STUDIES OF THE SURVIVAL OF *TREPONEMA HYODYSENTERIAE* AND THE EPIDEMIOLOGY OF SWINE DYSENTERY

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SUMMARY

The effects of temperature, dilution with tapwater, drying and exposure to disinfectants on T. hyodysenteriae in dysenteric pig faeces were studied. The organism was isolated on spectinomycin blood agar and its identity confirmed by using a specific fluorescent antibody test.

It was found to survive for a maximum of forty-eight days in faeces held at 0° C, forty-two days at 5° C, and thirty-eight days at 10° C, but disappeared more rapidly from faeces stored at temperatures higher than 10° C. Dilution 1:10 with tapwater appeared to enhance the survival time of the organism to a maximum of sixty-one days at 5° C. The organism could not be isolated from faeces after drying or after treatment with disinfectants at the levels recommended for farm use.

The survival of T. hyodysenteriae in common farm pests was studied by infecting laboratory mice, rats and flies with pure cultures of the organism. It could be reisolated from the faeces and large intestinal contents of infected mice for up to fourteen days after infection but was recovered only from the large intestinal contents of rats for two days and from the integument of flies for one hour after inoculation.

The faeces of pigs experimentally infected with swine dysentery were examined for T. hyodysenteriae by the culture of rectal swabs during the disease and after clinical recovery. T. hyodysenteriae was readily isolated for a period of up to sixty-two days after clinical recovery.

In three of the four recovered animals examined, faecal shedding of the organism was not demonstrated for periods of thirteen to fifty-five days before slaughter. These animals were found to have no lesions identifiable as those of swine dysentery at post-mortem examination but T. hyodysenteriae was isolated from their colonic mucosa in each case.

The findings described above and their relevance to the epidemiology of swine dysentery was discussed.

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Chapter One

Introduction and Review of the Literature

Swine dysentery is a disease of considerable economic importance in the major pig rearing areas of the world. The aetiology of the condition has only recently been described (Taylor and Alexander,1971 and Harris *et al*, 1972a)and the pathogenesis of the disease has not yet been determined in full. It is clear that the disease is transmitted from pig to pig in infective faeces containing the aetiological agent *Treponema hyodysenteriae*, but information about the survival of the agent in faeces is scarce. In addition, there is little information about the possible role of farm pests in the transmission and maintenance of the disease outside the pig.

The survival of the agent in the carrier pig has not yet been studied extensively.

This study was designed to examine the survival of *Treponema hyodysenteriae* in pig faeces under various conditions, in common farm pests such as mice, rats and flies and in the carrier pig.

History and Distribution

Swine dysentery was first recorded in 1921 by Whiting *et al* in Indiana, U.S.A. where heavy losses were occurring in fattening pigs. These authors described in detail the clinical signs and post-mortem findings of the disease and most subsequent descriptions and reports are based on their initial definition of the disease. Their

original observations were made in 1917 and 1918 when the traffic in feeder pigs was unusually haevy and were reported later after experimental studies.

The disease has since been recorded in most pig-rearing areas of the world, the remainder of the U.S.A., Canada, Central and South America, the Carribean, most countries in Europe, Africa, Australia and New Zealand, and Asia in which it has been reported from Korea, Laos, Japan, Taiwan and Malaysia (Roncalli and Leaning, 1976). In many of these reports the disease was described as bloody diarrhoea, bloody flux, black scour, haemorrhagic enteritis, necrotic enteritis or vibrionic dysentery. The name "vibrionic dysentery" resulted from the work of Doyle(1944) who claimed to have reproduced the disease using pure cultures of a vibrio. Under this name the disease was first recorded in Britain by Birrell in 1957. Since that time it has become widespread and a major problem in the British pig industry.

Clinical signs

Swine dysentery is a severe mucohaemorrhagic diarrhoeal disease of pigs. The clinical signs and post-mortem findings have been described by a number of authors including Whiting et al.(1921), Doyle(1944), Lussier(1962), Alexander and Taylor (1969), and Harris and Glock (1975).

The disease commonly affects weaned pigs between the age of 10-16 weeks but may also affect adult animals and more rarely, unweaned piglets. The onset of the disease is gradual and the early clinical signs are often overlooked. A transient rise in rectal temperature to 104-

105°F (40-40.5°C), twitching of the tail, signs of abdominal discomfort such as kicking at the belly, reluctance to move and loss of appetite may be noted before the onset of the diarrhoea. The onset of diarrhoea is preceded by the passage of loose or soft faeces (Olson,1974). Diarrhoea then persists throughout the course of the disease. At first it is grey, brownish or blackish in colour and gradually becomes more fluid and contains increasing proportions of mucus. In some cases this mucoid diarrhoea is passed for the whole course of the disease. In more severe cases fresh blood may be seen in the diarrhoea and this passage of dysenteric faeces is characteristic of the disease. In addition scraps of necrotic epithelium may be seen as pale flecks in the dysenteric material. The amount and duration of the passage of mucus and blood in the diarrhoea may vary within a group of affected pigs.

The onset of diarrhoea is accompanied by a marked loss of bodily condition. There is an initial hollowing of the flanks followed by increasing signs of dehydration such as sunken eyes, prominent backbones and ribs and a staring coat. Chronically affected animals remain stunted and hairy. The perineal region of affected animals may be stained with dysenteric faeces. Severely affected animals may be moribund with pallor of the skin and faint cyanosis of the extremities.

Death may result from dehydration, acidosis or hyperkalaemia or a combination of these factors resulting from the diarrhoea although sudden deaths may occur in

acutely ill animals. The cause of these peracute deaths is not known (Harris & Glock, 1975).

Affected pigs may recover completely. Recovery is accompanied by the disappearance of blood from the faeces and often by the passage of large amounts of mucus. The faeces may remain loose for a number of days, finally becoming firm and normal in appearance.

Chronically-affected animals may remain stunted and unthrifty. They may pass faeces containing varying amounts of mucus but in many cases mucus may not be obvious in the diarrhoeic motion. These animals may have a poor feed-conversion rate compared with non-affected animals (Taylor, 1976c).

Atypical cases of swine dysentery

The clinical signs of the disease may vary in their severity and duration in herds in which immunity has built up. Terpstra et al (1968) reported a reduction in severity and clinical signs of the disease in herds in which it had been present for sometime. A similar type of reduction in severity has been reported in experimental infections by Olson (1974) who re-infected recovered pigs and found that blood did not appear in the faeces in the second and subsequent infections.

Glock et al(1976), Schwartz and Glock(1976) also noted a reduction in the severity of clinical signs of the disease in animals which had been vaccinated parenterally with T. hyodysenteriae or passively protected by the parenteral injection of hyperimmune antiserum to T. hyodysenteriae.

Drug treatment or the use of feed medicated drugs active against T. hyodysenteriae may also affect the severity of the clinical signs of swine dysentery and this process was described by Olson and Rodabaugh(1976ab) who noted low levels of ronidazole reduced the severity of experimental swine dysentery. The incubation period of the disease in treated pigs was not reduced but haemorrhagic diarrhoea was less common. Where clinical signs were completely suppressed, they occurred within a few days of the removal of medication. Sometimes this recurrence of disease was severe and they named it "drug-delayed-augmented swine dysentery" (Olson and Rodabaugh,1976a).

Post-mortem findings

Animals which have died suddenly in the acute stage of the disease are usually in good bodily condition, but those which die after several days' illness or in the chronic stage of the disease are usually in poor bodily condition or appear dehydrated. The carcases of these chronically-affected animals have dirty skins and appear hairy, the eyes may be sunken and the abdomen drawn in. There may be faecal staining or excoriation of the perineal region. Most carcases appear pale with slight bluish congestion of the extremities.

The characteristic lesions of swine dysentery are restricted to the large bowel, the caecum, colon and to a lesser extent, the rectum (Whiting et al.1921). In pigs which have died from the disease there may also be hyperaemia of the fundus of the stomach (Whiting 1928) but this was considered by Warner(1965) to be a non-

specific change. Lussier (1962) also reported that the liver was swollen and friable in some cases.

Gross lesions

The large intestine is usually flaccid and the serosal surface may be inflamed and oedematous. There may be oedema of the folds of the mesentery of the spiral colon and the mesocolic lymph nodes may be pale and swollen in animals which have died in the early stage of the disease. Lussier (1962) found that the serosal surface of the large intestine was dry and appeared granular in animals which had died in the chronic stage of the disease.

The colonic contents are fluid and foul smelling, containing varying proportions of mucus, semi-digested feed, white flecks of necrotic material and sometimes fresh whole blood.

The mucosa may be swollen and congested and in the early stages of the disease the lesion is covered with clear mucus. In the later stages, the affected mucosa may be covered with a mixture of mucus and blood. Later still, a whitish diphtheritic membrane covers the mucosa and when removed may leave bleeding points. If animals are chronically affected at the time of death the mucosa may be covered with large areas of thick mucus.

Microscopic lesions

The histological changes seen in the mucosa of pigs with swine dysentery have been described by a number of authors including Whiting et al (1921), Lussier (1962), Taylor and Blakemore (1971), Taylor (1972), Glock and Harris (1972), and Harris and Glock (1975).

The earliest changes seen in the mucosa of the large intestine are oedema of the lamina propria, dilatation of the blood vessels and discharge of the goblet cells. This stage is quickly followed by loss of the mucosal epithelium covering the lumenal surface of the colon. Where cell loss is not complete the epithelium is cuboidal and abnormal in appearance. Blood, fibrin and desquamated cells form a diphtheritic membrane over the eroded areas. The crypts of the mucosa are dilated and the goblet cells are distended. In sections of tissue stained by silver-impregnation methods such as Levaditi's method, silver-stained spiral microorganisms and other bacteria may be seen. These bacteria are somethimes sufficiently thin and morphologically distinctive to be identified as spirochaetes. They may be present in large numbers in the crypts of the affected mucosa and have been demonstrated within the epithelial cells, particularly the goblet cells, lining the crypts. In some cases they may be seen at the eroded surface of the lamina propria accompanied by other large silver-staining organisms.

In the acute lesions and in the recovering mucosa massive numbers of leucocytes may be seen within the lamina propria. Necrosis of the superficial layers of the lamina propria may also occur.

Ultrastructural changes

Scanning electron microscopy of the surface of the affected colonic mucosa was carried out by Kennedy et al. (1973) who described spirochaetes in large numbers on the surface of the affected mucosa especially at the mouth of

the crypts. Other bacteria were seen in close association with these spirochaetes.

A number of studies have been carried out by transmission electron microscopy, especially those by Taylor and Blakemore(1971), Glock and Harris(1972), Glock et al (1974). The most striking ultrastructural feature of the lesions was the presence of large spirochaetes with many fibrils in the axial filament within cells lining the crypts of the colonic mucosa. Large numbers of similar spirochaetes were often present in the lumens of infected crypts. The spirochaete infected cells and others adjacent to them often appeared damaged, with swollen mitochondria, dilated endoplasmic reticulum and reduction in size or loss of the microvilli. Spirochaetes were rarely identified in the lamina propria. Other bacteria which could not be identified by their ultrastructure were often seen in or near the spirochaetes in these lesions.

Aetiology

Whiting et al (1921) defined the disease and showed by means of transmission experiments that the syndrome that they had described was transmissible. Their work showed that the infectious agent responsible for the disease was present in the faeces of affected pigs. Whiting(1924) found that the infectious agent was restricted to the gastrointestinal tract and that the disease was reproduced more consistently by feeding colonic and caecal material than by feeding stomachs from affected pigs.

They showed clearly that swine fever and salmonellosis were distinct from this condition and that the disease could not be reproduced by feeding bacteria-free filtrates. None of the bacterial cultures used to inoculate experimental pigs were found to reproduce the disease. They included E. coli, Fusobacterium necrophorus, Salmonella choleraesuis, Paracolon bacilli, Pseudomonas aeruginosa, an unidentifiable anaerobe and spirochaetes which had been noted in large numbers in the lesions and dysenteric faeces.

Although speculation as to the identity of the causal agent of the disease continued, no firm experimental evidence of its identity was provided until Doyle in 1944 claimed to have reproduced the disease by feeding pure cultures of a comma-shaped organism, Campylobacter(vibrio) coli to experimental pigs. Other workers found difficulty in reproducing his results. Some (James and Doyle 1947, and Roberts 1956) managed to reproduce the disease while others (Deas 1960, Davis 1961, Lussier 1962, Warner 1965, Andress et al 1968, and Andress & Barnum 1968, Terpstra et al 1968) failed to produce typical swine dysentery repeatably by feeding pigs with pure cultures of vibrios resembling those described by Doyle.

Filtrates of colonic material were found by Warner (1965) to produce dysentery when fed to experimental pigs when they had passed through filters with a pore size of 0.8μ but not when they had passed filters with a pore size of 0.22μ. His 0.45μ filtrates could be incubated in embryonated hens' eggs and these in turn would cause dysentery when fed to pigs. Cultures of the vibrionic

organism from this material failed to reproduce the disease. Further studies of this type of filtrate was carried out by Terpstra et al in 1968, who also studied other properties of the agent. It was found that the infectious agent was resistant to penicillin, sensitive to streptomycin, neomycin and tylosin and survived heating for 30 minutes at 50° C but not 60° C. Centrifugation at 10,000revs was found to precipitate the organism after 30 minutes but not centrifugation at 3,000 revs for 15 minutes. The most important part of their study was to focus attention on spiral micro-organisms by means of an indirect fluorescence test using serum from recovered animals on smears prepared from dysenteric faeces.

This work, in conjunction with that of Warner, focussed attention on the spirochaetes present in the large intestines and their contents in dysenteric pigs. Two teams of workers, Alexander and Taylor in Cambridge and Harris, Glock, Kinyon and others in Iowa carried out detailed studies of the disease and showed that a large spirochaete (Harris et al,1972a) or Type I spirochaete (Taylor & Alexander 1971) was consistently present in the faeces and lesions of pigs experimentally infected with swine dysentery. Both groups then demonstrated by electron microscopy that this large spirochaete was present in the colonic lesions of swine dysentery (Taylor & Blakemore 1971, Blakemore & Taylor 1970, Harris & Glock 1971, Glock & Harris 1972) and carried out isolation experiments. Both devised a filtration method for the isolation of the organism and cultured

it on blood agar under anaerobic conditions in the presence of carbon dioxide. Both groups reported the reproduction of the disease by feeding pure cultures of the spirochaete to experimental pigs (Taylor & Alexander 1971 and Harris et al.1972a) and Harris and his co-workers named the spirochaete Treponema hyodysenteriae. Since these original reports, a number of other workers (Akkermans & Pomper 1973, Hamdy & Glenn 1974, Hudson et al. 1974, Meyer et al. 1975) have confirmed that pure cultures of this organism are capable of initiating swine dysentery when fed to susceptible pigs.

The Characteristics and Identifications of Treponema hyodysenteriae

Treponema hyodysenteriae is a large spirochaete 6-10μ in length and 0.32-0.45μ in diameter. The number of axial fibrils present was found to be 7-9 (Harris et al. 1972a), 9-12 fibrils inserted in a V-shape (Taylor 1972). Live organisms are flexible, arranged in 2-4 open curves and with a snake-like motility.

The organism is an oxygen-tolerant anaerobe and grows well on enriched solid media such as tryptose soy agar or Blood Agar Base supplemented with 10 per cent horse or bovine blood. Colonies appear as hazy areas of beta-haemolysis after 48 hours incubation at 37°C in an atmosphere of 95 per cent hydrogen and 5 per cent carbon dioxide. The colonies may become larger after 72 hours when surface growth becomes obvious as a low, flattened, clear or whitish colony with an irregular edge 2-4 mm in diameter.

Kinyon and Harris(1974) described a liquid medium containing trypticase soy broth supplemented with 10 per cent foetal calf serum in which the organism could be grown. This enabled Kinyon(1974) to study the biochemical characters of T. hyodysenteriae already briefly listed by Harris et al.(1972a). T. hyodysenteriae produces acetic acid, butyric acid, hydrogen and carbon dioxide from glucose. The full biochemical characters were investigated by Kinyon(1974). She considered as a result of her studies that haemolysis patterns, indol production and fructose fermentation might be of value in separating pathogenic isolates from non-pathogenic isolates of this type of spirochaete.

T. hyodysenteriae is not the only spirochaete found in the porcine intestine. A number of other spirochaetes have been seen in the faeces and colon contents of both normal and diseased pigs(Taylor 1970&1972). Some of these spirochaetes connot easily be confused with T. hyodysenteriae because of their size, shape, motility or ultrastructure. Many are smaller, for example the 2:4:2 spirochaete identified in and isolated from a dysenteric pig by Harris et al.(1972c). Some, however, resemble T. hyodysenteriae in their morphology and ultrastructure. When cultivated on horse or bovine blood agar, these other spirochaetes produce less obvious haemolysis, and in many cases, more profuse surface growth. They appear not to be pathogenic, and when pure cultures of these organisms were fed to experimental pigs by Taylor(1972), Kinyon(1974) and Hudson et al.(1976), no disease resulted. Swine dysentery

was not produced. In general, workers in the field (Taylor 1971, Kinyon et al,1976) consider that the haemolysis pattern produced by T. hyodysenteriae(complete beta haemolysis with little surface growth appearing after 48 hours' incubation) is a good guide to the pathogenicity of the isolate. Non-pathogenic isolates generally produced weaker beta haemolysis and more surface growth.

Studies of the antigenic relationships of T. hyodysenteriae and other spirochaetes from the porcine intestinal tract have been carried out by Hunter & Clark in 1975 using a direct fluorescent antibody test for T. hyodysenteriae in the diagnosis of swine dysentery. This serological test was in routine use in diagnostic centres in Britain, but was shown to be non-specific by Hudson et al (1976) who submitted spirochaetal isolates obtained from outbreaks of post-weaning scour to the test and found that both T. hyodysenteriae and "non-pathogenic" spirochaetes flouresced. They therefore suggested that the reagent should be absorbed with the post-weaning scour isolates and this absorbed reagent is now used routinely for the diagnosis of swine dysentery in United Kingdom (Hunter & Saunders, in press) and in the confirmation of the identity of cultures of spirochaetes suspected on cultural grounds of being T. hyodysenteriae.

The role of T. hyodysenteriae and other bacteria in the aetiology of swine dysentery

The work of Taylor and Alexander(1971) and Harris et al(1972a) demonstrated that pure cultures of T. hyodysenteriae would produce swine dysentery when fed

to conventional susceptible pigs. Taylor(1972) found, however, that the lesions produced by feeding pure cultures of the organism to hysterectomy-derived, colostrum-deprived pigs were less severe than those seen in conventional pigs of similar age and genetic origin. This evidence of a requirement for the normal flora of the pig intestinal tract in the development of characteristic lesions was underlined by the work of Harris et al(1972b) who showed that T. hyodysenteriae could persist in the colon of the gnotobiotic pig but failed to produce lesions or to invade the colonic mucosa. A number of studies have been carried out since that time in an attempt to identify the other organisms concerned. Brandenburg(1974) and Meyer et al (1974a) failed to produce lesions in gnotobiotic pigs infected with T. hyodysenteriae or C. coli alone or in combination. The results of these two studies showed that C. coli alone was not capable of aiding T. hyodysenteriae in the development of the colonic lesions of swine dysentery. Subsequent work by Meyer and his co-workers(1975) showed that four enteric gram-negative obligate anaerobic bacteria could combine with T. hyodysenteriae to produce the lesions of swine dysentery in gnotobiotic pigs. The obligate anaerobes concerned were tentatively identified as Bacteroides melaninogenicus, another Bacteroides, and two fusiform bacteria. Isolation studies carried out on the large intestinal mucosa of affected and normal hysterectomy-derived pigs by Alexander et al(1976) showed that Fusobacterium sericola, Spirillum sulphurinigrans, Fusobacterium necrophorum and Bacteroides fragilis

vulgatus accompanied T. hyodysenteriae in the early lesions of swine dysentery. They speculated that these organisms would support the development of lesions in the colonic mucosa when fed to gnotobiotic pigs in combination with T. hyodysenteriae, although F.sericola and S. sulphurinigrans alone could not enable T. hyodysenteriae to produce typical lesions of swine dysentery.

It seems probable that T. hyodysenteriae produces the colonic lesions by cell invasion but require the presence of some or all of the gram-negative obligate anaerobes mentioned above to do this. There is little evidence for the secretion of any toxin by T. hyodysenteriae although Wilcock and Olander(1976) suggested that a vaso-active toxin might be present.

Diagnosis

A diagnosis of swine dysentery can be based on the clinical signs and post-mortem findings alone in most cases of the typical disease. Laboratory methods which are of value in confirming a diagnosis in complicated cases include histological examination of the colonic mucosa which is inflamed and in the crypts of which spirochaetes may be seen in silver-stained sections, the use of the absorbed fluorescent antiserum described above and cultural examination. Smears made from dysenteric faeces, colonic contents or mucosa are air dried, acetone-fixed and examined by the F.A.T. Wet smears may be prepared from the same material and viewed by phase contrast microscopy. The presence of numerous

motile snake-like spirochaetes is suggestive of swine dysentery, but because of the existence of non-pathogenic spirochaetes, is not confirmatory.

T. hyodysenteriae may be isolated from dysenteric faeces, colonic contents or mucosa by the filtration methods described by Taylor & Alexander(1971) or Harris et al (1972c). A less cumbersome method is the use of selective medium described by Songer et al(1976) in which 400 µg/ml of spectinomycin is incorporated in blood agar and inoculated plates are incubated at 42° C in anaerobic conditions in the presence of carbon dioxide (5%) and hydrogen (95%). T. hyodysenteriae may be distinguished from other spirochaetes by its colonial appearance and by the fluorescence tests outlined above.

Epidemiology

A number of authors, beginning with Whiting et al (1921) have described the epidemiology of swine dysentery in the field, but few studies of the epidemiology have been carried out since the discovery of the aetiological agent in 1972. The disease is infectious with an incubation period of 7-60 days (Whiting et al,1921). Most cases develop within 10-14 days of exposure to a dysenteric pig. In experimental studies, the incubation period after inoculation with cultures of T.hyodysenteriae or crude dysenteric material varies from two days to three months (Harris and Glock,1975) but in most cases the incubation period is 5-24 days with an average of 11 days (Olson,1974) or 4-12 days(Whiting et al,1921).

Clinical signs of swine dysentery may disappear

within 6-7 days in atypical cases but in the typical disease they may persist for 2-4 weeks in the absence of treatment. The course of the disease was studied by Olson(1974) in experimental pigs and found to average 6.8 days for pigs of eight weeks of age (range 3-19 days) and for 12-week-old pigs, four days, (range 2-5 days). This tendency for the course and severity of the clinical signs to be less pronounced in older pigs has been known since the original observations of Whiting et al(1921), but had not been examined previously in such detail.

The morbidity rate in groups of non-immune pigs may be as high as 90 per cent(Harris and Glock, 1975) but in herds where feed is medicated with a growth promoter or other therapeutic agent active against the disease the morbidity may be much less than this. Similar findings obtain in herds in which the disease has been present for sometime.

Death from swine dysentery is uncommon where effective treatment is given, but in cases in which ineffective treatment or no treatment is given, the mortality rate may be as high as 25 per cent. This mortality rate varies with the age of the pigs affected. Whiting et al (1921) noted that 40-60 per cent of affected weaner pigs died from the disease but only 10-20 per cent of affected fatteners died. Only 5-10 per cent of affected sows died (Whiting,1928).

In addition to the reduction in severity and duration of the disease associated with increasing age, immunity to the disease can also occur. Whiting(1924) stated that 13 per cent of pigs were resistant to the disease, but

Alexander and Taylor(1969) considered this phenomenon to be partially related to the dose of infectious material ingested.

Lussier(1962) suggested that active immunity to the disease did not protect against reinfection. Terpstra et al(1968) made similar observations but noted the progressive reduction in the severity of the disease in an infected herd with time. They did, however, demonstrate that circulating antibody to a spiral microorganism was present in recovered pigs. This spiral microorganism is now considered to have been T. hyodysenteriae and specific antibody to it has been demonstrated in the serum of recovered pigs by a number of workers. The tests used have included a serum agglutination test (Hunter and Saunders,1973) which was found to be effective in the diagnosis of swine dysentery on a herd basis. A similar test was described by Joens et al(1976a)who developed a microtitre agglutination test for serum antibody.

A different approach was used by Lee and Olson(1976b) who used an indirect fluorescent antibody test to detect serum antibody to T. hyodysenteriae in pigs which had recovered from the disease. A much more sensitive test was described by Jenkins et al(1976) who used a passive haemagglutination test with added complement to detect serum antibody to T. hyodysenteriae. As none of these workers used controls incorporating spirochaetes other than T. hyodysenteriae, the specificity for T.hyodysenteriae of the antibody detected is open to question.

Jenkins et al(1976) demonstrated a rise in serum antibody to spirochaetes (using T. hyodysenteriae as

antigen) after infection with pure cultures of T. hyodysenteriae. Antibody could be detected one week after infection (titre 1:16) and rose to a titre of 1:512 by the sixth week after infection. Antibody could still be detected four months after infection at a high level (titre 1:128). Similar findings were obtained by Lee & Olson(1977), using their less sensitive indirect fluorescent antibody test.

The role of immunity to T. hyodysenteriae in protection of recovered pigs against re-infection has not yet been fully elucidated. Glock et al(1976) described a study in which active parenteral immunisation of pigs with formalinised T. hyodysenteriae reduced the severity of the disease and delayed its onset after challenge when compared with controls. Protection against the development of the disease was not obtained. Similar results were obtained by Schwartz & Glock(1976) using pigs passively immunised against T. hyodysenteriae. Attempts by Hudson et al(1974) to immunise pigs by feeding cultures of an attenuated strain of T. hyodysenteriae failed to protect against challenge by virulent culture of the same isolate. These studies emphasize the limited value of serum antibody in the protection of pigs from swine dysentery and cast doubts on the value of local immunity in protection against the disease. Some protection against reinfection does eventually develop after recovery from experimental and natural infections. Olson(1974) showed that clinical signs attributable to swine dysentery failed to appear on the third consecutive attempt to re-infect pigs which had recovered from swine dysentery. It seems,

therefore, that protection against challenge resulting from recovery from swine dysentery is incomplete. It may be that protection may be complete against the low levels of infection experienced in the field in contrast to the artificially high levels used for challenge in the studies described above.

T. hyodysenteriae appears to be restricted to pigs. Although a syndrome resembling swine dysentery was produced by Joens et al(1976b)in the guinea pig by feeding pure cultures of T. hyodysenteriae there are no other reports in the literature of infection with T. hyodysenteriae in species other than the pig. It follows, therefore, that the infected pig is the major or only source of infection for susceptible animals. T. hyodysenteriae is present in large numbers (10^5-10^9 organisms/g) in the colonic mucosa (Kinyon 1974) and large numbers of organisms are present in the faeces of clinically affected pigs. The minimum number of organisms required to produce disease in a susceptible animal is not known.

Whiting et al(1921) transmitted the disease experimentally by feeding dysenteric faeces to experimental pigs. The faeces of infected pigs may reach susceptible animals directly or may contaminate food or drinking water, and may be carried from pen to pen in drainage channels (Taylor,1972) or on the footwear or implements used by attendants (Terpstra et al,1968). Infection disappears from pens within seven days if they are left unoccupied by pigs (Terpstra et al 1968). This finding suggests that T. hyodysenteriae cannot survive for long outside

the body although it has been shown that T. hyodysenteriae can survive for a mininum of 6-9 days at 4° C (Taylor, personal communication). At higher temperatures (22° C and 37° C) survival was two days and less than one day respectively.

In general, little other information exists as to the survival of the agent under various conditions in faeces and slurry. Glock et al.(1975) showed that the organism could be present in slurry and demonstrated infectivity by feeding contaminated lagoon effluent to pigs, but did not study the survival time of this infectivity. Harris and Glock(1975) recommended that premises should be left empty for sixty days in attempts to eradicate the disease from premises by depopulation and restocking with dysentery-free pigs.

Survival of the infectious agent in the body of carrier pigs has been suspected for many years. Whiting et al.(1921) mentioned the persistence of infection in the sows of breeding herds and its transmission to successive litters of piglets. A number of other accounts suggest that the carrier pig may be clinically normal. The long incubation period found in some experimental studies (Harris and Glock 1975, Whiting 1921) suggested that infected pigs which could be excreting the organism appear clinically normal. Recovered pigs have been shown to remain infectious up to 28 days after clinical recovery (Terpstra et al.1968) and the clinically normal recovered carrier animal is probably the most important reservoir of infection.

Epidemiological studies of the carrier pig have

rarely been accompanied by the isolation of T. hyodysenteriae, but Songer et al.(1976) showed that the organism could be isolated from a farrowing sow and from piglets on farms on which the disease was enzootic. These animals did not have clinical signs of the disease but no information as to the disease history of the infected sow or the subsequent disease status of the infected piglets was given. The development of a selective medium for the isolation of T. hyodysenteriae enabled this study to be carried out.

Treatment and Control

The control and treatment of swine dysentery has been reviewed by a number of authors, perhaps most comprehensively by Harris and Glock(1975). A number of studies of the in vitro activity of drugs against T. hyodysenteriae have been carried out by Taylor(1972) and Messersmith et al.(1973), Taylor(1976ab). All the agents used in the field for the treatment of swine dysentery have been shown to be active in vitro against T. hyodysenteriae. In addition, these agents may be active against other organisms present in the pig intestine and this may affect the course of the disease. This activity may account for some of the findings by Olson and Rodabaugh(1976b) and Taylor(1976c) who noted that clinical signs of the disease were less severe or delayed in onset after prophylactic medication had been given at low levels. The organism itself may be resistant in vitro to the drug used (Taylor and Alexander, 1971) and resistance of the disease to drug treatment may also occur

in the field (Harris and Glock,1975).

Control programmes involve the use of drug treatment on a whole-house or whole herd basis, usually by medication of the drinking water, but sometimes by medication of the feed. It appears to be necessary to clean out and disinfect individual pens or premises after such a course of treatment. Courses of treatment may be followed by low level medication of the feed and prophylaxis may be practised by medicating the feed of susceptible age groups of pigs.

In many control programmes eradication is not possible using medication alone. Control within a unit involves medication, depopulation and hygiene including the use of disinfectants. Depopulation of a whole farm, resting for a period of at least 60 days (Harris & Glock 1975), restocking with pigs from dysentery-free herds and subsequent isolation of the herd is generally successful. Particular attention must be paid to the prevention of the purchase of possible carriers and to the disinfection of vehicles, boots and implements coming on to the farm.

At present no reliable and tested method exists of proving that a given pig is free from infection with T. hyodysenteriae. The serological studies described by Hunter and Saunders(1973) were only applicable on a herd basis. The studies of Lee and Olson(1976a), although using a less sensitive technique than that developed by Jenkins et al(1976), showed that treated pigs did not develop antibody to T. hyodysenteriae after successful drug treatment, thus making it unlikely that

the serological detection of carrier pigs would be reliable on an individual basis. The fluorescent antibody test could be used to screen the faeces but Akkermans and Pomper(1973) suggested that it might not be of great value except in animals with clinical swine dysentery. The examination of wet smears of pig faeces for the organism by phase contrast microscopy is not sufficiently specific as Taylor(1972) showed that spirochaetes which were not pathogenic could not be distinguished from T. hyodysenteriae by phase contrast microscopy. The use of rectal swabs and selective medium (Songer et al.1976) may prove to be the most effective method of detecting carrier animals. A single negative result, however, may not be conclusive proof of freedom from infection.

It is therefore clear that further study of the survival of the organism with in the infected pig and in the environment and assessment of methods to detect infection are essential if successful control measures are to be adopted. This study of the survival of T. hyodysenteriae was carried out in an attempt to determine the answers to some of the problems outlined above.

Chapter Two

General Materials and Methods

The materials and methods used throughout this study are described in this chapter. Where materials or methods were only used in a particular experiment or were modified for use in a particular case, they are described in the appropriate chapter.

1. The cultivation of *Treponema hyodysenteriae*

Treponema hyodysenteriae was cultivated and maintained on 7 per cent horse blood agar prepared from Oxoid Blood Agar Base No. 2 (Oxoid Ltd. London) and Oxoid defibrinated Horse Blood. Cultures were inoculated onto the surface of blood agar plates by streaking and incubated for a mininum of 48 hours in anaerobic conditions at 42° C. Anaerobic conditions were produced using an all-metal anaerobe jar (Baird & Tatlock Ltd.) fitted with a palladinised asbestos cold catalyst. The jars were evacuated to 150 mm Hg and flooded with a gas mixture containing 95 per cent hydrogen and 5 per cent carbon dioxide (British Oxygen Company Special Gases Division). The procedure was repeated in order to flush out remaining oxygen.

Treponema hyodysenteriae was recognised in culture using the following criteria:

(a) Colonial appearance

Colonies of *T. hyodysenteriae* on blood agar resembled those described by Harris *et al.*(1972c) and Taylor and Alexander(1971). They were 2-4 mm in diameter and consisted of a beta-haemolytic zone

sometimes with a central opaque area 0.5-1.0 mm in diameter. Little surface growth was present in young cultures but in old cultures a clear spreading colony was formed.

(b) Morphology of the organism

Large spirochaetes resembling those described by Harris et al.(1972c), Taylor & Alexander(1971) were seen in wet smears made from these beta-haemolytic areas and viewed by phase contrast microscopy. In young cultures they were often arranged in 2-4 curves and were motile, moving in a snake-like way. Motility was less obvious and morphology less characteristic in older cultures. In these cultures the organisms were arranged in irregular folds or in ring forms. Granular forms were occasionally present.

(c) Antigenicity

Specific antiserum to T. hyodysenteriae strain S73/2 was available in the laboratory. It had been prepared in rabbits by using the method of Meyer and Hunter(1967) and stored at -20°C. This antiserum had been absorbed with non-pathogenic spirochaetes (isolate PWS/A Hudson et al. 1976) to eliminate non-specific reactions.

This antiserum was used to confirm the identity of spirochaetal isolates considered to be T. hyodysenteriae on the basic of criteria (a) and (b) using an indirect fluorescent antibody method.

Smears were made from the surface growth on culture haemolytic areas and air dried. They

were then fixed by immersion in cold acetone(+4° C) for ten minutes after which they were drained and allowed to dry in air at room temperature. Fixed smears were examined immediately after preparation or stored at -20° C until examination could be carried out.

The indirect fluorescent antibody test was carried out in the following way. Smears were placed in a moist chamber and the specific antiserum to T. hyodysenteriae (used at a working dilution of 1:16) was added to cover the area of the fixed smear. Treated smears were incubated at 37° C for ½ hour and then rinsed twice (5 minutes total immersion) with phosphate buffered saline (PBS) pH 7.4. Washed and treated smears were then incubated for 30 minutes at 37° C with fluorescent sheep anti-rabbit immunoglobulin(Wellcome) at a working dilution of 1:20. The smears were then rinsed twice with PBS (5 minutes total immersion) and prepared for examination by placing a coverslip on the PBS-covered smear. Smears were examined under utraviolet light using a Leitz Ortholux microscope fitted with an HBO 200 light source and a vertical fluorescence illuminator. All smears were screened for fluorescence using the X10 lens and X12.5 eyepieces and then examined in detail using the X25 lens.

T. hyodysenteriae was considered to be present when fluorescing spiral microorganisms were seen in any preparation. At all times saline controls

were included. Serum controls were not included as past experience in the laboratory with this system had shown it to be specific. In positive cases large numbers of organisms fluoresced, but when organisms considered on cultural grounds to be non-pathogenic to pigs were examined or when they were isolated from other species, no specific fluorescence was seen.

Smears were also made from faecal material and the large intestinal contents or mucosa from experimental mice and rats and were examined in the same way.

2. The isolation of Treponema hyodysenteriae from pathological material

T. hyodysenteriae was isolated from faecal specimens, large intestinal contents and mucosa using the selective medium described by Songer et al.1976. This medium was prepared by adding approximately 400 µg/ml of spectinomycin to the horse blood agar used in cultivation and described above. This medium was prepared by adding 1 ml of an injectable, water soluble spectinomycin solution (Spectam injectable Abbott) containing 100 mg/ml to 249 ml of molten agar containing horse blood just prior to pouring the plates.

Blood agar plates prepared by the method described above were inoculated with loopful of faeces or faecal suspension and then streaked for three further dilutions. Mucosal samples were taken using a stiff sterile loop inserted into the mucosal surface of the intestinal wall

and rotated in order to sample the material present in the mucosal crypts. In some cases swabs were used to sample the rectal faeces of pigs. These were soaked in sterile physiological saline before use and were then streaked over the first quadrant of the plate. Further dilutions were carried out in the normal way.

Inoculated spectinomycin blood agar plates were incubated as described above as soon as possible after inoculation and examined for colonies of spirochaetes after 48 hours' incubation then re-incubated for a further 48 hours or more if no growth was seen. Colonies of spirochaetes were examined for the production of haemolysis and surface growth. When there was no doubt of their identification as T. hyodysenteriae, the isolation plates were discarded. In cases in which positive identification was difficult or when porcine material was being examined, suspect colonies were subcultured onto spectinomycin blood agar and subsequently to horse blood agar. When grown on the latter, colonial appearance could be assessed more accurately and if identification was still difficult, smears were taken for examination by the fluorescent antibody test described above.

3. Bacterial counts of T. hyodysenteriae in saline and faecal suspensions

Saline suspensions of T. hyodysenteriae used as inoculum in transmission experiments were examined to determine the number of viable organisms per ml of suspension. The same methods were used to determine the number of viable organisms per gram of faeces.

The sample was diluted in sterile physiological saline in tenfold dilutions and drops of 0.1 ml were used to inoculate the surface of a blood agar or spectinomycin blood agar plate in a modification of the method described by Miles and Misra(1938). The size of the drops used meant that they could spread over a quadrant of the plate inoculated.

Inoculated plates were incubated for 48 hours and then examined. All plates were re-examined daily until no more areas of beta-haemolysis could be seen or until expansion of those present covered the area inoculated.

Areas of beta-haemolysis were counted and if large, the small raised plaques of growth within them were counted. This count was considered to give the number of colony forming units (Kinyon,1974) as no definite information exists about the number of organisms required to produce an individual colony. Numbers of organisms present in any sample counted in this study were always expressed in colony-forming units (c.f.u.).

4. Identity of the strain of *Treponema hyodysenteriae* used

All the studies described in this thesis were carried out using isolate S73/2 of *Treponema hyodysenteriae*. This strain was originally isolated from a pig which had died from swine dysentery in February 1973. The cultural characters of the isolate were typical of those *T. hyodysenteriae* and it had been shown to cross-react with antisera prepared against isolate B-78(type strain for *T. hyodysenteriae* Harris *et al.*1972a)and isolate A1(Taylor and Alexander,

1971). It has also been used in the experimental reproduction of swine dysentery (Taylor,1976c)and was pathogenic for pigs. The isolate was freeze-dried within 6 passages of isolation and has been maintained in the freeze-dried state since that time. All the cultures used in this study for pathogenicity tests or used in the pig experiments from which material was obtained for this study were prepared from freshly-reconstituted freeze-dried material. These cultures were grown for 2-3 passages after reconstitution and before use. In vitro studies were carried out using cultures of S73/2 maintained by serial passage on horse blood agar at intervals of 5-7 days.

5. Experimental animals

(a) Mice

The mice used in these studies were white mice, Porton strain or brown mice (strain C3HF) and were bred in the colony belonging to the Glasgow Veterinary School. Young adult mice of both sexes were used. They were reared on a purchased laboratory mouse diet (Diet 41, Angus Milling, Perth) which was also fed during the experiments. Experimental mice were housed in polythene cages containing no bedding. Feed and water were available ad libitum.

(b) Rats

Young adult rats of both sexes were used for the rat infection experiments. They had been reared on Diet 421 and were maintained on this diet during the experiments. Experimental rats were housed in

polythene cages with a wire mesh bottom placed over a tray designed to collect faeces. No bedding was provided for the rats. Feed and water were available ad libitum.

(c) Flies

Flies were obtained from two sources. In the first experiment Lucillia spp. were obtained from the Wellcome Institute of Comparative Parasitology, Glasgow University Veterinary School. They had been obtained as pupae and only fully-mature adult flies were used in this study.

The second group of fly studies was carried out using flies captured as adults in the vicinity of the Glasgow Veterinary School Post Mortem Room and animal housing. A gauze insect net was used and damaged specimens were rejected. Calliphora spp. and Musca domestica were identified in the catch and separated into generic groups. No specimens of Stomoxys calcitrans could be obtained.

Flies were housed in cardboard boxes with perforated clear polythene covers and fed on blood agar plates sprinkled with sugar until required. All flies were used within 2 days of capture or arrival.

(d) Pigs

The pigs used as sources of dysenteric faecal material were part of experiments being carried out by Dr. Taylor. They were Large White or hybrid animals obtained from the University of Glasgow Animal Husbandry Department. The herd

of origin was free from swine dysentery. They were all of 6-10 weeks of age at the time of first infection and were of mixed sex. All were individually identified by means of a numbered eartag. Pigs were housed in covered open-fronted concrete-walled and floored pens with feed and water ad libitum. The food was a mix standard to the Animal Husbandry department and did not contain any non-nutritive additives. It was composed of barley, soya and fish meal to give 16% crude protein. The pigs had straw bedding and were cleaned out daily.

6. The preparation of inocula

All the experimental animals were inoculated orally with cultures of T. hyodysenteriae isolate S73/2 prepared on 7 per cent horse blood agar. The plates used were inoculated with 2-3 drops of a suspension of T.hyodysenteriae in sterile physiological saline and spread using a glass spreader. The resulting bacterial lawns were incubated anaerobically for 48 hours at 42°C. When examined after incubation, these plates were found to be completely haemolysed. Plates free from obvious contaminants were used to prepare inocula.

The rats and mice were inoculated with a suspension prepared from the bacterial lawns described above by harvesting the growth in sterile physiological saline. 3-5 ml of saline was added to each plate and the organisms were harvested using a glass spreader. A drop of each preparation was examined by phase contrast microscopy

for motility and purity before use and inactive or contaminated preparations were discarded. A count was then carried out on the suspension by the method described above.

Flies were allowed access to thickly-inoculated incubated plates of cultures of isolate S73/2 on which 2-3 drops of sterile physiological saline had been used to moisten the surface. Pigs were inoculated with lawn cultures prepared as described above but chopped up and suspended in sterile physiological saline.

7. Inoculation

Food was withheld from all animals to be inoculated and from the controls for at least 3 hours (flies) to overnight (rats,mice and pigs). The inoculum was then presented as whole plate cultures (flies), a suspension of whole plate culture, force-fed (pigs) and spirochaetal suspension given by stomach tube under anaesthetic (rats, mice).

8. Routine examinations

All experimental animals were examined daily for signs of disease and samples were taken at intervals for bacteriological examination. Rectal swabs were used to sample pigs and whole faeces was obtained from mice, rats and pigs. The consistency and appearance of the faeces was recorded. Suspension of whole faeces were prepared in sterile physiological saline and examined for the presence of spirochaetes with the morphology of T. hyodysenteriae by phase contrast microscopy. All samples were examined for the presence of T. hyodysenteriae by

the cultural methods described above.

9. Euthanasia

The mice and rats were destroyed humanely by cervical dislocation. The flies were killed by crushing.

Pigs were killed at the Veterinary School by electric stunning and exsanguination.

10. Post-mortem examinations

Mice, rats and pigs were examined for the presence of gross lesions in the organs of the abdominal and thoracic cavities. In each case the appearance of the large intestine and its contents was noted. Samples of the latter were taken for bacteriological examination. The mucosa of the large intestines was rinsed in whole (pigs) or in part (mice and rats) in order that gross changes might be observed. The large intestinal mucosal surface of the mice and rats was examined by naked eye and using a dissecting microscope. Any changes seen were noted and photographed and samples were taken for histological and bacteriological examination.

11. Histological examination

Samples taken for histological examination were fixed in 10% formol saline and embedded in paraffin wax for sectioning. All sections were stained by Haemalum and Eosin (H&E) and those taken from the intestinal mucosa were stained by Young's method(Young,1969) in order to demonstrate spirochaetes. All sections were examined using the microscope used for the fluorescent antibody tests, screening using the x25 objective and making detailed

examinations using the x40 objective.

12. Bacteriological examination

Samples taken at post-mortem examination were examined for the presence of T. hyodysenteriae using the method described above.

The post-mortem examinations carried out on the flies is described in Chapter 4.

Chapter Three

Factors affecting the survival of *Treponema hyodysenteriae* in pig faeces

There is disagreement in the literature over the length of time required for depopulated infected premises to become safe for restocking with swine dysentery-free pigs. Terpstra *et al.*(1968) suggested that 7 days was sufficient but, more recently, Harris and Glock(1975) recommended leaving infected premises empty for sixty days.

The studies described here were carried out in order to determine the length of time that *Treponema hyodysenteriae* can survive in dysenteric faeces at different temperatures. The effects of drying, dilution in tapwater and the action of disinfectants were also studied in an attempt to provide some data which might be of use in the planning of control programmes.

Part 1 Studies of the effects of temperature on the survival of *T. hyodysenteriae* in dysenteric faeces

Materials and methods

Dysenteric pig faeces

Dysenteric pig faeces were collected from pigs experimentally infected with pure cultures of *T. hyodysenteriae* (isolate S73/2) as described in Chapter 2. Faeces used in this study were collected from the pigs as soon as blood was first seen in the faeces and for a

maximum of 5 days after this. Some of the samples taken later in the disease did not contain blood but all contained mucus, were liquid in consistency and were foul smelling. All the pigs from which the samples were taken had been diagnosed as cases of swine dysentery on the basis of clinical signs. Faeces samples were not taken from animals after clinical signs had continued for more than 5 days as it was considered that developing immunity might affect the survival of the organisms.

Samples were obtained in two ways. Most were taken directly from the rectum of clinically affected pigs using a gloved finger to stimulate the production of faeces. The remaining samples were collected from the pen floor, particularly in the case of severely-affected animals from which little rectal faeces could be elicited. All samples collected from the floor were freshly voided and in most cases were still warm when collected.

Each whole rectal faeces sample was placed in a sterile plastic or glass beaker covered with aluminium foil for transport to the laboratory. All samples reached the laboratory within one hour of collection.

Upon arrival at the laboratory, wet smears of each sample were prepared and examined by phase contrast microscopy for the presence of spirochaetes with the morphology of T. hyodysenteriae. An acetone-fixed air dried smear was also prepared from each sample in order to confirm by the fluorescent antibody test that any spirochaetes seen were T. hyodysenteriae.

Isolation technique :

Loopfuls of faeces were taken from the samples and cultured on spectinomycin blood agar using the method described in Chapter 2. Colonies thought to be those of T. hyodysenteriae were identified by their appearance, the morphology and motility of the spirochaetes present, and in case of difficulty, by the antigenicity of subcultures using the method described in Chapter 2.

T. hyodysenteriae was recorded as being present if a single colony was found on any isolation plate inoculated from a sample.

Experimental procedure :

Each sample of dysenteric faeces was cultured in order to confirm the presence of T. hyodysenteriae on day 0. After incubation, each culture was examined for suspected colonies of T. hyodysenteriae and a colony was subcultured for confirmation of the identity of the spirochaete as T. hyodysenteriae by the fluorescent antibody test.

As soon as the initial sample had been taken, the faeces was divided into aliquots of 3-5 grams using weighed sterile universal bottles. These aliquots were then stored at the appropriate temperature and examined by culture daily or at shorter intervals until 4 consecutive attempts at culture had proved negative. The samples were stored at 0°C, 5°C, 10°C, 20-22°C (room temperature), 25°C, 37°C, 42°C and 56°C, in various refrigerators and incubators in the Department of Veterinary Pathology. The sample kept at 0°C did not

appear to freeze. No samples were kept at temperatures below 0 C.

A loopful of the faeces was withdrawn daily and cultured. The aliquot was returned to its place of incubation within 15 minutes of removal in order to reduce the effects of temperature changes.

Results

(a) Preliminary experiments

The first two studies were carried out over the whole range of temperature given above (0°C-56°C). The results are shown in Table 1.

Table 1. Results of experiments to determine the survival of T. hyodysenteriae in dysenteric pig faeces at different temperatures.

Temperature °C	Survival time (days)	
	Sample 1	Sample 2
0	20	18
5	35	29
10	31	29
20-22	8	7
25	5	3
37	less than 1	less than 1
42	less than 1	less than 1
56	less than 1	less than 1

(b) Further studies

Six further studies were carried out. The results of the preliminary experiments had shown that the survival of T. hyodysenteriae was poor at temperatures above 25°C and aliquots were not incubated at these higher temperatures. In some cases, (Samples 3,4 and 5) insufficient faeces was available for survival to be studied at each of the remaining temperatures. The results are summarised in Table 2.

Table 2. Results of further experiments to determine the survival of T. hyodysenteriae in dysenteric faeces at different temperatures.

Temperature(°C)	Survival time (days)					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
0	34	22	ND	ND	ND	48
5	42	28	33	29	31	36
10	38	24	35	27	25	35
20-22	12	6	ND	ND	ND	8
25	5	3	ND	ND	ND	7

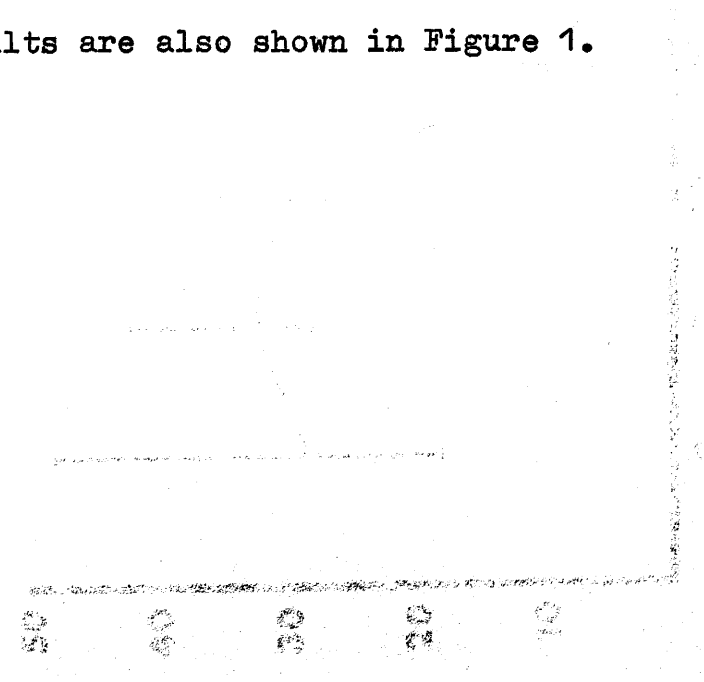
ND : Not done.

The results of these two groups of experiments are summarised in Table 3.

Table 3. Summary of results of the survival experiments described in Part 1.

Temperature °C	Number of Observations	range (days)	Mean
0	5	18-48	28.4
5	8	28-42	32.9
10	8	24-38	30.5
20-22	5	6-12	8.2
25	5	3-7	4.6
37	2	0	0
42	2	0	0
56	2	0	0

These results are also shown in Figure 1.



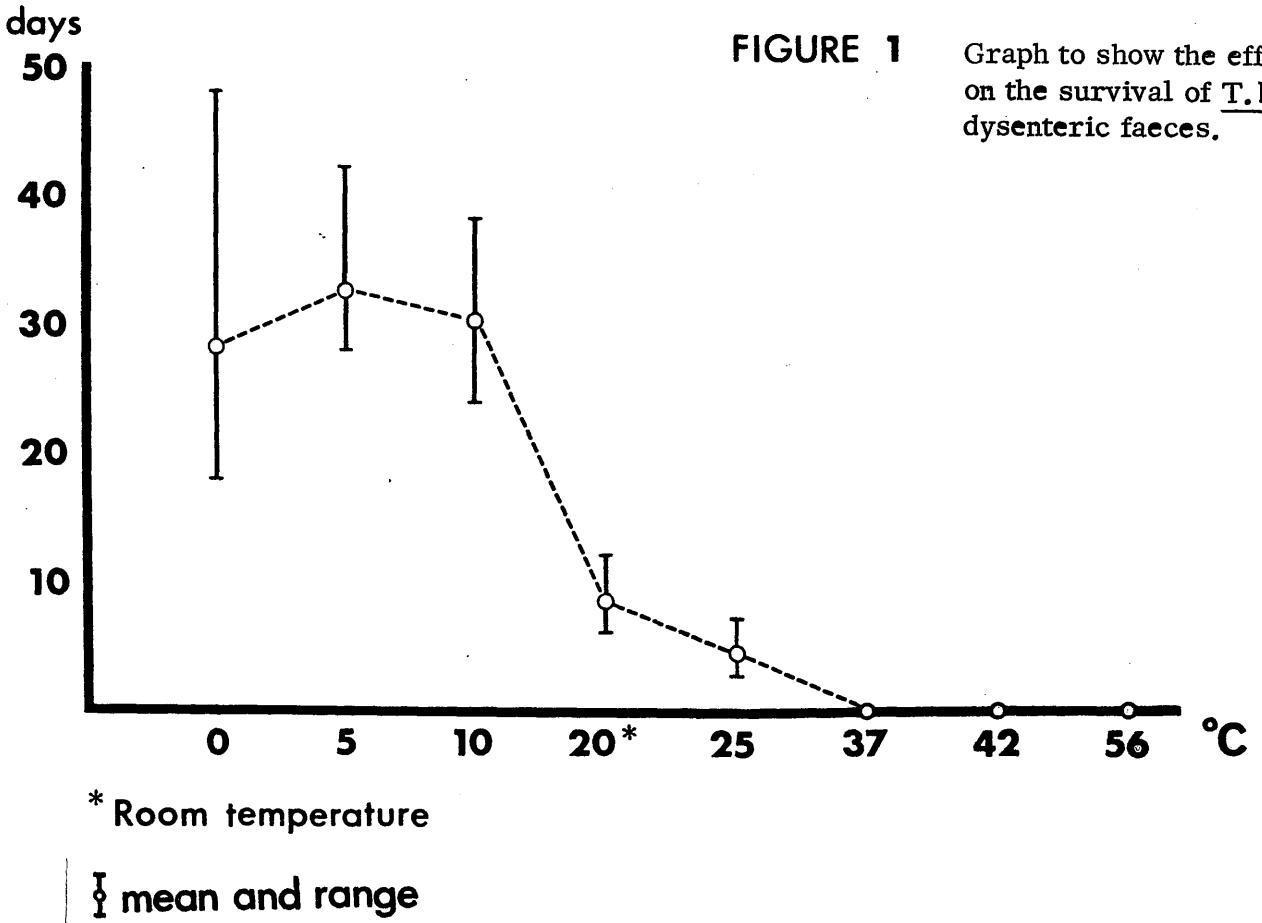


FIGURE 1 Graph to show the effect of temperature on the survival of T. hyodysenteriae in dysenteric faeces.

Part 2 Studies of the effect of dilution with tapwater on the survival of *T. hyodysenteriae* in dysenteric faeces at different temperatures

Materials and methods

The materials and methods used in these studies were the same as those described in Part 1 above. A weighed aliquot of a sample used in the study described in Part 1 was diluted in nine parts of Glasgow tapwater (pH 6.5-6.6) to give a final dilution of 1:10. After mixing thoroughly, one part of this was further diluted to give final dilutions of 1:50, 1:100 and 1:500. These diluted samples were incubated at temperatures of 0°C, 5°C, 10°C, 20-22°C (room temperature) and 25°C only.

Isolation techniques were carried out as described in Part 1 above. Counts were performed on diluted material using the method outlined in Chapter 2.

Results

(a) Preliminary experiments

Two preliminary experiments were carried out in this study using the dilution listed above. One was carried out at 5°C and the other at 10°C. The remainder of the dysenteric faeces sample was used as a control. The results are shown in Table 4.

Table 4. The effect of dilution in tapwater on the survival of T. hyodysenteriae in dysenteric pig faeces

Dilution	Survival time (days)	
	Sample stored at 5°C	Sample stored at 10°C
Undiluted control	42	38
1:10	50	41
1:50	32	19
1:100	Not done	10
1:500	10	3

(b) Further studies

The results of the preliminary experiments shown in Table 4 above indicated that T. hyodysenteriae survived longest in dilutions of 1:10 and 1:50 at 5°C. Further study of the survival of T. hyodysenteriae in 1:10 dilution was carried out in parallel with the studies described in Part 1 which formed an undiluted control sample in each case.

The results are shown in Table 5.

Table 5. The effects of dilution 1:10 in tapwater on the survival of T. hyodysenteriae in dysenteric faeces

Sample No.	Dilution	Temperature (°C)				
		0	5	10	20-22	25
1	Undiluted	34	42	38	12	5
	1:10	35	50	41	5	2
2	Undiluted	22	28	24	6	3
	1:10	15*	42	37	4	2
3	Undiluted	ND	33	35	ND	ND
	1:10	22	53	43	9	4
4	Undiluted	ND	29	27	ND	ND
	1:10	18*	44	46	4	2
5	Undiluted	ND	31	25	ND	ND
	1:10	ND	42	53	ND	ND
6	Undiluted	48	36	35	8	7
	1:10	ND	61	45	ND	ND

* It was noted that the diluted material held at 0°C froze on a few occasions while undiluted control material stored in adjacent bottles did not.

N.D. = not done.

The results of these dilution studies are summarised in Table 6 and the survival of T. hyodysenteriae in diluted faeces is compared with that in the undiluted controls at the same temperatures in Figure 2.

Table 6. Summary of the results of the survival of T. hyodysenteriae in dysenteric faeces diluted 1:10 with tapwater

Temperature °C	Number of Observations	Range (days)	Mean
0	4	15-35	22.5
5	6	42-61	48.7
10	6	37-53	44.2
20-22	4	4-9	5.5
25	4	2-4	2.5

During the course of these studies into the effects of dilution on survival it was noted that the number of colonies of T. hyodysenteriae formed did not appear to diminish until after at least 20 days' storage at 5° or 10°C. A count was therefore performed on one aliquot of faeces diluted 1:10 in tapwater and stored at 5°C to study the decline in the number of viable colony forming units with time.

The results are shown in Table 7 and Figure 3.

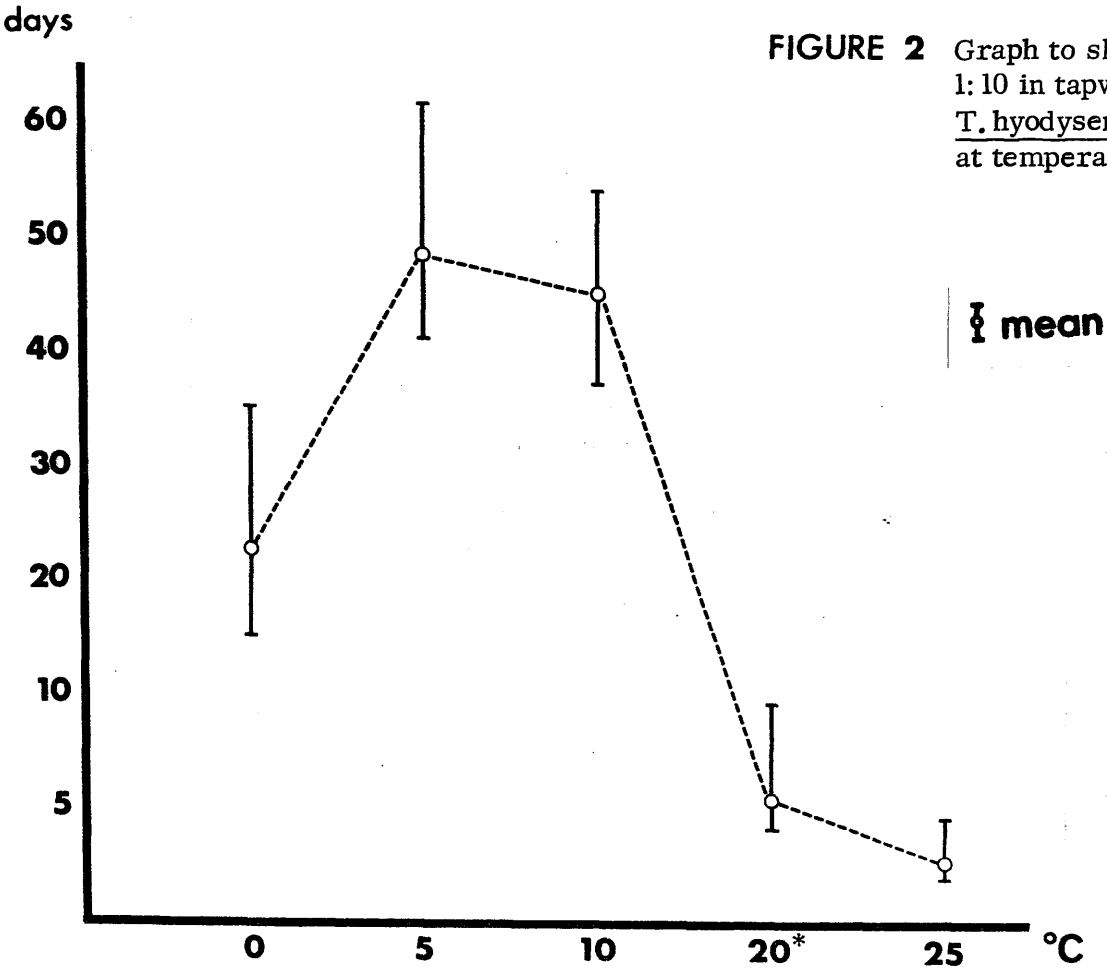


FIGURE 2 Graph to show the effect of dilution 1: 10 in tapwater on the survival of T. hyodysenteriae in dysenteric faeces at temperatures from 0^{o} - 25^{o}C.

*Room temperature

Table 7. The effects of storage at 5°C on the numbers of *T. hyodysenteriae* present in a sample of dysenteric faeces diluted 1:10

Day	Number of organisms (expressed as per gram of faeces)	Log
0	2.3×10^7	7.36
1	1.7×10^6	6.23
2	2.5×10^6	6.39
7	1.5×10^5	5.18
14	2.1×10^4	4.32
21	9×10^2	2.95

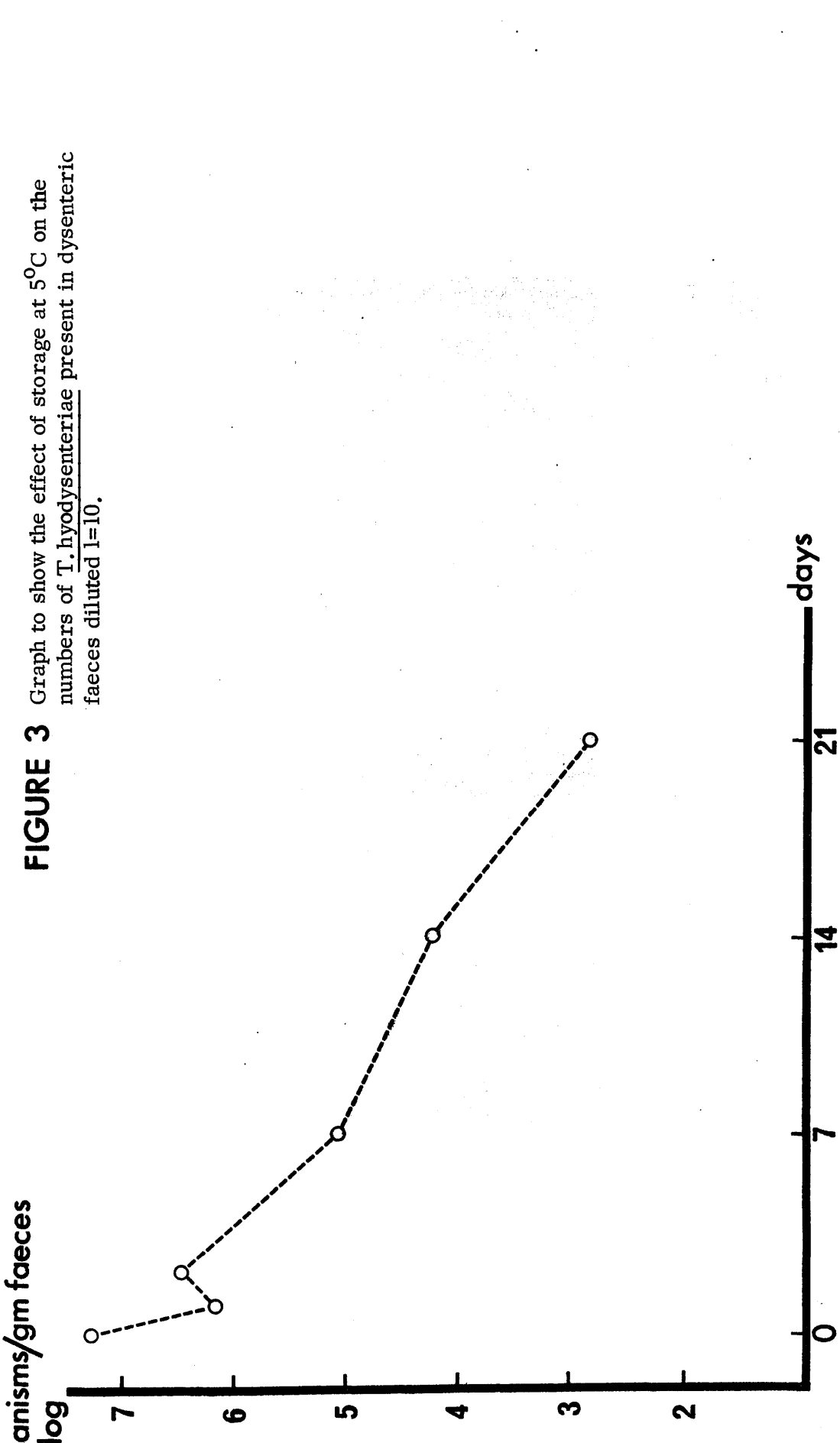


FIGURE 3 Graph to show the effect of storage at 5°C on the numbers of T. hyodysenteriae present in dysenteric faeces diluted 1=10.

Part 3. Studies of the effect of drying on the survival of T. hyodysenteriae in dysenteric faeces

Materials and methods

The faeces samples used in this study were taken using the methods described in Part 1 above and examined to confirm that T. hyodysenteriae was present in the same way.

Samples were divided into 3-5 gram portions and spread on a sterile plastic petri dish with no lid to form a thin layer. The samples were stored at 5°C, 20-22°C (room temperature) and 37°C and cultured at 12 and 24 hours after storage and then at daily intervals. The samples stored at room temperature and 37°C dried up within 12 hours of preparation but the sample stored at 5°C did not dry up completely until 24 hours after preparation. Sample 6 in Part 1 formed a control for this study.

Cultural examination was carried out by re-constituting a small fragment of dried faeces in sterile physiological saline to form a suspension which was sufficiently fluid to inoculate onto a blood agar plate. The cultural methods described in Part 1 above were used in this study.

Results

As a preliminary experiment, one sample was prepared as described above but although T. hyodysenteriae was isolated from the sample before storage it could not be isolated from any of the samples 12 hours after preparation or on any subsequent occasion. The study was discontinued after 4 days when the 12 hours culture results

were read.

This study was not repeated.

Part 4 The effect of disinfectants on the survival of T. hyodysenteriae in dysenteric faeces

A number of disinfectants were tested for their action against T. hyodysenteriae in faeces. In some cases it was possible to obtain disinfectants listed in the Diseases of Animals (Approved Disinfectants) Order (1970) of the Ministry of Agriculture, Fisheries and Food. In other cases, disinfectants containing the major chemical types were used.

Materials and methods

Dysenteric pig faeces was collected by the method described in Part 1 above.

The following disinfectants were used :

Formalin (40% W/V formaldehyde B.P.)

Dettol Hospital Concentrate (4.8% Chloroxylenol)

White Septol 'B' (50% phenol)

Chloros (sodium hypochlorite, available chlorine 10% by wt.)

Medipine (Methoxylated tertiary amine 0.5%, Terpenes 2%)

Savlon Hospital Concentrate (Chlorhexidine gluconate 1.5%, cetrimide 15%)

Cetrimide B.P.

Sodium carbonate decahydrate

2-4 grams of dysenteric pig faeces was placed in a weighed sterile universal bottle and weighed. To each faecal specimen was added three parts (by volume) of

disinfectant diluted with sterile deionised water. The specimens were mixed thoroughly with the disinfectant solution by shaking. The mixture was allowed to react and samples were with-drawn for cultural examination after 5, 10, and 35 minutes. 1 ml samples were taken for cultural examination and were diluted 1:4 in tapwater. 0.1 ml of this diluted mixture was dropped onto spectinomycin blood agar plates and incubated and examined using the methods described in Chapter 2.

All the disinfectants were used at the level of dilution recommended for use by the manufacturers or the Ministry of Agriculture, Fisheries and Food. Each disinfectant was then titrated to establish an end point dilution at which survival of the organism occurred.

In every test, a control sample was diluted with tapwater in the place of disinfectant and examined culturally by the method described above.

Results

a) The effect of recommended dilutions of disinfectant on the survival of T. hyodysenteriae in dysenteric faeces

The recommended level of all the disinfectants used (whether manufacturers' or the Ministry) were effective in inactivating T. hyodysenteriae or preventing its isolation under the conditions used with 5 minutes, except in the case of Savlon in which the organism could be isolated even after 35 minutes. The results are shown in Table 8.

Table 8. The effect of recommended dilutions of disinfectant on the survival of T. hyodysenteriae in dysenteric faeces

Disinfectant	Recommended dilution	Recovery of T.hyodysenteriae after		
	%	5 min.	10 min.	35 min.
Formalin	0.4	-	-	-
Dettol	2.5	-	-	-
White Septol 'B'	1.0	-	-	-
Chloros	2.0	-	-	-
Sodium carbonate	4.0	-	-	-
Savlon	0.5	+	+	+
Control	-	+	+	+

(b) Determination of the minimal inhibitory concentration of disinfectants for T. hyodysenteriae in dysenteric faeces

The results of this study are shown in Table 9.

Table 9. The determination of the minimal inhibitory concentrations of disinfectants for T. hyodysenteriae in dysenteric pig faeces

Disinfectant	Dilution (%)	Recovery of T. hyodysenteriae after		
		5 min.	10 min.	35 min.
Formalin	0.4	-	-	-
	0.2	+	-	-
	0.1	+	+	+
White Septol 'B'	1.0	-	-	-
	0.5	-	-	-
	0.2	-	-	-
	0.1	-	-	-
	0.05	+	+	+
Dettol	2.5	-	-	-
	1.0	-	-	-
	0.5	-	-	-
	0.3	+	+	-
Chloros	2.0	-	-	-
	0.5	-	-	-
	0.2	+	+	-
Savlon	0.5	+	+	+
	1.0	+	-	-
	2.0	-	-	-
Medipine	4.0	-	-	-
	2.0	+	+	+
Sodium carbonate	4.0	-	-	-
	2.0	+	+	+
Cetrimide B.P.	0.5	-	-	-
	0.2	-	-	-
	0.1	+	+	-
Control	-	+	+	+

Discussion

The length of time for which *T. hyodysenteriae* can survive under various conditions is obviously of importance in any study of the epidemiology of swine dysentery. The results of the four studies reported in this chapter provide some information from *in vitro* studies which may be of value in the design of control programmes and in the understanding of factors affecting the spread and survival of the disease.

One of the basis parameters investigated was the effect of storage at different temperatures on the survival of *T. hyodysenteriae* in faeces. The use of spectinomycin blood agar as a selective medium has made this type of study possible on small quantities of faeces. Previous studies (Taylor, personal communication) were carried out using the cumbersome filtration method of isolation and produced substantially lower figures for the survival of *T. hyodysenteriae* than were recorded in Part 1 of this study.

The results given in Part 1 showed that *T. hyodysenteriae* could not be cultured from samples of faeces kept at temperatures of 37°C or more for 24 hours. At lower temperatures, better survival was obtained (Table 2 and 3, Fig.1). *T. hyodysenteriae* could be recovered from faeces samples stored at 0°C for up to 48 days. The mean survival time at this lower temperature was less than that found at 5°C and 10°C, possibly because samples held at this temperature were exposed to freezing and thawing during which the delicate spirochaetal cell could be destroyed.

Survival at 25° C was similar to that at 20-22° C and survival at both of these temperatures was markedly less than at 0°, 5°, 10° C. It is possible that this decrease in survival time results from the effects of temperature on T. hyodysenteriae directly, but it is more likely that this decrease results from the increased activity of other bacteria in the faeces. This bacterial activity may act by using up substances present in the faeces and essential for the survival of T. hyodysenteriae, by direct digestion of T. hyodysenteriae cells by their extracellular enzymes, or by creating an adverse environment for survival by producing substances such as fatty acids and ammonia, acting directly or by means of pH changes.

Dilution of the faeces with tapwater was not found to inhibit the survival of T. hyodysenteriae except perhaps at the higher temperatures tested (20-22° C and 25° C) and at the higher dilutions (1:100 and 1:500) (Table 4). It had been thought that dilution might kill the organisms by osmotic disruption, but this proved not to be the case. The apparent increase in survival in samples diluted 1:10 at 5° C and 10° C over the survival of undiluted control samples is of interest and may be due to dilution of bacterial waste products harmful to the spirochaete. The apparent survival of large numbers of organisms for the first twenty days was so marked that the numbers of organisms surviving in a 1:10 dilution held at 5° C was titrated. The results shown in Figure 3 showed that the number of organisms present fell exponentially during the three-week storage period.

The brief attempt to recover organisms from dried

faeces was unsuccessful. The experiment performed was not repeated as insufficient dysenteric faeces was available at this point in the study. The reason for the failure to recover the organism was probably that it had died out, but it is possible that the method of reconstitution was unsuitable. Further study of this point is essential.

The studies described in Part 4 demonstrated that T. hyodysenteriae was destroyed within 5 minutes by all the classes of disinfectant used except chlorhexidine gluconate (Savlon), not normally used on farms. The method used allowed complete penetration of the faeces by disinfectant and exposed the organisms to the full concentration recommended. The survival monitored may have been less than the actual survival occuring in the mixture because of the method of monitoring employed. Inevitably, disinfectant remained on the surface of the isolation plates and continued to act although some dilution with tapwater was carried out in order to reduce this effect.

The dilution studies showed (Table 9) that the phenolic general farm disinfectant "White Septol B" was the most effective of all the disinfectants tested, followed by "Dettol" another phenolic compound. The remainder were comparatively less effective in terms of dilution from the recommended level. In terms of actual percentage of compound present, "White Septol B" was also the most effective with a minimal inhibitory concentration (M.I.C.) of 0.1%.

Chapter Four

The survival of *Treponema hyodysenteriae* in animals other than the pig

Very little is known about the persistence of *T. hyodysenteriae* in animals other than the pig. The purpose of this study was to investigate the possibility that *T. hyodysenteriae* might survive in farm pests such as birds, mice, rats and flies. As wild birds such as sparrows were not available as experimental animals, laboratory mice, rats and flies of three genera were used. The studies described in this chapter were intended to determine whether the organism could persist in or on the animal species and if so, for how long. Three studies were carried out in mice, two in rats and four in flies.

Part 1 Mouse inoculation experiments

Materials and methods

The mice and the inoculum used have been described in Chapter 2 above. Inoculation was carried out by Dr. Taylor who anaesthetised the mice with Trichlorethylene (Trilene, I.C.I.) in a two-litre glass beaker, the bottom of which was covered in cotton wool and the top of which was covered with perforated aluminium foil. When the mice were lightly anaesthetised, they were inoculated with 0.3 ml of the suspension of *T. hyodysenteriae* prepared as described in Chapter 2. The inoculum was given by stomach tube. For this purpose a nylon intravenous cannula (0.63 mm diameter, Portex Ltd.) was used, cut to 2.5-3 cm in length and

attached to a 1 ml graded disposable plastic syringe. Inoculated animals were allowed to recover and then placed in their experimental groups and housed as described in Chapter 2.

The faeces which had accumulated at the bottom of each cage was collected in a beaker, emulsified in sterile saline and samples were used to inoculate cultures and prepare wet smears for phase contrast examination. The presence or absence of spirochaetes resembling T. hyodysenteriae was recorded. The duration of the experiments is recorded below under the detailed experimental description.

Methods of euthanasia, post-mortem, histological and cultural examinations used in these experiments have been described in Chapter 2.

Experiment 1

24 white mice were divided into the groups shown in Tablbe 10.

Table 10. Arrangement of mice in Experiment 1

Group	Number of animals	inoculated
1	4	+
2	4	+
3	4	+
4	4	+
5	8	-(control)

Inoculation was carried out at Day 0 and the experiment lasted for 14 days. The concentration of T. hyodysenteriae in the inocular suspension was 1.2×10^7 organisms per ml giving a dose of approximately 3.6×10^6 organisms per mouse. Mice were killed and examined at the intervals shown in Table 11.

Table 11. Schedule of the post-mortem examination of mice in Experiment 1

Day	Infected mice		Group 5(control)
	Group No.	Number killed	number killed
0	-	-	2
1	1	2	-
2	2	2	-
4	3	2	-
5	-	-	2
6	4	2	-
8	1	2	-
10	2	2	2
12	3	2	-
14	4	2	2

Experiment 2

Experiment 1 was repeated with 14 white mice in an attempt to confirm the results found. This experiment also lasted 14 days. The concentration of T. hyodysenteriae in the inocular suspension was 1.2×10^8 per ml giving a dose of approximately 3.6×10^7 organisms per mouse. Mice were killed and examined at the intervals shown in Table 12.

Table 12. Schedule of the post-mortem examination of mice in Experiment 2

Group	Day				
	0	2	4	7	14
Inoculated (10) (number killed)	-	2	2	2	4
Uninoculated control (4) (number killed)	2	-	-	-	2

Experiment 3

This experiment was a repeat of Experiment 1 and 2 but was extended to 28 days in order to determine whether infection with T. hyodysenteriae could persist for more than 14 days.

39 brown mice were used in this experiment. They were divided into two groups: 29 mice were infected and 10 mice were not inoculated and were kept as controls. The concentration of T. hyodysenteriae in the inocular suspension was 1.8×10^7 organisms per ml giving a dose per mouse of 5.4×10^6 organisms. Mice were killed and examined at the intervals shown in Table 13.

Table 13. Schedule of the post-mortem examinations of mice in Experiment 3

Group	Day										
	0	1	2	4	6	8	10	12	14	21	28
Inoculated number killed	-	2	2	2	2	2	2	2	5	5	5
Uninoculated number killed	2	-	-	-	-	2	-	-	2	2	2

Results

Experiment 1

Clinical observations

The inoculated groups appeared healthy throughout the experiment and no difference in condition or behaviour could be distinguished between the inoculated and control groups.

The faeces of both groups remained normal throughout the period of the experiment. Faecal pellets which had been voided for sometime appeared dry and hard but freshly voided pellets passed by both groups were occasionally soft with a tail of clear mucus. This type of faeces appeared to be passed after handling.

Faecal examination for the presence of *T. hyodysenteriae*

(a) Direct examination

Spirochaetes with the morphology of *T. hyodysenteriae* were seen in samples of the pooled faeces from the cages containing inoculated animals. A thin,

flexible spirochaete which could be distinguished from T. hyodysenteriae on morphological grounds was seen on a few occasions in the faeces of inoculated and non-inoculated mice. This spirochaete was approximately 5-7μ in length and was often arranged in a simple 'S' shape. It was occasionally motile in faeces samples, moving with weak thrashing movements unlike those of motile T. hyodysenteriae. Spirochaetes with the morphological characters of T. hyodysenteriae were never seen in faeces samples from the control group.

The occurrence of spirochaetes in the pooled faeces samples in Experiment 1 is summarised in Table 14.

(b) Cultural Examination

T. hyodysenteriae was isolated from the pooled faeces of all four groups of inoculated mice (Fig.4) but not from the faeces of the control group. The first isolation occurred on the fourth day after inoculation but the organism could not be isolated every day from the faeces of each inoculated group. The last day on which isolation was made was on day 14 at the end of the experiment. The identity of the isolates was confirmed as T. hyodysenteriae by the morphological, cultural and antigenic characters outlined in Chapter 2.

The results of cultural examination are given in detail in Table 14.

Fig. 4 Spectinomycin blood agar inoculated with pooled faeces from experimentally infected mice. Note the haemolytic areas of growth of Treponema hyodysenteriae.

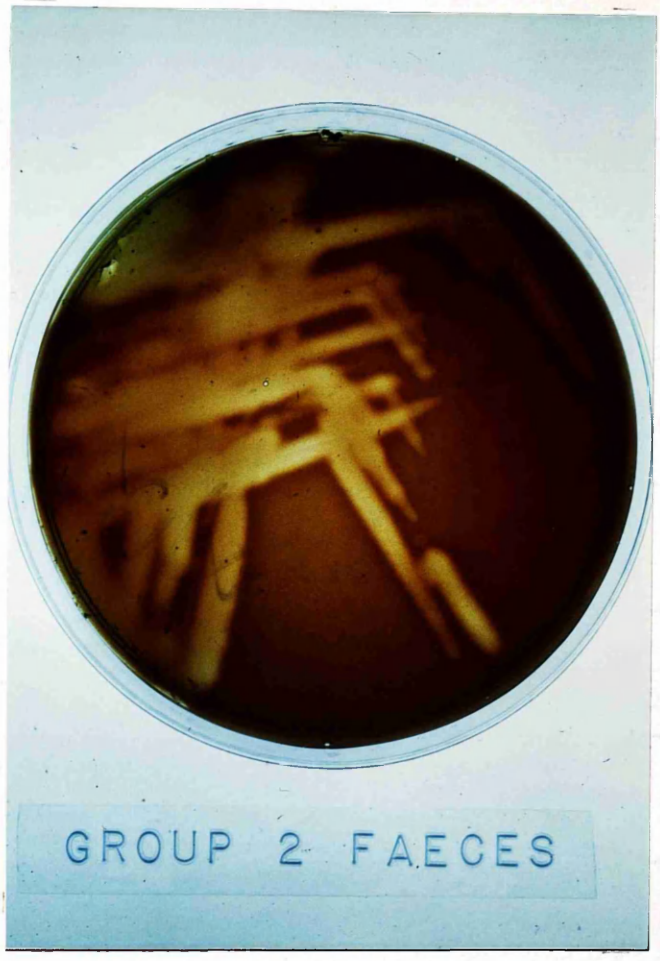

GROUP 2 FAECES

Table 14. The results of the daily examination for *T. hyodysenteriae* carried out on pooled faeces from all groups of mice in Experiment 1

Group	Type of examination	Day 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Direct	-	-	+	-	-	-	-	-	-	killed					
	Cultural	-	-	-	-	-	+	+	-	-						
2	Direct	-	-	-	+	-	-	-	-	-	-	killed				
	Cultural	-	-	-	-	+	+	-	+	-	-	+				
3	Direct	-	-	-	-	-	-	-	-	-	+	-	-	-	killed	
	Cultural	-	-	-	-	-	+	-	-	-	-	-	-	+		
4	Direct	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Cultural	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+
5	Direct	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Cultural	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Post-mortem examination of the mice

(a) Gross findings

The intestines and other organs of all the mice examined appeared macroscopically normal. No evidence of inflammation of the large intestinal mucosa was found in any of the mice. The contents of all the regions of the intestine were normal in consistency and appearance and no blood or excess mucus could be seen even when the tissue was examined under a dissecting microscope.

(b) Histological findings

Gastrointestinal specimens taken at the post-mortem were fixed in 10% formal saline and processed according to the method described in Chapter 2.

Sections stained by H & E

Samples of large intestinal wall were examined and no marked pathological changes were noted. Some shedding of cells from the mucosal epithelium was seen and some capillary dilatation was noted in the lamina propria. These changes were seen in both infected and uninoculated control animals. No difference could be detected between changes seen in material from the infected and control animals.

Sections stained by Young's Method

Spiral microorganisms were seen in the crypts of the large intestinal mucosa from both infected and control mice. Spirochaetes were tentatively identified by their diameter and appearance in longitudinal section (Fig.5). They were only identified in sections from the infected mice and were present amongst the intestinal flora adjacent or attached to the epithelial cells of the colonic and caecal mucosa. Heavy deposits of silver were present within some epithelial cells. No lesions were obvious at the site of attachment of the spirochaetes (Fig. 6).

Bacteriological findings

(a) Direct examination

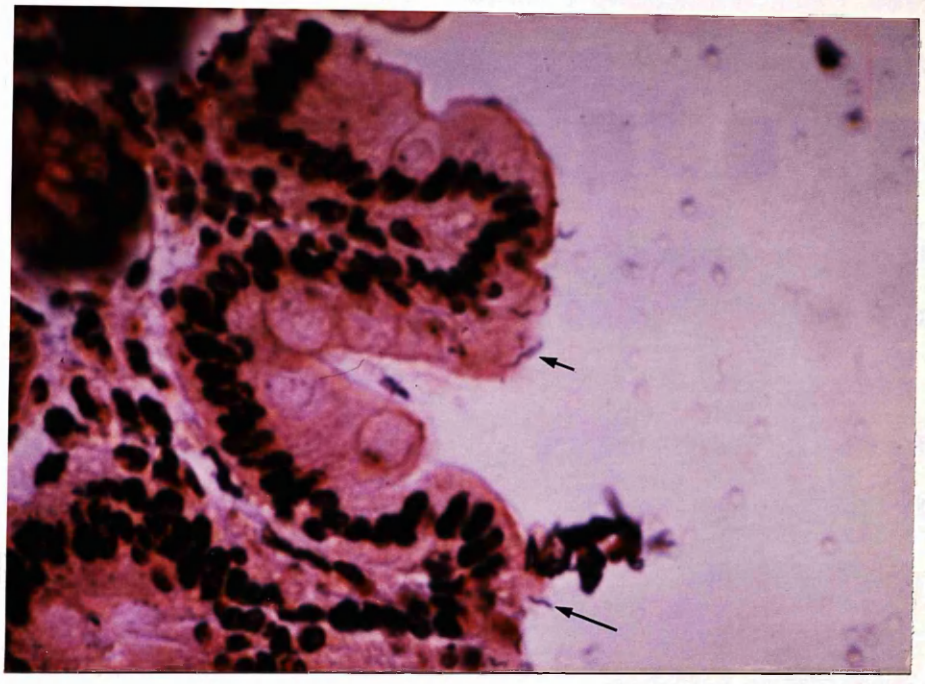
Spirochaetes with the morphology and motility of T. hyodysenteriae were seen in wet smears prepared

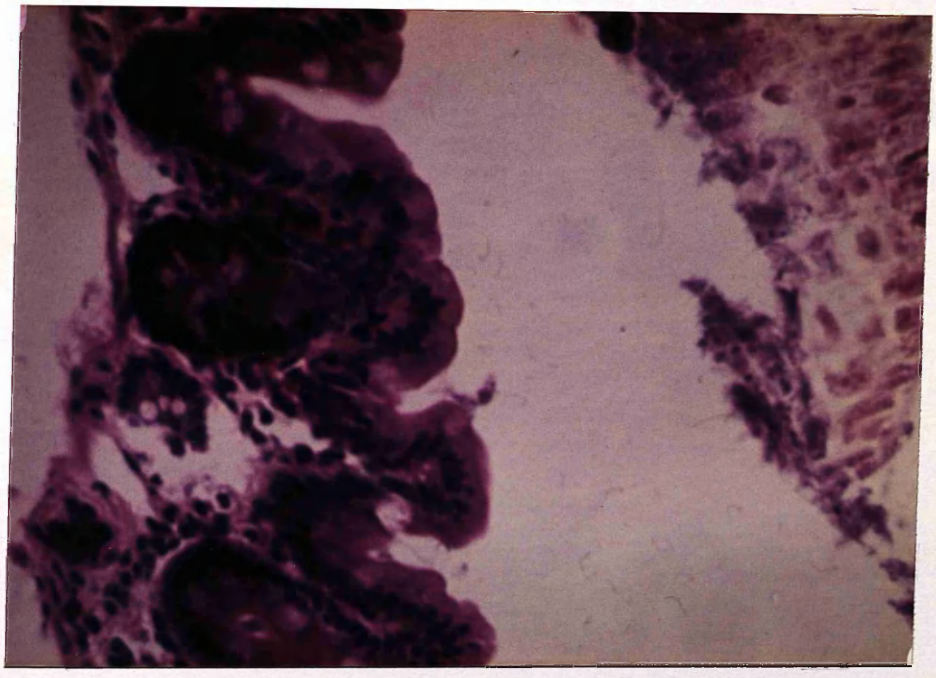
Fig. 5 Section of colonic mucosa from a mouse experimentally infected with Treponema hyodysenteriae.
Note the presence of spirochaetes(arrows) adjacent to the epithelium cells.

Young's x550

Fig. 6 Section of colonic mucosa from a mouse experimentally infected with Treponema hyodysenteriae.
The mucosa appears normal.

H & E x550





from the colonic and caecal contents of most of the inoculated mice. Fixed, Gram-stained smears of caecal contents are shown in Fig.7. They were also seen in preparations made by scraping the mucosa. None could be seen in material prepared from the uninoculated control mice.

The fine spirochaetes seen in faeces samples were also seen in samples of colonic and caecal contents from both groups of mice. Smears of faeces were examined by the fluorescent antibody method and no evidence for the presence of T. hyodysenteriae was found in either group of mice.

(b) Cultural examination

T. hyodysenteriae was isolated only from the caecal and colonic contents of the inoculated mice and not from the control mice (Figs.8 and 9). The organism was isolated from animals killed as early as Day 2 and as late as Day 14. Its identity was confirmed as T. hyodysenteriae by the use of the morphological, cultural and antigenic characters described in Chapter 2.

A detailed summary of these findings is given in Table 15.

Table 15. The results of examinations for *T. hyodysenteriae* carried out on large intestinal contents of the mice in Experiment 1

Group	Type of examination	Day													
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Direct	0/2[+]							1/2							
	Cultural	0/2							2/2							
2	Direct			1/2								0/2				
	Cultural			2/2								2/2				
3	Direct					0/2								1/2		
	Cultural					1/2								2/2		
4	Direct							1/2								0/2
	Cultural							2/2								1/2
5	Direct	0/2			1/2						0/2					1/2
	Cultural	0/2			0/2						0/2					0/2

[+] number positive is given as the numerator.

Fig. 7 Gram-stained smear of caecal contents from a mouse experimentally infected with T. hyodysenteriae.
A large number of spiral microorganisms are present. Spirochaetes with the morphology of T. hyodysenteriae(arrows) can be seen.

Gram x1350

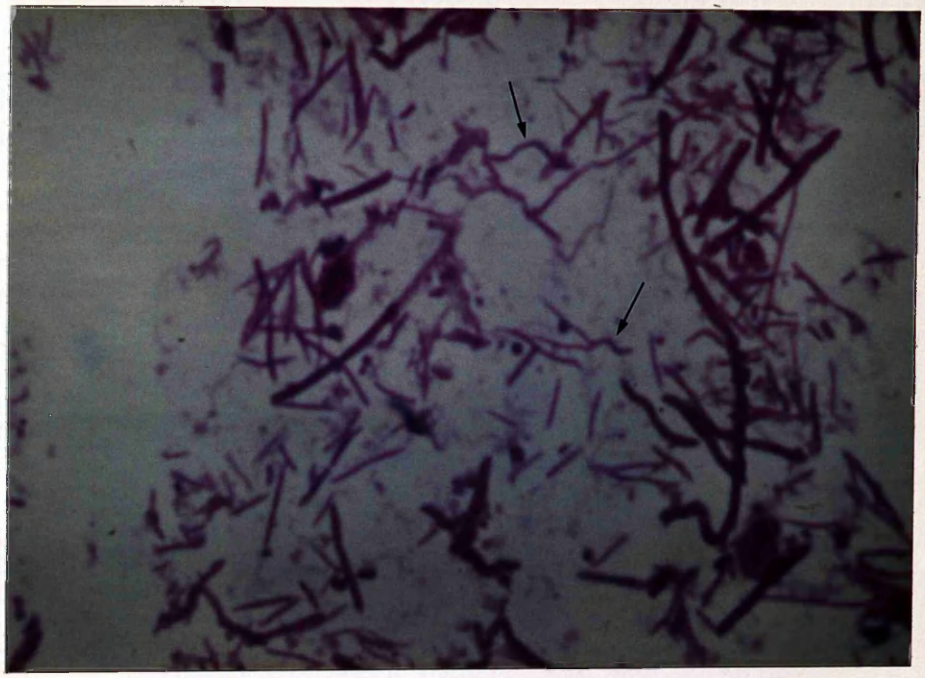
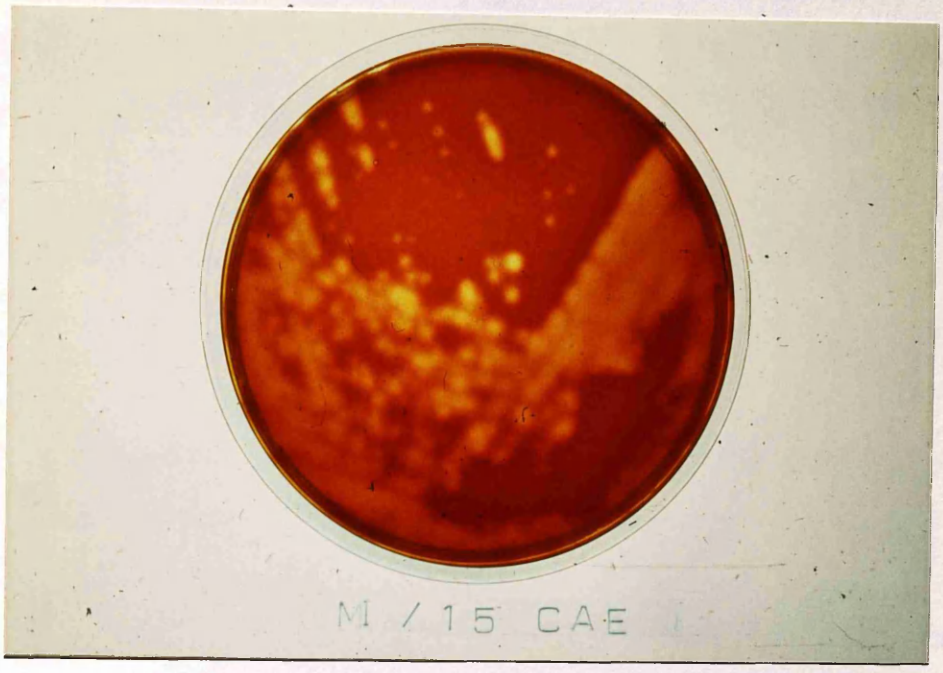


Fig. 8 Spectinomycin blood agar plate inoculated with caecal contents from an experimentally infected mouse.
Note the haemolytic areas of the colonies of T. hyodysenteriae.

Fig. 9 Spectinomycin blood agar plate inoculated with colonic contents from an experimentally infected mouse.
Note the faint areas of haemolysis along streak marks which indicate growth of T. hyodysenteriae.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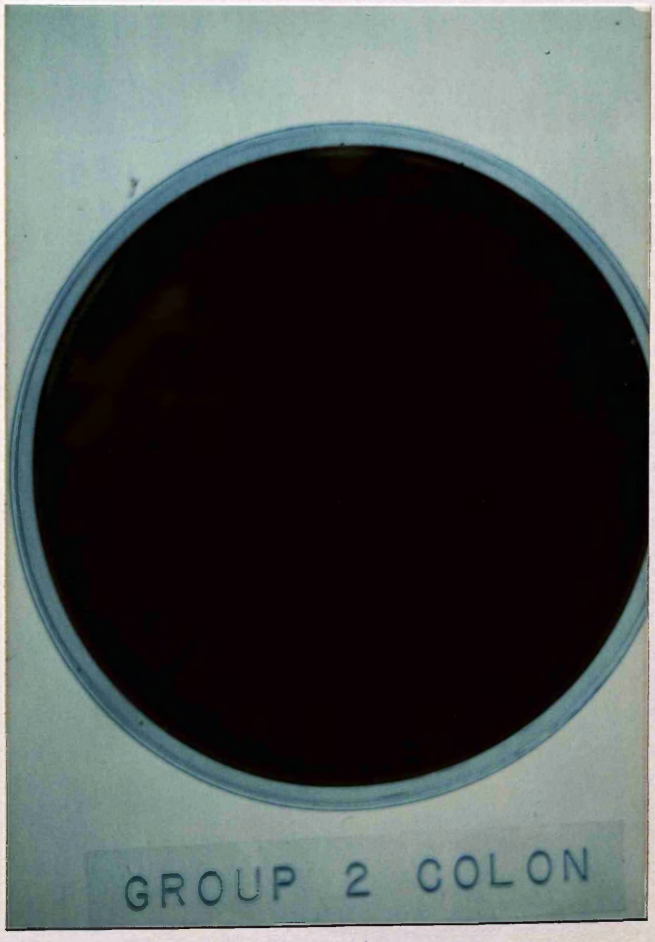

GROUP 2 COLON

Experiment 2

Clinical observations

The clinical observations resembled those made in Experiment 1. No abnormality was noted in either inoculated or control groups.

Faecal examination for the presence of *T. hyodysenteriae*

(a) Direct examination

Spirochaetes were only seen in faeces from the inoculated group of mice and appeared to have the morphology of *T. hyodysenteriae*. The occasions on which they were seen are recorded in Table 16.

(b) Cultural examination

T. hyodysenteriae was not isolated from the faeces of either group in this experiment.

Table 16. The results of the daily examination for *T. hyodysenteriae* carried out on pooled faeces from the mice in Experiment 2

Group	Type of examination	Day													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Infected	Direct	-	+	+	-	+	-	+	-	-	-	-	-	-	-
	Cultural	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Control	Direct	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Cultural	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Post-mortem examination

Gross findings

These were similar to those of Experiment 1 in that no gross changes were seen in the intestines of either inoculated or control groups.

Histological findings

The histological findings in this experiment resembled those of Experiment 1.

Bacteriological findings

(a) Direct examination

Spirochaetes with the morphology and motility of T. hyodysenteriae were seen in the colon and caecal contents of mice from both inoculated and uninoculated groups. The ones seen in the uninoculated control group could not readily be distinguished from T. hyodysenteriae by morphology. The occurrence of the organisms is shown in Table 17.

(b) Cultural examination

T. hyodysenteriae was isolated on one occasion only, on Day 2 from both mice killed on that day, and could not be recovered at any other time in the course of the experiment. The results are shown in detail in Table 17.

Table 17. The results of examinations for T. hyodysenteriae carried out on the large intestinal contents of the mice in Experiment 2

Group	Type of examination	Day				
		0	2	4	7	14
Infected	Direct*	-	2/2+	2/2	1/2	2/4
	Cultural	-	2/2	0/2	0/2	0/4
Control	Direct*	2/2	-	-	-	1/2
	Cultural	0/2	-	-	-	0/2

* Spirochaetes with the morphology of T. hyodysenteriae seen.

\+ number positive is given as the numerator.

Experiment 3

Clinical observations

The clinical observations resembled those made in Experiment 1 above. No abnormalities were noted in either the inoculated or uninoculated control groups.

(a) Direct examination

It was found that spirochaetes with morphology resembling that of T. hyodysenteriae were present in wet smears of the pooled faeces from both inoculated and uninoculated mice. The findings are given in Table 18.

(b) Cultural examination

T. hyodysenteriae was isolated from the faeces of the inoculated group on Day 3 only and never from the faeces of the uninoculated mice.

Small, clear, nonhaemolytic, spreading colonies

containing spirochaetes resembling T. hyodysenteriae were seen on spectinomycin blood agar plates inoculated with faeces from both inoculated and uninoculated groups. The colonies appeared within 48 hours of inoculation in some cases and as they aged, they expanded and produced faint haemolysis around the colonies. Surface growth was always very scant and difficult to see. The organisms were difficult to subculture and did not survive for more than two subcultures.

The colonial morphology and haemolysis produced by these spirochaetes was distinct from that produced by T. hyodysenteriae and when smears were stained with the specific antibody to T. hyodysenteriae in the fluorescent antibody test, there was no reaction. The results of the isolation studies are given in full in Table 18.

Post-mortem findings

(a) Gross findings

These were similar to those of Experiment 1 in that no gross changes were seen in the intestine of either the inoculated group or the uninoculated control group.

(b) Histological findings

The histological findings in this experiment resembled those of Experiment 1.

Bacteriological findings

(a) Direct examination

Spirochaetes with the morphology of T.hyodysenteriae

were seen in the large intestinal contents of mice of both groups. The detailed findings are given in Table 19.

(b) Cultural examination

T. hyodysenteriae was not isolated from the uninoculated control group but was isolated from the inoculated group between Days 2 and 14 inclusive. The mouse spirochaete described above was also isolated, from the large intestinal contents of both groups. The detailed results of the cultural examination are given in Table 19.

Table 18. The results of the positive examination for T. hyodysenteriae carried out on pooled faeces from mice in Experiment 3

Group	Type of examination	Day									
		2	3	4	8	12	14	19	20	22	26
Infected	Direct	*	-	*	*	-	*	-	-	*	*
	Cultural	-	+	MS	-	-	-	-	MS	-	-
Control	Direct	-	-	-	*	*	-	-	*	-	-
	Cultural	-	-	-	-	-	MS	MS	-	-	-

* : Large spirochaetes with the morphology of T. hyodysenteriae.

MS : mouse spirochaete isolated.

+ : T. hyodysenteriae isolated.

Table 19 The results of examinations for *T. hyodysenteriae* carried out on the large intestinal contents of mice from Experiment 3

Group	Spirochaetes	Day					
		0	1	2	4	6	8
Infected	*T. hyodysenteriae*	-	0/2+	2/2	2/2	0/2	1/2
	mouse spirochaete	-	1/2	0/2	0/2	2/2	0/2
Control	*T. hyodysenteriae*	0/2	-	-	-	-	0/2
	mouse spirochaete	1/2	-	-	-	-	0/2

(Table 19 continued)

Group	Spirochaetes	Day				
		10	12	14	21	28
Infected	*T. hyodysenteriae*	0/2	0/2	2/5	0/5	0/5
	mouse spirochaete	2/2	0/2	1/5	2/5	1/5
Control	*T. hyodysenteriae*	-	-	0/2	0/2	0/2
	mouse spirochaete	-	-	0/2	0/2	1/2

+ numerator = number of mice positive.

Part 2. Rat inoculation experiments

Materials and methods

The rat inoculation experiments were carried out using the materials and general methods described in Chapter 2 and in Chapter 4 Part 1 which describes the mouse inoculation experiments. A large intravenous cannula cut to 4-5 cm in length was used to stomach tube the rats which were also anaesthetised and inoculated by Dr. Taylor. The volume of inoculum used in this case was 0.8 ml of the saline suspension of T. hyodysenteriae described in Chapter 2.

Two experiments were carried out.

Experiment 1

28 rats were used in this experiment. They were divided into groups of 8 rats (uninoculated controls) and 20 rats (inoculated).

The concentration of T. hyodysenteriae in the inocular suspension was 1.0×10^9 organisms per ml and each rat received approximately 8.0×10^8 organisms.

The experiment lasted for 20 days and rats were killed according to the schedule given in Table 20.

Table 20. Schedule of the post-mortem examination of rats in Experiment 1

Group	Day									
	0	1	2	4	6	8	10	12	14	20
Inoculated (number killed)	0	2	2	2	2	2	2	2	2	4
Uninoculated (number killed)	2	-	-	-	2	-	-	2	-	2

Experiment 2

20 rats were used in this experiment, 14 were inoculated and 6 acted as uninoculated controls.

The concentration of T. hyodysenteriae in the inocular suspension was 1.2×10^8 organisms per ml giving about 9.6×10^7 organisms per rat.

The experiment lasted for 14 days and rats were killed according to the schedule given in Table 21.

Table 21. Schedule of the post-mortem examination of rats in Experiment 2.

Group	Day								
	0	1	2	4	6	8	10	12	14
Inoculated	-	-	2	2	2	2	2	2	2
Uninoculated control	2	-	-	-	-	-	-	-	4

Results

Experiment 1

Clinical observations

All rats appeared normal and healthy throughout the period of observation and no differences were noted in the condition, behaviour or faecal consistency of both inoculated and uninoculated groups.

Examination of the faeces for the presence of T. hyodysenteriae

No spirochaetes were seen by direct examination or detected by culture in the faeces of either group of rats in this experiment.

Post-mortem examination

(a) Gross findings

The gastrointestinal tracts and other internal organs of all the rats appeared macroscopically normal. There was no evidence of congestion or inflammation of the mucosa of the large intestines and no excess mucus was found. The contents of all the large intestines were normal in consistency and appearance.

(b) Histological findings

Sections stained by H & E

Desquamation of the cells of the mucosal epithelium of the large intestine was seen in sections from both groups of rats. There were no apparent differences between sections from both groups. Bacteria were present on the lumenal surface of the large intestine.

Sections stained by Young's Method

Spiral microorganisms were seen lying near the lumenal surface of the epithelium of the large intestinal mucosa and a few were seen in crypts. All those seen were shorter and larger in diameter than T. hyodysenteriae and there was no evidence for the presence of spirochaetes in these sections from both groups of rats.

Bacteriological findings

(a) Direct examination

No spirochaetes were seen in wet smears prepared from the colon or caecal contents of any rat in this experiment. Large numbers of spiral organisms distinct from spirochaetes were present.

(b) Cultural examination

T. hyodysenteriae was reisolated from the colon and caecal contents of rats from the infected group killed on the first and second days after inoculation. It was not isolated from any other rat in this experiment. No other spirochaetes were isolated. The results are summarised in Table 22.

Table 22. The results of examination for T. hyodysenteriae carried out on the large intestinal contents of the rats in Experiment 1

Group	Type of exam.	Day									
		0	1	2	4	6	8	10	12	14	20
Inoculated	Direct	-	0/2+	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/4
	Cultural	-	1/2+	2/2	0/2	0/2	0/2	0/2	0/2	0/2	0/4
Uninoculated control	Direct	0/2	-	-	-	0/2	-	-	0/2	-	0/2
	Cultural	0/2	-	-	-	0/2	-	-	0/2	-	0/2

+ numerator = number positive.

Experiment 2

No clinical differences could be observed between the inoculated and uninoculated control groups. No spirochaetes were seen in or isolated from the pooled faeces or the colon and caecal contents of these rats. There were no macroscopical or microscopical changes noted in rats of either group at post-mortem examination.

Part 3 Attempts to demonstrate the carriage of *T. hyodysenteriae* by flies

Three of the genera of flies commonly found in or near piggeries were used in this study. One species, *Stomoxys calcitrans* could not be obtained during the period of this study. Attempts to demonstrate the carriage of *T. hyodysenteriae* by these three genera were made in three separate experiments in the laboratory. In a further study, flies were captured in a piggery in which animals affected with swine dysentery were housed. These flies were then examined culturally for evidence of infection with *T. hyodysenteriae*.

Materials and methods

The flies in this studies were described in Chapter 2 and the method by which they were housed and maintained was also described. The flies used for Experiment 4 were captured in the experimental pig housing and killed on capture.

The inoculum and its preparation was described in Chapter 2 and the presentation of the inoculum was also described. When flies did not walk on the inoculum voluntarily, they were chilled to 5°C in a refrigerator and then, in a semi-comatose state, placed on the surface of the culture. *Musca domestica* in Experiment 3 were trapped against the culture plate and the polythene lid of the container in order to ensure that they were contaminated. Flies were exposed to the cultures for 30 minutes.

The presence or absence of carriage of *T. hyodysenteriae* by the flies was monitored by allowing flies to

have access to fresh spectinomycin blood agar plates. Flies would walk and feed on these plates which were exposed to them for 30 minutes at a time. The plates were then removed for incubation.

Flies were killed by crushing. The flies were then placed on a glass slide and an attempt was made to dissect out the gut for examination. Wet smears were prepared from this material and examined using phase contrast microscopy, and the remainder was used to inoculate spectinomycin blood agar plates. These were then incubated for growth of T. hyodysenteriae.

Experiment 1

12 flies of Lucillia spp. were divided into groups of 8 and 4. The group of 8 was exposed to T. hyodysenteriae and the group of 4 formed an uninoculated control group. The schedule for examination of surface contamination (by walking on plates) was as follows : each group was made to walk on sterile plates 30 minutes, 1, 2, 5, 10, 20 and 36 hours after exposure.

Post-mortem examinations were carried out at the intervals shown in Table 23.

Table 23. Schedule for the post-mortem examination of Lucillia spp. exposed to cultures of T. hyodysenteriae

Group	Number and time killed after exposure			
	5 hours	10 hours	20 hours	36 hours
Contaminated (8 flies)	2	2	2	2
Uncontaminated control(4 flies)	2	-	-	2

Experiment 2

Experiment 1 was repeated using 24 Calliphora spp. divided into a contaminated group of 20 and 4 controls. The presence of infection on the integument was measured at the times used in Experiment 1. Post-mortem examination was carried out at the times shown in Table 24.

Table 24. Schedule for the post-mortem examination of Calliphora spp. exposed to cultures of T. hyodysenteriae

Group	Number and time killed after exposure			
	5 hours	10 hours	20 hours	36 hours
Contaminated (20 flies)	5	5	5	5
Uncontaminated control(4 flies)	4	-	-	-

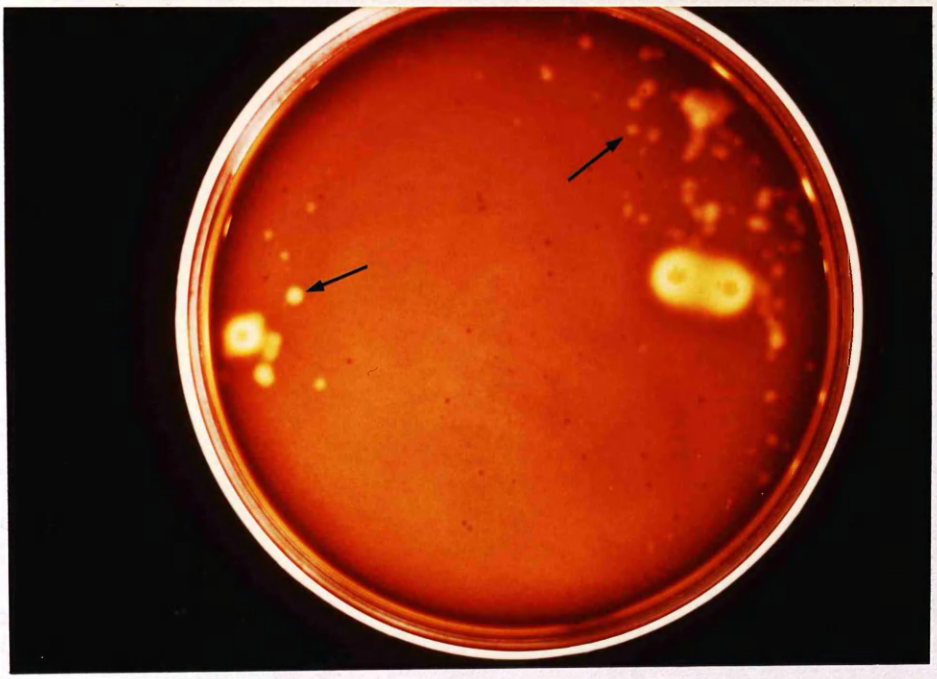
Experiment 3

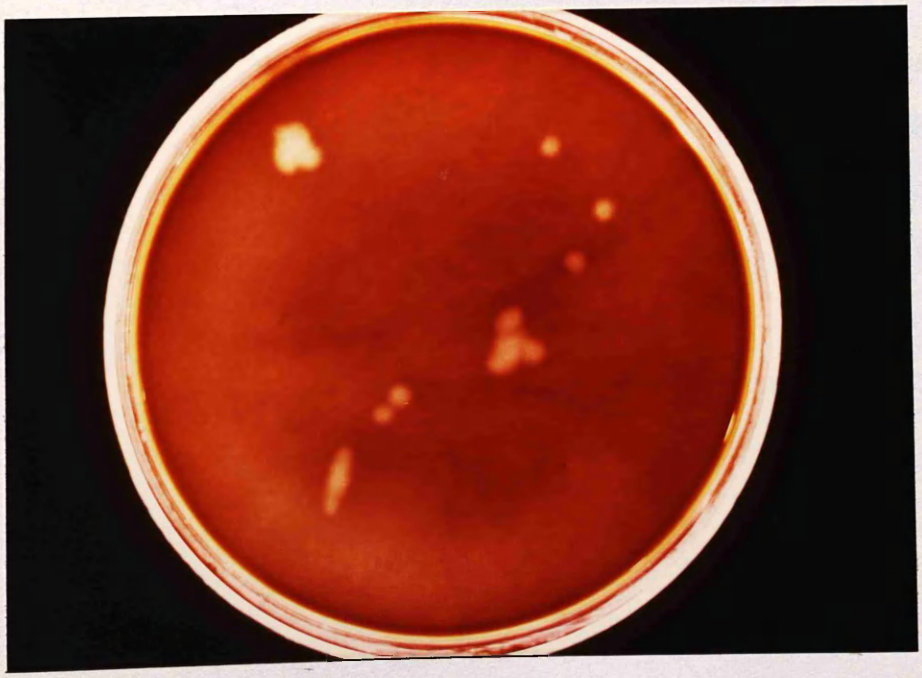
Experiment 1 was repeated using 16 Musca domestica divided into groups of 10 (contaminated) and 6 (control) flies. The presence of infection on the integument was measured at 30 minutes, 1, 2, 5,and 24 hours after exposure and post-mortem examination was carried out on half of each group at 5 hours and 24 hours after exposure.

Results

Experiment 1

T. hyodysenteriae was isolated from the integument of this group of flies 1/2 and 1 hour after exposure (Figs 10 and 11).





Immobile spirochaetes with the morphology of T. hyodysenteriae were seen in wet smears of the gut contents of these flies up to 24 hours after exposure but could not be cultured. The results are summarised in Table 25.

Table 25 Survival of T. hyodysenteriae in the gut of Lucillia spp. after exposure to pure cultures of the organism

Group	Type of examination	Time killed after exposure			
		5 hrs.	10 hrs.	20 hrs.	36 hrs.
Contaminated (8)	Direct	2/2 +	2/2	1/2	0/2
	Cultural	0/2	0/2	0/2	0/2
Control (4)	Direct	0/2	ND	ND	0/2
	Cultural	0/2	ND	ND	0/2

+ numerator = number positive.

Experiment 2

Similar results were obtained in this experiment. T. hyodysenteriae was isolated from the integument at 30 minutes and 1 hour after exposure and immobile spirochaetes resembling T. hyodysenteriae were seen in gut contents 5 and 10 hours after exposure. Motile spirochaetes were seen in the gut of 1 fly 5 hours after exposure. The results of this study are summarised in Table 26.

Table 26 Survival of T. hyodysenteriae in the gut of Calliphora spp. after exposure to pure cultures of the organism

Group	Type of examination	Time killed after exposure			
		5 hrs.	10 hrs.	20 hrs.	36 hrs.
Contaminated (20)	Direct	3/5+	2/5	0/5	0/5
	Cultural	0/5	0/5	0/5	0/5
Control (4)	Direct	0/4	ND	ND	ND
	Cultural	0/4	ND	ND	ND

+ numerator = number positive.

Experiment 3

In this experiment T. hyodysenteriae was reisolated from the contaminated Musca domestica 30 minutes after exposure to cultures of the organism but could not be demonstrated either directly or in culture after this period. It could not be seen in or cultured from the gut of exposed flies.

Experiment 4

This experiment involved the direct and cultural examination of flies caught in a piggery containing pigs with swine dysentery. Two flies of Calliphora spp. and twelve Musca domestica were examined. No spirochaetes were seen in their intestinal tracts or isolated from them.

Discussion

The results of the attempts to demonstrate the survival of T. hyodysenteriae in animals other than the pig met with mixed success. The organism was found to survive best in mice in which it persisted for up to 14 days. No clinical or pathological abnormalities were detected in the infected mice and it appears from the results of the experiments described in Part 1 that infection of the laboratory mouse with T. hyodysenteriae is asymptomatic.

T. hyodysenteriae was isolated from the caecum and colon of infected mice and less frequently, from the pooled faeces samples. Isolation of T. hyodysenteriae from the faeces was achieved up to 14 days after inoculation (Experiment 1) but failed in Experiment 2 and was successful only once in Experiment 3 (on Day 3). These uneven results may reflect the few occassions on which infection was demonstrated in the mice of these two experiments when compared with the number of infected mice found in Experiment 1. In addition, the procedure adopted for monitoring the faeces was probably unsatisfactory in the light of the results of the drying experiment described in Chapter 3. Faeces used for sampling were almost all dry and the number of fresh, moist ones in which T. hyodysenteriae may have survived was probably quite small. In spite of these technical problems, the experiments demonstrated conclusively that laboratory mice could pass T. hyodysenteriae in their faeces for up to 14 days after infection.

The recovery of T. hyodysenteriae from the caecum and colon of infected mice was more successful than recovery from the pooled faeces samples. The reasons for the larger

number of infected mice in Experiment 1 was uncertain as the inoculum contained approximately the same number of organisms in each case. It is possible, therefore, that the difference in batches of mice was important in determining the success of the infection, particularly in Experiments 1 and 2 in which the same strain of mice was used.

A spirochaete distinct from T. hyodysenteriae was found in the mice in Experiments 2 and 3 but was either absent from or was not noticed in the mice of Experiment 1. This organism could not always be distinguished from T. hyodysenteriae by its appearance in wet smears, but in culture it produced very different colonies, non-haemolytic with more surface growth and extremely difficult to subculture. In smears stained by the fluorescent antibody technique it did not fluoresce, either in material from culture or in gut smears. It is probable that this spirochaete is similar to that described in normal mice by Gordon and Dubos(1970), Lee et al.(1971) and Savage et al.(1971). Whether the presence of this organism affected the success of the experimental infections is not clear, but it could not be confused with T. hyodysenteriae except in wet smears and in silver-stained histological sections.

The histological sections prepared from the caecae and colons of the infected mice appeared to be normal and no differences could be detected between sections prepared from control mice or from those from which T. hyodysenteriae was isolated. Spirochaetes could be identified adjacent to the mucosal epithelium in some sections but there was no evidence to suggest that the organisms seen

were T. hyodysenteriae and they were never identified in the close association with tissue cells found in swine dysentery. It appears, therefore that T. hyodysenteriae did not invade the mucosa of infected experimental mice but that it remained within the lumen of the large intestine.

The attempts to infect rats with cultures of T. hyodysenteriae were less successful than the mouse experiments described above. T. hyodysenteriae was recovered from inoculated rats until Day 2 of Experiment 1 but could not be recovered from any rat in Experiment 2.

The organism was not recovered from faeces, perhaps for the reasons discussed above for mouse faeces, in particular the dry nature of the faecal pellets and the low number of infected rats in the group. It was, however, of interest to note that T. hyodysenteriae could survive for 48 hours in the gut of the rat even though permanent colonisation did not take place in this study. It is possible that the inoculum given contained insufficient organisms to allow adequate infection to take place or the rat may be relatively resistant to colonisation by this organism.

The results of the fly experiments indicated that T. hyodysenteriae could be recovered from the integument for up to 1 hour in the case of the large flies, Calliphora and Lucillia spp. and for 30 minutes in the case of the smaller fly, Musca domestica. The flies were exposed to cultures of T. hyodysenteriae only and it may be that exposure to faeces would have resulted in longer survival of the organism on the flies' integument. No

spirochaetes could be cultured from the intestinal tract of any fly exposed to plate cultures, perhaps for technical reasons. In one case a motile spirochaete was seen in gut contents of a Calliphorine fly 5 hours after exposure to T. hyodysenteriae cultures and it may be that a small number of organisms persist in the gut. Ingestion of dysenteric faeces might possibly improve this chance of survival in the gut. On the one occasion on which flies could be captured in an infected piggery, T. hyodysenteriae could not be recovered from their bodies, again possibly for technical reasons or because the number of flies caught and examined was too small.

Chapter Five

Survival of *Treponema hyodysenteriae* in the pig

The evidence for the survival of *T. hyodysenteriae* in pigs recovered from swine dysentery has been reviewed in Chapter 1. There is little information about the persistence of the organism in recovered animals although the persistence of infectivity is well documented and has been reviewed. The work of Songer *et al.*(1976) is the only cultural study described in the literature todate in which the isolation of *T. hyodysenteriae* from asymptomatic carrier animals has been carried out.

The use of spectinomycin blood agar has facilitated the isolation of *T. hyodysenteriae* from faeces and allowed the limited study described here to be carried out.

This study was intended to monitor the clinical signs of swine dysentery and the persistence of *T. hyodysenteriae* in the faeces of pigs with the acute disease and for some time after recovery. The small number of pigs used was limited by the cost of the animals and of their maintenance.

Materials and Methods

The pigs used, the method of infection, and the housing and diet used are described in Chapter 2. Five animals were used in this study, numbered 138, 139, 142, 145 and 146. They were inoculated at the same time as other animals used in another trial and formed part of a group of 29 animals. These five animals were the last members of the group to develop swine dysentery and were surplus to the requirements

of the main study.

The affected group was observed daily and their clinical condition noted. The condition of their faeces was recorded on the days on which cultures were taken (Table 27).

Rectal swabs were taken from each animal and examined by culture for the presence of T. hyodysenteriae.

The study was terminated 96 days after the last inoculation. Euthanasia and post-mortem examination were carried out by the methods described in Chapter 2.

Results

Clinical observations

All five pigs developed clinical signs of swine dysentery although these were mild in the case of pig 146. The duration of the clinical signs in the group is shown in Table 27, beginning on the 9th day post inoculation and ending with the death of pig 145 on the 62nd day after inoculation. The other four animals had recovered within 42 days of the appearance of the disease in the group.

Recovered animals were vigorous, had a good appetite and passed faeces of normal consistency. Their bodily condition improved throughout the period following clinical recovery.

Examination of rectal swabs for the presence of T. hyodysenteriae

T. hyodysenteriae was isolated from the pigs on the days shown in Table 27. The organisms were identified by the criteria outlined in Chapter 2.

Table 27 The results of clinical observations and the cultural examination for *T. hyodysenteriae* on the experimental pigs

Pig No.	Faecal examination	Day after last inoculation							
		9	12	18	20	24	27	32	34
138	Appearance	MD	MD	MD	LF	LF	MD	LF	F
	Culture for *T. hyodysenteriae*	+	+	+	+	+	+	+	+
139	Appearance	F	F	LF	MD	MD	MD	MD	MD
	Culture for *T. hyodysenteriae*	+	-	+	+	+	+	+	+
142	Appearance	F	MD	MD	MD	MD	MD	BMD	MD
	Culture for *T. hyodysenteriae*	+	+	+	+	+	+	+	+
145	Appearance	LF	MD	MD	LF	LF	MD	MD	MD
	Culture for *T. hyodysenteriae*	+	+	+	+	+	+	+	+
146	Appearance	MD	MD	MD	LF	LF	F	F	LF
	Culture for *T. hyodysenteriae*	+	+	+	+	+	+	-	+

(continue Table 27)

	41	52	54	62	68	76	80	83	87	90	94	96
138	MD	LF	F	F	F	F	F	F	F	F	F	F
	+	+	+	-	-	-	-	-	-	-	-	-
139	LF	F	F	F	F	F	F	F	F	F	F	F
	+	+	+	+	+	+	+	+*	-	-	-	-
142	LF	LF	F	F	F	F	F	F	F	F	F	F
	+	+	+	+	+	+	+	+	+	-	+	+
145	BMD	BMD	BMD	died								
	+	+	+									
146	F	F	F	F	F	F	F	F	F	F	F	F
	+	-	-	-	-	-	-	-	-	-	-	-

F : firm faeces LF : loose faeces M : mucus present
B : blood present D : diarrhoea
* : Non-pathogenic spirochaetes isolated

On one occasion (Pig 139, Day 83), a mixed culture of T. hyodysenteriae and another spirochaete was isolated. The other spirochaete produced patches of slight discolouration in the blood of the medium after 48 hours incubation. The colonies were clear and flat with more surface growth than was seen in adjacent colonies of T. hyodysenteriae from which they were readily distinguished by the marked beta-haemolysis produced by the latter. When examined by the indirect fluorescent antibody test using the reagents described in Chapter 2 they did not fluoresce. Upon subculture they maintained the colonial characters noted in the isolation plate.

There did not appear to be any morphological differences between these organisms and T. hyodysenteriae in wet smears.

Post-mortem examination

Gross findings

Pig 138 The condition of this animal was fair and no changes were noted in the internal organs except for the presence of a few fibrin or fibrous tags on the serosal surface of the colon. The colonic contents and mucosa were normal in appearance.

Pig 139 This pig was in good condition. There were old lesions of enzootic pneumonia in the dependent anterior lobes of the lung but all other organs appeared normal. The large intestine and its contents and mucosa were normal in appearance.

Pig 142 This animal was in fair condition and the lungs, liver and other internal organs all appeared normal. The

serosa of the large intestine and its contents appeared normal, but faeces was found adhering to the mucosal surface (Figs 12 & 13) and in one area 10 x 15 cm, there was slight prominence of individual blood vessels.

Pig 145 This animal was emaciated and had been frozen before examination. The lungs, liver and other abdominal organs appeared grossly normal but the large intestine was flaccid and the contents were reddish-grey and liquid in consistency. The mucosa was swollen and covered in thick layers of whitish mucus.

Pig 146 The condition of this animal was good and all the internal organs appeared to be normal. The large intestinal serosa, contents and mucosa all appeared normal.

Histological findings

Pig 138

H & E The most prominent feature of the mucosa of this animal was the cellular infiltrate present in the lamina propria. This infiltrate was composed mainly of macrophages and plasma cells. There was some dilatation of the mucosal capillaries and in some areas there was loss of cells from the mucosal epithelium, in particular at the lumenal surface. In places this epithelium was low and cuboidal. A general low power view of the mucosa may be seen in Fig. 14.

Young's stain A few dilated crypts near the muscularis mucosae were seen to contain spirochaetes (Fig. 15). No spirochaetes were seen at the mouth of the crypts along the lumenal epithelium or in the cells lining the crypts.

Pig 139

H & E The colonic mucosa of this animal was also infiltrated with mononuclear cells and some slight capillary dilatation was also noted. No epithelial cell desquamation from the lumenal surface was seen in the sections examined but a layer of debris comprised of bacteria and desquamated cells was lying adjacent to the mucosal epithelium in one of the four sections examined (Fig. 16).

Young's stain Thin faint spirochaetes and some thicker, well-stained spirochaetes were seen both at the mouths of some crypts and lower down in others. Spirochaetes were very localised (Figs 17 & 18).

Pig 142

H & E Considerable cellular infiltration with mononuclear cells was present in all sections from the colonic mucosa of this pig. Capillary dilatation was not marked. The luminal surface of the mucosa was covered with flattened cuboidal cells in some areas and in others desquamation of epithelial cells was seen, appearently associated with bacterial invasion. A thick layer of bacteria and debris was adjacent to the lumenal epithelium.

Young's stain Faintly-staining spirochaetes were seen in 1 or 2 crypts but none could be identified in association with the bacterial layer adhering to the mucosal epithelium.

Pig 145 No histological sections were prepared from this animal as it had died and had then been frozen pending examination.

Pig 146

H & E Marked cellular infiltration of the lamina propria was present in sections from the colon of this animal. In some areas desquamation of the lumenal epithelium may have occurred, there was slight capillary dilatation and a surface layer of bacteria and cell debris was also present adjacent to the mucosal epithelium.

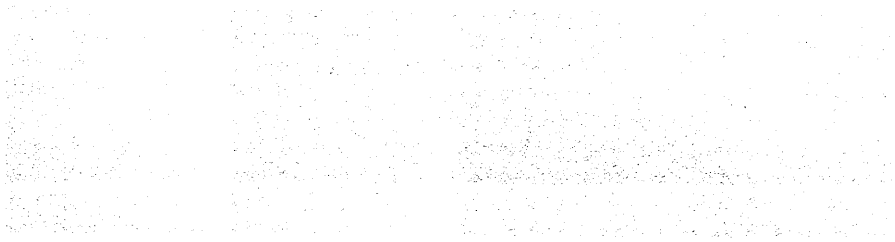
Young's stain Spiral microorganisms could be seen in some crypts but none could be identified as spirochaetes.

Examination for T. hyodysenteriae

T. hyodysenteriae was isolated from the mucosa of all five animals. The results of examinations of the colonic mucosa of these animals for T. hyodysenteriae are shown in Table 28.

Fig. 12 General view of the washed colonic mucosa of Pig 142.
Note the pale, normal appearance of the mucosa and the attached faeces.

Fig. 13 Close up view of an area of washed colonic mucosa from Pig 142.
Note the patches of adherent faeces.



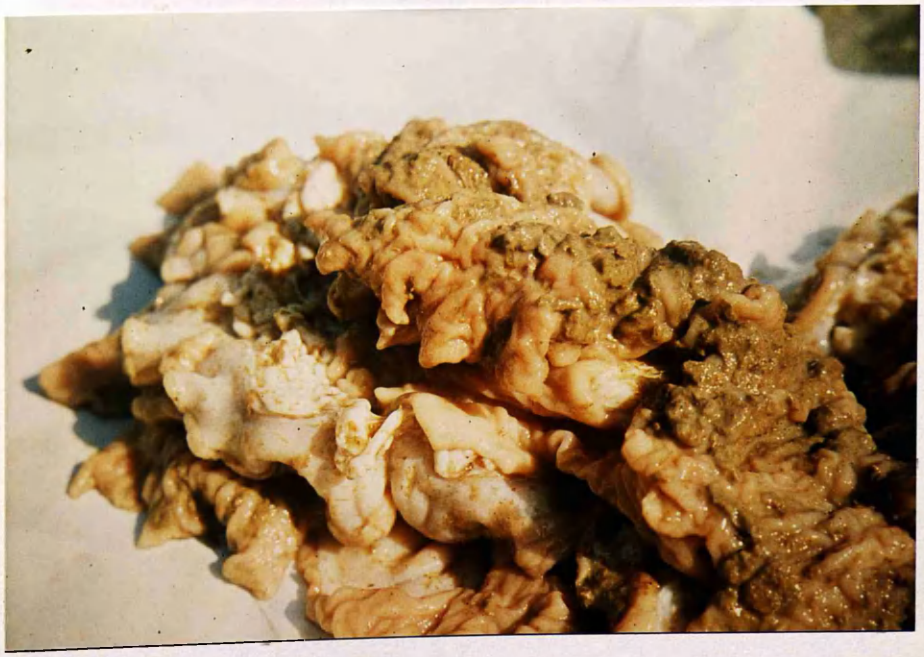
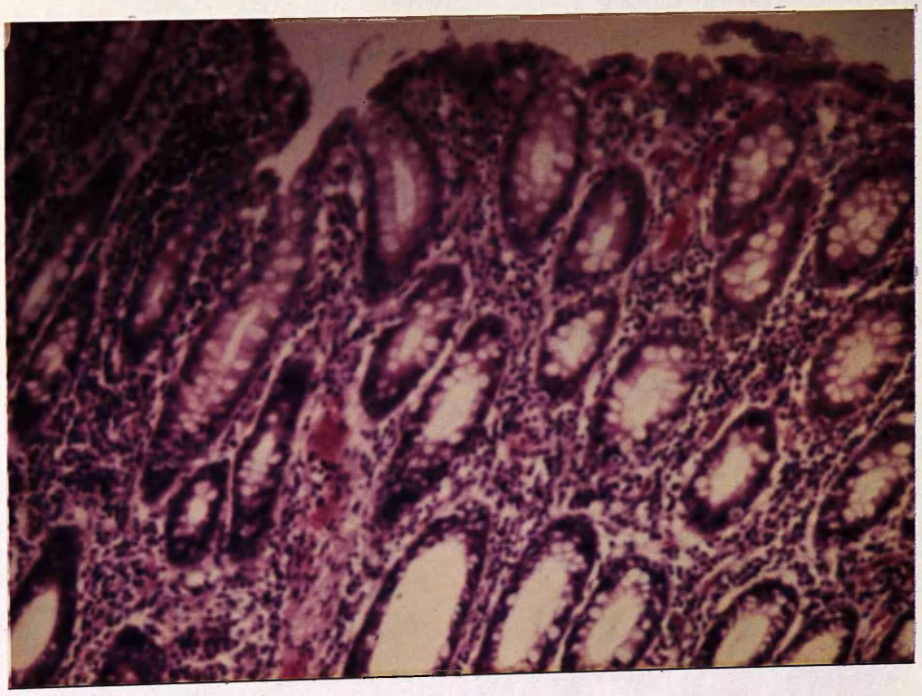


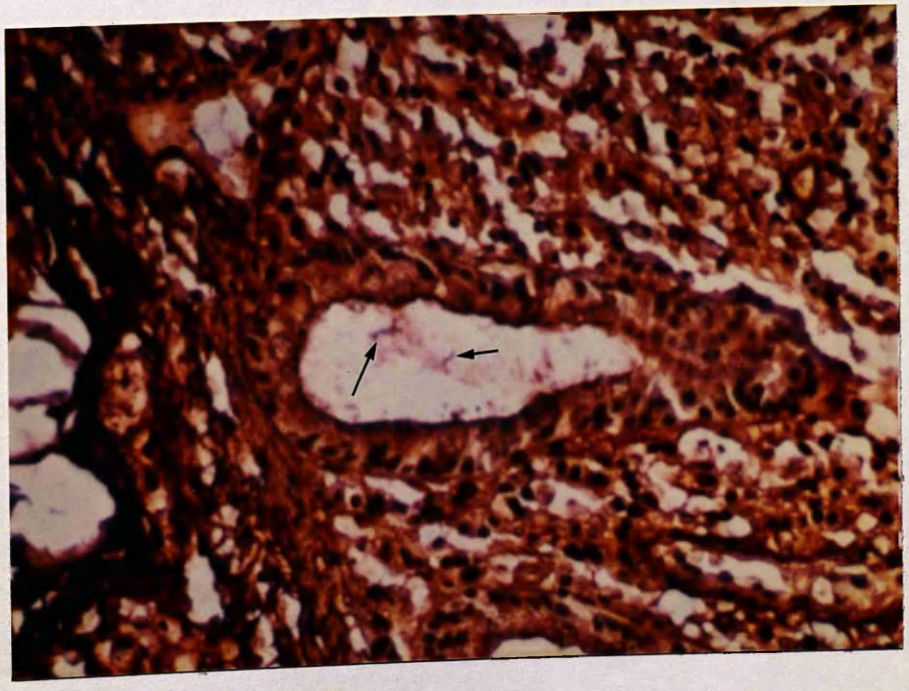
Fig. 14 Low power view of a section of colonic mucosa from Pig 138.
Note the areas of capillary dilatation, and the dilated crypts. Cell shedding is present but is difficult to identify at this point.

H & E x135

Fig. 15 Section of colonic mucosa of Pig 138.
Note dilated crypt with spirochaetes (arrows) inside.

Young's x340





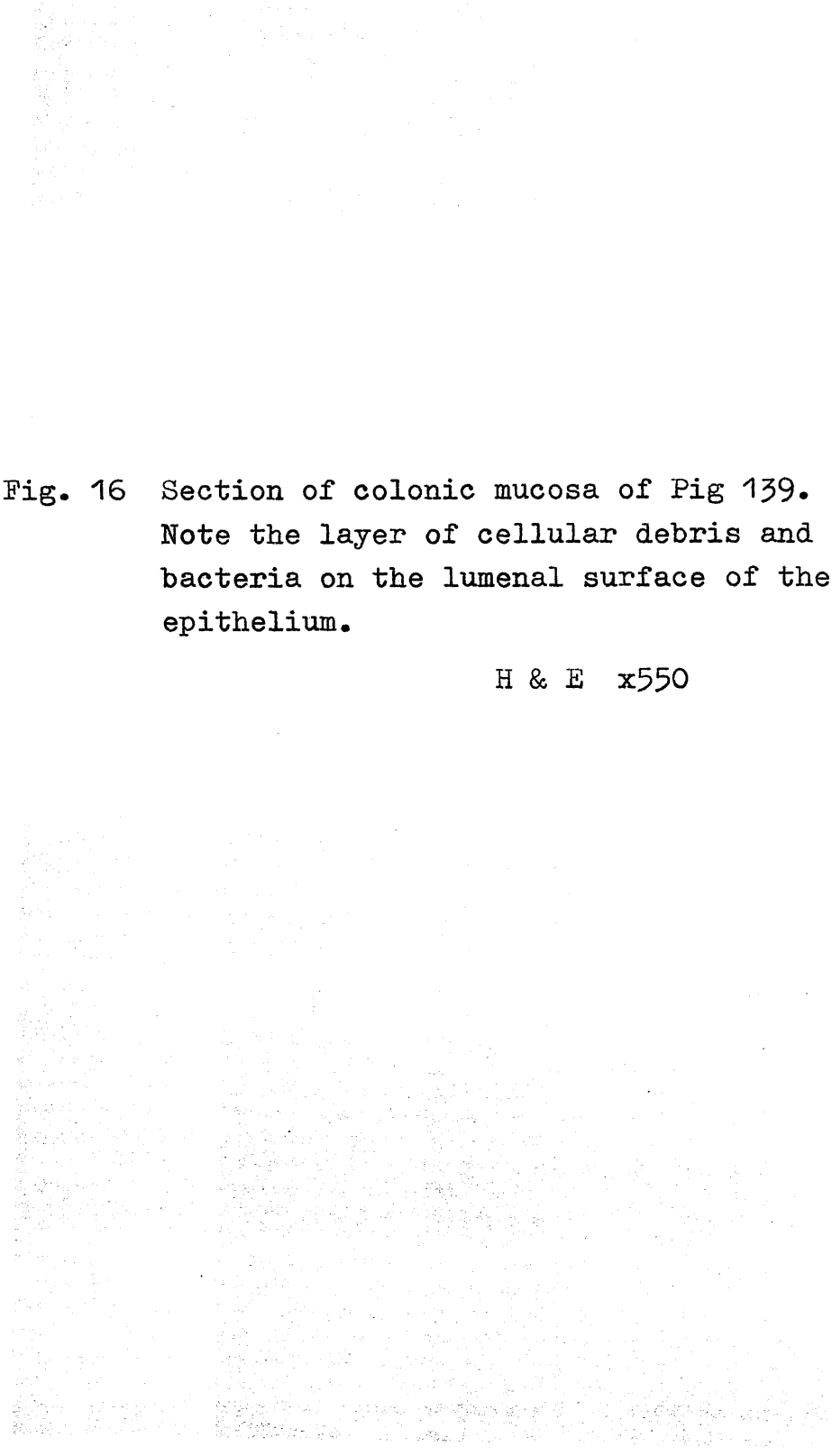


Fig. 16 Section of colonic mucosa of Pig 139. Note the layer of cellular debris and bacteria on the lumenal surface of the epithelium.

H & E x550

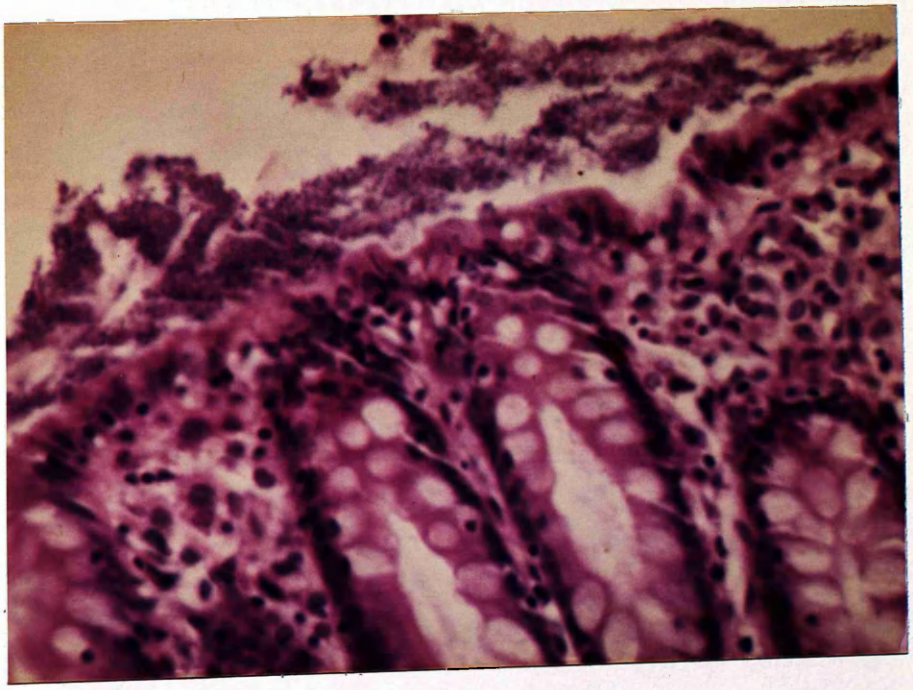
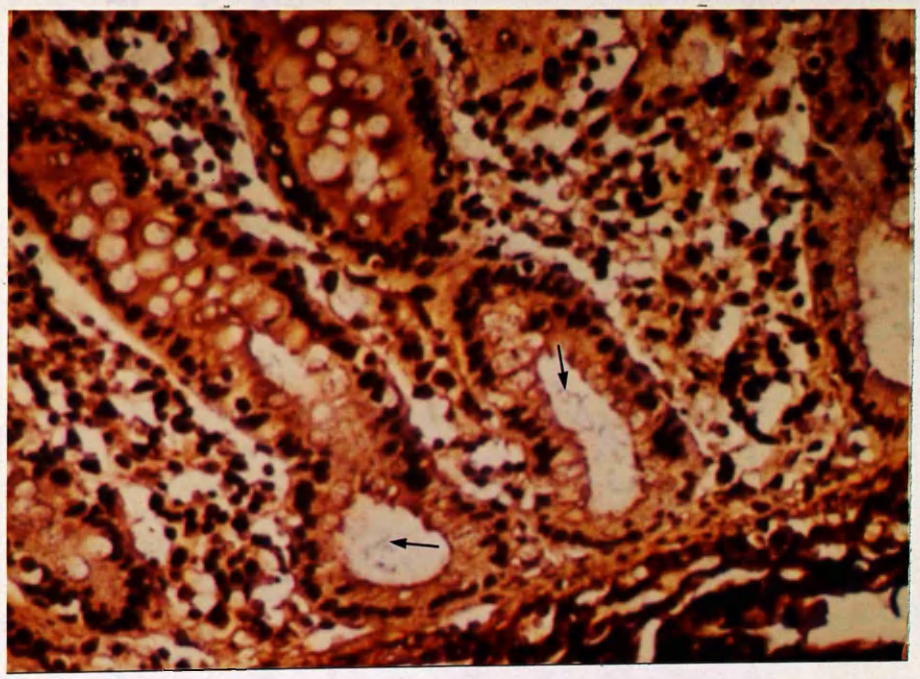


Fig. 17 Section of colonic mucosa of Pig 139.
Note dilated crypts with spirochaetes
(arrows) inside.

Young's x340

Fig. 18 A high power view of the section shown
in Fig. 17.
Note the presence of spirochaetes(arrows)
within the dilated crypts.

Young's x550



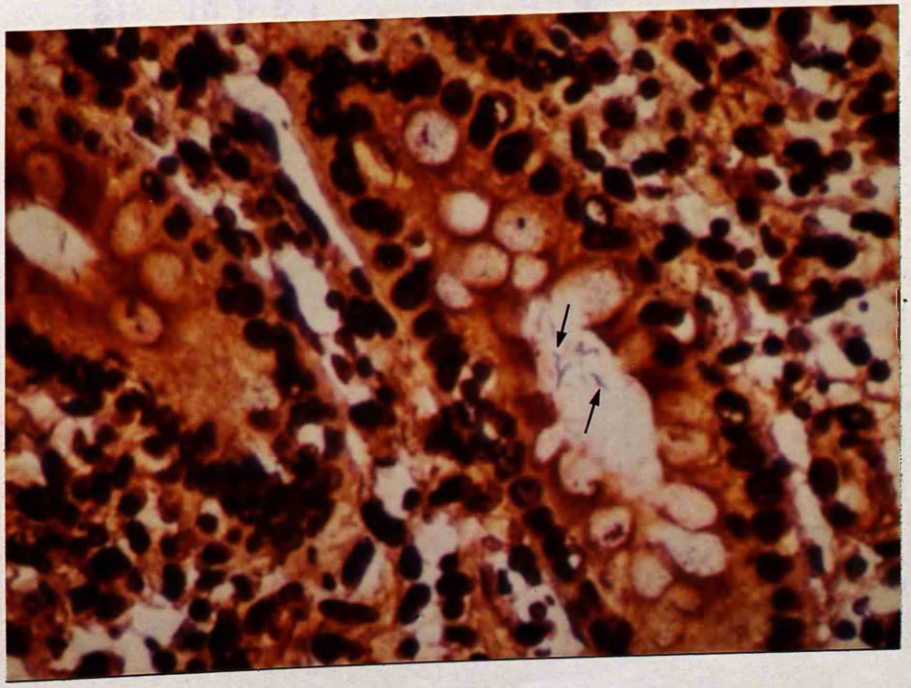


Table 28. Summary of the findings in the experiment to determine the survival of T. hyodysenteriae in the pig.

Pig No.	Period of observation (days*)	Incubation period (days)	Course of diarrhoea (days)	Type of diarrhoea.	Period during which T. hyodysenteriae isolated (days)	Isolation of T. hyodysenteriae from large intestine
138	96	9	9	M	54	+
139	96	20	14	M	83	+
142	96	12	22	MB	96	+
145	62	12	50+	MB	62	+
146	96	9	8	M	41	+

* = days after last inoculation

M = mucus present

B = blood present

\+ = died in the course of disease

Discussion

The results of the study described in Chapter 5 above were obtained from a small number of pigs only, but are of general interest in the study of the carriage of T. hyodysenteriae by the recovered pig.

The isolation of T. hyodysenteriae from clinically recovered pigs was particularly interesting. Four untreated pigs in this study survived the disease and recovered completely. The results given in Table 27 and summarised in Table 28 showed that T. hyodysenteriae could still be isolated from the faeces for the following length of time : 36 days(Pig 138), 49 days(Pig 139), 62 days(Pig 142), and 23 days(Pig 146). These findings were based upon single rectal swabs taken at the intervals shown above, but the results emphasis that every recovered pig does not remain a persistent excretor of T. hyodysenteriae.

Further studies using samples taken daily, perhaps on more than one occasion, would demonstrate whether the low frequency of sampling in this study was responsible for the poor recovery of the organism noted above.

T. hyodysenteriae could be recovered from the faeces of one pig(142) throughout the period of observation, i.e. for 62 days after clinical recovery had occurred. This animal appeared healthy but at post-mortem examination was found to be in poorer condition than pigs 139 and 146 which were in extremely good condition. The continued recovery of T. hyodysenteriae from the faeces of this pig indicates that shedding of the organism in the faeces may continue for more than 62 days after clinical recovery, but the enforced termination of the experiment at this point made

it impossible to determine the maximum possible duration of faecal shedding of the organism.

The recovery and identification of a 'non-pathogenic' type of spirochaete from one of these animals on the same plate as T. hyodysenteriae was of considerable significance in the interpretation of results from cultural examinations using spectinomycin blood agar. The colonies of the 'non-pathogenic' spirochaete were distinctive under the conditions of isolation used in this study, but in less optimum conditions, for example where haemolysis of the blood had occurred or where no T. hyodysenteriae colonies were available for comparision, this finding might have been mistaken for an isolate of T. hyodysenteriae. The use of the fluorescent antibody test proved of considerable value in establishing the difference between the two isolates.

Pig 145, which died 62 days after inoculation, was found at post-mortem examination to have lesions characteristic of chronic swine dysentery. In contrast to the findings in Pig 145, the four recovered pigs did not have gross or microscopical lesions suggestive of previous infection with swine dysentery. In Pig 142 the presence of faeces adherent to the mucosa suggested that some disturbance in large intestinal function had occurred, but none of the features of classical swine dysentery lesions were seen. In the histological sections of large intestinal mucosa examined, individual features indicating slight abnormality of the mucosa were seen. These included the marked infiltration of the lamina propria with mononuclear cells, the cuboidal appearance of the lumenal epithelium, the adherence of bacteria to this epithelium

and the occasional shedding of cells from the lumenal surface. Dilated crypts with low epithelium were seen deep in the lamina propria in some cases. In silver-stained sections spirochaetes were seen in the crypts of the colonic mucosa from three pigs (Pigs 138, 139 and 142). It was not possible to identify these as T. hyodysenteriae and they were not associated with any obvious lesions.

In contrast to the non-specific findings of the gross and histological post-mortem examinations, T. hyodysenteriae was isolated from the colonic mucosa of all recovered pigs. Only one of the four animals (Pig 142) was found to be shedding the organism in its faeces at the end of the experiment. T. hyodysenteriae had last been demonstrated in the faeces of the other three recovered pigs 55 days (Pig 146), 42 days(Pig 138), 13 days(Pig 139) prior to slaughter. It thus appears that culture of the colonic mucosa is a more reliable method of identifying pigs infected with T. hyodysenteriae than is the culture of rectal faeces swabs.

The post-mortem results discussed above also indicate that all four recovered pigs were still infected with T. hyodysenteriae and that all were still carrying the organism at the end of the experiment, i.e. 78 days after clinical recovery(Pig 138), 62 days(Pig 139 and Pig 142) and 79 days(Pig 146).

Chapter Six

General Discussion

The ability of T. hyodysenteriae to survive under different conditions is of importance in understanding the spread of the disease and in designing efficient programmes for its treatment and control. The results of the studies of the survival of T. hyodysenteriae in faeces under various conditions, in farm pests and in the carrier pigs reported in Chapters 3, 4, and 5 allow a re-appraisal of the epidemiology of swine dysentery to be made.

It is clear that from the literature reviewed in Chapter 1 that the faeces of infected pigs is the source of infection in swine dysentery. The majority of outbreaks result from the introduction of infected pigs to a farm or a pen. These animals are rarely clinically affected and are usually incubating the disease or are recovered carriers. The infected carrier pig is probably the major reservoir of infection within the herd and some authors such as Whiting et al.1921 have emphasised the persistence of infection in sows, and that recovered pigs remain infectious for up to 28 days after clinical recovery (Terpstra et al.1968). In most of these cases, susceptible pigs have access to freshly voided faeces from infected animals and the question of the ability of T. hyodysenteriae to survive outside the animal body does not arise. In some circumstances, however, the survival of the agent in faeces is important, especially when infected faeces contaminates drainage channels or drinking

water (Taylor,1972) or when infection is transmitted on boots or implements (Terpstra et al.1968). Another critical time in transmission is the period required for a depopulated pen, building or farm to become free from infection and the survival of T. hyodysenteriae under conditions which may be found during cleaning is discussed below.

Finally, the survival of T. hyodysenteriae in hosts other than the pig has only been recorded once, in the guinea pig (Joens et al.1976b). The significance of the findings described and briefly discussed in Chapter 4, that T. hyodysenteriae can survive for varying lengths of time in mice, rats and flies is of considerable theoretical interest but is probably only of minor importance in the transmission and maintenance of swine dysentery in this country.

The survival of T. hyodysenteriae in the recovered pig.

T. hyodysenteriae is shed in large numbers in the faeces of pigs with clinical swine dysentery. There is little or no information on the numbers of organisms present in dysenteric faeces except that Kinyon(1974) gave a figure of 10^5-10^9 organisms per gram of colonic mucosa from pigs with swine dysentery. The one count performed in this study(Chapter 3) gave a figure of 10^7 colony-forming units per gram of faeces, suggesting that the number of organisms present in faeces may be similar to those present in the colonic mucosa. No attempt was made in this study to monitor the faecal shedding of T. hyodysenteriae by pigs in the incubation period.

The recovered pig was studied in most detail as it is particularly important in the transmission and maintenance of the disease. The only previous information about the presence of T. hyodysenteriae in clinically-normal animals is the work of Songer et al.(1976) in which the organism was isolated from faeces of farrowing sows and their piglets. The isolation of T. hyodysenteriae from the faeces of recovered pigs described in Chapter 5 indicates that this organism is shed for a variable period after clinical recovery, and that some pigs may shed the organism for up to 62 days after recovery. Any animal shedding T. hyodysenteriae in this way must be a source of infection for other animals. The shedding of T. hyodysenteriae by subclinically-affected animals could not be examined in this study because all the animals used developed clinical signs of swine dysentery. In the field, the presence of immunity in herds where the disease is enzootic and the widespread use of drugs in the prevention and treatment of swine dysentery means that subclinical infection is widespread and that further study of this type of animal should be carried out.

T. hyodysenteriae could not be demonstrated in the faeces of three recovered pigs after varying periods following recovery. These animals were found to be infected when the colonic mucosa was examined at post-mortem examination. This result implies that single rectal faeces swabs may not identify all infected carrier animals and suggests that the cultural examination of the colonic mucosa of carrier pigs may be a more

reliable method of demonstrating infection with the organism. The cultural and post-mortem findings described in Chapter 5 suggest that gross pathology and the examination of histological sections of the colon may be of some use but the absence of the specific lesions renders this type of examination unreliable in the absence of cultural findings.

It may be that the tests for serum antibody described by Jenkins et al.(1976) and by Lee and Olson(1976a) could be of considerable use in the detection of carriers, but in view of the latter's finding that treated animals had no demonstrable serum antibody, this may not be reliable. The presence of spirochaetes other than T. hyodysenteriae in infected pigs has not been taken into consideration in the development of the tests mentioned above, and the fluorescent antibody test using an absorbed serum (Hunter and Saunders, in press) may be more specific as a diagnostic tool but is subject to the limitations discussed above for the culture of faecal swabs.

Tests for the possible diagnosis of swine dysentery on a farm basis include the serological tests outlined above with the appropriate safeguards for specificity, the culture of rectal faecal swabs and the use of Hunter and Saunders' absorbed fluorescent antibody test. In addition to these methods, the cultural examination of colonic mucosa samples taken at slaughter may be a useful method of monitoring infection with T.hyodysenteriae in pig herds. This procedure would be particularly valuable in herds in which medication is widely used. The technique would probably repay further study.

The isolation of T. hyodysenteriae and other spirochaetes on the same spectinomycin blood agar plate has not previously been reported although Kinyon et al. (1976) used this medium to isolate spirochaetes other than T. hyodysenteriae. This finding suggests that if cultural methods such as those described here were adopted in a herd test, the identity of the isolates should be checked by the use of fluorescent antibody test using antiserum absorbed with the 'non-pathogenic' spirochaetes.

The effect of drug treatment on the ability to recover T. hyodysenteriae by culture is not known, although the work of Songer et al.(1976) suggested that this may still be possible on a herd basis.

The survival of T. hyodysenteriae in faeces outside the host

Few studies of the ability of T. hyodysenteriae to survive in faeces have been reported. The study described in Chapter 3 showed that environmental temperature and humidity were important factors in affecting the survival of the agent. Previous studies (Taylor,1972, Kinyon,1974) had shown that T. hyodysenteriae could survive freezing especially in colon contents and in culture and several authors have shown that the infectivity of faeces or colonic material can be preserved by freezing (Olson,1974). In this study, T. hyodysenteriae survived best at 0° C, 5° C and 10° C and less well at 20-22° C and 25° C. The effects of freezing on faeces were not studied.

The ambient temperature in the interior of British piggeries is normally in the range of 15-25°C and survival will normally be poor at this temperature. In cold weather, where outside dunging passages are present, in depopulated houses and in extensive husbandry systems, temperatures of 10°C or less may be common, particularly in the winter months. It is possible, therefore, that T. hyodysenteriae may survive in infective faeces under farm conditions for the times shown in Chapter 3, i.e. up to 48 days.

Drying appears to have affected the survival of T. hyodysenteriae more drastically than temperature. Cleaned pig accommodation usually dries out after cleaning and before re-stocking and this may be an important feature in the elimination of T. hyodysenteriae from cleaned pens or buildings.

Dilution 1:10 with tapwater seemed to have no deleterious effect on the survival of T. hyodysenteriae at the temperatures tested and even to enhance its survival (Table 5). In any system in which water is used to wash or rinse infected pens and in which this contaminated water then comes in contact with susceptible pigs, the organism may survive and produce infection. An example of this type of survival of infection is quoted by Glock et al. (1975). Another method of survival would be in uncleaned contaminated water bowls or troughs in which long-term survival of T. hyodysenteriae could occur and account for the persistence of infection in a depopulated pen or building.

Common farm disinfectants appear to be uniformly effective against T. hyodysenteriae and their use should

be an adequate method of ensuring freedom from the organism in cleaned and disinfected premises. In dirty or contaminated premises poor penetration of the disinfectant into organic residues may render it ineffective against T. hyodysenteriae as with other better-known organisms.

The survival of T. hyodysenteriae in farm pests

The finding that T. hyodysenteriae can survive in mice for long periods (up to 14 days) and can be shed in their faeces, and can survive in rats and on flies for even shorter periods of time may be relevant to the survival or spread of the agent on a farm. The findings may be of little practical significance, because the pests concerned may have little access to infective faeces and in turn, pigs may have little opportunity to ingest contaminated or infected pests or material contaminated by them. It is probable that flies are the most likely pests to contact infective pig faeces and in the studies recorded in Chapter 4, the survival time of T. hyodysenteriae on flies was short. The large numbers of flies present in some piggeries in the summer months may, however overcome the effect of the small number of organisms carried and could play a part in the transmission of the disease. There is as yet no evidence that this is so.

The infection in mice may be of importance as a model for the future study of swine dysentery and for the screening of compounds in in vivo studies of their activity against T. hyodysenteriae. Mouse studies may be more reliable than in vitro tests and would be cheaper than using pigs. This possibility needs to be examined further, as also does

the possibility that T. hyodysenteriae can establish itself in species as yet unstudied e.g. man.

Conclusion

The studies described and discussed above suggested that T. hyodysenteriae can survive both inside and outside the pig for longer and in more situations than previously suspected. The findings suggest that control of the spread of swine dysentery by means of hygiene must include thorough cleaning of pens to remove faeces, the drying of pens and floors and the use of phenolic disinfectants. It is advisable on the basis of the findings reported here to remove infected slurry and manure in order to prevent the carriage of infective faeces back into cleaned areas on boots, implements, vehicles and by vectors such as flies, rats and mice. It is possible that the mice present in a building may act as a reservoir of infection and attempts should be made to eliminate them. In any disinfection or depopulation programme, infected pigs should be kept away from the cleaned premises.

It is clear from these studies that the period of 60 days' freedom from pigs recommended by Harris and Glock (1975) in depopulation programmes is probably unnecessary if the procedures mentioned above are carried out. When slurry or manure cannot be removed from pens or farms and in extensive systems, the period of rest should approach this figure, especially in cold weather.

The major problem in any control programme is the identification of swine dysentery-free stock for replacement purposes. Hysterectomy-derived pigs are free from

swine dysentery but others could be tested individually on entry to a farm, although the cultural studies described in Chapter 5 suggest that carrier pigs do not always shed the organism and its detection by any method in faeces may not always be reliable. The limitations of serological tests have already been discussed, and it may be that a herd test perhaps by culture of the colonic mucosa of slaughter pigs may be the most satisfactory method of proving that infection is present in any herd or source of pigs.

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