-TSB after 3 days, 4 days, and 10 days incubation) did not produce H_2.

DISCUSSION

The transmission experiments in these studies with ThLO indicate overwhelmingly the primary role of Treponema hyodysenteriae in the etiology of SD. Twenty-two of 24 dysentery isolates produced SD when orally inoculated into susceptible 2-week-old swine from 2 Iowa SPF herds. The isolates were from geographically separated outbreaks of SD (Colorado, Florida, Indiana, Illinois, Iowa, Kansas, Minnesota, Nebraska, North Carolina, Canada, and Great Britain).

Pure cultures of T. hyodysenteriae, strain B78, had been previously used to produce SD in 6-8-week-old pigs (Harris et al., 1972a; Glock and Harris, 1972). In the present studies strain B78 produced SE in 2-week-old pigs, establishing the model for the determination of pathogenicity of the isolates. This model is perhaps not ideal for study of a disease which occurs primarily in 7-14-week-old pigs, however, SD does occur naturally in the suckling piglet (Lussier, 1962; Alexander and Taylor, 1969; Harris and Glock, 1973).

Clinically and pathologically, the disease produced was like the natural infection (Whiting et al., 1921; Lussier, 1962; Alexander and Taylor, 1969; Harris and Glock, 1971, 1972, 1973).

Clinical signs of SD did not appear as gradually as compared to 6-8-week-old pigs infected with crude SD inoculum (Glock et al., 1974). However, the incubation period for production of SD by pure cultures of ThLO in the present studies was similar to the incubation periods reported for: 1) the natural infection (Whiting et al., 1921; Lussier, 1962; Harris and Glock, 1971, 1972); 2) crude SD infection (Davis, 1961; Harris and Glock, 1973; Glock et al., 1974; Olson, 1974); and 3) pure culture infection (Taylor and Alexander, 1971; Harris et al., 1972a; Hamdy and Glenn, 1974).

Diarrhea of normal composition was usually observed for less than 1 day before mucus and blood were seen. The pigs then became rapidly dehydrated and gaunt. No pigs in this study developed the infection to the point of mortality, primarily because the first pig to reach the acute stage of the disease was killed and necropsied. This fact also prevented the second pig from being continually exposed to SD, and probably increased the number of second pigs in each pair inoculated with pure cultures of a single isolate which did not succumb to the disease.

Macroscopic lesions were confined to the large intestine and consisted of catarrhal, mucohemorrhagic, hemorrhagic, and necrotic colitis. Pseudomembranous accumulations were also observed and the colonic mucosa appeared edematous and hyperemic. The serosal surface was

frequently inflamed and in a few instances nodules were seen.

Microscopically the crypts appeared dilated, there was congestion and hemorrhage in the lamina propria, and congestion in the submucosa. The epithelial covering was often pushed away from the lamina propria by exudate including cellular components and bacteria. The lumen was filled with exudate consisting of fibrin, mucus, red and white blood cells, and bacteria. Phase microscopic examinations of the exudate and deeper tissue scrapings revealed the presence of large numbers of ThLO. These organisms were frequently observed penetrating epithelial cells.

Several pigs which had developed SD became moribund, some recovered to remain normal, another frequently exacerbated, and some continued to show signs of SD and ThLO in the feces over a 30 day observation period. These facts confirm the production of SD in all its ramifications by oral inoculation of SPF pigs with pure cultures of ThLO. Reports concerning the oral inoculation of susceptible pigs with pure cultures of *V. coli* have never satisfactorily demonstrated its role in the etiology of the disease (Doyle, 1944; James and Doyle, 1947; Davis, 1961; Warner, 1965; Andress *et al.*, 1968; Terpstra, 1968; Harris *et al.*, 1972a; Glock and Harris, 1972; Sofrenovic *et al.*, 1973; Hamdy and Glenn, 1974).

Pigs which had resisted pure culture challenge were

susceptible to crude SD challenge 4 weeks later. This susceptibility could be age dependent; perhaps some of the 2-week-old pigs had not developed the necessary microbial flora to allow ThLO to ellicit the dysentery response (Taylor, 1972; Harris et al., 1972c; Brandenburg, 1974). On the other hand, perhaps the pure culture challenge did not reach the individual minimal infective dose (Alexander and Taylor, 1969). It is also possible that the pigs were naturally resistant similar to the observations of Gorrie (1946), Harris et al. (1972b), Taylor (1972), and Olson (1974).

Isolate B179 was not orally inoculated into pigs in this study, however, it did produce SD in pigs in a study by Hamdy and Glenn (1974). Isolate A-1 is the isolate which was pathogenic for MD pigs in Great Britain (Taylor and Alexander, 1971; Taylor, 1972). It is interesting to note that isolate A-1 produced SD in 1 of the shortest incubation periods (5 DPI) compared to other isolates in this study (average = 8 DPI). Two isolates (B171 and B259) which did not produce SD in susceptible pigs, were at low passage and the inoculum supposedly contained sufficient numbers of ThLO to produce the disease. Perhaps an older pig would have developed the disease from oral inoculation with isolates B171 or B259. One must also consider the possibility that animals in the herds where those outbreaks originated, may have

harbored nonpathogenic ThLO as did normal pigs studied by Taylor (1972). If so, these nonpathogenic types may not have been eliminated in the cloning procedures used to isolate the organism.

This is the first report of the isolation of spirochetes (Puppy isolate) from enteritis in dogs. Previous workers (Jungherr, 1937; Craige, 1948; Pindak et al., 1965; Zymet, 1969; Mortensen, 1970; Goudswaard and Cornelisse, 1973; van Ulsen and Lambers, 1973) have noted ThLO in the stools of diarrheic dogs but have not successfully cultured the organisms. The inability of the Puppy isolate to produce disease in either pigs or puppies is a preliminary indication that dogs are not a source of infection in SD outbreaks. The exact role of spirochetes in canine diarrhea could be determined by studies on the sequential lesion development of affected animals, similar to the work on SD in pigs of Taylor and Blakemore (1971) and Glock (1971), and Glock et al. (1974).

The reports of van Ulsen and Lambers (1973) and Goudswaard and Cornelisse (1973) that spirochetes with ThLO morphology were observed by FAT of diarrheal stools of affected dogs are not surprising, as cross reactivity of rabbit antisera to T. hyodysenteriae and antigen prepared from the Puppy isolate has been observed (Glock, R. D., Iowa State University, personal communication, 1974).

The observation and isolation of ThLO (isolate B256) from a Duroc uninoculated control pig affected with colibacillosis is somewhat contradictory to the report by Akkermans and Pomper (1973). They studied 169 cases of diarrhea in swine other than SD, and did not detect ThLO by the FAT. It has not been determined if isolate B256 is antigenically similar to T. hyodysenteriae.

On the other hand, the isolation of B256 is in partial agreement with a theory by Leach et al. (1973). The ThLO appeared simultaneously with diarrhea and could have been there as a result of dislodgement from crypts, however, other clinically normal swine from the same herd did not have ThLO in deep scrapings of the colonic mucosa observed by phase microscopy. The isolate was obtained from a Duroc pig and did not produce SD in either Yorkshire or Duroc pigs.

Isolate 4/71 did not produce disease either in pigs in Great Britain (Taylor, 1972) or in the pigs used in these studies. Furthermore it did not appear to establish in the colons of Duroc pigs orally inoculated with pure cultures of the isolate.

Isolation of ThLO by titration methods from fresh tissue of pigs acutely affected with the disease was easier and faster, and just as successful as isolation by filtration methods. Kast and Kolmer (1940) isolated oral spirochetes

from cultures contaminated with other bacteria by serial dilution methods. The use of serial dilutions of the original material has been reported by Bryant (1952) in the successful isolation of spirochetes from the bovine rumen.

A frequent observation noted on isolation by titration from pigs in the acute stage of the disease was the relative absence of oxygen-tolerant anaerobes at the 10^{-8}, 10^{-9} dilutions. This observation is consistent with the results of a study by Harris (Harris, D. L., Iowa State University, unpublished data, 1973). Increased length of incubation (as long as 18 days) of blood agar media for primary isolation of ThLO was helpful in obtaining zones of hemolysis free of contaminating organisms.

It was noted on several occasions that the growth of ThLO on primary isolation media streaked with filtrate from the 0.45 μm cellulose acetate filter was less at filtrate volumes of 0.5 ml compared to volumes of 0.1 ml or 0.01 ml. This could be an indication of the inhibitory influence of the colonic filtrate or contaminants on growth of the ThLO. Filtration methods are more reliable than titration methods for the isolation of ThLO from field specimens of pigs affected with dysentery, especially when the tissue has been held for long periods of time.

All of the isolates of ThLO grew at 42° C in PRAS-TSB with FCS under deoxygenated CO_2. This ability to grow at

higher temperature was considered as a possible aid in isolation[1] of ThLO.

The trypticase and phytone nutrients of TSA (and TSB) and the H_2 stimulation of ThLO were apparently important factors in the isolation of ThLO from the colonic mucosa of pigs and in the development of a liquid medium. The supplementation of TSB (PRAS or aerobically prepared) with FCS (not heat inactivated) was also important. The supplementation of PRAS-TSB with heat inactivated NRS would not support similar growth of ThLO. Cocarboxylase did not improve growth of ThLO in PRAS-TSB with NRS and TEM-4T, however, cocarboxylase was not evaluated in PRAS-TSB with FCS.

The exact role of H_2 (it both stimulates growth of ThLO and is produced by ThLO) and the mechanism of O_2 tolerance in cultivation of ThLO should be further investigated. Stimulation of ThLO by H_2 was repeatedly observed in liquid media culture systems which were not adequate for the nutrition of ThLO. This was most obvious in experiments to

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[1]A preliminary study of isolation of ThLO from the colon of a pure culture infected pig was conducted. The organisms incubated on TSA blood agar at 42° C grew on titration plates at every dilution to 10^8 (although some were overgrown or contaminated) after 2 days incubation, as compared to the titration on media incubated at 37° C where organisms grew to 10^8 after 4 days incubation. The apparent advantage to isolation at 42° C was not elimination of contaminants, but more rapid growth of ThLO.

determine the minimal amounts of FCS and inoculum required to obtain growth of ThLO in PRAS-TSB. Adequate growth of ThLO was obtained for 6 passages with as little as 0.1% FCS and 1% inoculum in H_2:CO_2 atmospheres; and some growth was obtained without FCS for 6 passages under H_2:CO_2. No growth beyond the first passage was possible in either system under CO_2 alone.

It is possible that ThLO uses H_2 as the electron donor in cytochrome-mediated electron transport coupled to oxidative phosphorylation as does Vibrio succinogenes (Wolin et al., 1961; Niederman and Wolin, 1972). Although oxidase was not detected in isolates of ThLO, Kawata (1967) has reported the presence of cytochromes in the Reiter treponeme. His methods were based on the spectrophotometric observations of washed suspensions of the treponeme and should be applied to cultures of ThLO. It is also possible that electron transport is mediated in ThLO by other coenzyme and enzyme systems.

Energy derived from electron transport coupled with oxidative phosphorylation has been postulated for Treponema pallidum (Cox and Barber, 1974). These workers believe that O_2 serves as an electron acceptor.

The use of 10% FCS and 7% inoculum in PRAS-TSB under H_2:CO_2 or CO_2 alone for growth of ThLO is still not an absolutely predictable system. This should improve with the

definition of optimal eH and pH values, optimal temperature, and nutrients. It should be noted that this study defined a lower temperature and alkaline pH at which growth of ThLO was not obtained. It did not define the upper temperature or acidic pH limits of ThLO. The optimal temperature and pH for growth of ThLO have not been determined.

Treponema hyodysenteriae-like organisms have been grown on agar media supplemented with bovine, horse, and sheep blood (Taylor and Alexander, 1971; Harris et al., 1972a, d; Taylor, 1972; Akkermans and Pomper, 1973). This study indicates that a quantitative superiority of TSA supplemented with horse and sheep blood may exist. This possibility should be further investigated.

Preservation of viability and virulence of isolates of ThLO for at least 2 years was obtained by freezing at -80° C and by lyophilization. The organisms were not protected in either instance except by small quantities of the culture medium and supplement. Hollander and Nell (1954) have shown the influence of cryoprotective agents for preservation of viability and virulence of T. pallidum.

Characteristics of isolates of ThLO support of the placement of T. hyodysenteriae in the genus Treponema (Smibert, 1974) based on its location in the host, helical morphology, motility, negative Gram's stain, and fermentative metabolism. Furthermore, ThLO are anaerobic; catalase negative; oxidase

negative; urease negative; acetylmethylcarbinol negative; nitrate reduction negative; and not inhibited by iodoacetic acid.

This treponeme appears to require long chain fatty acids available in FCS but cannot utilize the short chain fatty acids of rumen fluid. Rumen fluid and TEM-4T are not acceptable fatty acid sources for growth of ThLO. Whether or not ThLO requires CO_2 remains to be determined as the organism was capable of growth in TSB with cysteine hydrochloride and resazurin prepared and inoculated under N_2 alone. However, the atmospheres over supplemented, uninoculated, control media did contain slight amounts of CO_2 as detected by gas chromatography.

A goal of characterization was to establish the definite classification of these spirochetes as a separate species within the genus Treponema. By using Smibert's (1973b) key (Appendix, Table A1) ThLO would be classified as T. refringens btp refringens. However, further comparisons of ThLO with T. refringens btp refringens (Smibert, 1973b, 1974) reveal several conflicting characteristics: most of the isolates of ThLO fermented fructose, glucose or maltose; ThLO did not digest gelatin; ThLO grew in presence of bile; ThLO were H_2S negative; ThLO produced gas, and ThLO did not produce NH_3. The end products of fermentation of amino acids (it does not ferment carbohydrate) by T. refringens are acetic,

lactic and succinic acid, and those of carbohydrate fermentation by ThLO are acetic and butyric. This comparison and speciation would be more reliable if ThLO had grown in PYS. Treponema refringens and ThLO should be characterized in the same differential media.

Isolates of ThLO are distinct from the small spirochetes (Harris et al., 1972d) pig feces treponemes (Smibert, 1971; Smibert and Claterbaugh, 1972) and PN-5 spirochetes (Saheb and Berthiaume (1973) and Saheb and Richer-Massicotte (1972) primarily by morphology and motility and anaerobic requirement. In addition the pig feces strains and small spirochetes are able to metabolize more carbohydrates and produce acetic, lactic, and succinic acid (Harris and Kinyon, 1974).

The majority of the characteristics of isolates of ThLO (59 of 68) indicate the similarity of isolates to one another and to strain B78. It was hoped that characterization would establish in vitro parameters which would separate morphologically identical ThLO into pathogenic and nonpathogenic types. Preliminary data indicate this may be possible using hemolysis, indol production, and fructose fermentation. The most stable of these characteristics is the hemolytic pattern, a fact first noted by Taylor and Alexander (1971). A more sensitive method for the quantitation and description of the hemolysins of ThLO should be devised. The production

of indol was studied in SIM and chopped meat broth but inconsistencies between the two media and between different trials of the same isolate indicate more sensitive and reliable methods must be developed. The production of acid from fructose (and all carbohydrates) will be more easily and accurately determined in media supplemented and inoculated under N_2 because of wider separation of pH values of media with and without substrate under N_2.

SUMMARY

Thirty-four isolates of Treponema hyodysenteriae-like organisms were compared in their ability to produce swine dysentery, requirements for growth, and characteristics in differential media.

Of the 31 isolates of Treponema hyodysenteriae-like organisms of dysentery origin, 24 were inoculated into pigs. Typical, acute swine dysentery was produced in 47 of 63 susceptible SPF pigs orally inoculated with pure cultures of 22 isolates. Three of the isolates were of nondysentery origin and did not produce disease when orally inoculated into susceptible SPF pigs. One of these isolates was from a puppy with catarrhal enteritis. It did not produce signs of enteric disease in puppies orally inoculated with pure cultures of the isolate. In addition, one isolate of dysentery origin which had produced disease in pigs, did not produce disease when orally inoculated into puppies.

Growth of the organisms was stimulated in anaerobic atmospheres containing high concentrations of H_2. The solid medium for growth of the organisms was trypticase soy agar supplemented with 5% bovine blood. The liquid medium, for most studies, was PRAS-trypticase soy broth (TSB) supplemented with 10% fetal calf serum (FCS). The organisms did not grow in liquid media used for cultivation of other treponemes.

The comparison of *in vitro* characteristics of isolates of *T. hyodysenteriae*-like organisms to other cultivatable treponemes indicate the definite separation of *T. hyodysenteriae* from the other members of this genus. All isolates were similar in 59 of 68 morphologic and metabolic characteristics, to strain B78, the type species of *T. hyodysenteriae*. Isolates of *T. hyodysenteriae*-like organisms from dysentery and nondysentery origin may be differentiated on the basis of type of hemolysis, production of indol, and fermentation of fructose.

In conclusion, *T. hyodysenteriae* is currently the only cultivatable pathogenic treponeme. It is distinct morphologically and metabolically from the other cultivatable treponemes. The ability of several isolates of *T. hyodysenteriae* from geographically separated outbreaks of swine dysentery to produce the disease when inoculated into susceptible pigs is accepted as evidence for the primary role of the organism in the disease.

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I dedicate this manuscript to the memory of Monsieur Cocoa Godot--a never-forgotten friend.

APPENDIX

Table A1. Key for *Treponema*[a] (Smibert, 1973b)

cell with 0.25–0.35 μ	mannitol fermentation	sucrose fermentation	esculin hydrolysis	glycine (1%) gr	indol production	lactate → prop	Products from PYG	Suggested Species
+	W		+				ABpls[b]	*T. phagedenis* btp. *kazan*
			−				ABpls	*T. phagedenis* btp. *reiterii*
	−		+	+			Als	*T. refringens* btp. *calligyrum*
				−			Als	*T. refringens* btp. *refringens*
			−				ABls	*T. vincentii*
−		+					Als	*T. macrodentium*
		−	+		+	+	Apls	*T. oralis*
						−	Als (pb)	*T. denticola* btp. *denticola*
					+		Als (pb)	*T. denticola* btp. *comondonii*
			−				Apbs	*T. scoliodontum*

[a] Symbols: + = possess characteristic; − = does not possess characteristic; W = weak.

[b] Symbols: A = acetic acid; B = butyric acid; P = propionic acid; L = lactic acid; S = succinic acid; ABPLS = major amounts; abpls = minor amounts.

Table A2. Measurements of Treponema hyodysenteriae-like organisms from electron photomicrographs of phosphotungstic acid negatively stained isolates

Isolate No	Passage	Culture[a]	Diameter in nm $\bar{x}$	Range	No Obser
B78	70	PRAS-TSB	305	233-449	10
B137	8	PRAS-TSB	316	297-351	5
B140	15	PRAS-TSB	392	298-501	7
B153	10	BAP	321	313-346	4
B163	11	Aer-TSB	308	242-355	8
B169	11	PRAS-TSB	414	383-445	6
B171	13	BAP	364	274-484	8
B173	10	BAP	318	298-351	7
B179	13	PRAS-TSB	301	279-336	5
B204	8	Aer-TSB	336	244-374	6
B234	10	Aer-TSB	357	250-473	5
B254	10	BAP	265	200-307	6
B256	13	Aer-TSB	362	313-401	7
B259	8	BAP	292	212-361	6
Puppy	8	PRAS-TSB	282	208-314	4
A-1	5	Aer-TSB	303	276-317	4
4/71	7	BAP	334	288-365	4
N-1	40	BAP	337	295-392	4
N-2	25	BAP	271	246-329	5
300/8	7	BAP	308	296-336	5
1037	7	BAP	381	347-447	5
Totals			327 nm		

[a]Isolates were grown in pure culture in: PRAS-TSB or aerobically prepared TSB (Aer-TSB); with 10% FCS under H_2:CO_2; or on TSA with blood.

Length in μm			No Axial Fibrils		
$\bar{x}$	Range	No Obser	$\bar{x}$	Range	No Obser
8.9	83 -98	4	8- 9	7-10	9
10.9	12.8- 7.7	3	8- 9	7-10	7
10.4	7.6-14.3	3	7- 8	7-11	11
8.0	7.7- 8.3	2	8- 9	7- 9	5
6.5	5.6- 8.2	5	8- 9	7-10	4
7.6	6.4- 9.7	5	8- 9	7-10	10
7.9	5.6- 9.1	8	7- 8	6- 9	3
5.8	5.4- 6.5	4	7- 8	7- 9	5
11.3	11.3	1	7- 8	7-10	9
8.6	5.9-10.5	4	8- 9	7-10	10
8.3	7.8- 9.0	4	7- 8	7- 9	8
6.2	3.8- 8.2	3	9	6-10	11
8.8	7.4-14.1	7	9	7-10	8
8.4	6.6- 9.2	4	7- 8	7- 9	5
6.1	5.6- 6.5	2	5- 6	4- 7	7
10.9	8.7-15.0	3	10-11	9-12	2
6.3	5.3- 7.4	4	12-13	10-14	5
9.5	7.8-10.5	3	8- 9	6- 9	8
10.4	8.0-12.0	4	7- 8	7- 8	5
8.6	6.0-11.2	2	8	7- 9	8
8.4	6.5-11.2	3	7- 8	6- 8	5
8.5 μm			8-9 axial fibrils		

Table A3. Characteristics of isolates of Treponema hyodysenteriae-like organisms in differential media inoculated under H_2:CO_2[a]

Differential Test	Isolate No B78	B140	B169	B204	B234
Fructose	-	-	ND	ND	-
Glucose	+	-	-	+	-
Lactose	+	ND	-	+	+
Mannitol	-	-	-	-	-
Sucrose	-	ND	-	-	-
Indol	+	-	+	+	-
Hemolysis	+	+	+	+	+
Motility	+	+	+	+	+
H_2S	-	-	-	-	-
1% Glycine	-	-	-	-	-
Lactate	-	-	-	ND	-
Products from Glucose[b]	a,b	a,b	a,b	a,b	a,b

[a]Symbols: + = possesses characteristic (pH > 0.25 units lower than control); - = does not possess characteristic; ND = not done.

[b]Symbols: a = minor amounts of acetic acid; b = minor amounts of butyric acid.

Table A4. Characteristics of isolates of Treponema hyodysenteriae-like organisms in differential media inoculated under CO_2[a]

Differential Test	Dysentery Isolates								
	B78	B140	B153	B163	B169	B171	B173	B179	B204
Amygdalin	-1/1	ND	-1/1	-1/1	-1/1	-1/1	ND	ND	-1/1
Arabinose	-1/1	ND	-1/1	-1/1	-1/1	-1/1	ND	ND	-1/1
Cellobiose	-1/1	ND	-1/1	-1/1	-1/1	-1/1	ND	ND	-1/1
Erythritol	-1/1	ND	-1/1	-1/1	-1/1	-1/1	ND	ND	-1/1
Esculin pH	-1/1	-1/1	-1/1	-1/1	-2/2	-1/1	ND	-2/2	-2/2
Esculin hyd	+2/2	+1/4	+1/1	+1/1	+2/2	+1/1	ND	+1/1	+2/2
Fructose	-3/3	-4/4	-1/1	-1/1	-1/1	-2/2	-1/1	-2/2	+1/2
Glucose	+2/3	-4/4	+1/1	+1/1	-2/2	-2/2	-1/1	-2/3	+1/2
Glycogen	-1/1	ND	-1/1	-1/1	-1/1	-1/1	ND	ND	-1/1
Inositol	-1/1	ND	-1/1	-1/1	-1/1	-1/1	ND	ND	-1/1
Lactose	-1/1	-4/4	-1/1	-1/1	-2/2	-2/2	-1/1	-2/2	-2/2
Maltose	+1/1	-3/3	+1/1	+1/1	+1/1	-1/1	-1/1	-3/3	+1/1
Mannitol	-3/3	-4/4	-1/1	-1/1	+2/2	-2/2	-1/1	-2/2	-2/2
Mannose	-1/1	ND	-1/1	-1/1	-1/1	ND	ND	ND	-1/1
Melezitose	-1/1	ND	-1/1	-1/1	-1/1	ND	ND	ND	-1/1
Melibiose	-1/1	ND	-1/1	-1/1	-1/1	ND	ND	ND	-1/1
Raffinose	-1/1	ND	-1/1	-1/1	-1/1	ND	ND	ND	-1/1
Rhamrose	-1/1	ND	-1/1	-1/1	-1/1	ND	ND	ND	-1/1
Ribose	-1/1	ND	-1/1	-1/1	-1/1	ND	ND	ND	-1/1
Salicin	-1/1	ND	-1/1	-1/1	-1/1	ND	ND	ND	-1/1
Sorbitol	-1/1	ND	-1/1	-1/1	ND	ND	ND	ND	-1/1
Starch	-1/1	ND	-1/1	-1/1	-1/1	ND	ND	ND	-1/1
Sucrose	-3/3	-4/4	-1/1	-1/1	-1/1	-2/2	-1/1	-2/2	-2/2

[a]Symbols: + = possesses characteristic (pH >0.25 units lower than control); - = does not possess characteristic; ± = weak +; ND = not done; numerator = number of attempts which gave characteristics; denominator = number of attempts.

Dysentery Isolates								Nondysentery Isolates		
B234	B254	B259	N-2	N-1	A-1	300/8	1037	B256	Puppy	4/71
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	-1/1	-1/1	-1/1	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	-1/1	-1/1	-1/1	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	-1/1	+1/1	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	-1/1	-1/1	ND
-2/2	-1/1	-1/1	-1/1	-1/1	-2/2	-2/2	-3/3	-1/1	-1/1	-3/3
+1/1	+1/1	ND	61/1	+1/1	+2/2	+2/2	+3/2	+2/2	+1/1	-3/3
-2/3	-1/1	-1/1	ND	-2/2	-2/2	-1/1	+3/3	+2/2	+1/1	+2/3
-3/4	+1/1	+1/1	-1/1	+1/2	-2/2	-2/2	+3/3	ND	-3/3	+3/3
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	-1/1	-1/1	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	-1/1	ND	ND
-2/2	-1/1	-1/1	ND	-2/2	-2/2	-2/2	-3/3	-2/2	-3/3	+2/3
+2/3	+1/1	+1/1	-1/1	-2/2	+1/2	-2/2	-3/3	+1/2	-3/3	-3/3
-2/2	ND	-1/1	-1/1	-2/2	-2/2	-2/2	-3/3	-2/2	-3/3	-3/3
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	-1/1	ND	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	-1/1	ND	ND
-2/2	ND	-1/1	ND	ND	-1/1	ND	ND	-1/1	ND	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	+1/1	ND	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	-1/1	ND	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	-1/1	ND	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	-1/1	ND	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	-1/1	ND	ND
-2/2	-1/1	-1/1	ND	ND	-2/2	-2/2	-3/3	-2/2	-1/1	-3/3

Table A4 (Continued)

Differential Test	Dysentery Isolates								
	B78	B140	B153	B163	B169	B171	B173	B179	B204
Trehalose	ND	ND	-1/1	-1/1	-1/1	ND	ND	ND	-1/1
Xylose	-1/1	ND	ND	-1/1	ND	ND	ND	ND	-1/1
Gelatin	±1/1	-1/1	-1/1	-1/1	-1/1	-1/1	-1/1	-1/1	-1/1
Meat	-3/3	-4/4	-1/1	-1/1	-1/1	ND	-1/1	-2/2	-1/1
Indol	+3/3	+3/4	+1/1	+1/1	+1/2	-2/2	-1/1	-2/2	+1/2
Nitrate	-1/1	-2/2	-1/1	-1/1	-1/1	-1/1	ND	-1/2	-1/1
Catalase	-1/1	-2/2	-1/1	-1/1	-1/1	-1/1	-1/1	-2/2	-1/1
Bile gr	+1/1	+2/2	+1/1	+1/1	+1/1	+2/2	ND	+1/1	+1/1
Lecithinase	-1/1	-3/3	-1/1	-1/1	-1/1	-2/2	-1/1	-2/2	-1/1
Lipase	-1/1	-3/3	-1/1	-1/1	-1/1	-2/2	-1/1	-2/2	-1/1
Hemolysis	+3/3	+4/4	+1/1	+1/1	+2/2	+2/2	+1/1	+2/2	+2/2
Motility	+3/3	+4/4	+1/1	+1/1	+2/2	+2/2	+1/1	+2/2	+2/2
H_2S	-3/3	-4/4	-1/1	-1/1	-2/2	-2/2	-1/1	-2/2	-1/1
Adonitol	-1/1	ND	-1/1	-1/1	ND	ND	ND	ND	-1/1
Dulcitol	-1/1	ND	-1/1	-1/1	ND	ND	ND	ND	-1/1
Galactose	-1/1	ND	-1/1	-1/1	ND	ND	ND	ND	-1/1
Glycerol	-1/1	ND	-1/1	-1/1	ND	ND	ND	ND	-1/1
Glycine	-3/3	-4/4	-1/1	-1/1	-2/2	ND	ND	-2/2	-2/2
Inulin	-1/1	ND	-1/1	-1/1	ND	-2/2	ND	ND	-1/1
Sorbose	-1/1	ND	-1/1	-1/1	ND	ND	ND	ND	-1/1
Gas	+3/3	+2/2	+1/1	+1/1	+1/1	ND	+1/1	+2/2	+2/2
NH_3	-1/1	-2/2	-1/1	-1/1	-1/1	+2/2	ND	-1/1	-1/1
AMC	-1/1	-2/2	-1/1	-1/1	-1/1	-2/2	ND	-1/1	-1/1
Hippurate	±1/1	-2/2	±1/1	-1/1	-1/1	±1/2	ND	-1/1	-1/1
NR Reduction	+1/1	+2/2	+1/1	+1/1	+1/1	+1/1	ND	+1/1	+1/1

Dysentery Isolates								Nondysentery Isolates		
B234	B254	B259	N-2	N-1	A-1	300/8	1037	B256	Puppy	4/71
-3/3	-1/1	-1/1	ND	ND	-1/1	ND	ND	ND	ND	ND
ND	ND	-1/1	ND	-2/2	-1/1	ND	ND	ND	ND	ND
-2/2	±1/1	-1/1	ND	-2/2	-1/1	-2/2	-3/3	-1/1	-1/1	-1/1
-2/2	-1/1	-1/1	ND	-2/2	-1/1	ND	-3/3	ND	-1/1	-1/1
±2/3	+1/1	+1/1	ND	-2/2	+2/2	±2/2	+3/3	-2/2	-2/3	-3/3
-2/2	-1/1	-1/1	ND	-1/1	-1/1	-2/2	-3/3	-1/1	ND	-2/2
-2/2	-1/1	-1/1	ND	-1/1	-2/2	-1/1	-3/3	-1/1	-2/2	-2/2
+2/2	+1/1	+1/1	ND	+2/2	+2/2	+2/2	+3/3	+1/1	+3/3	+3/3
-2/2	-1/1	-1/1	-1/1	-2/2	-2/2	-2/2	-3/3	-2/2	-3/3	-3/3
-2/2	-1/1	-1/1	-1/1	-2/2	-2/2	-2/2	-3/3	-2/2	-3/3	-3/3
+4/4	+1/1	+1/1	+1/1	+2/2	+2/2	+2/2	+3/3	+2/2	+3/3	+3/3
+4/4	+1/1	+1/1	+1/1	+2/2	+2/2	+2/2	+3/3	+2/2	+3/3	+3/3
-3/3	-1/1	-1/1	-1/1	-2/2	-2/2	-2/2	-3/3	-2/2	-2/3	-3/3
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	ND	ND	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	ND	ND	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	ND	ND	ND
-2/2	-1/1	-1/1	ND	-2/2	-2/2	-2/2	-3/3	-2/2	-3/3	-3/3
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	ND	ND	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	ND	ND	ND
+2/2	+1/1	+1/1	ND	+2/2	+2/2	+2/2	+3/3	+1/1	+3/3	+2/3
-2/2	-1/1	-1/1	ND	-2/2	-1/1	-1/1	-3/3	-1/1	-1/1	-2/2
-2/2	-1/1	-1/1	ND	-2/2	-1/1	-1/1	-3/3	-1/1	-1/1	-2/2
±2/2	±1/1	-1/1	-1/1	-2/2	-1/1	-1/1	-1/1	-1/1	-1/1	+1/1
+2/2	+1/1	+1/1	ND	+1/1	+1/1	+1/1	ND	+1/1	ND	ND

Table A4 (Continued)

Differential Test	B78	B140	B153	B163	B169	B171	B173	B179	B204
Lactate	-3/3	-3/3	-1/1	-1/1	-2/2	-2/2	ND	-1/1	-1/1
Pyruvate	+1/1	+1/1	+1/1	+1/1	+1/1	-2/2	ND	-1/1	+1/1
Threonine	ND	ND	±1/1	±1/1	±1/1	ND	ND	±1/1	±1/1
Pectin	-1/1	ND	-1/1	-1/1	ND	ND	ND	ND	-1/1
3.0 NaCl	-1/1	-2/2	-1/1	-1/1	-1/1	ND	ND	ND	-1/1
6.5 NaCl	-1/1	ND	-1/1	-1/1	ND	ND	ND	ND	-1/1
Tartaric	-1/1	ND	-1/1	-1/1	ND	ND	ND	ND	-1/1
Dextrin	-1/1	ND	-1/1	-1/1	ND	ND	ND	ND	-1/1
Mucin pH	-1/1	ND	-1/1	-1/1	ND	ND	ND	ND	-1/1
Mucin color	-1/1	ND	-1/1	-1/1	ND	ND	ND	ND	-1/1
Oxidase	-1/1	-2/2	-1/1	-1/1	-2/2	-2/2	ND	-2/2	-1/1
25° C	-1/1	ND	-1/1	-1/1	-1/1	ND	ND	ND	-2/2
30° C	-1/1	ND	-1/1	-1/1	-1/1	ND	ND	ND	-2/2
37° C	+3/3	+4/4	+1/1	+1/1	+2/2	+2/2	+1/1	+2/2	+2/2
42° C	+3/3	+3/3	+1/1	+1/1	+2/2	+2/2	+1/1	+2/2	+2/2
pH 6.0	ND	-1/2	+1/1	+1/1	+1/1	ND	ND	ND	+1/1
pH 9.6	ND	-1/2	-1/1	-1/1	+1/1	ND	ND	ND	±1/1
Urease	-1/1	-1/1	-1/1	-1/1	-1/1	ND	ND	ND	-1/1
Iodoacetate +	+1/1	+1/1	+1/1	+1/1	+1/1	ND	ND	ND	+1/1
Galacturonic	-1/1	ND	ND	ND	ND	ND	ND	ND	-1/1
Gluconic	-1/1	ND	-1/1	+1/1	ND	ND	ND	ND	-1/1
Glucuronic	-1/1	ND	-1/1	ND	ND	ND	ND	ND	-1/1
Products[b]	a,b	a,b,r	a,b	a,b	a,b	a,b	a,b	a,b	a,b

[b]Symbols: a = minor amounts of acetic acid; b = minor amounts of butyric acid; r = alcohol produced from lactate.

Dysentery Isolates								Nondysentery Isolates		
B234	B254	B259	N-2	N-1	A-1	300/8	1037	B256	Puppy	4/71
-2/2	-1/1	ND	-1/1	-1/1	-2/2	-1/1	-2/2	-1/1	-2/3	-3/3
±1/2	+1/1	±1/1	±1/1	+2/2	+1/1	+1/1	+2/2	+1/1	-1/3	+3/3
-2/2	ND	ND	-1/1	-1/1	+1/1	ND	ND	+1/1	ND	-1/1
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	ND	ND	ND
-2/2	-1/1	-1/1	ND	-1/1	-1/1	ND	-3/3	-1/1	ND	-2/2
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	ND	ND	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	ND	ND	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	ND	ND	ND
-2/2	ND	-1/1	ND	ND	-1/1	ND	ND	ND	ND	ND
-2/2	ND	-1/1	ND	ND	-1/1	ND	ND	-2/2	ND	ND
-4/4	-1/1	-1/1	-1/1	-2/2	-2/2	-2/2	-3/3	-2/2	-3/3	-3/3
-2/2	-1/1	-1/1	ND	ND	±1/1	ND	ND	-1/1	ND	ND
-2/2	-1/1	-1/1	ND	ND	ND	ND	ND	-1/1	-1/1	-1/1
+2/2	+1/1	+1/1	+1/1	+2/2	+2/2	+2/2	+3/3	+2/2	+3/3	+3/3
±2/2	-1/1	-1/1	±1/1	±2/2	±2/2	±2/2	±3/3	±2/2	±3/3	±3/3
+2/2	+1/1	-1/1	ND	ND	+1/1	ND	±1/1	+1/1	ND	+1/1
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	-1/1	-1/1	ND	+1/1
-2/2	-1/1	ND	ND	ND	-1/1	ND	-1/1	-1/1	-1/1	-2/2
+2/2	+1/1	+1/1	ND	ND	-1/1	ND	+1/1	+1/1	+1/1	+2/2
-2/2	ND	ND	ND	ND	-1/1	ND	ND	ND	ND	ND
-2/2	-1/1	-1/1	ND	ND	-1/1	ND	ND	ND	ND	ND
ND	-1/1	ND	ND	ND	-1/1	ND	ND	ND	ND	ND
a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b	a,b,r	a,b,r

Table A5. Characteristics of isolates of Treponema hyodysenteriae-like organisms in differential media inoculated under N_2[a]

Differential Test	B78	B204	Differential Test	B78	B204
Amygdalin	-	-	Xylose	-	-
Arabinose	-	-	Gelatin	-	-
Cellobiose	-	ND	Meat	-	-
Erythritol	-	ND	Indol	+	+
Esculin pH	-	-	Nitrate	-	-
Esculin hyd	ND	ND	Catalase	-	-
Fructose	+	++	Bile	+	+
Glucose	+	++	Lecithinase	-	-
Glycogen	-	-	Lipase	-	-
Inositol	-	-	Hemolysis	+	+
Lactose	+	+	Motility	+	+
Maltose	++	++	H_2S	-	-
Mannitol	-	-	Adonitol	-	-
Mannose	-	-	Dulcitol	-	-
Melezitose	-	-	Galactose	-	+
Melibiose	-	-	Glycogen	ND	-
Raffinose	-	-	Glycine	-	-
Rhamnose	-	-	Inulin	-	-
Ribose	-	+	Sorbose	-	+
Salicin	ND	+	Gas	+	+
Sorbitol	-	-	NH_3	-	-
Starch	-	-	AMC	-	-
Sucrose	-	-	Hippurate	±	-
Trehalose	-	++	NR	+	+

[a]Symbols: + = possesses characteristic (pH > 0.25 units lower than control); ++ = pH > 0.5 units lower than control; - = does not possess characteristics; ND = not done; ± = weak +.

Table A5 (Continued)

Differential Test	B78	B204
Lactate	-	-
Pyruvate	+	+
Threonine	+	±
Pectin	-	+
3.0 NaCl	-	±
6.5 NaCl	-	-
Tartaric	-	-
Dextrin	-	-
Mucin	-	+
Mucin color	-	-
Oxidase	-	-
25° C	±	-
30° C	+	0
37° C	+	+
42° C	+	+
ph 6.0	+	+
pH 9.6	±	-
Urease	-	-
Iodoacetate	+	+
Galacturonic	-	ND
Gluconic	-	-
Glucuronic	-	ND
Products[b]	a,b	a,b

[b] Symbols: a = minor amounts of acetic; b = minor amounts of butyric.