BACTERIA ASSOCIATED WITH INFLAMMATORY LESIONS OF THE ENTERIC TRACT OF CATTLE.

Thesis submitted for the degree of
Doctor of Philosophy in the
Faculty of Veterinary Medicine,
University of Glasgow.

by

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December, 1981.

D E D I C A T E D

T O

M Y W I F E

F A T E I N

FOR HER UNFAILING PATIENCE.

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Acknowledgements

I am greatly indebted to my supervisor, Dr. D.J. Taylor, for his help, advice and encouragement at all times. Any procedures described in this thesis which required Home Office licence were carried out by him.

I would like to thank Professor W.F.H. Jarrett, head of the Department of Veterinary Pathology for allowing me to work in his department.

I would also like to thank Mrs. N. Schivas, Miss C. Oldacre, Mr. D. Ramsay, Mr. R. Stevenson, for technical assistance and for the preparation of some of the media.

I must thank Dr. H. Laird for the electron-microscopy, especially for figures 48, 49, 50, 51, 52, 53, and other members of the staff of the Department of Veterinary Pathology who provided assistance at various times throughout the studies described here.

I must also thank Mr. A. Finnie, Mr. A.H. May and Mr. G.S. Cameron for assistance with the photography.

I am also indebted to Mrs. M. Riddell for the quality of her work and her patience in typing this thesis in its many drafts and in its final form.

I wish to thank Basrah University and the Ministry of Higher Education of the Republic of Iraq for their financial support for the past three years.

Finally, I would like to express my deepest love and thanks to my beloved parents and my brothers, sisters and friends for the support and constant encouragement they have given me throughout.

Preface

The work described in this thesis is original and has not been submitted in any form to any other university.

It was carried out by the author in the Department of Veterinary Pathology, Glasgow University from Martinmas term 1978 to Martinmas term 1981 under the supervision of Dr. D.J. Taylor, M.A., Ph.D., Vet. M.B., M.R.C.V.S.

Unfortunately the Microbiology laboratories were badly damaged by fire on 28th May 1981 and many of my results were damaged by fire or water and all the photographs connected with the work were destroyed. The location of this thesis and all my experimental results are indicated in the photograph below. The thesis which follows was based on the results which survived under the ashes at the site indicated.

Microbiology laboratories May 29th 1981.
The site of this thesis is indicated by the arrow.

Summary

A survey of bacteria associated with inflammatory lesions of the bovine intestinal tract was carried out on 64 cattle (41 calves and 23 adults) examined post-mortem.

A number of bacterial species were isolated from the lesions and identified. Some were associated with specific pathological changes.

Thirty-four isolates of campylobacter were obtained from 28 cases. Eighteen of these were identified as C.f. ss. jejuni, six as C.f. ss. intestinalis, six as C. fecalis and four remained unidentified. Sometimes, more than one campylobacter was isolated from the same lesion. Typical pathological changes were seen at sites from which campylobacters were isolated. The mucosa was often inflamed and covered with excess mucus. Microscopical changes included villous atrophy, cellular infiltration of the lamina propria, the presence of inflammatory cells in the mucosal crypts and proliferation of the submucosal lymphoid tissue particularly in the ileum.

Thirty-five isolates of clostridia were recovered from 32 cases. Thirty were identified as Cl. perfringens Type A and were isolated from sites at which congestion, ulceration or haemorrhage were present. Localised disruption of the epithelium, oedema of the lamina propria and other inflammatory changes were seen histologically. Two cases yeilded Cl. sordellii from congested or haemorrhagic mucosa with marked necrosis and cell shedding in histological sections. Cl. bifermentans was recovered from similar lesions in one case.

Fusobacterium necrophorum was isolated from a case in which necrosis of the lamina propria was a feature and Actinobacillus lignieresi from granulomatous lesions in one case. Mycobacterium paratuberculosis was demonstrated in three classical cases of Johne's disease.

The 57 isolates of E. coli were not tested for enteropathogenicity but in two cases β-haemolytic isolates were obtained from typical enteric lesions.

The remainder of the bacteria could not be clearly linked with any specific changes but were in most cases isolated from lesions or areas with microscopic changes. Some of these were bacteria normally considered as commensals or opportunist pathogens of the respiratory tract Alcaligenes odorans (2) Acinetobacter lwoffii (1) Branhamella catarrhalis (6) Moraxella non-liquefaciens (1) Pasteurella multocida (1) Streptococcus zooepidemicus (1) Aerococcus viridans (8) Corynebacterium pyogenes (2). Pneumonic lesions were present in many of these animals and the relationship of their presence to such lesions is discussed.

The other bacteria found included Bacillus licheniformis (20) Bacillus coagulans (1) Bacteroides fragilis (8) Bacteroides vulgatus (4) Bacteroides melaninogenicus (1) Bacteroides oralis (1) Streptococcus bovis (10) Staphylococcus epidermidis (10) Corynebacterium bovis (5) Pseudomonas aeruginosa (5) Aeromonas hydrophila (3) Veillonella sp. (3) Actinomyces bovis (2) Peptostreptococcus productus (1) Enterobacter aerogenes (1) Eubacterium aerofaciens (1) and Clostridium butyricum (1), one unidentified Clostridium and one unidentified Fusobacterium.

Pure cultures of C.f. ss. jejuni isolated from a calf were used to inoculate experimental calves in three controlled experiments. Ruminating animals were used in two studies and milk-fed calves in another. The inoculated animals developed clinical signs which included a rise in rectal temperature to 39.4°C - 41°C, diarrhoea and occasional dysentery within one to three days of inoculation. The faeces contained varying amounts of mucus and blood and varied in consistency from diarrhoeic to firm. C.f. ss. jejuni was isolated from the faeces of the inoculated animals and from the faeces of two control animals in one experiment.

The small intestine was flaccid and the terminal ileum thickened at post-mortem examination. The ileal contents were dark and mucoid and the wall of the ileum was thickened. The mucosa of the small intestine was inflamed. Enlargement of the mesenteric lymph nodes was common. Fewer changes were seen in the large intestine. Stunted villi, dilated crypts, some of which were filled with inflammatory cells, dilated capillaries and mononuclear cell infiltration were seen in the affected

small intestinal mucosa. C.f. ss. jejuni was isolated from the ileum, caecum and colon of all the inoculated animals and less frequently from the jejunum and gall bladder and abomasum. Antibody to the inocular strain of C.f. ss. jejuni was demonstrated in the serum of all inoculated animals at titres of up to 1:1280 and was absent from all the control sera.

The pathogenesis of C.f. ss. jejuni infection was studied in six calves killed at daily intervals. Lesions developed 48 hours after infection and resembled the typical lesions within four to five days. The organism was recovered from organs outside the gastrointestinal tract only within 48 hours of infection when it was also present in the abomasum and jejunum. No microscopic or ultrastructural evidence was found for invasion of the mucosal epithelium or lamina propria. This failure to demonstrate invasiveness was discussed with reference to the development of serum antibody at three days post-infection and in the context of the literature.

Pure cultures of C.f. ss. intestinalis and C. fecalis isolated from animals in the survey were used to inoculate ruminating and milk-fed calves in controlled experiments. Both organisms produced syndromes resembling that initiated by C.f. ss. jejuni. The pathological changes were indistinguishable but C. fecalis produced fewer clinical changes particularly in milk-fed calves. Both organisms were more commonly isolated from sites outside the gastrointestinal tract. Serum antibody to the inocular strain was detected in each case.

Pure cultures of Cl. sordellii initiated a slight rise in rectal temperature and the passage of faeces containing blood and mucus within one to six days of infection in six calves in two controlled experiments. Cl. sordellii was isolated from the faeces of all affected animals and from their large intestines at slaughter. Mild inflammatory changes were seen in the small intestine. The pathogenesis of Cl. sordellii infection was studied in experimental calves and culture filtrate was found to initiate similar clinical and pathological changes to those produced by whole culture .

The relationship of the bacteria isolated during the survey to the lesions in which they were found was discussed and it was concluded that C.f. ss. jejuni, C.f. ss. intestinalis, C. fecalis and Cl. sordellii could all initiate clinical and pathological changes. The pathogenesis of these infections was discussed.

Chapter 1.

Review of the literature concerning enteric disease in cattle with particular reference to bacteria and their involvement.

Enteric disease is important as a cause of loss in domestic animals and is particularly severe in calves kept under intensive systems of management. A number of agents have been shown to cause enteric disease in cattle by experiment and others have been strongly associated with enteric conditions seen in the field. It is clear from the literature that more than one of these agents may be present in the enteric tract of an animal with enteritis. For this reason a review of each condition is presented here with particular reference to the bacteria.

1. Bacteria in enteric disease in cattle

The bacteria commonly accepted as causes of the inflammatory lesions of the gastrointestinal tract and of enteritis in cattle include Escherichia coli, Salmonella spp. and Myocbacterium paratuberculosis and, to some extent, clostridia and Campylobacter (Vibrio) fetus subsp. jejuni. Others have been described as being present in enteritis but in many cases experimental evidence of their pathogenicity is not available. In this section the clinical signs, pathology and other relevant details of enteric infections with bacteria are described as a basis for the discussion of the results of the survey in Chapter 3.

Escherichia coli infection in cattle

Some serotypes of E. coli are normal inhabitants of the intestine, and may have little or no adverse effect on health. Some serotypes may, however, initiate disease, particularly in the absence of immunity to a specific strain especially following deprivation of colostrum, (Logan, 1974). For many years E. coli has been considered to be a major cause of diarrhoea in neonatal, unweaned and recently weaned animals.

Two distinct syndromes associated with E. coli infection in cattle commonly occur in Britain. The septicaemic form (colisepticaemia), which usually occurs in neonates, is associated with certain serotypes of E. coli which invade the blood and tissue and cause sudden death following invasion. The organism can be isolated as a pure culture of a single serotype from organs throughout the body. Involvement of the gut is not a feature of this syndrome. The enteric form occurs in calves, particularly in neonates, and is characterised by diarrhoea and dehydration. It does not involve systemic invasion and is associated with massive proliferation of enterotoxigenic strains of E. coli within the proximal small intestine where they adhere to the mucosal epithelium. In some cases invasion and enterotoxin production may occur but this form is not commonly reported in Britain.

Enterotoxins cause the intestinal mucosal epithelial cells to secrete water and electrolyte. This excess secretion results in diarrhoea. Two major forms of E. coli enterotoxin are recognised, heat-labile and heat-stable enterotoxin (Bywater, 1976).

The "K" antigens of E. coli have for a long time been considered to have a role in pathogenicity. K99 antigen was observed by Ørskov et al. (1975) who suggested that this common "K" antigen was important in the enteropathogenicity of E. coli strains in calves. It appears to be responsible for adhesion to the brush borders of the intestinal epithelium.

In a study of 51 diarrhoeic calves one to 15 days of age made by Morin et al. (1978), it was found that enterotoxigenic E. coli were isolated from the intestinal tracts of 15 of the 51 (29 per cent) diarrhoeic calves and that all isolates possessed the K99 antigen. It therefore appears to be widespread.

According to Gay (1965), Moon (1974) and Blood et al. (1979), several clinical syndromes due to E. coli infection in calves have been described. Those associated with the gut are described here.

Enterotoxaemic colibacillosis affects newborn calves from three to five days of age. Its severity depends on the presence of an enterotoxigenic strain of E. coli and it may cause a state of collapse usually designated as enteric toxaemia. It is characterised by severe diarrhoea. Sudden changes in serum electrolyte levels and movement of electrolyte into the lumen of the gut result in signs such as dehydration, severe weakness, coma, subnormal temperature, a cold clammy skin, pale mucosa, wetness around the mouth, collapse of superficial veins, slowness and irregularity of heart, mild convulsive movements and periodic apnoea.

Post mortem findings in the enterotoxaemic form usually include marked petechiation and ecchymoses of the heart, spleen and kidneys. E. coli can usually be isolated from the small intestine and mesenteric lymph nodes but not from other viscera (Gay et al., 1964 and Logan and Penhale, 1971).

The enteric form of colibacillosis is the commonest form of E. coli infection encountered in Britain. It normally occurs during the second and third weeks of life and death occurs in only a proportion of affected calves. When death occurs, it usually does so after two or three days of profuse diarrhoea and during this time there is a marked loss of body weight and increasing dullness. The faeces become progressively more watery, are usually pale yellow to white in colour, and very foul-smelling. Defaecation is frequent and effortless and the tail and hindquarters are soiled. Some calves become reluctant to feed early in the course of the condition. As the condition progresses, calves become recumbent and eventually become comatose. Death usually occurs in three to five days. In calves which recover, it is common to find that the hair is lost in regions where faecal staining has occurred.

Young calves which have died as a result of a severe E. coli diarrhoea are dehydrated. The intestinal tract is distended with yellow watery contents and gas. The abomasum is usually grossly distended with fluid and may or may not contain milk clot. The abomasal mucosa may contain numerous small haemorrhages. Wray and

Thomlinson (1974) described the gross lesions in 25 calves aged between two hours and four weeks and found varying degrees of hyperaemia in the abomasal mucosa, and oedema of the submucosa of the abomasum in a few. Haemorrhagic ulcers were present in the abomasal mucosa of the two calves which had shown profuse diarrhoea. The intestinal mucosa was hyperaemic in most cases but hyperaemia was irregular in distribution and occurred mainly in the small intestine which was flaccid and dilated. The mesenteric lymph nodes were enlarged and oedematous and, in a few calves, were congested and showed petechial haemorrhages.

Histological studies were described by Wray and Thomlinson (1974) on calves which were suffering from E. coli infection. In this study the submucosa of the abomasum was oedematous and numbers of lymphocytes, neutrophils, plasma cells and eosinophils were present in lamina propria of the abomasum and small intestine. Focal congestion of intestinal villi and dilatation of lacteals occurred in most of the calves and, in a few, cellular debris and neutrophils were observed in the lamina propria of the abomasal glands and in the crypts of Lieberkühn. In some calves, the lymphoid follicles of the Peyer's patches were depleted of cells. No abnormalities in the large intestine were seen in any calves. The mesenteric lymph nodes appeared hyperplastic and neutrophilic polymorphonuclear leucocytes were commonly seen in the medullary cords. Their findings were supported by Pearson and Logan (1979) who described the pathological changes which occurred in calves between six and 36 hours after inoculation of a known enteropathogenic strain of E. coli. There was a reduction in villous length in the small intestine which was accompanied by infiltration of the lamina propria of the villi with reticular cells and polymorphonuclear leucocytes. The epithelial surface of many of the villi in the distal small intestine had irregular outlines resulting in distortion at 12 and 16 hours after inoculation. A group of epithelial cells were seen bulging from the intact epithelium into the lumen before their separation from the villus. Fusion of the villi at their epithelial surface was also seen at 21 and 36 hours after inoculation. Exudation of polymorphonuclear leucocytes into the lumen was marked.

It was seen also within some of the crypts. Rod-shaped bacteria were observed by light microscopy on the villi of the small intestine from six hours after inoculation.

Both septicaemic and enteric colibacillosis have been produced experimentally by the administration of relevant specific serotypes of E. coli (Osborne,1967; Osborne and Watson, 1967; Smith and Halls, 1967; Smith and Halls, 1968; Logan and Penhale, 1971 and Logan and Penhale, 1972). Recently, Pearson et al. (1978) showed that enteropathogenic forms of E. coli produce severe diarrhoea 24 to 48 hours after challenge, increasing to maximum severity by day 3. The inocular strain was reisolated from the faeces of all infected calves but never from controls. It was found in the small intestine and mesenteric lymph nodes of calves at post mortem. Pathological lesions were seen in the small intestines. Histological changes were also seen associated with adherence of E. coli in the distal small intestine.

E. coli diarrhoea may be suspected on clinical or pathological grounds. Final diagnosis depends upon the isolation of E. coli in profuse numbers and almost pure culture from the intestinal tract and contents, especially in the anterior intestinal lumen in cases of the enterotoxic disease. Certain serotypes, especially those bearing K99 antigen, can be identified as pathogens by haemagglutination and by the brush border attachment test (Burrows et al., 1976). Enterotoxin production can be demonstrated by the inoculation of ligated intestinal loops of suitable hosts such as calves in which they cause fluid accumulation (Smith and Halls, 1967).

Enteropathogenic strains of E. coli may be isolated from faeces and from the lumenal mucosal surface and other internal organs, using five to seven per cent sheep blood agar and MacConkey media. After incubation for 24 hours at 37°C, cultures may be examined for the presence of Beta-haemolytic organisms which may be pathogens. Representative numbers of colonies resembling E. coli should be tested by further biochemical tests to confirm their identity and examined serologically and by the methods outlined above to confirm their pathogenicity.

Salmonella infections in cattle

Many serotypes of Salmonella cause salmonellosis in cattle. Those of major importance in Britain are usually either Salmonella dublin or S. typhimurium although the so-called exotic serotypes are being isolated more frequently (Sojka and Field, 1970; Hughes et al., 1971; Sojka et al., 1975 and Wray and Sojka, 1977). Enteric disease is a common consequence of infection in cattle of all ages but septicaemia may also occur particularly in neonates and young calves. Abortion, mastitis and arthritis may also occur. Cattle of all ages may be affected. Infection usually occurs by the ingestion of contaminated pasture or other food and water (Williams, 1975).

Infection with Salmonella species may occur more commonly in young or adult cattle, whose resistance has been decreased by intercurrent infection such as parasitic infestation, poor nutrition and the stress of transport. Following ingestion of the organism, enteric lesions result from invasion of the Peyer's patches and of the mucosal epithelium. Blood et al. (1979) state that in newborn calves S. dublin can be found in the blood stream within 15 minutes of oral infection, and in older calves the bacteria can be isolated from the intestinal lymph nodes within 18 hours of oral administration. In lambs it was shown experimentally by Brown et al. (1976) that S. typhimurium, when fed orally as pure culture produced a febrile reaction within 24-48 hours, and the acute phase of the disease, resembling that seen in natural cases, was present three to nine days later. A similar course of events may occur in calves.

Bovine salmonellosis normally gives rise to a syndrome in which fever, and diarrhoea frequently with dysentery may result in death, recovery or chronic infection.

In the septicaemic form which affects calves up to four months of age, there is depression, dullness and a rise of body temperature (105-107°F) with or without diarrhoea. Affected calves become anorexic, recumbent and weak. Death usually occurs 24-27 hours after onset (Blood et al.,1979).

Acute enteritis affects calves and adult animals. There is a high fever (104-106°F), with severe watery diarrhoea, sometimes dysentery, and,in some cases tenesmus. The faeces have a putrid smell and contain mucus, shreds of mucous membrane, and in less severe cases, casts of intestinal mucosa. Pregnant animals may abort. The mortality rate is about 75 per cent in untreated cases (Blood et al., 1979; Wray and Sojka, 1977).

Severe dehydration and toxaemia develops, the animal loses condition, quickly becomes recumbent and dies after two to five days. Some calves which survive this initial acute stage may subsequently develop a painful polyarthritis.

Sub-acute enteritis is less dramatic, a fever of 103°F continues for four to five days or is absent, and the other signs are less severe. Chronic enteritis is often preceded by an attack of the acute enteric form of the disease. It occurs occasionally in cattle. There is persistent diarrhoea, severe emaciation and intermittent fever. At intervals the faeces contain spots of blood, mucus, and are occasionally firm (Blood et al., 1979).

There may be an absence of gross lesions in animals which have died peracutely but there is usually evidence of septicaemia in the form of extensive submucosal and subserous petechial haemorrhage.

In acute enteritis,lesions may be found both in the small and large intestine. Inflammation is evident and varies from a mucoid enteritis with submucosal petechiation to diffuse haemorrhagic enteritis. In calves, multiple mucosal erosions and petechiation of the abomasal wall accompanied by abomasitis may be seen. Small intestinal involvement is inconstant. The intestinal contents are watery, have a putrid odour and contain mucus, are blood-tinged or contain whole blood. Superficial necrosis may proceed to the development of an extensive diphtheritic pseudo-membrane in some cases of Salmonella typhimurium infection. Normally the organism is readily isolated from the intestinal tract (Gibson, 1961 and Tutt and Hoare, 1974).

The mesenteric lymph nodes are enlarged, oedematous and haemorrhagic. The wall of the gall bladder may be thickened and inflamed. Enlargement and pallor of liver occurs and the serous cavities may contain blood-stained fluid. Varying degrees of jaundice, splenomegaly and pneumonia occur. Joints are affected, the joint cavities and adjacent tendon sheaths contain a gelatinous or sero-fibrinous fluid in infected calves (Hughes et al., 1971 and Wray and Sojka, 1977).

In the chronic form lesions are most common in the caecum and colon. The wall is thickened and covered with a yellow-grey, necrotic material overlying a red, granular surface. Chronic pneumonia may also be present. Salmonella may be isolated from the heart blood, spleen, liver, gall bladder, mesenteric lymph nodes, and intestinal contents in both septicaemic and acute enteric forms. In the chronic form, the bacteria may be isolated from intestinal lesions and less commonly from other viscera.

Robinson (1966) described the histological appearance of sections of small intestine in which desquamation of the tips of villi with pseudomembrane formation was seen. Peyer's patches were oedematous and there was considerable neutrophilic infiltration of crypts, sinusoids and the reticular net. The medullary sinusoids of the mesenteric lymph nodes were distended and packed with neutrophils and monocytes.

Salmonellosis in calves is readily reporoduced experimentally with S. dublin (Henning, 1953 and Smith and Jones, 1967) and with Salmonella typhimurium infection (Rankin and Taylor, 1966 and De Jongand Ekdahl, 1965).

Diagnosis in bovine salmonellosis may be based on clinical grounds, especially if the disease is known to be present in the area and by its epidemiological pattern. Only bovine salmonellosis and mucosal disease are likely to spread through all age groups in a susceptible herd. Also, the age incidence in calves differs from that of colibacillosis (Blood et al., 1979). The post-mortem findings

are also suggestive.

Final diagnosis rests on bacteriological examination which includes:

(1) Culture of faeces of rectal swabs from live animals, to confirm clinical salmonellosis. Isolation may be carried out using selective media such as sodium selenite or tetrathionate broth. Material from the incubated broths is cultured on differential media such as SS, D.C.A. or brilliant green agar. Non-lactose fermenting colonies are examined biochemically and positively identified and grouped by serological tests. Direct isolation from parenchymatous organs is also possible.

(2) Culture of intestinal contents and viscera such as liver with attached gall bladder and a length of small intestine with the mesenteric lymph nodes at post-mortem examination.

(3) Bacterial agglutination tests on sera may be of diagnostic value when used in conjunction with bacteriological examination of faeces, especially if paired sera are examined and the serotype involved is known.

Mycobacterium paratuberculosis infection in cattle

Johne's disease (paratuberculosis) is a chronic enteric disease of cattle and other ruminants. The causative organism is a slow growing acid-fast bacillus, Mycobacterium paratuberculosis (syn. M. johnei), the direct pathological effects of which are confined to the intestinal tract and associated lymph nodes. The intestine is the site of primary infection and the main site of bacterial proliferation and action (Gilmour, 1965). Lesions are produced within one to two months of infection in the intestinal mucosa and lymph nodes of experimental calves and large numbers of organisms may then be shed for a varying length of time (Gilmour et al., 1965). Following multiple oral dosing, Payne and Rankin (1961) found that lesions and excretion of organisms reached a peak at three months in calves and two months in cows. Following the development of lesions, the organism may be carried in macrophages from the intestinal mucosa and

perhaps from the tonsils, retropharyngeal lymph nodes and mesenteric lymph nodes to other parts of the body (Payne and Rankin, 1961; Gilmour et al., 1965; Kluge et al., 1968). The method by which diarrhoea is produced in Johne's disease is poorly understood, but there have been several suggestions. Merchant and Barner (1971) felt that pockets of organisms appearing in the mucosa act as foreign bodies producing a specific inflammatory reaction. Patterson et al.(1967) and Patterson and Berrett (1969) suggested that the massive cellular infiltration in the mucosa and submucosa resulted in increased gut motility, decreased transit time, decreased absorption and an increased loss of protein from the intestine. Merkal et al. (1970) demonstrated that diarrhoea may result from antigen-antibody reactions in the tissue.

Payne and Rankin (1961) confirmed that M. paratuberculosis can enter the tissues of the adult cow and that the lesions of the "primary complex" pattern developed at the portals of entry and the associated lymph nodes in both cows and calves.

Surveys have revealed that, in Britain, Johne's disease was of great economic importance (Doyle and Spears, 1951), and the most important disease of cattle in certain areas.

In the series of surveys carried out by Withers (1959) the overall incidence of clinical Johne's disease was 0.84 per cent although these figures may not be correct now. Breed incidence was found to be higher in Jerseys at 4.78 per cent. However this survey on breed incidence was done in three areas, namely, Surrey, Berkshire and Wiltshire and hence does not represent the whole country. The incidence of latent infection, as determined by culture of the ileo-caecal lymph nodes from samples of apparently normal adult cattle slaughtered at an abattoir was found to be much higher and to vary from 6 to 17 per cent.

Johne's disease is a condition which develops slowly. The incubation period which varies widely depending, perhaps, on individual susceptibility, breed, nutritional status and the degree of challenge to which the animal has been exposed, is rarely less than 12 months and may even extend to many years (Doyle, 1953). The clinical signs do not

usually appear before two years of age and are commonest between two and six years (Blood et al., 1979).

Julian (1975) described the typical clinical picture of Johne's disease as being characterised by progressive unthriftiness, weight loss, decreased milk production and development of continuous or intermittent diarrhoea with a faecal consistency varying from soft to watery. Once profuse watery diarrhoea occurs, animals tend to weaken quickly and die. Marked muscle wasting and inappetance may be observed as the syndrome progresses. Fever and anaemia are not always present.

An excellent account of the gross and microscopic pathology of Johne's disease has been provided by Jubb and Kennedy (1970). The intestinal lesions occur from the duodenum to the rectum but are usually best developed in the lower jejunum and ileum. The classical intestinal lesion is a diffuse hypertrophy with the mucosa folded into thick transverse rugae like the convolutions of the cerebral cortex. The mucosal changes are due to the accumulation of epithelioid and other cells in the submucosa. The crests of rugae are often slightly reddened by congestion and the mucosal surface is velvety, but there is no excess of mucus. The same authors stated that necrosis sometimes occurs.

Transmission under natural conditions is usually by the oral route, and infection usually follows ingestion of the organism in water or feed contaminated with infected faeces (Julian, 1975; Blood et al., 1979.) Infected animals may excrete organisms in the faeces for 15 to 18 months before clinical signs appear. Animals reared in an infected environment may become temporary or permanent carriers.

Diagnosis of Johne's disease should be considered under two distinct headings, diagnosis of clinical disease and of M. para-tuberculosis infection (Gilmour, 1976). In the case of clinical disease, the herd or individual history together with clinical signs are a useful aid to diagnosis by emphasising a drop in milk production and the presence of diarrhoea in individual adult animals. In certain circumstances, particularly when confusion is likely to occur because

of other conditions in which diarrhoea occurs, laboratory confirmation is necessary. This can be done by the microscopic examination of faeces for the acid-fast bacilli and by serology.

In 30 to 50 per cent of clinically affected cows, typical clumps of acid-fast bacilli can be found and provide confirmation of diagnosis (Gilmour, 1976). Merkal et al. (1968) recommended cultural examination of faecal samples for the detection of Johne's disease in cattle prior to the development of clinical signs. The same author and others (1970) isolated the organism from intestinal mucosa or lymph nodes at necropsy by using an egg yolk medium containing mycobactin. The method is laborious and cultures must be incubated at least for three months.

The complement fixation test is positive in about 90 per cent of cattle with advanced disease (Gilmour, 1976) and the use of indirect immunofluorescence is also of equivalent value (Gilmour and Angus, 1976 a and b). Gilmour (1976) reviewed the efficacy of these diagnostic tests and suggested that the complement fixation test, immunofluorescence and faecal culture should all be used. Other tests such as allergic tests using intradermal Johnin or avian tuberculin may be used in living animals and were considered to be of value by Hole and McClay (1959) in the identification of positive animals before clinical disease developed.

Post-mortem diagnosis can be based on the presence of gross lesions such as exaggerated corrugation of the mucosa of the small intestine or the presence of areas in which villous or other mucosal architecture is destroyed. The examination of smears from the mucosa of the ileum, the ileocaecal value area, the caecum and the colon is useful in confirmation of the diagnosis (Julian, 1975) as is culture from suspected areas of the mucosa. Histopathological examination of gastrointestinal tissue from cattle with suspected clinical Johne's disease, in which acid-fast bacilli were not identified in mucosal smears was also recommended in the diagnosis of Johne's disease. (Summers, 1981).

Clostridia in enteric infections of cattle

Clostridia are associated with a wide range of diseases in cattle, both as primary causes of disease and as secondary invaders.

Pathogenic clostridia have two primary habitats in nature, soil and the intestinal contents of apparently normal animals and cause disease only in special circumstances.

Cl. perfringens is considered a natural inhabitant of the intestinal lumen of the calf but has also been incriminated as a cause of enteric disease. There are at least six toxigenic types of Cl. perfringens which have been designated A, B, C, D, E and F, and the organism produces 12 toxic substances of which the most important are Beta toxin produced by groups B and C and epsilon toxin produced by types B and D.

Surveys of Cl. perfringens types present in 379 animals submitted for post-mortem examination in Alberta and Saskatchewan in 1962 (Niilo and Avery, 1963) and in 100 fattened cattle (Vance, 1967) revealed 201 Type A isolates and only one culture of any other Cl. perfringens type (Type D).

Clostridium perfringens Type A:

Cl. perfringens Type A has been associated with disease in a number of case reports. It was associated with a highly fatal haemolytic disease in calves two weeks to four months of age by Rose and Edgar (1936). This disease was marked by intense icterus, haemoglobinuria, and haemoglobinaemia. Most cases were acute but some animals survived for a few days. The urine was reddish or brown in colour. On post-mortem examination, all tissues were of a brilliant yellow colour. The liver was friable and jaundiced. The kidneys were swollen and dark brown and degeneration after death was extremely rapid. The red blood count dropped very rapidly in the course of the disease and, in some cases became as low as 1×10^6/cumm. They also demonstrated Type A toxin in the ileum and anterior portion of small intestine. Cl. perfringens was isolated from different sites along

the intestine and Cl. perfringens Type A antitoxin was found to protect against natural infection. A similar condition in cattle has been reported by Quin (1954). Histological sections of icteric, granular livers revealed coagulative necrosis around the central veins and haemoglobulin casts were present in the kidney tubules.

Enterotoxaemia in calves associated with Cl. perfringens Type A has also been reported by Macrae et al. (1943) and Schofield (1955). Schofield (1955) found the susceptible age to be between six and ten weeks although cases occurred in the first week of life. Two forms have been described in very young calves.

In the peracute form, sudden death occurred in animals with no history of previous indisposition and showing no evidence of diarrhoea. In the acute syndrome, a staring coat is noted on the third day of life followed the next day by the appearance of diarrhoea. The diarrhoeic faeces were straw-coloured, white or even black. Rectal temperatures varied from 101°F to 104° F. Depression followed rapidly. The animals became recumbent and weaker, finally entering a coma which usually lasted for only a few hours before death. At post-mortem examination enteritis or gastroenteritis (often patchy) was present. In some cases the alimentary tract was practically devoid of solid or liquid matter but distended with gas. Congestion of the alimentary blood vessels, the presence of straw-coloured pericardial pleuritic and peritoneal transudate and petechia of various internal organs such as the liver, heart and spleen were also reported (Macrae et al., 1943). Cl. perfringens Type A was isolated from the alimentary tract of affected animals and from their faeces.

Similar findings but with no icterus were reported by Schofield (1955). Type A toxin could not be demonstrated in the intestine, but Cl. perfringens was thought to be responsible because of the large numbers found in the intestine. The histological findings in his cases included desquamation of the epithelium of the small intestine, with necrosis of the denuded villi and some histological changes in other organs such as the heart, kidneys, spleen, lung, liver and lymph nodes. Oedema, congestion and slight haemorrhage were present.

Clostridial enterotoxaemia was also reported in Britain in adult cattle by Shirley (1958)., who described an acute and rapidly fatal gastroenteritis caused by Cl. perfringens Type A associated with the period shortly after calving (within the first 48 hours) and he suggested that parturition might act as a trigger mechanism. The post-mortem findings in this condition included congestion of the abomasum and both local and general severe acute enteritis. The mucosa resembled red velvet with fluid and blood stained contents which could also be dark brown in colour.

Some experimental evidence for Cl. perfringens Type A in enteric disease has been found. A similar disease has been produced experimentally by feeding pure cultures of Cl. perfringens Type A to lambs (Hauschild et al. 1967) while the culture supernatant fluids had no apparent enteric effects. Following oral and intraduodenal inoculation, most challenged animals developed diarrhoea within 6-12 hours of feeding which subsided within 12 hours from the onset of signs. They also produced diarrhoea in lambs by the intraduodenal introduction of cultures of isolates from normal animals as well as food poisoning strains of Cl. perfringens Type A. Slight congestion of the duodenum, jejunum and small intestine was seen at post-mortem examination. Histological examination of sections from these tissues revealed no significant lesions and no invasion of the mucosa by bacterial cells was apparent.

Enteric reactions to cultures of Cl. perfringens Type A have been demonstrated in ligated intestinal loops of lambs (Hauschild et al., 1968). A characteristic finding in the experimental disease is transitory diarrhoea in intact animals and the accumulation of clear, straw-coloured or blood-tinged fluid in ligated intestinal loops. The intestinal mucosa remains intact and is not invaded by Cl. perfringens Type A during diarrhoea.

The enteric response has been found to be due to an entero-pathogenic factor associated with growth and sporulation of Cl. perfringens Type A under suitable conditions (Hauschild et al., 1970 a and b) and clinical responses can be reproduced by cell free extracts

of the causative organism (Hauschild et al., 1970 a). Niilo and Dorward (1971) examined two entero-toxigenic strains of Cl. perfringens Type A which produced pathological response in calves and five classical strains of the same organism. Extracts of ruptured cells of the former strain grown in sporulation medium caused illness, and diarrhoea occurred within five to six hours after intra-duodenal inoculation. The animals returned to apparent normality 8 to 10 hours later.. Fluid accumulation was produced in intestinal loops of calves by these strains. Strains isolated from normal animals did not produce any noticeable clinical or pathological effects.

Niilo (1971) injected an enterotoxin prepared from ruptured cells of Cl. perfringens Type A intravenously into lambs, rabbits and guinea pigs. This procedure caused lassitude in most of the animals and, in addition, salivation, lacrimation and diarrhoea in some of the lambs. Vascular congestion, particularly of the mucosa of the small intestine was seen in all animals that died from this intoxication. Further investigation in sheep (Niilo, 1972) showed that intravenous injection of enterotoxin rapidly lowered the systemic blood pressure, but that the clinical effects resulting from small and moderate doses of enterotoxin were reversible. These findings suggested that the toxins had a predilection for the intestinal mucosa and that vaso-dilation was one of the possible mechanism of action by which this toxin caused illness.

Further investigation shown by Niilo (1973) in five to nine month-old calves that intravenous injection of enterotoxin, caused lassitude, dyspnoea, recumbency, hypotension, tachycardia and death. Lesions of visceral hyperaemia, particularly in the small intestine, petechial haemorrhage, ascites and pulmonary oedema were recorded. He suggested that the minimum effective dose was 2.28 erythema units per kg/body weight and the minimum lethal dose was 4.96 erythema units per kg/body weight. The effects from sub-lethal doses were reversible. Histological examination revealed dilatation of capillary blood vessels in the intestinal mucosa especially at the tips of villi and was accompanied by detachment of the surface epithelium, congestion in the liver was also observed. The effect of the erythemal factor on gut

loops was completely neutralised by antisera to it (Hauschild et al., 1971).

Clostridium perfringens Type B.

Cl. perfringens Type B produces Beta toxin which can cause a haemorrhagic enteritis and ulceration of the intestinal mucosa.

Enterotoxaemia caused by Cl. perfringens Type B has been reported in calves in Great Britain by Hepple (1952). Blood et al., 1979 suggested that disease occurs in calves 7-10 days old although calves up to 10 weeks of age might be affected. The clinical signs included diarrhoea, dysentery and acute abdominal pain accompanied by violent bellowing and aimless running. There may be additional nervous signs including tetany and opisthotonus. In very acute cases, death occurs in a few hours, sometimes without diarrhoea being evident. In less severe cases, the illness lasts for about 4 days and the slow recovery usually lasts 10-14 days.

Post-mortem findings in one case were described by Hepple (1952). Lesions were located in the small intestine which showed severe generalized enteritis with extensive necrosis of the mucous membrane, particularly that of the ileum. Small yellowish diphtheritic patches were distributed irregularly in these necrotic areas. The contents of the small intestine were liquid and blood-stained. Slight congestion of the mucous membrane of the caecum and colon were also noted. There was some gelatinous oedema of the mucous membrane lining the abomasum. The mesenteric lymph nodes were swollen and severely haemorrhagic. Liver, spleen and kidneys were intensively congested but the lungs appeared normal. A large haemorrhagic patch covered much of the epicardium of the left ventricle but no endocarditis was observed.

Extensive inflammatory changes were present in histological sections of the necrotic and inflamed areas. The mucous membrane overlying the villi was necrotic with some villous destruction. An active vascular reaction with hyperaemia of the venules and extensive cellular infiltration was present in the mesenteric lymph nodes and

there were changes in the kidneys. The characteristic toxin could be demonstrated in the liquid, blood-stained intestinal contents. The same type of haemorrhagic enteritis with ulceration of the mucosa has been found in other animals with Cl. perfringens Type B infection.

Many haemolytic colonies of Cl. perfringens were isolated from ileal contents and the necrotic lesions in this case.

Clostridium perfringens Type C.

Haemorrhagic enterotoxaemia of calves caused by Cl. perfringens Type C was first reported by Griner and Bracken in (1953)and has subsequently been reported by Lozano et al. (1970) and Niilo et al. (1974). The former described typical acute clinical signs and post-mortem lesions in many neonatal calves from central Colorado. Post-mortem lesions included an acute haemorrhagic enteritis in the jejunum and ileum with necrosis and desquamation of the mucosa. The intestinal contents were deep red in colour. They also observed consistently petechial or ecchymotic haemorrhages on the epicardium and thymus, and inconsistently in the diaphragm, abomasum and parietal pleura.

Marked pathological lesions of the mucosa and submucosa were present in histological sections. Extensive haemorrhage and necrosis of the mucosa were also noted and blood and necrotic tissue were present in the intestinal lumen. Numerous rod-shaped bacilli were seen singly and in chains among the debris and red blood cells in the intestinal lumen. Hyperaemia and haemorrhage were also reported in other organs. Their diagnosis was based on the isolation of Cl. perfringens Type C and the demonstration of its toxin in the intestinal contents. They also reported the successful production of a typical case of haemorrhagic enteritis by feeding a pure, live culture of Cl. perfringens Type C mixed with cornmeal and milk to a calf.

Clostridium perfringens Type D.

Enterotoxaemia due to Cl. perfringens Type D has been demonstrated by Keast and McBarron (1954) who described it in the case of an adult cow in Australia and Griner et al. (1956) determined it as

the cause of a small outbreak of the disease in 7 to 10 week old calves in Colorado. It was also implicated as a cause of mortality in 3-month old calves investigated by Blood and Helwig (1957) and was reported in cattle of all ages in New South Wales (Mumford 1961).

In most cases, the course of the disease was peracute or acute and the animals were found dead (death having occurred quickly) or on the point of death. Bloating was sometimes noted. Calves were sometimes heard to cry out as if in severe pain and usually succumbed very quickly. Few other clinical signs were noted apart from transient nervous signs before death. Diarrhoea occurred sometimes. In adult animals there was extreme depression, dullness, listlessness, inappetance, aimless wandering or standing with a lowered head, champing of the jaws and strings of saliva hanging from the lips.

Post-mortem examination revealed an excess of straw-coloured (later sanguineous) abdominal fluid. The rumen was filled with feed of varying consistency, there was patchy congestion of the abomasum and patchy enteritis throughout the small intestine. There was intense congestion of the small intestinal blood vessels which showed evidence of diffusion of blood pigments into surrounding tissues. The contents of the small intestine were generally sparse and consisted mainly of semifluid material of creamy-brown colour and of mayonnaise consistency. In some cases in calves, an acute haemorrhagic enteritis has been recovered. The small intestines were often digested to such a degree that it was quite easy to tear sections apart with the fingers.

The liver showed patchy degeneration and was mottled in appearance. The gall bladder was distended and the surrounding tissues were stained with bile. The spleen was often soft and haemorrhagic. The kidneys in most cases showed some changes although in the pulpy kidney seen in sheep has only been seen rarely in cattle.

Intestinal changes included superficial desquamation of the mucosal epithelium with congestion and the presence of numerous typical bacilli in the contents. Changes were also reported in the kidney, spleen and heart.

Clostridium perfringens Type E.

Enterotoxaemia caused by Cl. perfringens Type E has been found to be associated with diarrhoea in calves (Bosworth, 1943). It has been isolated from the small intestine of a 4-day old calf found dead after an illness of about 12 hours during which it showed dullness, inappetance, a subnormal temperature and signs of general intoxication and collapse.

The post-mortem findings shown were of marked enteritis of the small intestine, caecum and colon. Petechial haemorrhages in the abomasal mucosa, mesentery and epicardium were noted. The small intestine contained a brown, slightly mucoid fluid. The intestinal contents of two of the three animals were also tested by him and found to be lethal to mice.

Unsuccessful attempts were made to reproduce the disease in calves a few days old by administering the organism orally. No gross lesions can be considered to be specific for this infection. Inflammation of the intestinal tract and small haemorrhagic areas in the intestine and on the endocardium have been recorded and a dilatation of the forestomachs is common.

Clostridium botulinum infection.

The toxin of Cl. botulinum has been identified in the ileal contents of cattle (Simmons and Tammemagi, 1964) but no enteric lesions have been reported in either natural (Davis et al., 1974) or experimental infections (Simmons and Tammemagi, 1964).

Clostridium chauvoei

Clostridium chauvoei has properties resembling those of Clostridium septicum and causes black leg in cattle and sheep. It is normally considered to be a soil organism but may be found in spleen, liver (Kerry, 1964) and alimentary tract of normal animals and contamination of the soil and pastures may occur from infected faeces or decomposition of carcases of animals dying of the disease. There appears to be no association between infection with this organism and

enteric lesions.

Clostridium septicum

Cl. septicum is a normal inhabitant of the soil and is frequently present in the faeces of herbivores and other animal species.

It is associated with a condition known as braxy in sheep and is the cause of a small proportion of blackquarter cases in cattle and sheep, as well as gas gangrene infection arising from wounds in both animals and man. It does not appear to have been associated with enteric lesions in cattle.

Clostridium oedematiens

Cl. oedematiens is not generally recognised as an important pathogen of cattle in Britain. The only records in cattle are those of Soltys and Jennings (1950) who reported a case of Type D infection in the English Lake district and Williams (1964).

The disease is an acute, highly fatal toxaemia of cattle and sheep and is characterized clinically by high fever, haemoglobinaria and jaundice, and at post-mortem by the presence of necrotic infarcts in the liver. Enteric involvement was described by Soltys and Jennings (1950) in a heifer which had died fairly suddenly. Blood-stained peritoneal fluid was present in the abdominal cavity and a patchy catarrhal gastroenteritis with a few areas of acute haemorrhagic inflammation was also seen. The mesenteric lymph nodes were enlarged, oedematous and congested. The liver showed a large, sharply demarcated infarct, approximately three inches in diameter, the edge of which was congested and contrasted strongly with pale toxic liver. Bloody faeces were recorded in an outbreak described by Williams (1964).

Enteric lesions were not a feature of experimentally-reproduced disease (Olander et al., 1966).

Clostridium sordellii

A single report on a case of enterotoxaemia probably caused by Cl. sordellii was published in Britain by Brooks et al. (1956).

Definite association of this organism with the cause of death could not be established, although toxin of high potency was found in the intestine.

The disease could not be reproduced experimentally in calves dosed orally either with toxin or with the organism. Only one limited outbreak was seen.

Cl. sordellii has also been isolated from sheep in which it has been associated with lesions resembling blackquarter and bighead disease in rams (Smith et al., 1962). but no other reports of the isolation from enteric lesions appear to exist.

Campylobacters in enteric infections in cattle

For many years Campylobacters (formerly Vibrio) have been associated with enteric disease in cattle. The original description of the enteric campylobacter was as "Vibrio jejuni" but extensive changes have occurred recently in the classification and nomenclature of this group. This section of the literature review therefore begins by considering the classification of the genus Campylobacter/Vibrio.

The microaerophilic vibrios have been recently removed from the genus Vibrio, and reclassified in the genus Campylobacter (Sebald and Veron, 1963). More than one campylobacter species is found in animals and man and it is not always easy to establish a clear borderline between pathogenic and saprophytic or commensal organisms on biochemical grounds. The classification of these campylobacters has been extensively reviewed by Smibert (1974 and 1978). The genus Campylobacter is included in the family Spirillaceae. All campylobacter species are curved Gram-negative rods, oxidase-positive and some produce catalase, a property that serves to divide the genus into two groups. The majority are microaerophilic.

I. Catalase-negative group. These are represented by a single species Campylobacter sputorum. The type species (C.s. subsp. sputorum) has been isolated from the normal reproduction organs of cattle and is considered to be a non-pathogenic. It also found in the human oral

cavity and in about 3 per cent of faecal samples from normal people (Skirrow, 1979a).

Two other subspecies have been recognised: C.s. subsp. mucosalis and C.s. subsp. bubulus. C.s. subsp. mucosalis has been associated with intestinal adenomatosis of pigs (Lawson and Rowland, 1974 and Lawson et al., 1975) and is also associated with proliferative haemorrhagic enteropathy in pigs (Rowland and Lawson, 1975a and Love et al., 1977). C.s. subsp. bubulus can be recovered from the preputial sac of normal bulls (Florent, 1953) and has also been isolated from the small intestine of calves with some degree of inflammation (Lederle, 1963).

II. The catalase-positive group have been classified in the ways shown below.

Florent and others.	Veron and Chatelain (1973)	Smibert (1974)
V.f.ss. veneralis (Florent 1959)	C.f.ss. veneralis	C.f.ss. fetus
V.f.ss. intestinalis (Florent 1959)	C.f.ss. fetus	C.f.ss. intestinalis
V.f. subtype 1. (Bryner et al.,1962)	C.f.ss. veneralis biotype intermediate	
V. coli (Doyle, 1948)	C. coli	
V. jejuni (Jones et al., 1931)	C. jejuni	C.f.ss. jejuni
Related vibrio (King, 1957)		
V. fecalis (Firehammer, 1965)	C. fecalis	C. fecalis

The differentiation, characterisation and pathogenicity of Campylobacter species

Campylobacter fetus subsp. fetus has been known and associated with disease in cattle and sheep for many years. It is an inhabitant of the bovine prepuce and a pathogen of the female genital tract causing infertility and occasional abortion. It is characterised by the production of catalase, failure to produce hydrogen sulphide either in triple sugar iron agar or in sensitive media containing 0.02 per cent cystine (using lead acetate strip), fails to grow in one per cent glycine or in 3.5 per cent sodium chloride and grows at 25°C but not at 42°C. It is venereally transmitted. No association between this organism and enteric disease has been described.

Campylobacter fetus subsp. intestinalis has been isolated from the large intestine and faeces of experimentally infected cows (Florent, 1959) and causes outbreaks of abortion in sheep and sporadic abortion in cattle and is probably spread by the ingestion of contaminated food or water. El Azhary (1968) isolated C.f.ss. intestinalis from bovine faeces but did not consider it to be a pathogen. The literature on the subject is confused by lack of distinction between isolates of "Vibrio fetus". Allsup and Hunter (1973) isolated what now appears to be C.f.ss. intestinalis, a catalase-positive campylobacter which produced hydrogen sulphide only in sensitive media, growing at 25°C but not at 42°C, in one per cent glycine but not in 3.5 per cent sodium chloride, from the small intestine and faeces of both diarrhoeic and healthy calves. On serological grounds they considered that it was either V.f. intestinalis or V. coli. They also recorded that no histological lesions were associated with the isolation of vibrios, and claimed that the superficial changes they saw in the mucosa may have been caused by early autolysis. The experimental oral infection of two calves with an isolate of this type resulted in slight pyrexia, unthriftiness and intermittent scouring. The organism was present in the faeces for six weeks after challenge and was isolated from the jejunum and mesenteric lymph nodes (Allsup et al., 1972).

Campylobacter fetus subsp. jejuni. (V. jejuni) was incrimated as a causal agent of infectious diarrhoea (Winter scours) by Jones et al. (1931), who isolated it from the small intestine of unthrifty scouring calves and in (1932) Jones et al. isolated a similar organism from the faeces of scouring calves. It was also isolated from healthy calves by Florent (1959). El Azhary (1968) recovered this organism from bovine faeces and considered that C.f.ss. jejuni was a normal intestinal inhabitant not associated with any enteric condition in the ox. C.f.ss. jejuni has recently been associated with experimental mastitis in cattle (Lander and Gill, 1979) and they also suggested that infection might occur naturally.

C.f.ss. jejuni is catalase-positive, produces hydrogen sulphide in sensitive media, grows at 42°C but not at 25°C, and in one per cent glycine but not on 3.5 per cent sodium choride.

'Winter dysentery' or 'winter scour' resembling the disease described by Jones and his coworkers was observed in the autumn and winter in southern England by Rollinson (1948). The cause was not determined. Similar disease was seen in Sweden by Hedstrom and Isaksson (1951) who described outbreaks of epizootic enteritis in cattle which they suggested might be caused by a virus as no campylobacters were detected in the faeces. They failed to transmit the disease with filtrates of what they considered to be potentially infected material. Similar disease has also been reported in Canada by Macpherson (1957) who produced winter dysentery by subcutaneous inoculation of animals with filtered faeces and by oral inoculation of two animals with diluted faeces and suggested a viral aetiology. A sterilising Seitz filter was used and it is unlikely to have allowed bacteria through. Finally Scott et al. (1973) recently claimed that C.f.ss. jejuni was not involved in bovine winter dysentery and the aetiology of this disease was, at the beginning of this project, quite obscure.

The position of C.f.ss. jejuni was summarised by Blood et al. (1979) who considered that the organism may play only a secondary role in winter dysentery. The clinical signs of infectious diarrhoea (winter scours) or winter dysentery have been described by

a number of authors since the original description by Jones and Little (1931 a and b) and Jones (1933). Descriptions include those of Rollinson (1948); Merriman (1953); Udall (1954); Roberts (1957); Fincher and Fox (1963) and Blood et al. (1979).

The onset of the disease is usually sudden, and the milk yield drops markedly in adult milking cows. Whole herds may be affected with a mild to severe diarrhoea.

Young stock from six months to two years of age and bulls may also become affected, but the disease is usually milder than in milking or adult cattle. Winter dysentery is seldom observed in calves, and when it is present, symptoms are mild and the course is short. The animals become dull, refuse food and may exhibit signs of abdominal pain. There may be slight elevation in body temperature from 103°F to 105°F. The onset has been associated with the occurrence of a dry cough and excessive salivation. During the period of scouring, the temperature is usually normal and the pulse rate is normal except in severe cases in which it may become elevated. The respiratory rate is usually normal. The outstanding clinical sign is diarrhoea, the faeces are liquid to porridge-like in consistency, profuse, granular to smooth in texture, depending upon the amount of mucus present, and dark brown to greenish black in colour. They have a characteristic odour that may occasionally be foetid. In a few cases the faeces may be yellowish colour. In severe cases the faeces are often blood-tinged or contain large amounts of free blood and blood-stained mucus. Small amounts of the mucus are more common. In certain cases mucus casts one metre or more in length have been found in the stools.

The disease may run a relatively short course (a few hours) or diarrhoea may persist for four to five days. The rapidity of recovery and the loss in general condition depends on the length and severity of the diarrhoea. The morbidity in a herd affected with winter dysentery may be high, but the mortality is very low. Gross lesions have been described by Jones and Little (1931 a and b); Jones et al. (1932); Jones (1933); Rollinson (1948) and Blood et al. (1979).

The most noticeable pathological findings in both the natural and experimental disease were in the alimentary tract especially the mucosa of the lower duodenum, jejunum and the ileum in which the upper half and an area one metre above the ileocaecal valve were affected. The intestinal walls were thickened and the mucosa swollen, wrinkled and congested. The intestinal contents were fluid, usually dark brown in colour and mixed with mucus. Oedema and congestion of the mucosal folds of the abomasum were seen. Few abnormalities were observed in the large intestine.

In fatal cases the musculature was dry; the heart muscle was excessively dry and of brownish red colour. The liver was orange coloured and dry and when broken by bending, the fracture was straight and the surfaces dry and granular. The gall bladder was engorged with dark bile. The mesenteric lymph nodes were enlarged, pale and oedematous. On the whole the severity of the enteritis was not pronounced but its location was characteristic.

C.f.ss. jejuni (V. jejuni) can usually be isolated from the small intestine but not from other viscera (Jones and Little 1931 a and b).

The histological appearance of the lesions in both spontaneous and experimental studies have been described by Jones and Little (1931 a and b). The earliest changes were observed in the mucosa of the small intestine. The superficial mucosa was degenerate and overlain with mucus and disintegrated cells. The vessels of the mucosa and submucosa were congested. There were increases in cellular infiltration of the cores of the villi and the lamina propria with lymphocytes and neutrophils. Oedema of the lymph nodes and leucocytic infiltration were seen. The liver cells were degenerate and infiltrated with fat.

Infectious diarrhoea (winter scour) or winter dysentery is transmitted by contact between affected and healthy calves and cows. Jones and Little (1931 a and b) and Jones et al. (1932) showed that the the disease is reproducible and from both the spontaneous and the experimentally induced disease they cultivated 'Vibrio jejuni'. Such

organisms reproduced the disease when fed to calves and cows. Jones and his coworkers, Macpherson (1957) and Scott et al. (1973) also showed that transmission of the disease was due to the ingestion of the faeces of affected animals by healthy susceptible animals.

The incubation period of the disease varies from 3 to 7 days, Roberts (1957) and Blood et al. (1979). The latter considered that winter dysentery is highly contagious, and that the carrier of the infection usually is a recently purchased animal or a human recently in contact with an outbreak. Roberts (1957) considered it possible that birds, dogs, or other vectors might carry infective material. Diagnosis of winter dysentery is based on the clinical signs described above. A careful history should be taken because the outbreak of diarrhoea might be due to sudden feed changes or toxic chemicals. The post mortem findings are also useful in diagnosis but are rarely available.

Winter dysentery can be readily differentiated from mucosal disease or bovine virus diarrhoea on clinical grounds, as there are no mouth lesions in winter dysentery and blood is absent from the faeces of virus diarrhoea cases. The presence of blood in the faeces in some individual cases of winter dysentery may lead one to the suspicion of coccidiosis or salmonellosis but both of these diseases are more severe and usually affect only one or two animals at a time. Their absence can be confirmed by faecal examination and faecal cultures.

The microscopic examination of the faeces from a patient with diarrhoea may suggest the presence of enteric campylobacter infection (Butzler and Skirrow, 1979). Dark-ground or phase contrast microscopy can be used to recognise campylobacters by their extremely rapid darting and corkscrew motility. Despite their small size they can be seen by dark-ground illumination with a lower power objective. This method is likely to be of less value in the ox, in the faeces of which other motile vibrios may be seen. Isolation of campylobacters from faeces or from the mucosal surface of gastrointestinal and other organs using Thioblood agar (TBA), Brewer's medium and Albimi brucella broth (ABB) was described by Morris and Park (1971) for the isolation of campylobacter species from faeces, gastrointestinal mucosa and other organs.

Sheep blood agar or horse blood agar may also be used. More recently, Skirrow (1979a) described a selective medium with 5-7 per cent horse blood containing Vancomycin (5 mg); Trimethoprim lactate (2.5 mg) and Polymyxin B 1250 i.u. in 500 ml blood agar. All cultures were isolated in microaerophilic conditions.

Campylobacter colonies present on media were recorded and subcultured for subsequent identification.

Campylobacter fecalis has been isolated from the faeces of sheep (Firehammer, 1965) and was considered by him and by Smibert (1978) to be a non-pathogen. The characteristic features of this organism are its ability to produce hydrogen sulphide in triple sugar iron agar and in sensitive medium, growth in one per cent glycine, at 42° C not at 25°C or in 3.5 per cent sodium chloride. The distribution and importance of C. fecalis in the intestinal tract of cattle has not been assessed.

Miscellaneous bacteria associated with bovine enteric infections

A number of other bacterial species have been associated with lesions of the gastrointestinal tract. Some of them have been shown to produce enteric disease experimentally.

Actinobacillus species.

Actinobacillosis is a chronic infectious disease of cattle and sheep caused by A. lignieresi. Phillips (1961) isolated A. lignieresi from the ruminal content of 31 of 306 healthy adult cattle and suggested that A. lignieresi is a commensal in the rumen. A generally held view is that the organism enters the tissue through wounds of the skin and of the oral and ruminal mucous membrane (Gerring, 1947).

A. equuli has also been isolated from the faeces of a calf diarrhoea (Osbaldiston and Walker 1972). One calf was necropsied showing a necrotic rumenitis, inflammation of the intestine and pleuropneumonia. Their findings resembled those of Du Plessis et al. (1967) in calves with severe diarrhoea, dehydration, dyspnoea and nervous symptoms from one of which A. equuli was isolated. They produced a

profuse watery diarrhoea within 12 hours and, later, death in one of four calves following intravenous injection. A. equuli was isolated from calves with diarrhoea. Three other calves showed a mild rise in rectal temperature over two to four days, a serous to mucocatarrhal nasal discharge and slightly blood stained,mucus covered softened faeces.

Post-mortem examination revealed severe oedema and congestion of the lung as the cause of the death. In addition severe hydrothorax, oedema of bronchial, mediastinal and mesenteric lymph nodes and superficial necrosis of the mucosa in the blind end of the caecum was also reported. Histological findings included the presence of karyorrhexis in the lymphoid follicles at the ileo-caecal junction, in the mesenteric lymph nodes and patchy necrosis of the superficial parts of the ileal mucosa. Necrosis of the mucosa of the blind end of the caecum, varying in depth from the epithelium to the muscularis mucosa was also present.

Providencia stuartii

P. stuartii has been isolated from calves with neonatal diarrhoea and all of 14 isolations made by Waldholm et al.,(1969) belonged to biochemical group B. The pathogenicity of one isolate was tested by oral inoculation of a pure culture into 18 calves aged less than 30 minutes. (None was more than one hour old), 15 calves were also used as normal controls. Diarrhoea occurred in 12 of 18 inoculated calves as compared with the occurrence of mild diarrhoea in two control calves. Their results suggested that P. stuartii may be a cause of calf diarrhoea.

Pasteurella spp.

Pasteurella were cultured from the intestinal tract of four per cent of dead calves which had been extensively treated with antibacterial sera, sulphonamides, and antibiotics (Smith, 1965).

Acute enteritis has frequently been recorded in P. multocida haemorrhagic septicaemia, and P. multocida has often been isolated from cases of gastroenteritis (Blood et al., 1979).

Yersinia spp.

Y. enterocolitica was isolated from the intestinal contents of 9 out of 115 slaughter cows and 15 of 61 fat cattle (Inoue and Kurose, 1975). Wooley et al. (1980) isolated Y. enterocolitica from bovine faecal samples. Mittal et al. (1980) attempted to study the serological response produced by the oral inoculation of three month old calves, but failed to produce one although serum antibodies could be demonstrated following intramammary inoculation in cows.

Pseudomonas spp.

Barr et al. (1955) isolated Ps.aeruginosa together with E. coli and a salmonella from cases of calf diarrhoea which had developed following treatment with broad spectrum antibiotics for other diseases. Enteritis in a dairy herd with severe diarrhoea containing mucus and blood in adult cows and calves has been reported. Ps.aeruginosa was isolated in large numbers from the faeces during life and from necrotic enteric lesions after death of both calves and adult cows (El Nasir, 1959).

Diarrhoea associated with mastitis from which Ps.aeruginosa was isolated was recorded in Britain by Gardiner and Craig (1961). Diffuse reddening, congestion of the abomasum and patches of the small intestine throughout its length were found at post-mortem examination. Matthews and Fitzsimmons (1964) demonstrated Ps.aeruginosa in rectal swabs from clinically normal calves aged from 1-8 weeks. The organism was found throughout the intestinal tract in young healthy calves but it was confined to the posterior part in older calves slaughtered between the ages of 4 and 59 days. In two clinically abnormal, colostrum-deprived calves artificially reared up to 80-97 days, Ps.aeruginosa was isolated consistently from rectal swabs taken during life and the organism was also isolated from the small intestine at slaughter. They suggested that the highest incidence of Ps.aeruginosa appeared to extend from the first week of life to about the third or fourth week and thereafter there was a gradual diminution of incidence with advancing age.

Smith (1965) isolated Pseudomonas from 21 per cent of diarrhoea calves and Hoadley and McCoy (1968) reported that the incidence of calf mortality was greater in a farm with a high incidence of Ps.aeruginosa than in farms free from the bacterium. They also failed to isolate Ps.aeruginosa from rectal swabs after four weeks of age.

Fusobacterium (Sphaerophorus) spp.

Fusobacteria are common inhabitants of the alimentary tract of animals (Kanoe et al., 1975) and are also associated with necrotic and gangrenous lesions. In many instances the organism is considered to be a secondary invader rather than a primary cause of disease. F. necrophorum has commonly been isolated in pure culture from hepatic abscesses and rumenitis (Mullen, 1970 and Weiser et al., 1966) in ruminants, conditions which can be reproduced experimentally (Jensen et al., 1954).

Bacteroides melaninogenicus

B. melaninogenicus has been isolated from cases of traumatic pericarditis, traumatic peritonitis, suppurative arthritis and fibrinous pneumonia. It is invariably found in association with other micro-organisms (Biberstein et al., 1968).

Various other bacterial species.

Aerobacter aerogenes, Klebsiella spp., Shigella spp., Staphylococcus spp., Streptococcus spp., Corynebacterium spp., have been isolated from intestinal contents and internal organs, but are of minor importance compared with E. coli and Salmonella spp. (Amstutz, 1965), although Smith (1965) suggested that bacterial isolates of this type might be a significant cause of death.

2. Mycotic agents associated with enteric disease in cattle.

Candida albicans has been identified histologically in lesions in the liver, forestomach and other organs and has been recovered in pure culture from some of these organs in young calves with severe diarrhoea following repeated antibiotic therapy. (Mills and Hirth, 1967). In another report (Morin et al., 1976), three cases of mycotic abomasitis were identified amongst 55 calves which had died from severe neonatal diarrhoea. The abomasal mucosa showed consistent lesions of focal areas

of necrosis and haemorrhage in the mucosa with occlusion of mucosal and sub-mucosal vessels by thrombi. Periodic acid-Schiff stain revealed large irregular branching non-septate hyphae either free in the necrotic areas of the mucosa or in the thrombi. It was also recorded in the rumen and reticulum of one calf.

3.Viruses in enteric diseases of cattle

The involvement of viruses in bovine diarrhoeas has been recognised more frequently in recent years. The use of electron microscopical examination of negatively-stained preparations of faeces and the preparation of specific antisera to rota and coronaviruses has enabled the presence of viruses to be confirmed. Experimental work in conventional, colostrum deprived and gnotobiotic calves has shown that these viruses can initiate changes in the gastrointestinal tract. The disease seen in the field is not, however, always the same as that seen in experimental animals and other factors may sometimes be involved. The viruses present in the enteric tract of cattle in the U.K. are reviewed briefly below.

Rotaviruses

Rotavirus particles are 60-66 nm in diameter and have a characteristic wheel-like morphology. Flewett et al. (1974) and can be readily identified by their appearance in negatively-stained preparation of faeces viewed by electron microscopy. They have been associated with diarrhoea in calves (Turner et al., 1973; Woode et al., 1974; Bridger and Woode, 1975) and have also been demonstrated in the faeces of calves and yearling cattle with diarrhoea (Durham and Burgess, 1977; Durham et al., 1979).

The virus is widespread in cattle in the U.K. In a survey of the incidence of rotavirus associated with diarrhoea of calves in the United Kingdom and Northern Ireland, rotavirus was demonstrated in 80 per cent of outbreaks and from 66 per cent of faecal samples (Woode, 1976). The herds studied included both dairy and beef herds with a history of enzootic diarrhoea or sporadic and acute epizootics of the disease. Woode (1978) reported that infection could be transmitted by direct

contact or indirect contact through the agency of human handlers or contaminated utensils under intensive housing systems.

Infections may occur in animals of all ages including adult cattle (Woode and Bridger, 1975). Rotavirus infection in older animals may be clinically dramatic. There is a marked drop in milk production but the syndrome does not result in serious illness in the individual concerned (Woode and Crouch, 1978).

Rotavirus has been associated with enteric infection and death in calves aged 0-9 weeks and in yearling cattle (Bridger and Woode, 1975). Infection may be endemic on individual farms. Anderson (1980) suggested that infection could become obvious as a result of conditions such as overcrowding, poor sanitation improper feeding of calves, deficient colostrum intake, or failure to isolate newly acquired animals. He also stressed that adverse weather conditions seemed to trigger outbreaks of disease. Maternal immunity appears to prevent the clinical disease in the first few days after parturition (Woode et al., 1975), during which time the colostrum contains specific rotavirus antibody active in the lumen of the intestine. Virus is present in, and may be isolated from such infected calves. They suggested that passively derived maternal antibody in the blood does not protect the calf against oral challenge and confirms field observations of the prevalence of the disease.

Experimental studies have shown that rotavirus is pathogenic for calves and that it can induce diarrhoea and pathological lesions in non-immune animals challenged with both animal-passaged and cell culture passaged virus. The virus may be recovered from all such animals. (Woode and Bridger, 1975).

The incubation period of the experimental disease in both colostrum deprived and gnotobiotic calves was 18-24 hours following oral inoculation (Mebus et al., 1971; Woode et al., 1974). It has been suggested that natural disease varies from relatively mild to severe with death in field cases, the severity probably depending on the dose of virus and the nature of the bacterial flora (Woode and Bridger, 1975).

The studies of Mebus et al. (1971) in which they found that non-pathogenic E. coli enhanced the severity of rotavirus infection in gnotobiotic calves confirms this supposition.

According to Mebus (1976 a and b) clinical signs in individual calves usually progressed in the following order: depression, anorexia, a few strings of thick saliva hanging from the lips and then diarrhoea. The onset of depression was rapid and in some cases calves progressed from apparent normality to recumbency in two hours. The diarrhoeic period lasted five to six hours during which time the animal passed up to 300 ml of liquid, yellowish faeces. The amount passed appeared to depend on the previous level of milk intake.

The sequential pathology of experimental infections in gnotobiotic calves was studied by Mebus (1976 a). At about 30 minutes and four hours after the onset of diarrhoea, tissue appeared to be macroscopically normal. However, on histological examination of tissue obtained 30 minutes after infection, the small intestine was found to be lined by tall columnar villous epithelial cells which showed immunofluorescence when stained specifically. Within four hours these cells were lost and replaced by low cuboidal cells and stunting of the villi was seen. Anderson (1980) reported that some disruption of the normal villous architecture might be seen upon examination of fresh specimens of small intestinal mucosa under the dissecting microscope.

Rotavirus infection may be confirmed by demonstration of the agent in faeces or in small intestinal contents either by direct electron microscopical examination, immunoelectronmicroscopy or else by immunofluorescent staining of faecal smears or infected cell cultures (Woode and Bridger, 1975; Flewett, 1978). However, the virus is known to be widespread in calves and other neonates (Woode and Bridger, 1975; Snodgrass et al., 1977) and has also been demonstrated in the faeces of apparently normal calves so the mere finding of the virus is not without interpretative problems.

Coronaviruses

Coronaviruses were thought by Stair et al. (1972) to play a part

in the neonatal calf diarrhoea syndrome. This virus has been identified by electron microscopic examination of the faeces of diarrhoeic calves in Britain (Woode et al., 1974) and resembles that which causes transmissible gastroenteritis (TGE) virus of pigs. They have been recognised in the faeces of scouring calves, cattle 3 months or more of age and in adult cattle (Horner et al., 1975; Sharpee et al., 1976; Durham et al., 1979).

The virus may be recognised by its distinctive morphology when viewed by electron microscopy. It appears as a round body surrounded by numerous small projections resembling a crown and measured about 80-120 nm in diameter.

The primary lesion observed in calves infected with both rotavirus and coronavirus is very similar to lesions observed with transmissible gastroenteritis (TGE) of pigs. Loss of function of the epithelial cells of the small intestine results in malabsorption, and diarrhoea. Death may follow as a consequence of ionic, bicarbonate and serum protein loss, acidosis and dehydration. Much of this results from the loss of the membrane bound enzyme, lactase. Undigested lactose then causes high osmotic pressure in the gut lumen (Woode and Bridger, 1975). Bacteria may multiply in this rich medium and contribute to the disease (Mebus, 1976 b).

The clinical signs of coronavirus infection in calves have been described by Anderson (1980). Severe depression, non-bloody diarrhoea and dehydration progressing over four to five days to a moribund state and death are seen. The disease most frequently occurs between 5-21 days of age, although calves two or three days old could be affected.

At post-mortem examination no gross diagnostic changes can be ascribed to coronavirus infection. Villous atrophy of the small intestinal mucosa is visible using a dissecting microscope. Gills et al (1977 suggested that both jejunum and ileum should be examined since the damage usually begins high in the tract and progresses distally. The examination should be carried out on fresh tissue floating in physiological saline solution.

Experimental studies have shown that in both colostrum deprived and gnotobiotic calves, diarrhoea developed within 19-24 hours following oral inoculation with coronavirus (Mebus et al., 1973 and 1975). Infected calves were moderately depressed and slow to eat and passed liquid yellow faeces. In gnotobiotic calves,lesions were present in the colon, mesenteric lymph nodes and in all segments of the small intestine. Calves killed four hours after the onset of diarrhoea had specific immuno-fluorescence in the epithelial cells of the villi of the small intestine and on the surface of the colon. Calves killed at 34-44 hours post infection had shortened and some fused intestinal villi and cuboidal epithelial cells. The crypt:villus ratio in the lower small intestine averaged 1.0 compared with 5.3 in a control calf. Atrophy of the colonic mucosal ridges were seen. Specific immunofluorescence was present in cells of the tips of the villi and in the mucosal epithelium of the colon.

Parvovirus infections

Bovine parvovirus has repeatedly been reported to cause diarrhoea and has been isolated from the faeces of sick calves (Storz et al., 1978). It has also been demonstrated by immunofluorescence in cells in sections of small intestine from dead calves (Storz and Bates, 1973). They inoculated bovine parvovirus into normal colostrum-fed calves by the oral and intravenous routes. All the calves developed diarrhoea 24-48 hours after inoculation which was first mucoid and then watery in consistency. Bovine parvovirus was first excreted in their faeces 24 hours after inoculation and then excreted continuously. It was found in highest concentration within the mucosal cells of the jejunum and ileum in immunofluorescence studies. The calves inoculated intravenously also excreted parvovirus in the diarrhoeic faeces. Enterocytes of all parts of the intestinal tract were infected and this infection was cytocidal. Different organs became infected during the viraemic stage but most pronounced replication of bovine parvovirus occurred in the lymphatic tissues. Their findings suggested that bovine parvovirus could be pathogenic for newborn calves because of the unusual nature of these agents and because of their affinity for actively growing cells.

Serological studies were carried out on serum samples collected from 35 herds of cattle that had a history of abortions or enteric

disease in newborn calves. Twenty-nine herds had antibody titres indicating that parvovirus infection is widespread in the United States.

Bovine enteroviruses

Bovine enteroviruses have been isolated from apparently normal calves and adult cows as well as from those suffering from clinical disease (Moscovici and La Placa, 1962; Spradbrow, 1963; Rovozzo et al., 1965; Van Der Maaten and Packer, 1967).

Enteroviruses appear tohave some pathological potential in bovine enteric disease as they have been shown to cause neonatal diarrhoea in calves when given experimentally (Van Der Maaten and Packer, 1967). Dunne et al. (1974) infected 10 colostrum-fed and 9 colostrum-deprived calves aged one day to eight weeks with enterovirus. Fever occurred in 13/19, diarrhoea in 8/19 and leukopaenia in 6/10. They suggested that the disease was more severe in calves which had been colostrum-deprived.

Astroviruses and calicivirus

Small viruses have been found in faeces from calves infected with bovine rotavirus or bovine coronavirus (Woode et al., 1974), and were also isolated from three samples of calf diarrhoea (Woode and Bridger, 1978). Samples of these viruses caused diarrhoea when given orally to gnotobiotic calves. Anorexia occurred after an incubation period of 1-3 days. Astrovirus-like and calicivirus-like particles were seen in faecal preparations from infected gnotobiotic calves. The calicivirus-like agent (Newbury agent) was 33 nm in diameter with reticulate surface appearance and projecting capsomeres. The virus mixture caused diarrhoea in 12 gnotobiotic calves. Those infected with Newbury agent were found to have lesions confined to the small intestine. Haemorrhagic foci occurred and the villi appeared to be shortened when viewed under a dissecting microscope. In histological sections the villi were shortened and thickened with an increased cellularity of the lamina propria and capillary dilatation, especially in the mid-small intestine. Most villi had normal columnar epithelial cells with normal brush borders, but on some villi the epithelium was lost from the top third and on the other villi epithelial cells a cuboidal appearance.

The astrovirus-like agent was 28 nm in diameter and was distinct from the Newbury agent. On some particles a six-pointed star pattern was visible and they occurred in clumps of varying size. This virus did not cause diarrhoea in two gnotobiotic calves although one of them had mild villous atrophy in the middle region of the small intestine.

Adenovirus infection in cattle

Several serotypes of adenovirus have been isolated or serologically identified in naturally infected cattle (Bibrack and McKercher, 1971). More recent, Mattson (1973 a) suggested that adenovirus has been incriminated as a causative agent in naturally occurring pneumonoenteritis of calves aged 1-4 weeks. Although the infection occurs in calves from cows of all age groups, both morbidity and mortality are higher among calves delivered by first-calf heifers. He also reported the initial clinical signs included excessive ocular and nasal discharge and tympanitis, colic and diarrhoea. The virus was isolated from the conjunctiva and nasal cavity.

Experimentally induced infection of colostrum-deprived calves has resulted in a clinically and pathologically recognisable syndrome (Mattson, 1973 b). The calves were examined at post mortem 7 to 16 days postinfection and gross pathological changes were found in the lungs and the lymphatic tissue of the respiratory and enteric tract.

Bulmer et al. (1975) found evidence of enteric adenovirus infection in two 2-week old calves suffering from pyrexia, diarrhoea and subsequent dehydration. At post-mortem examination foci of necrosis were found in the abomasum and rumen of each calf. The small and large intestine was seen to be dilated by greyish, turbid fluid. Numerous large amphophilic intranuclear inclusion in endothelial cells of blood vessels in the abomasum and rumen, in endothelial cells of the adrenal cortical sinusoids and renal glomeruli. Adenovirus particles were identified in the intestinal epithelial cells.

Bovine viral diarrhoea - mucosal disease (B.V.D. - M.D.).

Bovine viral diarrhoea or mucosal disease is characterised by diarrhoea, fever, leukopaenia, nasal discharge, depression,

dehydration and subsequent abortion in pregnant animals. It has been described in neonatal calves (Schipper and Eveleth, 1957) in which it caused death within 96 hours of onset, but occurs most commonly in animals aged between 6 and 20 months of age (Johnston, 1959). The disease has been identified in cattle of all ages in Scotland (Dow et al., 1956) and in England (Huck, 1957) where attention was drawn to the presence of blood in the faeces of affected animals. There appears to be a higher incidence in winter (Blood et al., 1979).

The post-mortem findings include enteric lesions varying from a mild enteritis to a haemorrhagic enteritis with free blood in lumen of the bowel. Ulceration of the lips, the mucous membranes of the mouth, tongue, pharynx, oesophagus, small intestine and large intestine may all be found. In many regions of the alimentary tract (especially the oesophagus) these ulcers are linear and haemorrhagic. Lymph nodes may sometimes be enlarged and oedematous or mildly congested when sectioned. The haemal lymph nodes are frequently prominent.

Infection with the virus or suspensions of infected material such as urine, faeces or nasal secretions gives rise to clinical signs after an incubation period of 4-10 days. Experimental studies by Lambert et al. (1969) showed that following oral or intranasal infection with virus, infected calves developed diarrhoea within 24 hours remained diarrhoeic for 7-10 days. The virus was isolated from nasal and rectal swabs for up to 103 days post infection. One of eight colostrum-fed calves and four of 13 specific-pathogen-free, colostrum-deprived calves died of neonatal enteritis attributed to bovine viral diarrhoea virus after 48 hours. Post-mortem findings included ulcerations of the digestive tract and severe lymphoid depletion.

At necropsy bovine viral diarrhoea virus was isolated from the heart, spleen, mesenteric lymph nodes, small intestine and caecum. Diagnosis is based on the clinical features, and the post-mortem findings in particular the characteristic linear ulcers of the oesophagus and small intestine. It may be confirmed by virus isolation from rectal and nasal swabs and by demonstrating a rise in serum antibody in recovered cases.

Malignant Catarrhal Fever

Malignant catarrhal fever (MCF) is an acute, generalised disease of cattle characterised clinically by high fever, with severe inflammatory and degenerative lesions in the mucosa of the upper respiratory tract and throughout the alimentary tract. It is associated with infection with a cell-associated herpes virus.

Malignant catarrhal fever of cattle frequently occurs as a sporadic disease affecting one or several animals in a herd (Plowright, 1968). The mortality is extremely high, usually greater than 95 per cent and death commonly occurs five to 12 days after onset of pyrexia.

Pierson et al. (1974) recorded an epizootic of malignant catarrhal fever occurred in cattle and recognised three forms, peracute, head and eye and the intestinal forms. Typical clinical signs included anorexia, depression, nasal discharge, hyperventilation and rectal temperature of 40.6 to 42.2°C. Superficial lymph nodes were enlarged and easily palpable. Copious lacrimation and photophobia occurred and partial or complete opacity of the cornea developed within 36 hours of the onset of the disease. Visible mucous membranes were hyperaemic, profuse salivation was present and the faeces were watery, foetid and bloody. Rapid and severe loss of body weight also occurred. Moderate leucopaenia occurred. The post-mortem findings included enlargement of somatic and visceral lymph nodes and erosion, ulceration, oedema or hyperaemia in the mucosa of the oral cavity, oesophagus, forestomachs, distal jejunum, ileum, caecum and proximal colon. Fibrinopurulent exudate was consistently found in the nasal cavities and occasionally elsewhere in the respiratory tract. Lesions were present in most parts of the body. The basis of these lesions is a necrotizing vasculitis (Pierson et al., 1974).

Diagnosis of this syndrome is based on the clinical signs, blood picture and lesions observed at necropsy.

4. Chlamydial infections of the bovine enteric tract.

Chlamydia have been isolated from the intestinal mucosa of clinically normal young calves (York and Baker, 1951) and from the faeces of newborn calves with diarrhoea and polyarthritis (Storz et al., 1966), It was also isolated from faeces of a healthy cow (Smith et al., 1973) and caused acute tracheitis, interstitial pneumonia and mild diarrhoea when inoculated intratracheally and orally into neonatal colostrum-deprived calves. Fever and transient mucoid diarrhoea occurred.

After oral inoculation with specific chlamydial strains from bovine polyarthritis, young calves developed severe diarrhoea and polyarthritis and the infection became localised in the lower portion of the small intestine and multiplied in mucosal epithelial cells of the gastrointestinal tract. Local spread to the lamina propria occurred and it could be isolated from the regional mesenteric lymph nodes and liver (Eugster and Storz, 1971).

Pathological changes have been studied in newborn, colostrum-deprived or colostrum-treated calves infected orally with bovine chlamydia by Doughri et al. (1974). Calves showed a slight rise of temperature, diarrhoea within 24 hours, became dehydrated, weak and then moribund when killed four to five days after infection.

Gross lesions were found in the abomasum and throughout the intestinal tract, the terminal part of the ileum being most consistently severely affected. The lesions affected the mucosa and comprised oedema, congestion, petechial haemorrhage, epithelial erosions and ulceration. Histopathological features included desquamation of the surface epithelium, lacteal dilatation, occlusion and dilatation of the crypts of Lieberkuhn and infiltration of the lamina propria by neutrophils and mononuclear cells. Ultrastructural changes in infected cells were degenerative and characterised by irregular alterations in the size and shape of microvilli, swelling of the mitochondria, dilatation of the endoplasmic reticulum, loss of ribosomes, ballooning of cells and rupture of plasma cell membranes. Supranuclear intracytoplasmic chlamydial inclusions stained by Wolback's Giemsa stain could be demonstrated in the

epithelial cells covering the tips of the villi and intravillous zone but were rare in the base of the villi.

Foggie (1977) outlined the methods used to isolate and to diagnose chlamydial infections.

a. Microscopical examination of smears stained by modified Ziehl-Neelsen or Machiavello methods may confirm the diagnosis. This method used when a heavily infected material is available.

b. Fluorescent antibody techniques can also be used.

c. Isolation techniques should also be used and the most common method is yolk sac inoculation in five to seven day incubated hens' eggs.

d. The most commonly used serological technique is the complement fixation (CF) test in which sera are tested against a stable, heat-treated antigen prepared from suspensions of chlamydia.

5. Protozoal infections of the bovine enteric tract.

Cryptosporidial infections in cattle

The first description of bovine cryptospiridiosis was in 1971 (Panciera et al., 1971). There have been several subsequent reports on cryptosporidiosis in calves with neonatal diarrhoea (Meuten et al., 1974; Schmitz and Smith, 1975; Powell et al., 1976; Pohlenz et al., 1978). Morin et al. (1976) demonstrated cryptosporidium in 11 of 55 calves with diarrhoea. In six of the 11 calves, cryptosporidia was found along with rotavirus or coronavirus or both. The changes in the histological appearance of the lesion have been described by Meuten et al., 1974; Schmitz and Smith, 1975; Powell et al., 1976; Morin et al., 1976). The lesions occur in the ileum and in the colon. The lamina propria of the ileum was infiltrated with plasma cells and lymphocytes. Neutrophils and eosinophils were also noted. The villi appeared shortened and blunted. Colonic lesions were focal with reduction in the thickness of the mucosa with mononuclear cell infiltration of the lamina propria and inflammation of the crypts. Columnar epithelial cells were replaced by shortened cuboidal cells. Cryptosporidial organisms were observed in

various stages of their life cycle in the crypts of Lieberkuhn and embedded in the microvillous border of the epithelium of both small and large intestines.

Cryptosporidia could be seen adhering closely to the surface of intestinal epithelial cells (Meuten et al., 1974; Powell et al., 1976; Pearson and Logan, 1978) and microvilli were absent from the site of parasitic attachment.

Experimental transmission of cryptosporidiosis (along with rotavirus and coronavirus) was described by Pohlenz et al. (1978). All calves developed profuse watery diarrhoea and had raised rectal temperatures within 48 hours of inoculation. Faeces were ringed with flecks of blood 48 to 72 hours after inoculation, and also contained masses of fibrin and mucus. Diarrhoea persisted in all four calves until they were killed or had died. Cryptosporidia were demonstrated in histological sections in the ileum of all four infected calves and less frequently in the jejunum and large intestine. Cryptosporidia were also seen in smears from faecal samples stained with Giemsa stain daily from four to 12 days post-inoculation. Rotavirus and coronavirus were also present on days 3 and 6 after inoculation but not on days 9 and 10 after inoculation. These findings suggest that

(1) Cryptosporida can be regarded as enteric pathogens of calves.

(2) Diagnosis can be based on finding the organism in Giemsa stained smears of faeces or scrapings of ileal mucosa supplemented when possible by histological examination of the ileum.

The histological diagnosis of bovine cryptosporidiosis is based on the discovery of ovoid or round sporozoans approximately 3.0 by 2.5μ in the microvillous border of the small and large intestinal epithelium cells. Multiple stages of the life cycle of the organism can be identified, other histological changes mentioned above could also be useful.

Examination for the organism is aided by using special stain such as toluidine blue and Wolback-Giemsa applied to very thin 3.0 μ

tissue sections.

Coccidial infections in cattle

Coccidiosis is a major disease problem of domestic ruminants in many parts of the world. It is a contagious enteritis caused by infection with Eimeria spp. Subclinical infection may occur or there may be diarrhoea and dysentery. In some cases there is anaemia and the chronic form of the disease is characterised by reduced growth rates. (Blood et al., 1979).

In cattle, infection occurs mainly in animals three weeks to six months of age kept under crowded, unhygienic conditions, but occasionally disease occurs in yearling or even adult cattle, especially if massive infections are acquired (Soulsby, 1968). The two major pathogenic species are E. zurnii and E. bovis, the former being the principal parasite in Europe. Other species such as E. auburnesis, may, at times, contribute to the general clinical picture.

In Britain, coccidiosis has been recorded as a sporadic disease in the late summer and autumn in the south-west of England and in Northern Ireland (Soulsby, 1968).

The life cycle of bovine coccidia passes through asexual and sexual stages in 18 to 21 days. In the two common species mentioned above, two asexual generations occur, the first stage schizonts occurring in the epithelial cells of the small intestine and the second stage in those of both the small and large intestine. The sexual stages or gametocytes are found in both small and large intestine and are more numerous and pathogenic than the asexual forms. In severe infections the majority of the intestinal crypts are destroyed, the epithelial layer is denuded and haemorrhage occurs. In calves experimentally infected with E. zurnii, first generation schizogony occurs in the lamina propria of the lower ileum and second generation schizogony and gametogony occurs in the epithelial cells of the caecum and proximal colon (Stockdale, 1976).

Oocyst production in calves infected with E. zurnii reaches peak numbers on the 19th and 20th day after experimental infection (Stockdale

and Niilo, 1976).

The clinical signs of coccidiosis depend on the severity of infection. In mild cases, severe diarrhoea with foul-smelling fluid faeces containing mucus and blood occurs suddenly. The blood may appear as dark tarry staining of the faeces or as streaks or clots, or the motion may consist entirely of large clots of fresh red blood. There is a decrease in the appetite of most animals with clinical coccidiosis. The course of the disease is usually 5-6 days but some animals undergo a long convalescent period during which feed consumption and body weights are subnormal (Fitzgerald and Mansfield, 1972).

The acute disease is characterised by haemorrhagic diarrhoea and the condition may become so intense that the faeces are dark and contain frank blood. Tenesmus is marked, there is anaemia, weakness and inactivation. In severe infection, death may occur as early as seven days after the onset of clinical signs. The gross pathological changes have been recorded by Soulsby (1968), Julian et al. (1976) and Blood et al. (1979). Gross lesions were present only in the digestive tract. The large intestine and ileum were congested and slightly oedematous. The mucosa had a granular appearance with scattered flecks of fibrin on the surface and yellow brown watery contents. Some mucus and casts of fibrin were present in the colon. Ulceration or sloughing of the mucosa has been recorded in severe cases. Whole blood or blood-stained faeces may be present in the lumen of the large intestine and the carcase may show profound anaemia.

Histological changes include necrosis and desquamation of epithelial cells with haemorrhage in parts of the colon. Merozoites may be present in some cells. Schizonts and gametocytes are present in the cells of some crypts. Some crypts contain oocytes, necrotic material and debris. Coccidial forms are absent from some sections. Hyperaemia and oedema are also present in the lamina propria and increased numbers of eosinophils, lymphocytes and neutrophils are present, particularly in the villi and in areas where the epithelium is lost. Lymphatic tissue in the ileum, caecum and colon is oedematous.

Diagnosis is based on clinical signs especially the haemorrhagic diarrhoea in acute cases and demonstration of large numbers of oocysts in the faeces. In peracute cases, especially those due to E. zurnii, oocysts may be few, the marked pathogenic effects being produced by the development stages prior to the shedding of the oocysts.

History, herd morbidity, seasonal occurrence and histological examination may also be helpful in diagnosis.

6. Nematode infections of the bovine gastrointestinal tract.

A number of nematode species have been recorded from cattle and nematode infection in cattle is the norm unless stringent measures to eliminate or prevent infection have been carried out. In general calves reared indoors are free from many nematode infections and all grazing cattle are infected, although the degree of infection varies. Nematodes may be found in all regions of the gut. Those commonly present in cattle in Britain are as follows (Armour, 1974): Ostertagia ostertagi in the abomasum and commonly involved in outbreaks of bovine gastroenteritis.

Trichostrongylus axei in small numbers in the abomasum and as a primary pathogen in at least one outbreak of parasitic gastritis in young calves in the early spring.

Cooperia oncophora in the small intestine.

Nematodirus helvetianus in the small intestine and as a cause of outbreaks of parasitic enteritis in calves in south of England in mid-summer (Rose, 1972).

Bunostomum phlebotomum in the small intestine as a cause of parasitic enteritis in cattle (Soulsby et al., 1955).

Strongyloides papillosus and Oesophagostomum radiatum have also been recorded from British cattle.

The only significant nematode parasite of the abomasum is Ostertagia ostertagi which is the most pathogenic and economically important in Britain and in several areas of the world (Armour, 1970, 1974).

Ostertagia ostertagi infection occurs in two clinical forms in calves (Anderson et al., 1965; Armour 1970, 1974), Type I, and Type II. Type I disease is the classical type of clinical parasitic gastritis in which calves at grass for the first time show a loss of weight and diarrhoea. This may occur at any time from late July until the end of the autumn grazing season and results from the ingestion of vast numbers of infective larva (L3) which enter the gastric glands and mature within 3-4 weeks to young adults which are pathogenic. Adults emerge after two months to lie on the mucosal surface and eggs then appear in the faeces. The complete life cycle in the host normally takes 21 days except in late autumn and winter when many of the ingested L3 become inhibited to give rise to Type II disease at a later date.

Type II disease is preceded by a Pre-Type II clinical syndrome. Large populations of Ostertagia ostertagi are present, of which over 80 per cent were inbibited in the early fourth stage (EL4). Type II disease results from the later maturation of sufficient numbers of these inhibited larva to cause a clinical condition characterised by weight loss and diarrhoea. It occurs in both outwintered and housed stock following the first grazing season.

The presence of large numbers of Ostertagia ostertagi in the bovine abomasum gives rise to severe clinical signs and extensive pathological and biochemical changes.

Two factors have been suggested by Armour (1970) as the direct cause of diarrhoea in bovine ostertagiasis, the direct effect of the emergent parasite per se or its cytolytic action on the gastric epithelium and an indirect effect in which hyperplasia and loss of differentiation of the gastric gland epithelium produces a loss of parietal cells, elevation in abomasal pH and increased numbers of bacteria.

The main clinical sign in both Type I and Type II Ostertagiasis in cattle is a profuse watery diarrhoea. In the Type I form, it tends to be continuous and of a characteristic bright green colour due to failure of the abomasum to denature chlorophyll. In the Type II form of the disease it is more intermittent.

Pathological abomasal lesions have been described by Armour (1974). The lesion produced in Ostertagia ostertagi infection is a raised nodule with a visible central orifice; in heavy infections these nodules overlap and coalesce to produce an effect reminiscent of morocco leather. Sometimes necrosis and sloughing of the mucosal surface occur and oedema and hyperaemia are usually present; the regional lymph nodes are enlarged and reactive. The adult worms are reddish-brown and about 1 cm in length and may be seen on the mucosal surface. Diagnosis is based on the seasonal incidence of the disease, the previous grazing history of the animals involved and the clinical signs. Faecal egg counts in Type I disease are normally greater than 1000 eggs per gram but in Type II disease the count is highly variable and is of limited value.

In clinically affected animals up to two years of age plasma pepsinogen levels are usually in excess of 3000 I.U. The test is less reliable in older cattle where high values are not necessarily indicative of heavy current infection. Larval counts may be obtained by tryptic digests of affected mucosa.

7. Other causes of enteritis and diarrhoea.

Bovine renal amyloidosis

Bovine renal amyloidosis is a condition of individual middle-aged to old cows in which deposition of amyloid, an inert, proteinaceous material in the renal glomeruli results in a disease characterised clinically by profuse diarrhoea, subcutaneous oedema and renal enlargement.

The clinical signs and pathology of this disease have been described in detail by Murray et al. (1972). The disease usually arises in dairy cows within a few weeks of calving and the presenting signs are a very profuse, homogenous diarrhoea and marked subcutaneous oedema. Once the clinical signs become evident, the disease is of relatively short duration. In all cases the carcases are thin with widespread subcutaneous oedema. The abomasal folds, intestinal walls and mesentery are thickened by oedema fluid. There may be a slight increase in free abdominal fluid in most cases. Both kidneys are two to three times larger than normal and pale, granular, yellowish-brown with a waxy appearance.

Microscopical changes in the intestine include subepithelial oedema, with distortion of villous architecture and the presence of amyloid in the lamina propria, submucosa and the muscularis.

Their findings suggested that the development of diarrhoea in this syndrome was probably related to the widespread deposition of amyloid around lymphatics and blood vessels in the gastrointestinal tract and to the distortion of small intestinal villi by general oedema.

Diagnosis rests upon recognition of the clinical signs especially those of profuse diarrhoea, marked subcutaneous oedema and enlargement of the left kidney and the characteristic pathological lesions in the kidney and intestine.

Nutritional deficiency and the occurrence of bovine enteric disease.

Nutritional factors have commonly been incriminated in the pathogenesis of diarrhoea. Amstutz (1965) stated that deficiencies of Vitamin A, D and E contributed to the incidence of diarrhoea and Withers (1953) concluded that absence of sunshine was among the factors contributing to much of the calf mortality in the early months of the year. Murphy (1955) provided evidence that the administration of Vitamins D3 and A to the dam before calving did not prevent the incidence of calf diarrhoea.

When gastrointestinal disturbance occurs, however, it may lead to multiple deficiencies. Among the first to develop are deficiencies of several B vitamins. These in turn may cause diarrhoea. In calves, diarrhoea follows deficiencies of thiamine, riboflavine, pantothenic acid, nicotinic acid and folic acid (Anon, 1971).

Anderson (1980) reported that the occurrence of calcium deficiency was more reasonably attributed to diarrhoea initiated by improper feeding, which resulted in poor assimilation of calcium.

Acute enteritis may also occur as a result of lactic acid formation after engorgement on grain in ruminants and has been reported to occur following the feeding of grass silage with high lactic acid content (Tutt, 1972).

Physical agents associated with bovine enteric disease.

Physical agents are uncommon causes of enteritis in farm animals but may result from the ingestion of large quantities of sand or soil in feed contaminated during dust storms (Blood et al., 1979).

Trauma by roughage has also been reported to cause gastric ulcers in calves in the first three months of life. An incidence of approximately 80 per cent in calves four-five weeks old and approximately 95 per cent in calves two -three months of age has been found. This syndrome only occurs in calves which have access to roughage. The incompletely digested fibres pass directly to the abomasum, as significant rumen digestion of such fibres does not begin until about the fourth month (Blood et al., 1979.) These ulcers are discrete but multiply and involve the pyloric mucosa near its junction with the spiral folds. They are flat, irregular defects and progressively increase in size and erode blood vessels. The non-ulcerated pyloric mucosa is slightly oedematous and multiple petecchiae have also been seen. Most ulcers heal after a while, but scarring may still be evident at six months of age.

Local irradiation of the gut gives rise to ulceration of the intestinal lining, the most radiosensitive is the mucosa of the first part of the small intestine but it normally does not ulcerate deeply. Ulcers are most commonly found after irradiation in the lower ileum in Peyer's patches and in the caecum, when bacterial invasion is common (Radeleff, 1970).

It has been suggested that overfeeding, irregular feeding and underfeeding of young calves have also been thought to act as predisposing factors (Amstutz, 1965) and developing diarrhoea.

Poisons

A number of poisons cause enteric signs and lesions in cattle. Arsenic is an example of such a corrosive poison. The clinical signs include an acute watery greenish diarrhoea, tenesmus with a little mucoid faeces and signs of acute abdominal discomfort. In subacute forms the faeces may contain shreds of intestinal mucosa and blood in flecks or in quantities sufficient to give the faeces a dark colour (Radeleff, 1970).

Erythema of the mucosa of the abomasum and small intestine occur and the contents become fluid. Other lesions are usually present in the carcase and arsenic or the appropriate poison may be demonstrable.

Plant poisons may cause enteric lesions and an example is Ragwort. Ataxia, frenzy, clinical signs referrable to liver damage are often accompanied by scouring and tenesmus. In addition to the liver lesions and subcutaneous petechial haemorrhages, enteric changes including petechial haemorrhages and oedema of the mucosa of the abomasum have been recorded. Diagnosis rests on a history of access to ragwort, the clinical signs and the characteristic histopathological findings in the liver.

Dietary deficiencies such as primary deficiency of copper or the intake of high dietary levels of molybdenum may result in persistent diarrhoea containing gas bubbles (Clark and Clark, 1975). The low blood levels of copper and the lack of figmentation of the hair allow a diagnosis to be reached. Enteric changes are poorly documented but erythema of the jejunal mucosa has been recorded (Hallgren et al., 1954).

From the literature reviewed above it was clear that the information about bacterial diseases of the bovine enteric tract was less well documented than that about viral and parasitic diseases. This study of the bacteria associated with lesions of the bovine enteric tract was carried out in two main parts. The first was a study of the bacteria present in lesions and normal mucosa of animals presented for post-mortem examination at Glasgow University Veterinary School. The second part was to study the relationship between some of the bacteria found and the bovine host by means of animal inoculation studies.

It was hoped that these two approaches would provide much needed information about the bacteria present in lesions and their significance.

Chapter 2.

General materials and methods

Many of the materials and methods described in this chapter were used throughout the study.

Bacteriological materials and methods.

a. Media and reagents used.

The following media and materials were used throughout the study for general cultivation and maintenance and were prepared according to the manufacturers' instructions and used in the form given below.

1. Tetrathionate broth (Oxoid).
2. Salmonella shigella agar plates (SS) (Oxoid).
3. Desoxycholate citrate agar plates (DCA) (Oxoid).
4. Tryptone soya agar slopes (Oxoid).
5. Nutrient agar plates (Oxoid).
6. MacConkey agar plates (Oxoid).
7. Cooked meat medium (Oxoid).
8. 7 per cent sheep blood agar.

   7 ml formalised sheep blood (Gibco-Biocult Ltd.) were added to 100 ml blood agar base No. 2 (Oxoid).
9. 7 per cent horse blood agar.

   7 ml defibrinated horse blood (Oxoid) were added to 100 ml blood agar base No. 2 (Oxoid).
10. Basal media for identification of campylobacter species.

For the first five cases in the survey the following media were prepared using Thioglycollate broth (Difco). From that stage to the end of the study Brucella broth (Difco) was used. Similar reactions were obtained in materials prepared using both basal media. The two basal media in common use were:

i. Semisolid medium.

Brucella broth (Difco) was added to 0.12 per cent Oxoid agar

No. 3. This semisolid medium was dispensed in 15 ml amounts in 16 mm screw cap universal bottles.

ii Solid medium.

Brucella broth (Difco) was used with 1.2 per cent Oxoid agar No. 3.

11. Selective medium for catalase-positive campylobacters.

Freeze dried antibiotic supplement for the selection of Campylobacter fetus (campylobacter supplement SR69 Oxoid) with added 1 per cent nystatin (Mycostatin suspension, Gibco Biocult), was incorporated in 7 per cent horse blood agar prepared as described above to give a final concentration of 100 IU/ml.

12. Reinforced clostridial medium (Oxoid) was prepared with the addition of 2.5 per cent Oxoid agar No. 2, and 7 per cent horse blood.

13. Nutrient broth.

Nutrient broth No. 2 (Oxoid) was prepared and was used when broth media were required.

14. Serum broth.

1 per cent horse serum (Gibco-Biocult) was added to the nutrient broth described above.

15. Physiological saline

Physiological saline was used throughout the study. The sodium chloride concentration was 0.85 per cent in deionised water. It was sterilised before use.

b. Conditions of cultivation

1. Atmospheric conditions.

Cultures were incubated aerobically under microaerophilic and anaerobic conditions.

Microaerophilic atmospheres were produced by evacuating anaerobic jars with no catalyst (Gallenkamp Ltd., Whitley Scientific Ltd) to a negative pressure of 20 inches of mercury using a vacuum pump and flooding with a 5 per cent carbon dioxide, 95 per cent hydrogen mixture (British Oxygen Co. Ltd. Special Gases Division).

Anaerobic conditions were produced by evacuating anaerobe jars fitted with cold catalysts to a negative pressure of 24 inches of mercury using a vacuum pump. The evacuated jars were then filled with the carbon dioxide:hydrogen gas mixture. Gassed jars were evacuated once more and gassed again in order to flush out any remaining oxygen. The inoculation of plates for anaerobic culture was performed rapidly and inoculated plates were returned to an anaerobic atmosphere as soon as possible in order to reduce the period of time for which cultures were exposed to oxygen.

2. Temperature of incubation.

Cultures were incubated at 37° C unless specified. Other temperatures of incubation were used routinely in the identification of bacteria. They were 4°C, (refrigerated), 22°C (room or bench temperature), 25°C, 42°C, 43°C, 45°C and 65°C. The five latter temperatures were produced by incubation of cultures in accurately-adjusted water baths. Cultures of microaerophilic or anaerobic organisms were placed in anaerobe jars containing the appropriate atmosphere and the jar was placed in the water bath for the appropriate period.

3. Period of incubation.

All primary cultures on routine non-selective media were incubated for 24 hours examined and then reincubated for 48 hours and 72 hours incubation. Cultures were not discarded until five days after inoculation. Selective media were incubated for 24 hours prior to examination (Reinforced clostridial medium) and 48 hours (campylobacter medium).

c. <u>Identification of bacterial isolates.</u>

1. Colonial morphology.

Many different colony types were noted in the initial cultures and each colony type seen was recorded and subcultured to provide pure sub-cultures. The examination of colonies was made by unaided eye and by low power microscopy (x 80). The morphological characteristics of the colonies (size, elevation, consistency etc.) were noted.

2. Methods of identification

All isolates were identified using the methods outlined by Cowan and Steel (1974), supplemented where necessary by reference to Bergey's Manual (1974) and the Anaerobe Laboratory Manual (1977). Specialist publications were consulted for the identification of campylobacters. The tests used and the materials used in each test are given here and variations from the methods and criteria for assessment of Cowan and Steel , Bergey and the Anaerobe Laboratory Manual are given below where appropriate. Biochemical tests were carried out in the atmospheric and temperature conditions appropriate to the organism under test. The tests used in identification were as follows:

A. Morphology of bacterial cells.

This was determined by the microscopic examination of air-dried heat-fixed smears made from cultures and stained by Gram's method. The modification of Moeller's spore stain described by Cowan and Steel (1974) was also used.

B. Motility test.

The hanging drop technique was used on suspensions prepared from broth cultures or from growth on solid media after both 24 hours' and 48 hours' incubation. It was also tested at room temperature in some of the isolates.

C. Growth at temperatures other than 37°C.

The ability of organisms to grow at temperatures other than 37°C was determined by culture of the organism under test on blood agar or in nutrient broth under the appropriate atmospheric conditions described by Cowan and Steel . The presence or absence of growth was recorded.

D. Biochemical tests.

Oxidase tests were carried out using filter paper dipped in the reagent (1 per cent NNN'N - tetramethyl-P-phenylenediamine dihydrochloride B.D.H. Ltd.). Cultures were streaked on to the paper using a platinum wire.

Catalase tests were carried out using loopfuls of 24 hour or 48 hour cultures of the organism under test grown on nutrient agar or the blood medium described above emulsified with a few drops of 3 per cent hydrogen peroxide (B.D.H. Ltd.) on a microscope slide. Suspensions were examined for bubbles of gas immediately and after five minutes.

Oxidation and fermentation tests (O/F) were carried out to determine the effect of the organism on OF basal medium (Difco) containing 1 per cent added glucose (Analar). Results were assessed after 2, 4 and 8 days.

Utilisation of sugars. This was tested using sugar broth base No. 2 prepared using the following ingredients in the basal medium. 5g Lab Lemco beef extract (Oxoid); 10g Oxoid bacteriological peptone; 3g sodium chloride (May and Baker) and 2g disodium hydrogen phosphate (May and Baker) were dissolved in 1 litre of deionised water and steamed for 30 minutes. When the medium was cool, the pH was adjusted to 7.2 to 7.3 and 12 ml of indicator (0.1g Bromothymol blue, May and Baker; 2.5 ml 0.1 N NaoH, Analar and 47.5 ml of distilled water) were added per litre. This medium was dispensed in 100 ml volumes. One gram of the appropriate sugar was added to 100 ml of this medium. When each sugar was dissolved it was dispensed in 4 ml volumes into bijou bottles containing durham tubes. The bottles were then sterilised by tyndallisation.

Indole production was determined by growing the culture in 1 per cent peptone water (Oxoid) and the testing with Kovacs reagent.

Gluconate oxidation was determined by growth in medium containing potassium gluconate (B.D.H. Ltd.).

Decarboxylase tests were carried out using Bacto Decarboxylase Medium Base (Difco) with the addition of 0.5 per cent L. lysine, L. ornithine, L. arginine (Analar) where appropriate.

Methyl red (MR) and Voges-Proskauer (VP) tests were carried out after cultures were grown in M.R.V.P. medium (Oxoid) for two days

at 37°C. The production of acid was tested for by adding two drops methyl red solution. After completion of this test, the VP test was carried out by adding 0.6 ml of 5 per cent ∝-naphthol (Analar) in absolute ethanol) and 0.2 ml 40 per cent KOH (Analar) aqueous solution to another aliquot of culture.

Nitrate reduction was determined on cultures grown in nutrient broth supplemented with 0.1 per cent potassium nitrate (Analar) and incubated for five days. They were then tested for the presence of nitrite.

Arginine hydrolysis was examined for by the inoculation of cultures into Arginine broth prepared by the method described by Cowan and Steel incubated with a control tube at 37°C for two days, followed by test with Nessler's reagents (B.D.H. Ltd.).

Hydrogen sulphide production was assessed in two ways.

i. On Triple sugar iron agar (TSI) - Oxoid.
Slopes of TSI were inoculated and incubated in aerobic, micro-aerophilic and anaerobic conditions where appropriate for 24 and 48 hours. The production of hydrogen sulphide was indicated by a black colour.

ii. The sensitive of hydrogen sulphide test.
This was conducted by suspending filter paper strips saturated with lead acetate (B.D.H. Ltd.) in universal bottles of the basal medium described above containing 0.02 per cent cystine-HCl (B.D.H. Ltd.). If any portion of the paper stiips became blackened, the isolate was considered hydrogen sulphide positive by the sensitive hydrogen sulphide test.

Hippurate hydrolysis was determined by growing cultures in hippurate broth prepared by the method of Cowan and Steel using sodium hippurate (Hopkins and Williams Ltd.) and incubated along with a control tube at 37°C for four days in the appropriate atmospheric conditions.

Coagulase tests were carried out by the slide method using citrated rabbit plasma collected in the department.

Citrate utilisation was determined using the modified Koser's citrate described by Cowan and Steel .

Phosphatase production was determined using Phenolphthalein phosphate agar prepared using Phenolphthalein phosphate (B.D.H. Ltd.). This was lightly inoculated to obtain discrete colonies, incubated for 18 hours at 37°C and examined. The ammonia solution used was 0.1 ml (sp. gr. 0.880, B.D.H. Ltd.).

Starch hydrolysis was determined on nutrient agar containing 1 per cent starch (B.D.H. Ltd.) sterilised at 115°C for 10 minutes.

Haemolysis was determined by streaking cultures on 7 per cent sheep blood and 7 per cent horse blood agar plates. These were incubated at 37°C for 24 or 48 hours and haemolytic activity around discrete colonies was recorded.

Growth on MacConkey agar was recorded after incubation for 18-24 hours at 37°C in the appropriate atmospheric conditions.

Litmus milk (Oxoid) was used in 10 ml quantities and incubated for seven days at 37°C when the result was recorded.

Gelatin liquefaction was determined using nutrient gelatin (Oxoid) after 15 days of incubation at 37°C or at room temperature. Cultures were placed in a refrigerator at 4°C for 1 hour before being recorded as positive.

Aesculin hydrolysis was determined by inoculation of 10 ml quantities of aesculin broth prepared by the method described by Cowan and Steel .

Urease production was determined following inoculation of urea broth medium (Oxoid) with urea solution 40 per cent (Oxoid) added was incubated and examined after 18-24 hours.

ONPG tests were prepared and carried out by the method of Cowan and Steel .

Growth in potassium cyanide medium was carried out using nutrient broth and nutrient broth with potassium cyanide (May and Baker).

Digestion of insipissated serum was determined using Loeffler's serum slopes prepared using horse serum and examined for liquefaction after inoculation and incubation in the appropriate atmospheric conditions.

Malonate utilization was determined using Malonate-phenylalanine medium (Oxoid).

Appearance on egg yolk agar was determined on medium prepared as follows:

5 per cent egg yolk suspension (Oxoid) was added to sterile molten nutrient agar base No. 2 (Oxoid) at 55°C and plates were poured immediately. Cultures were inoculated onto plates and incubated at 37°C in the appropriate atmospheric conditions. The changes in the medium were recorded.

Digestion of cooked meat was determined following the inoculation of synthetic cooked meat medium (Oxoid) and incubation for up to 14 days.

Casein hydrolysis was assessed on milk agar prepared using 100 ml blood agar base No. 2 (Oxoid) and 35 ml of sterile skimmed milk.

Growth in media with increased sodium chloride concentration was assessed on nutrient agar plates containing the required concentration of sodium chloride (May and Baker).

E. Lancefield test for streptococcus species.

The test employed was that in use in the Bacteriology Department at Glasgow Veterinary School and was carried out as follows:

1. Pure cultures were inoculated into Lancefield broth composed of nutrient broth No. 2 (Oxoid) and 1 per cent glucose (Analar) and incubated at 37°C overnight.

2. The bacteria were removed by centrifugation at 1500g for 10 minutes.

3. The deposit was suspended in 2 ml of N/20 hydrochloric acid in 0.85 per cent sodium chloride solution.

4. The suspension was heated in a boiling water bath for 5 minutes, cooled and centrifuged at 1500g for 10 minutes.

5. The supernatant fluid was removed and several drops of 0.02 per cent phenol red solution were added. Sufficient N NaoH (May and Baker) were added carefully to turn the indicator red.

6. Tests were carried out in Dryer tubes (Microtube).

7. A drop of each grouping serum (A, B, C, D, E, F ... etc) (Wellcome Reagents Ltd) was placed in each tube and overlayed by a drop of the antigen prepared as above.

8. Tubes prepared by the method described above were left on the rack for 10 minutes. A positive reaction consisted of the formation of a white, flocculent precipitate at the junction of the extract and the homologous serum after 10 to 30 minutes at room temperature.

F. Tests for the identification of campylobacters (Veron and Chatelain, 1973).

Glycine tolerance was assessed by the inoculation of 15 ml of the semisolid brucella medium described above containing 1 per cent glycine (Sigma Ltd.). This was incubated aerobically for six days at 37°C, and any growth was considered glycine positive.

Sodium chloride tolerance was assessed on semisolid brucella medium containing 3.5 per cent sodium chloride (May and Baker) inoculated and incubated aerobically for six days at 37°C. Any growth was recorded.

Selenite reduction was determined on the solid brucella medium containing 0.1 per cent sodium selenite (Sigma Ltd.), inoculated and incubated microaerophically. The reduction of selenite was indicated by the colour change of the medium to a deep orange colour. The result was recorded after two - three days' incubation.

Glucose tolerance was assessed by the inoculation of semisolid brucella medium containing 8 per cent glucose (B.D.H. Ltd.). Cultures

were incubated aerobically for six days at 37°C. Any growth was recorded.

Bile tolerance was determined using the semi-solid brucella medium supplemented with 1 per cent ox bile (Oxoid). Inoculated medium was incubated aerobically for six days at 37°C. Growth was recorded.

Nalidixic acid (NA) tolerance was determined on 7 per cent horse blood agar plates containing 40 µg of nalidixic acid (Sigma Ltd.) per ml. Plates were inoculated and incubated microaerophilically. Any growth on this medium after two - three days was recorded.

2, 3, 5 triphenyltetrazolium chloride (TTC) tolerance was determined by the ability to grow after two - three days microaerophilic incubation at 37°C on 7 per cent horse blood agar plates containing 1 mg of TTC (Sigma Ltd.) per ml.

Nitrate and nitrite reduction was determined by the inoculation of semi-solid medium containing 0.1 per cent potassium nitrate (Analar). Inoculated cultures were incubated aerobically for six days at 37°C and were then tested for the presence of nitrite and then nitrate.

The growth of isolates at 43°C (Skirrow, 1977) was employed in addition to the other growth temperatures used by Veron and Chatelain.

d. Maintenance of cultures

Each pure culture isolated was inoculated on to tryptone soya agar or blood agar slopes in universal bottles as appropriate to the isolate, incubated for 24 hours in appropriate conditions and stored at room temperature. Duplicate cultures were made of each isolate, one being stored at +4°C.

Campylobacter isolates were maintained by subculture onto blood agar at eight-day intervals. All cultures were maintained in the microaerophilic conditions described above at room temperature following initial inoculation at 37°C for 48 hours. Duplicate cultures were maintained in cooked meat medium treated in the same way. Cooked meat

medium was also used to maintain Clostridium species and Bacteroides species. Selected cultures were freeze dried using the method described by Garvie (1967) within the number of passages specified in the appropriate chapter and then stored at +4°C.

Sources of animals used in the survey.

Twenty three cattle aged one year or more and 41 calves aged 8 months or less were examined in this study, a total of 64 animals. Thirty four were clinical cases admitted to the veterinary hospital and 30 were examined post mortem in the course of husbandry or other experiments being carried out at the veterinary school.

The history of the animal and the clinical diagnosis were recorded in each case. All but 11 animals were killed immediately prior to post-mortem examination. The other 11 cases died within 24 hours preceding post-mortem examination.

Euthanasia

All animals that were killed were stunned using a captive bolt pistol and then exsanguinated. Blood was collected into sterile universal bottles for serum preparation by the method described below.

Post-mortem techniques

At post-mortem examination the gross appearance of the abdominal and thoracic organs was noted and the gut was investigated in detail. Within 10 minutes of slaughter or as soon as possible after death, samples of bowel wall were collected from a series of standard sites in the gastrointestinal tract. These were the abomasum, the mid jejunum, the ileum, the caecum, the proximal end of the colon and mesenteric lymph node. The samples were rinsed in sterile physiological saline or sterile distilled water and divided into two portions. One was fixed in 10 per cent formol saline for histological examination within five minutes of death and one was kept at +4°C until bacteriological examination could be carried out usually within 30 minutes of sampling. The remainder of the intestinal tract was opened and examined for the presence of gross lesions. Any lesions seen were recorded and additional samples were taken and processed as previously described. The lesions

were photographed in a few cases. In some cases lesions in other organs were also examined in this way. Any deviation from this treatment is considered under the appropriate section in Chapter 3.

Histological examination

a. By light microscopy

Tissue sections were prepared from wax embedded blocks and stained with Haematoxylin and Eosin. Some sections were stained by Gram's method (Twort) (Drury and Wallington, 1967) to demonstrate gram-positive and gram-negative bacteria. An attempt was made to demonstrate campylobacters using silver staining by Young's method (Young, 1969). All sections were examined by light microscopy and the findings recorded.

b) By electron microscopy

0.5 cm square portions of the whole thickness of the abomasum, jejunum, ileum, caecum and proximal end of colon were excised with a sharp scalpel within two minutes of death and were immediately fixed by immersion in 3 per cent paraformaldehyde/glutaraldehyde (Karnovsky, 1965). The fixed samples were gently agitated in order to remove large particles of debris from the mucosal surface and to enhance even fixation. The tissue was trimmed after two hours' fixation and small 1 mm square blocks were selected for embedding. They were post fixed in osmium tetroxide, dehydrated in ethanol and embedded in ARaldite for sectioning. The most suitable sites for electron microscopical examination were selected after examination by light microscopy of full face 1µ plastic sections stained with methylene blue / AZURII).

Thin sections of the Araldite embedded tissue were stained with uranyl acetate and lead citrate and examined in the electron microscope.

Methods used in Bacteriological examinations in the survey

Samples of rinsed mucosa, parenchymatous organs and faeces were examined bacteriologically in the studies described here.

Mucosal samples.

The lumenal surface of the mucosa of each gut sample and

parenchymatous organ samples were seared with a hot spatula and material was taken from the mucosa using a stiff bacteriological loop for cultural and direct examination. Smears were made from this material, air dried and heat fixed. They were stained by Gram's method and by Ziehl Neelsen's method where necessary and examined by light microscopy. The findings were recorded. Cultural examination was carried out as follows:

Inocula were streaked on one 7 per cent horse blood agar plate and one MacConkey plate for aerobic incubation and on one horse blood agar plate for both microaerophilic and anaerobic cultivation.

Faeces samples were only examined in the experimental series and were examined for specific organisms by the methods outlined below and by general methods during screening prior to infection and when clinical signs appeared in experimental animals.

Examinations for specific bacteria.

a. Salmonella.

Samples of the colonic contents of the calves, the ileal contents of the adult cattle and the faeces of experimental animals were inoculated into tetrathionate broth and incubated for 24 hours at 37°C, then subcultured onto SS and DCA plates and incubated aerobically for 24 hours at 37°C. Colourless colonies on these media were examined biochemically by the methods described by Cowan and Steel and serologically using specific O and H agglutinating antisera (Wellcome Reagents Ltd.) to confirm their identity as Salmonella species.

b. Escherichia coli.

The material obtained from the ileum of all cases and from the faeces of experimental animals was used to inoculated 7 per cent sheep blood agar plates and MacConkey agar and incubated aerobically to detect the presence of β-haemolytic E. coli.

c. Campylobacters

The selective medium for campylobacters described above were inoculated with loopfuls of faeces, faecal swabs or mucosal samples in

the isolation of campylobacters in each experiment and in some of the cases described in the survey (Chapter 3). Inoculated plates were incubated at 37°C for 48 hours in microaerophilic conditions. Smears of colonies with the morphology of Campylobacter spp. and all the other colony types growing on the medium were stained by Gram's method to ascertain whether they contained vibrios. All colonies which did contain vibrios were examined by the tests described above and listed in full in Chapter 3 to confirm their identity as campylobacters and allot them to species if possible.

d. Clostridia.

Clostridia were detected on anaerobic plates inoculated from the samples described above. In the experimental animals inoculated with Clostridium sordellii samples of faeces, gut contents and mucosa were streaked on reinforced clostridial medium plates and incubated anaerobically.

All colony types on these plates were used to make smears and all those containing Gram-positive rods were examined further by the methods of Cowan and Steel and the Anaerobe Manual to confirm their identity. Clostridia isolated in the survey were tested for toxin production by mouse inoculation as described by Anaerobe Laboratory Manual (1977) after overnight culture in cooked meat medium.

Serological studies

a. Serum samples

Blood samples collected at slaughter or by venepuncture were allowed to clot and the serum was withdrawn, centrifuged at 750 g for 10 minutes to remove cellular debris and stored in glass universal or bijou bottles sealed with plastic film at -20°C until required.

b. Fluorescent antibody technique

Smears were made from the surface growth of cultures and air dried. They were then fixed by immersion in cold acetone (+4°C) for 10 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 made from cultures obtained from the animals from which the serum was obtained were placed in a moist chamber and covered and tested for specific antibodies to the organisms at dilutions of 1/10, 1/100, 1/500. The area of the fixed smear was covered. Treated smears were incubated at 37°C for 30 minutes and then rinsed twice (5 minutes total immersion) with phosphate buffered saline (PBS) (Oxoid) pH 7.4. Washed and treated smears were then incubated for 30 minutes at 37°C with fluorescent antibovine immunoglobulin (Wellcome Ltd.) diluted 1/20 with PBS. The smears were then rinsed twice more with PBS (5 minutes total immersion) and prepared for examination by placing a coverslip on the PBS-covered smears.

Smears were examined under ultraviolet light using a Leitz Ortholux microscope fitted with a vertical fluorescence illuminator.

All smears were screened for fluorescence using the x 10 lens and x 12.5 eye pieces and then examined in detail using the x 25 water immersion lens. Antibodies to bacteria were considered to be present when the organisms in the smear were seen to fluoresce. Control smears were also used in parallel but untreated with serum.

c. Agglutination tests for antibody to campylobacters

The general method followed was that described by Butzler and Skirrow (1979). Horse blood agar cultures were inoculated overnight or for 48 hours at 37°C. The growth was suspended in 0.5 per cent buffered formol saline and left overnight at +4°C. Cells were washed by centrifugation and resuspended in 0.25 per cent buffered formol saline. The antigen suspension was standardised at tube 8 using Wellcome opacity tubes (Wellcome Reagents Ltd.). In the tube agglutination test this suspension was placed in round bottomed agglutination tubes in the ratio 0.5 ml to 0.5 ml of phenol serum in 10 fold dilutions and incubated at 37°C in a water bath for 18 hours. The end point was taken to be the last tube with a clear supernate and a deposit.

d. Agglutination tests for antibody to Clostridium sordellii

Similar tests were carried out using 24 hour cultures of Cl. sordellii.

Virological examination

Portions of the anterior jejunum of the calves which had died or were killed were excised, opened, placed in a petri dish containing physiological saline and examined under a disssecting microscope for evidence of villous atrophy. Samples of rectal faeces from some of the experimental calves were negatively stained and examined by electron microscopy for the presence of virus particles by Dr. H.M. Laird.

Parasitological examination

In each experiment, faecal samples from each animal were examined for the presence of nematode eggs and coccidia during the period of experiment. The examination was carried out by the Department of Veterinary Parasitology at the veterinary school.

Transmission experiments

a. Source of the experimental animals

The calves used in this study were purchased direct from the farm when they were reared or were purchased from a market. They were three weeks to six months of age at the time of first infection and were of mixed sex and mixed breed. Details of the calves used in each experiment are given in the appropriate section. All were individually identified by means of numbered eartags.

b. The maintenance of the experimental animals

Groups of calves were housed in separate open-fronted pens and bedded on straw which was not cleaned out during the experiment but merely littered down. Groups of experimental animals were fed by the same attendant who always moved from control to infected pens, wearing an outer protective coat and rubber boots and disinfecting his boots with antiseptic solution (White Septol B, Young's) before and after entry to the pens. Water and hay were freely available, and barley based ruminant rations containing no non-nutrient additives were fed

daily. The milk fed calves were given a milk replacer diet containing no non-nutrient additives (Easymix, Volac) at a final volume of approximately two pints twice daily. No antibiotics were used to treat any clinical disease noted.

c. Experimental technique

All animals were kept for a minimum of one week before being used in the experimental studies in order that any enteric disease contracted in the market or in transport should become obvious. The animals were divided into groups appropriate to the experiment.

i. Routine examination before and after inoculation

Faecal samples were taken from each animal at least three times before inoculation for gross and bacteriological examination.

Faeces samples were taken using rectal swabs (Exogen Ltd.) soaked in sterile physiological saline which were examined after sampling and rectal faeces which was obtained manually and examined. The consistency and appearance of faeces was recorded and photographed. Samples were examined for salmonellae, haemolytic E. coli, campylobacters and, where appropriate, clostridia by the methods described above. General bacteriological examination was also carried out. Parasitological and virological examinations were also carried out where indicated.

ii. Blood samples

Blood samples were collected from the jugular vein with sterile needle size 18G 1/2 T.W. (B-D, Yale Microlance) with disposable sterile plastic syringes into sterile universal bottles before inoculation of the animals. Blood was also obtained from all calves at the end of each experiment at the time of slaughter. Serum was separated and treated as described earlier.

iii. The preparation of inocula

Campylobacter experiments

All inoculated calves were given cultures of the appropriate campylobacter isolate, grown on 7 per cent horse blood agar.

The plates used were inoculated with 2-3 drops of a suspension of campylobacter isolate in sterile physiological saline and spread using a glass spreader. The resulting bacterial lawns were incubated microaerophilically for 48 hours. Plates free from obvious contamination were used to prepare inocula. The calves were inoculated with a suspension prepared from the bacterial lawns by harvesting the growth in sterile physiological saline. 4 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 below.

Clostridium sordellii experiments.

Bacterial cultures were prepared by the method described above but the resulting bacterial lawns were incubated anaerobically for 24 hours at 37°C.

Preparation of Cl. sordellii toxin

Cl. sordellii colonies were picked from fresh culture and inoculated into cooked meat medium which was incubated at 37°C overnight. The resulting cultures were filtered using a sterile acetate filter, pore diamter 0.22 μ (Millipore, U.K.).

Bacterial counts

Saline suspensions of Campylobacter species and Clostridium sordellii used as inoculum in transmission experiments were examined to determine the number of viable organisms per ml of suspension.

The samples were diluted tenfold in sterile physiological saline in dilution and drops of 0.1 ml were used to inoculate the surface of a blood agar plate as described by Miles and Misra (1938). Inoculated plates were incubated for 48 hours for the Campylobacter spp and 24 hours for Cl. sordellii and then examined. Colonies of Campylobacter species and Cl. sordellii were counted.

iv. Inoculation procedure

Animals were inoculated with 20 ml of bacterial suspension by stomach tube after feed (including roughage) had been withheld for 24 hours. 200 ml of sterile physiological saline was then given in order to ensure that the complete dose entered the rumen.

v. Observations

All experimental animals were examined daily and their appearance, appetite, the presence or absence of rumenal movements, respiratory rate, rectal temperature and the consistency of their faeces were recorded. Experimental animals were killed at the end of each experiment by the methods described above.

After death, samples of bowel wall were collected from a series of standard sites in the gastrointestinal tract. These were the abomasum, jejunum, ileum, caecum and the proximal end of the colon. Histological and bacteriological examination was carried out on these samples and on samples of liver and lung by the methods described above for the survey. Bacteriological examination alone was carried out on the gall bladders and spleens.

Chapter 3.

Results of the Survey

Introduction

A number of bacterial species were isolated from the gastro-intestinal mucosa of the animals examined in the survey. The results of the survey are presented here under the names of the bacterial species isolated from the lesions. The lesions from which they were isolated are described, the presence of both bacterial and other agents in the lesions are given and the final diagnosis reached in each case is recorded in Appendix 1.

The bacteria isolated were as follows:

Campylobacter spp.	C.f. subsp. jejuni, C.f. subsp. intestinalis, C. fecalis and unidentified campylobacters.
Clostridium spp.	Cl. perfringens Type A, Cl. sordellii, Cl. bifermentans, Cl. butyricum, unidentified Clostridium.
Bacillus spp.	B. licheniformis, B. coagulans.
Bacteroides spp.	B. fragilis, B. vulgatus, B. melaninogenicus, B. oralis.
Fusobacterium spp.	F. necrophorum, Fusobacterium unidentified.
Escherichia coli	
Enterobacter aerogenes	
Pseudomonas aeruginosa	
Aeromonas hydrophila	
Alcaligenes odorans	
Acinetobacter lwoffii	
Actinobacillus lignieresi	
Branhamella catarrhalis	
Moraxella non-liquefaciens	
Pasteurella multocida	

Veillonella spp.

Staphylococcus epidermidis

Streptococcus spp. S. bovis, S. zooepidemicus.

Aerococcus viridans

Corynebacterium spp. C. bovis, C. pyogenes.

Peptostreptococcus productus

Eubacterium aerofaciens

Actinomyces bovis

Acid fast bacteria resembling
Mycobacterium paratuberculosis were demonstrated in smears and sections.

The findings are discussed at the end of the chapter.

Campylobacter spp.

Campylobacters were isolated from seven of the 23 adults and 21 of the 41 calves in the survey Thirty four isolates of campylobacter species were obtained and identified by the methods described in Chapter 2. The isolates were assigned to three subspecies or species and an unidentified group (Table 1).

C.f.ss. jejuni

Eighteen isolates were identified as C.f. ss. jejuni.

C.f. ss. intestinalis

Six isolates were identified as C.f. ss. intestinalis.

C. fecalis

Six isolates were identified as C. fecalis.

Unidentified campylobacters

Four isolates could not be identified to species using the tests described above.

Selected biochemical findings for each isolate are given in Table 1.

Table 1.

Selected biochemical characters of the 34 isolates of Campylobacters spp. obtained during the survey.

Species of isolate	Number of isolates with biochemical characters shown	Case number(s) from which derived	Biochemical characters							Growth at		
			H_2S TSI	H_2S Sensitive	1% Bile	8% Glucose	NA	TTC	Hippurate	25℃	42℃	43℃
C.f.ss.jejuni	5	74589, 11 50, 291 44P	-	+	+	-	-	±		-	+	+
	1	75257	-	+	+	-	-	-		-	+	+
	1	75258	-	+	±	-	±	±		-	+	+
	1	45	-	+	+	-	±	±	+	-	+	+
	9	22P,31P ,34P 37P ,38P,46P 82773, 83444 83613	-	+	+	±	-	±		-	+	+
	1	49	-	+	+	±	+	+		-	+	+

Table 1.(Continued).

Species of isolate	Number of isolates with biochemical characters shown	Case number(s) from which derived	Biochemical characters							Growth at		
			H_2S TSI	H_2S Sensitive	1% Bile	8% Glucose	NA	TTC	Hippurate	25℃	42℃	43℃
C.f.ss.intestinalis	2	75258 , 75715	-	+	+	±	+	-		+	-	-
	3	25, 33, 36	-	+	+	+	+	-		+	-	-
	1	83555	-	+	+	±	±	-		+	-	-
C.fecalis	6	75205, 78530 80958, 83444 79894, 83613	+	+	+	+	+	±		-	+	+
C.unidentified	1	75067	+	+	±	±	+	-		+	+	+
	3	31P , 37P 46P	+	+	+	+	+	+		-	-	-

All isolates producedcatalase, oxidase, reduced selenite and nitrate, grew on 1 per cent glycine and failed to grow on 3.5 per cent sodium chloride.

NA = Nalidix acid 40 µg per ml. TTC = Triphenyltetrazolium chloride 1 mg per ml.

± = Weak. + = Positive. - = Negative.

Campylobacter fetus subsp. jejuni.

Colonies subsequently identified as C.f.ss. jejuni were found to be of two morphological types on primary isolation. One was low, flat, greyish, finely granular and translucent with an irregular edge, spreading along the direction of the streak and tending to swarm and coalesce (Fig. 1). The second type was round, 1-2 mm in diameter convex, entire smooth and glistening. Both colony types could be seen in the same primary culture or on different halves of the same plate in sub-culture. The morphological appearance of C.f. ss. jejuni in smears is shown in (Fig. 2).

It is a comma or S-shaped bacillus and has the appearance of a flying seagull. Longer forms of the organism with four or five coils are sometimes seen. C.f. ss. jejuni was isolated from three adult cattle and from 15 calves. It was isolated from the gastrointestinal tract of 13 animals with diarrhoea (numbers 74589, 75257, 75258, 45, 22P, 31P, 37P, 44P, 46P, 82773, 83444, 83613 and 49). The diarrhoeic faeces contained excess mucus and streaks of blood in some cases. The remaining five animals (numbers 11, 50, 291, 34P and 38P) did not have obvious diarrhoea at the time of slaughter.

Gross changes were recorded in the mucosa at the sites from which C.f. ss. jejuni was isolated in 15 cases. They varied from slight congestion to ulceration and acute inflammation with oedema of the mucosa (Fig. 3). In two cases (50, 291) it was isolated from macroscopically normal mucosa and in one case (83613) from apparently normal large intestinal mucosa when macroscopic changes were present in the small intestinal mucosa. Sites from which C.f. ss. jejuni was isolated and its relative abundance are summarised in Table 2.

Microscopical changes were seen in the gastrointestinal mucosa at all sites from which the organism was isolated (animals 83444 and 49 were not examined). The lesions seen were attributed to mucosal disease (74589) to parasitic infection (11, 22P, 31P, 34P, 37P, 38P, 44P and 46P) and to rotavirus infection (45) which was known to be present in the group as a result of electron microscopical examination of faeces samples.

Fig. 1: Colonial morphology of C.f. ss. jejuni isolated from calf 45. 48 hours' incubation on horse blood agar at 37°C in micro-aerophilic conditions.

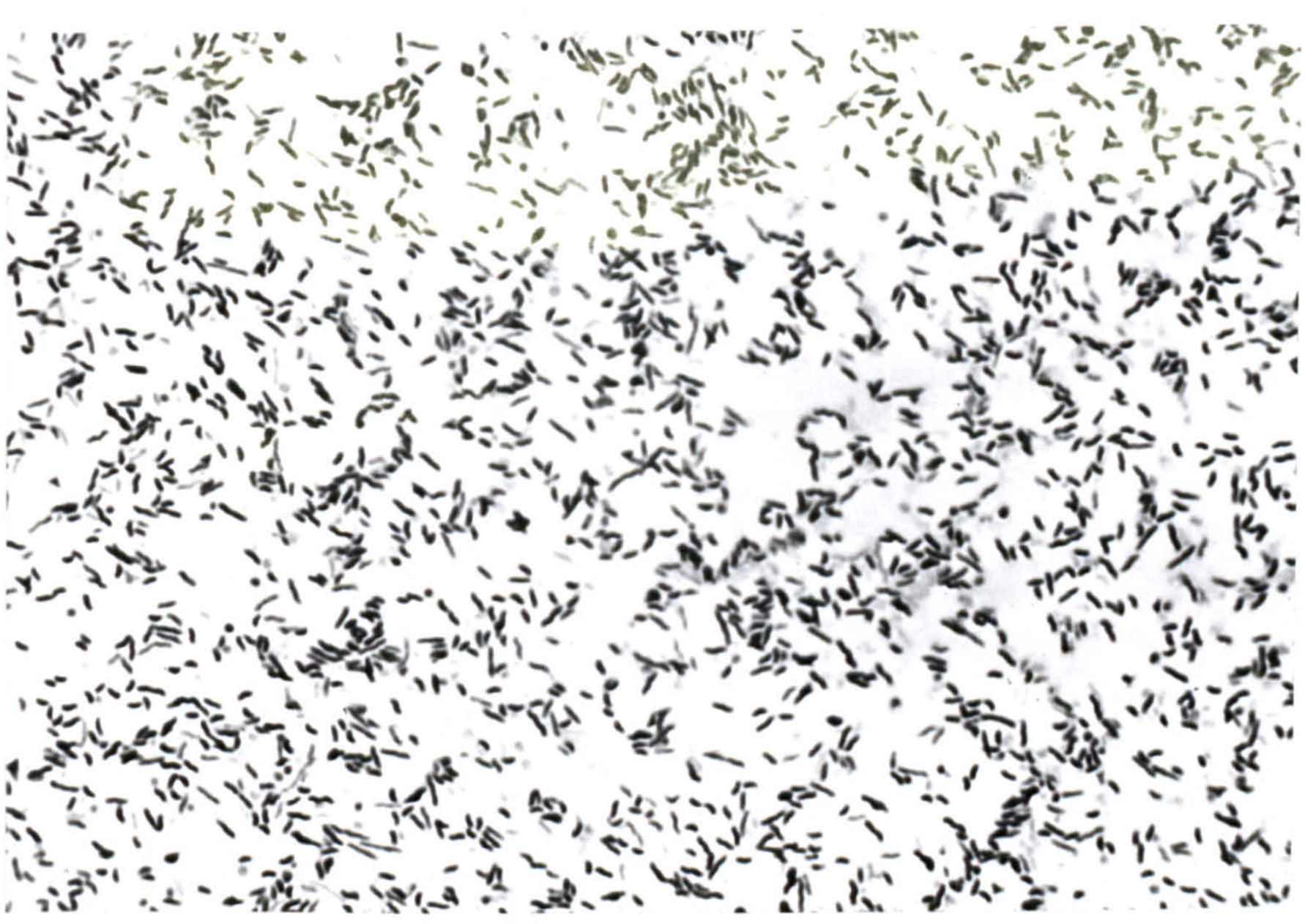


Fig. 2: Smear of a colony of C.f. ss. jejuni from the plate shown in Figure 1.

Gram X 1200.

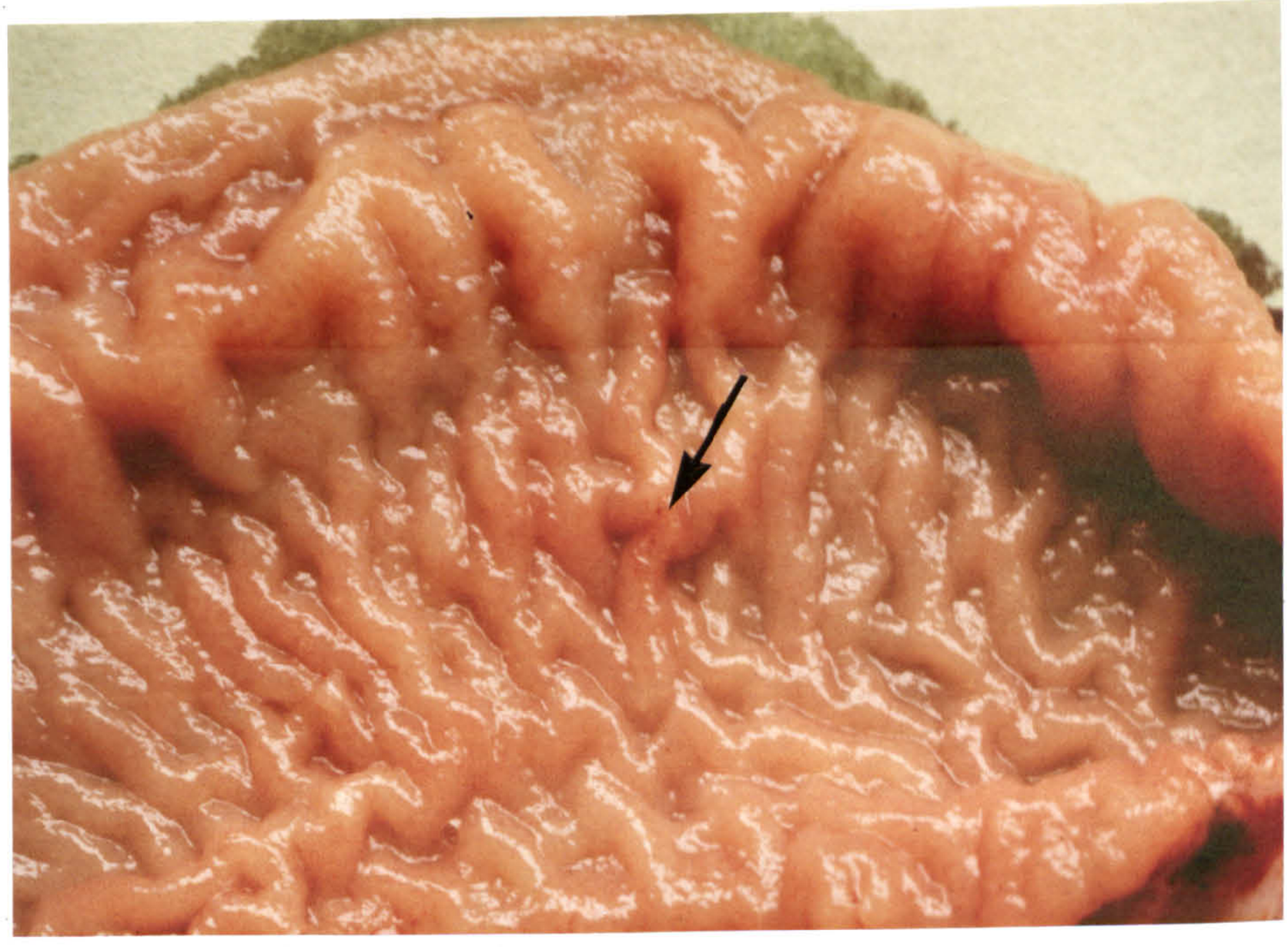


Fig. 3: Macroscopic appearance of the jejunal mucosa of calf 49 at a site from which C.f. ss. jejuni was isolated. B. licheniformis and E. coli were also isolated.

Note mild inflammation (arrow).

Table 2.

Sites from which C.f.ss. jejuni was isolated in 18 cases and the relative abundance of colonies

Site of isolation	No. of cases positive	Quantity of C.f.ss. jejuni isolated	Media used Non-selective	Media used selective	Case Number
Abomasum	7	Moderate	ND	+	22P,31P,34P 37P,38P, 44P;46P
Small intestine	2	Profuse	ND	+	75257,75258
	1	Moderate	ND	+	83444
Large intestine	2	Scanty	+	ND	50, 74589
Small and Large intestine	3	Scanty	+	ND	11, 291, 45
	2	Profuse	ND	+	83613, 49
Abomasum Small and Large intestine	1	Scanty Moderate Profuse	ND	+	82773

Scanty = Less than 5 colonies seen.

Moderate = 5-10 colonies seen.

Profuse = More than 10 colonies seen.

ND = Not done.

In other cases, the lesions seen could not be attributed to other agents. In the small intestine, varying degrees of stunting of the villi, capillary dilatation and cellular infiltration of the mucosa were seen. The cellular infiltrate consisted of large numbers of plasma cells and other mononuclear cells, a few neutrophil polymorphonuclear leucocytes and eosinophils within the mucosa. In sections from the ileum of case 75257, cell debris or inflammatory cells were present in some crypts (Fig. 4).

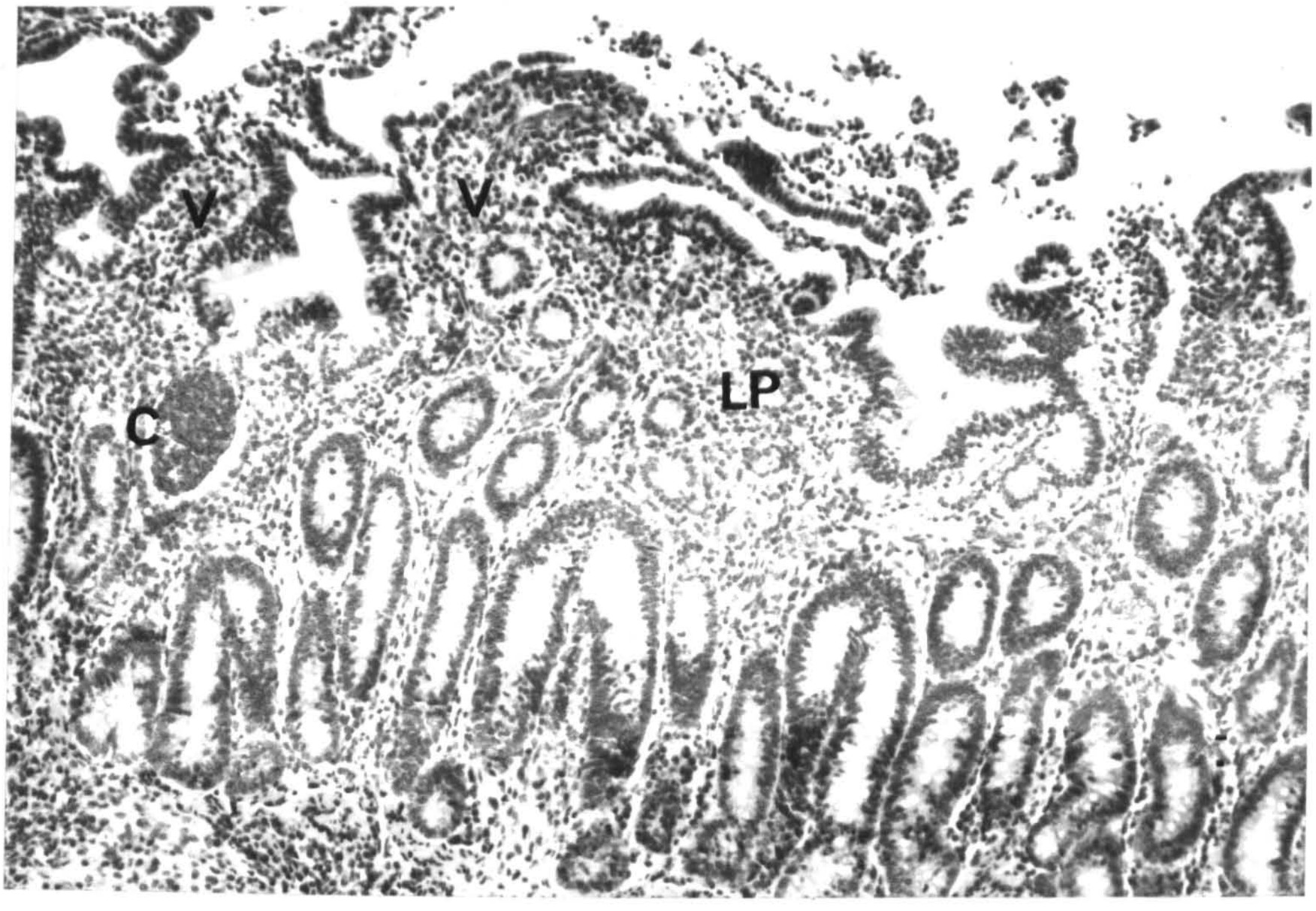


Fig. 4: Histological section of the ileal mucosa of calf 75257 from which C.f. ss. jejuni was isolated.

Note the stunting of the villi (V) the inflammatory cells in a crypt (C) and the cellularity of the lamina propria (LP).

H & E X 110.

The histological appearance of the mucosa of the large intestine at sites from which C.f. ss. jejuni was isolated varied from mild inflammatory change to desquamation of the mucosal epithelium with capillary dilatation. Some crypts were dilated. A cellular infiltrate similar in composition to that seen in the small intestine was often present in the lamina propria. Other agents such as coccidial oocysts or gametocytes or cryptosporidia were not found in this series.

Curved or spiral Gram-negative bacteria were seen in smears from the sites from which C.f. ss. jejuni was isolated in 15 cases (Fig. 5) but were not seen in sites in three cases (11, 50, 74589) from which the organism was subsequently isolated.

Campylobacter fetus subsp. intestinalis.

Colonies subsequently identified as C.f. ss. intestinalis were identical to those of C.f. ss. jejuni and the organisms were indistinguishable in Gram-stained smears.

C.f. ss. intestinalis was isolated from the intestinal mucosa of one adult animal and five calves (numbers 25, 33, 36, 75258, 75715 and 83555) all of which had soft faeces with excess mucus. The site from which C.f. ss. intestinalis was isolated and its relative abundance are given in Table 3. The macroscopical changes resembled those recorded for sites from which C.f. ss. jejuni was isolated. In case 75258 in which macroscopic changes and C.f. ss. jejuni were present in the small intestine, it was isolated from apparently normal large intestinal mucosa. Microscopic lesions were present, however (Fig. 6). More severe changes including necrotic enteritis were noted in case 83555.

Microscopic lesions were seen at the site of isolation in the intestinal mucosa in five cases. They were attributed to rotavirus infection (confirmed by electron microscopical examination of faeces) in cases 25 and 33, post mortem change (36) and necrosis associated with the isolation of F. necrophorum (83555). In case 75715, the lesions resembled those seen in cases from which C.f. ss. jejuni had been isolated.

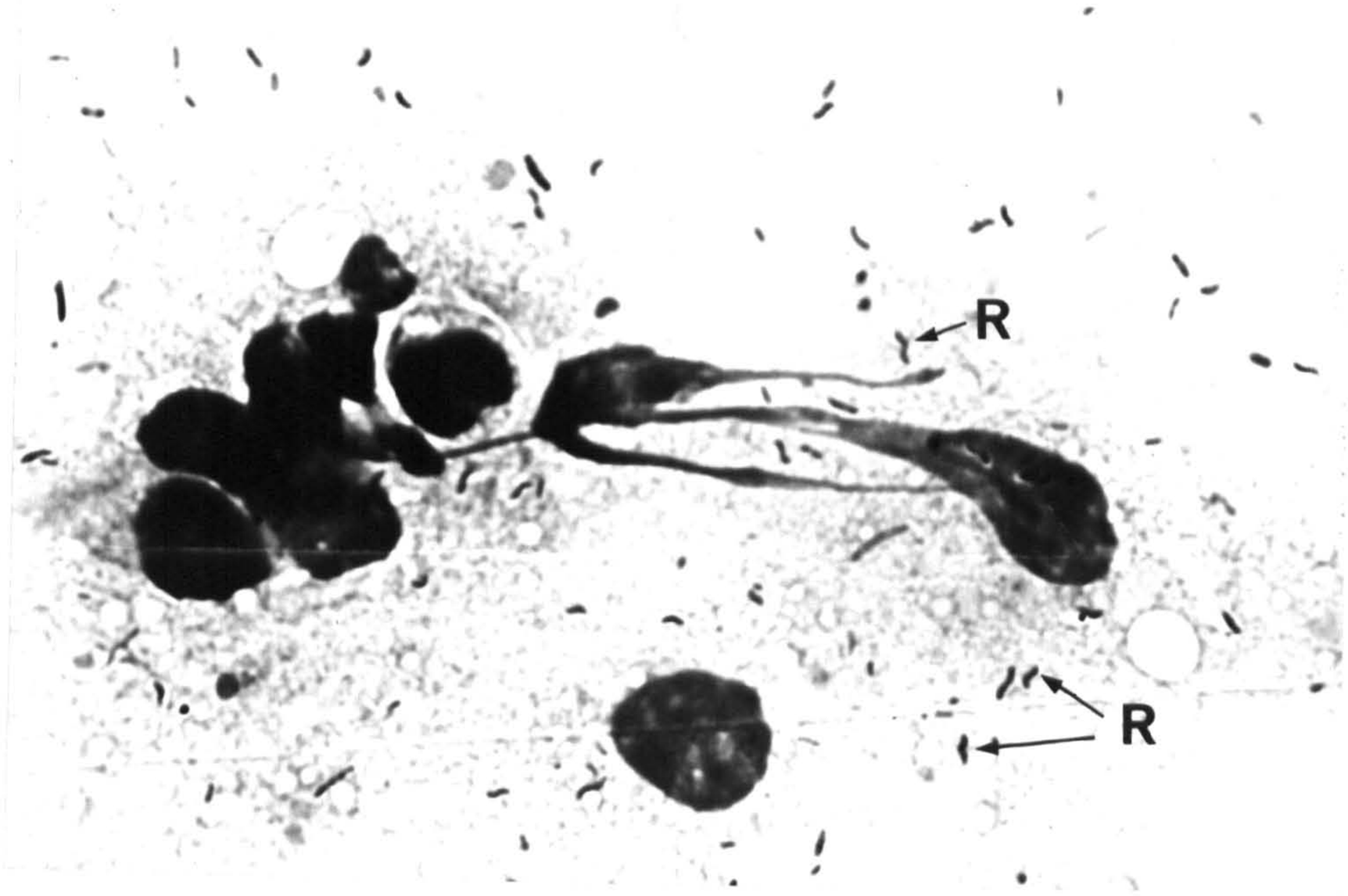


Fig. 5: Smear from the ileal mucosa of calf 75257 at a site from which C.f. ss. jejuni was isolated.

Note the curved rods (R).

Gram X 1200.

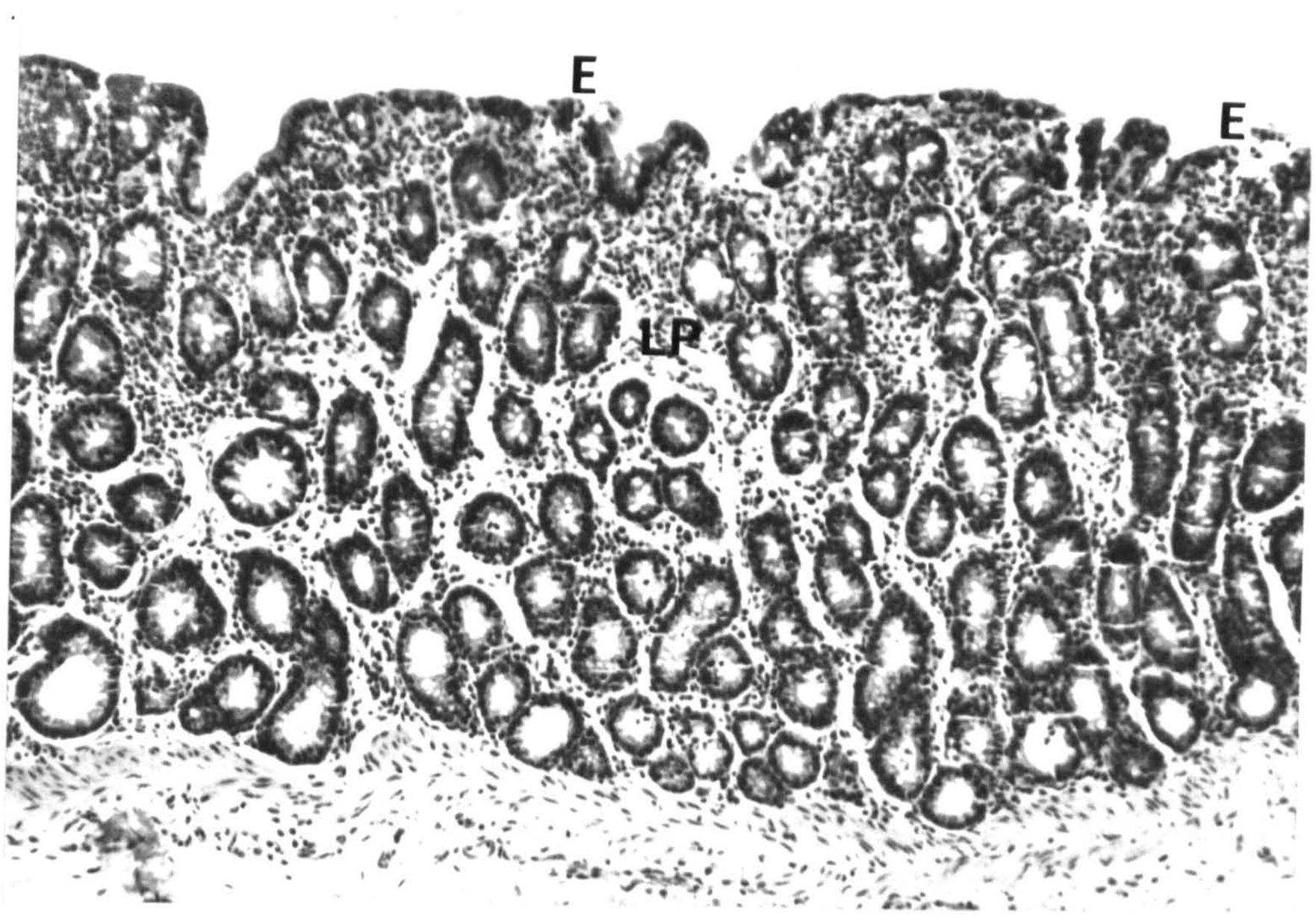


Fig. 6: Histological section of the colonic mucosa of calf 75258 from which C.f. ss. intestinalis was isolated.

Note the disruption of the lumenal epithelium (E) and oedema of the lamina propria (LP).

H & E X 110.

Table 3.

Sites from which C.f.ss. intestinalis was isolated in 6 cases and the relative abundance of colonies.

Site of isolation	Number of cases positive	Quantity of C.f.ss. intestinalis isolated	Media used Non-selective	Media used selective	Case Number
Small and Large intestine	4	Moderate	+	ND	25,33,36 75715
Small intestine	1	Profuse	ND	+	83555
Large intestine	1	Profuse	+	ND	75758
Spleen	1	Scanty	ND	+	83555

For key to amount of growth etc., see Table 2.

Curved or spiral Gram-negative bacteria were seen in smears from the sites from which C.f. ss. intestinalis was isolated in all six cases.

Campylobacter fecalis.

The colonial morphology of C. fecalis differs from that of C.f. ss. jejuni in that the colony is either a pinpoint or less than 3 mm in diameter. The colonies appear to be shiny, smooth, convex, greyish and round with entire edges (Fig. 7).

The morphology of C. fecalis when seen in Gram-stained smears (Fig. 8) differs from that of C.f. ss. intestinalis and C.f. ss. jejuni (Fig. 2). The cells are larger, longer, thicker and have rounded ends. C. fecalis was isolated from the gastrointestinal tracts of five adult cattle and one calf (75205, 78530, 79894, 80958, 83444 and 83613) all of which had soft, watery faeces.

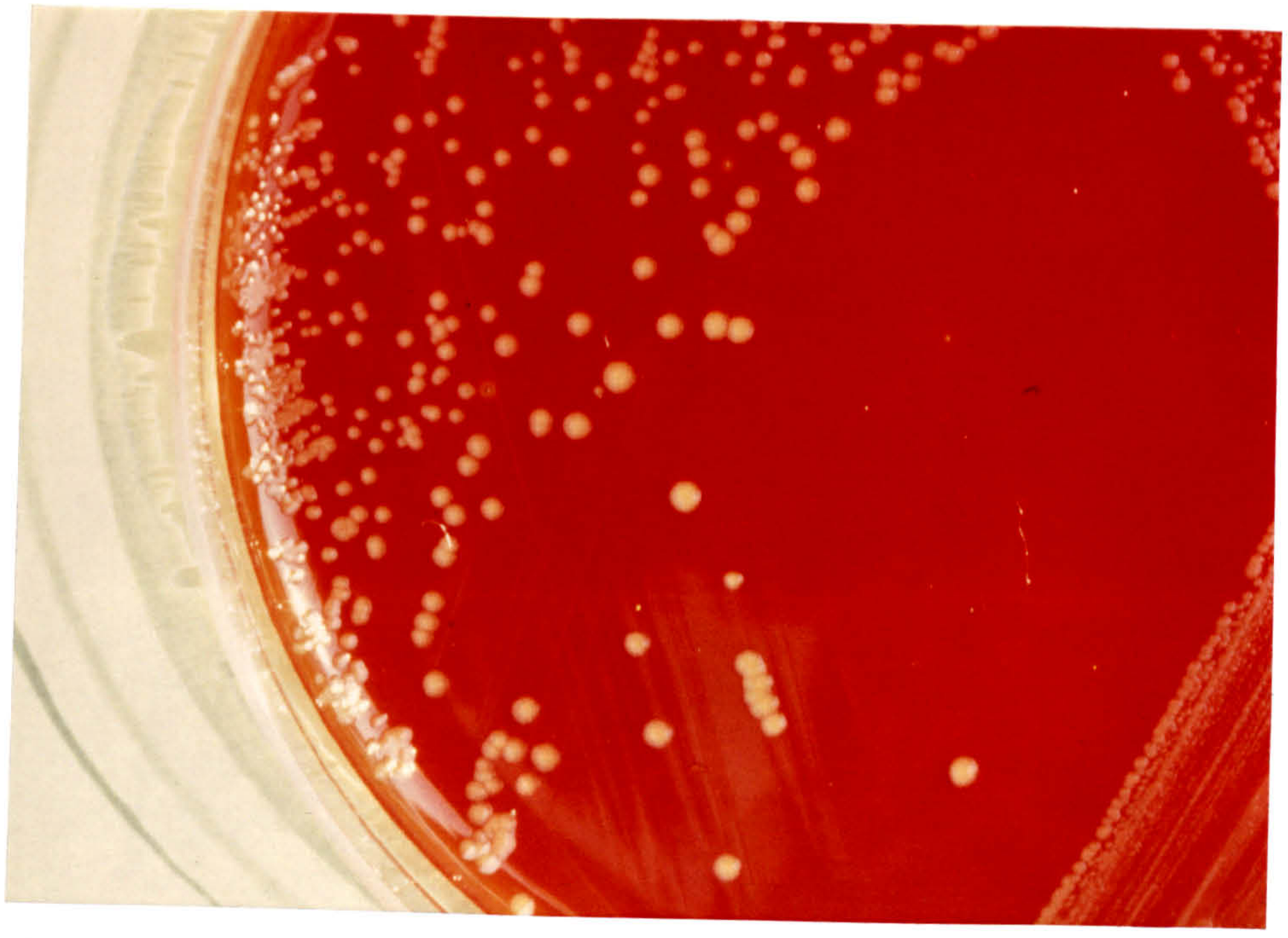


Fig. 7: Colonial morphology of C. fecalis isolated from cow 75205. 48 hours' incubation on horse blood agar at 37°C in micro-aerophilic conditions.

Fig. 8: Smear of a colony of C. fecalis from the plate shown in Figure 7.

Note that the cells are larger and coarser than those of C.f. ss. jejuni (Fig. 2).

Gram X 1200.

Macroscropical changes were seen at the site of isolation with the exception of case 83613 (see p.76 above). They varied from mild to severe congestion with oedema of the mucosa. The contents of the region were frequently mucoid. Abomasitis was frequently seen. The sites from which C. fecalis was isolated and its relative abundance are given in Table 4.

Table 4.

Sites from which C. fecalis was isolated in 6 cases and the relative abundance of colonies

Site of isolation	Number of cases positive	Quantity of C.fecalis isolated	Media used Non-selective	selective	Case Number
Abomasum	1	Scanty	+	ND	75205
	1	Moderate	ND	+	78530
Abomasum and Small intestine	1	Scanty Moderate	ND	+	79894
Small intestine	1	Profuse	ND	+	83444
Abomasum Small and Large intestine	1	Profuse	ND	+	80958
Abomasum and Large intestine	1	Profuse	ND	+	83613
Spleen and Gall Bladder	1	Scanty	ND	+	

For key to amount of growth etc., see Table 2.

Microscopic changes were attributed in part to post mortem change in cases 78530 and 80958. In case 83444 which died, no histological examination was undertaken. In the other cases (75205 and

83613) the lesion of the abomasal mucosa consisted of erosion of the mucosal surface, oedema and mild capillary dilatation of the lamina propria, blockage of some glands with neutrophilic polymorphonuclear leucocytes and mononuclear cells. Similar changes were also found in the small and large intestine in which infiltration of the lamina propria with polymorphs, eosinophils and increased numbers of mononuclear cells and plasma cells was seen. In case number 79894, stunting of the villi of the small intstine, dilated lacteals, crypts blocked with mononuclear cells and cuboidal lumenal epithelial cells were seen. There was a marked cellular exudate and much cellular debris at the lumenal surface. Curved Gram-negative bacteria with the morphology of C. fecalis were seen in small numbers in smears from the sites of which C. fecalis were isolated in all cases.

Unidentified campylobacters

The colonial morphology of the campylobacter isolates which could not be attributed to species was similar to that of C. fecalis (smooth, round and 1-2 mm in diameter) but the organism was intermediate in size between C. fecalis and C.f. ss. jejuni/intestinalis when smears were examined. Unidentified campylobacters were isolated from the abomasal mucosa of three calves (31P, 37P and 46P) with soft faeces containing excess clear mucus and streaks of blood and from the small intestinal mucosa of calf (75067) which had firm faeces.

Macroscopic changes consisting mainly of congestion were recorded at sites from which these unidentified campylobacters were isolated. The sites from which unidentified campylobacters were isolated and their relative abundance are summarised in Table 5.

Microscopic lesions were seen in abomasal mucosa in three cases (31P, 37P and 46P). These lesions were attributed to parasitic infection as they formed part of a parasitological experiment. In the other case (75067) stunted villi, patches of oedema, infiltration of neutrophilic polymorphs and increased numbers of mononuclear cells of the lamina propria were seen in the small intestine. Some crypts were dilated and filled with mononuclear cells.

Table 5.

Sites from which unidentified campylobacters were isolated in 4 cases and the relative abundance of colonies.

Site of isolation	Number of cases positive	Quantity of C.unidentified isolated	Media used Non-selective	Media used Selective	Case Number
Abomasum	3	Moderate	ND	+	31P,37P,46P
Small intestine	1	Moderate	+	ND	75067

For key to amount of growth etc., see Table 2.

Curved or spiral Gram-negative bacteria were seen in smears from the sites from which the unidentified campylobacters were isolated in three cases but were not seen in those from the site in case 75067 from which the organisms were cultured.

Clostridium spp.

Clostridia were isolated from the gastrointestinal mucosa of 12 of the 23 adult and 20 of 41 calves in the survey. Thirty five isolates were obtained and identified by the methods described in Chapter 2. The isolates were assigned to four clearly-defined species and one isolate remained unidentified.

They were:

Cl. perfringens Type A	30 isolates
Cl. sordellii	2 isolates
Cl. bifermentans	1 isolate
Cl. butyricum	1 isolate
Unidentified	1 isolate

Clostridium perfringens Type A.

Colonies of Cl. perfringens Type A could easily be distinguished from those of the other clostridia isolated by their morphology (Fig. 9). Cl. perfringens Type A appeared in Gram-stained smears as a straight Gram-positive rod with no obvious spores. None of the isolates produced toxins lethal for mice and all fulfilled the criteria of the Anaerobe Laboratory Manual for Cl. perfringens Type A.

It was isolated from the gastrointestinal tract of 11 adults and 19 calves. Diarrhoea was present in 22 of the 30 animals from which Cl. perfringens Type A was isolated (numbers 25, 33, 36, 45, 74331, 75478, 75911, 76217, 76457, 24, 29, 30, 79383, 79894, 80958, 22P, 31P, 44P, 46P, 83444, 83555 and B). The diarrhoeic faeces were soft to watery in consistency and contained excess clear mucus and streaks of blood in cases 31P, 44P, 46P. In case B, haemorrhagic diarrhoea was noted and Cl. sordellii was also isolated (see p.90 below). The remaining eight animals (10, 22, 31, 34P, 42P, 43P, 75186 and 76585) were passing normal faeces at the time of slaughter.

The gross changes recorded at the sites from which Cl. perfringens Type A was isolated, varied from slight to severe congestion and ulceration. In three cases (76457, 76585, B) haemorrhagic enteritis was seen. In another case (number 10) the organism was isolated from the apparently normal small and large intestinal mucosa and in case 76217 from apparently normal large intestinal mucosa when macroscopical changes were present in the small intestinal mucosa. The sites from which Cl. perfringens Type A was isolated and its relative abundance are given in Table 6.

Microscopical changes were seen in the gastrointestinal mucosa. These were contributed to by rotavirus (24, 25, 29, 30, 33, 36 and 45) post mortem change (24, 29, 30, 36, 79383, 80958, B) parasitic infection (10, 22, 31, 22P, 31P, 34P, 42P, 43P, 44P and 46P) and in some cases other organisms were isolated whichmay have initiated or maintained the lesions (Campylobacters 79894, 80958 and 83555, Cl. sordellii B and 80958). In the remaining cases (74331, 75186, 75478, 75911, 76217, 76457 and 76585) histological changes were seen. Localised disruption of the surface epithelium of the abomasal mucosa was seen in many, and

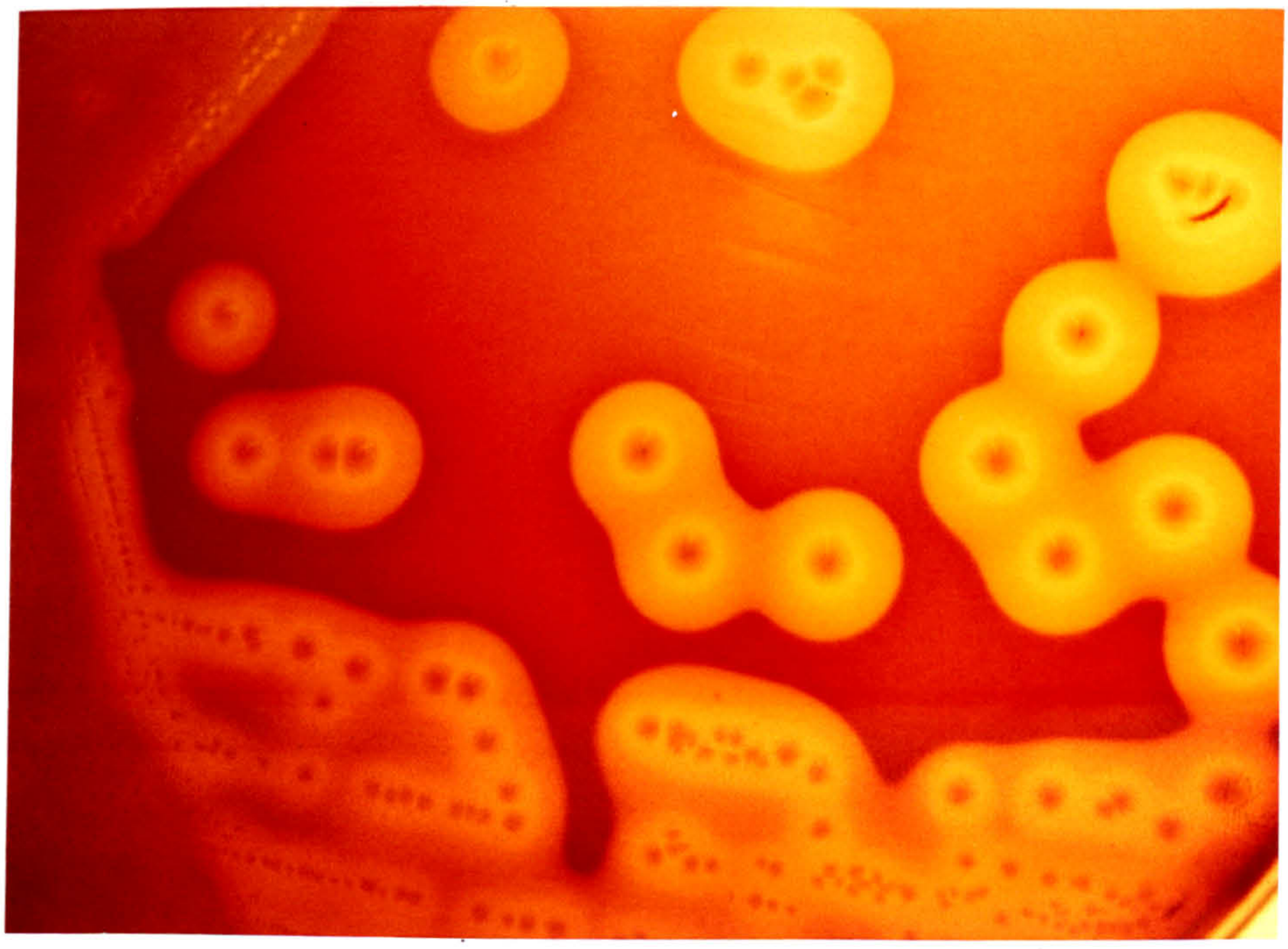


Fig. 9: Colonial morphology of Cl. perfringens Type A isolated from cow 79894. 24 hours' incubation on horse blood agar in anaerobic conditions at 37°C.

Note the double zone of haemolysis around the colonies.

mild dilatation of the mucosal capillaries was also common. Infiltration by neutrophils, increased numbers of mononuclear cells and plasma cells and local areas of oedema were seen in the abomasal lamina propria. Dilated glands containing mononuclear cells and inflammatory cells were also present. Rod shaped bacteria could be seen on the mucosal surface in case 74331. Similar changes were found in the small and large intestine. In the small intestine stunting and fusion of villi was present and some crypts were dilated and filled with inflammatory cells (Fig. 10). Coccidial oocysts were seen in the lamina propria of the jejunum in case 76457.

Thick straight Gram-positive rods were seen in smears from the sites from which Cl. perfringens Type A was isolated in all cases (Fig. 11). Other bacteria were often present in the same lesions (see Appendix 1).

Clostridium sordellii

Colonies of Cl. sordellii could be recognised by their morphology. They were 3-4 mm in diameter, greyish white, circular with markedly irregular edges and points of growth following the line of the streak, making the colonies appear longer in the direction of streak (Fig. 12). In Gram-stained smears the organism appeared as a short Gram-positive rod with an oval, subterminal or central spore. (Fig. 13).

Cl. sordellii was isolated from two animals. In one animal (B), an adult, haemorrhagic diarrhoea had been present before death. In the other animal, (80958) the faeces were soft.

Gross changes were seen at the sites from which Cl. sordellii were isolated. In case B the wall of the small intestine was thickened and oedematous and there was a necrotic haemorrhagic enteritis in which the mucosal surface was dark, velvety with streaks of clotted blood and dark, blood-stained mucus. In case 80958 the small intestine was slightly congested and covered in mucoid, pale-yellowish-grey foetid material. The underlying mucosa was oedematous but necrosis was not seen. Cl. sordellii was isolated in moderate numbers from the small intestine of case B and from both small and large intestine in case

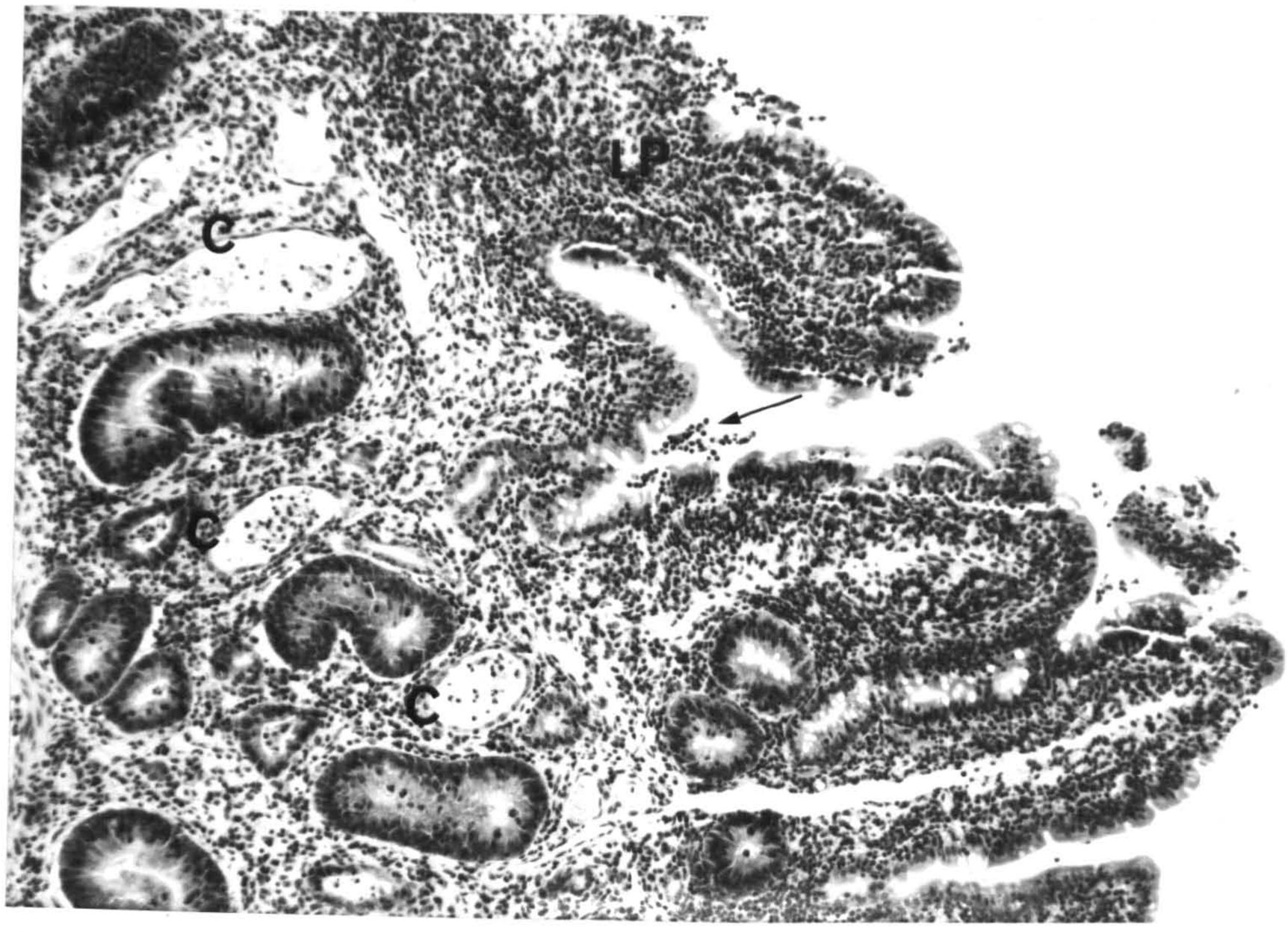


Fig. 10: Histological section of the small intestine of cow 79894 from which Cl. perfringens Type A was isolated.

Note the dilated capillaries (C), the hypercellularity of the lamina propria (LP) and the presence of inflammatory cells in the crypt lumen (arrow).

H & E X 110.

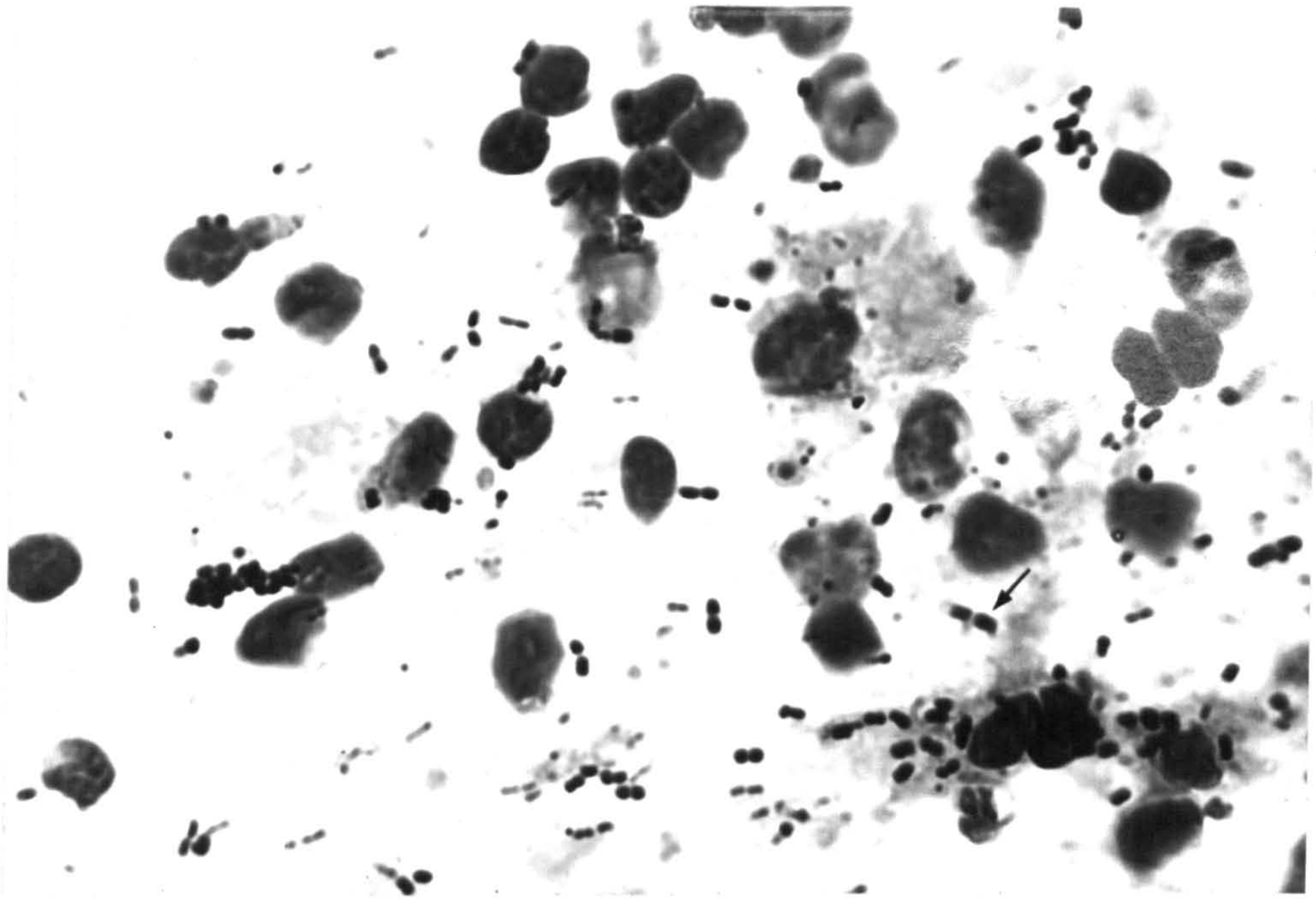


Fig. 11: Smear of the small intestinal mucosa of cow 79894 at a site from which Cl. perfringens Type A was isolated. Gram-positive rods with the morphology of Cl. perfringens can be seen (arrow). Other bacteria were also isolated (see Appendix I).

Gram X 1200.

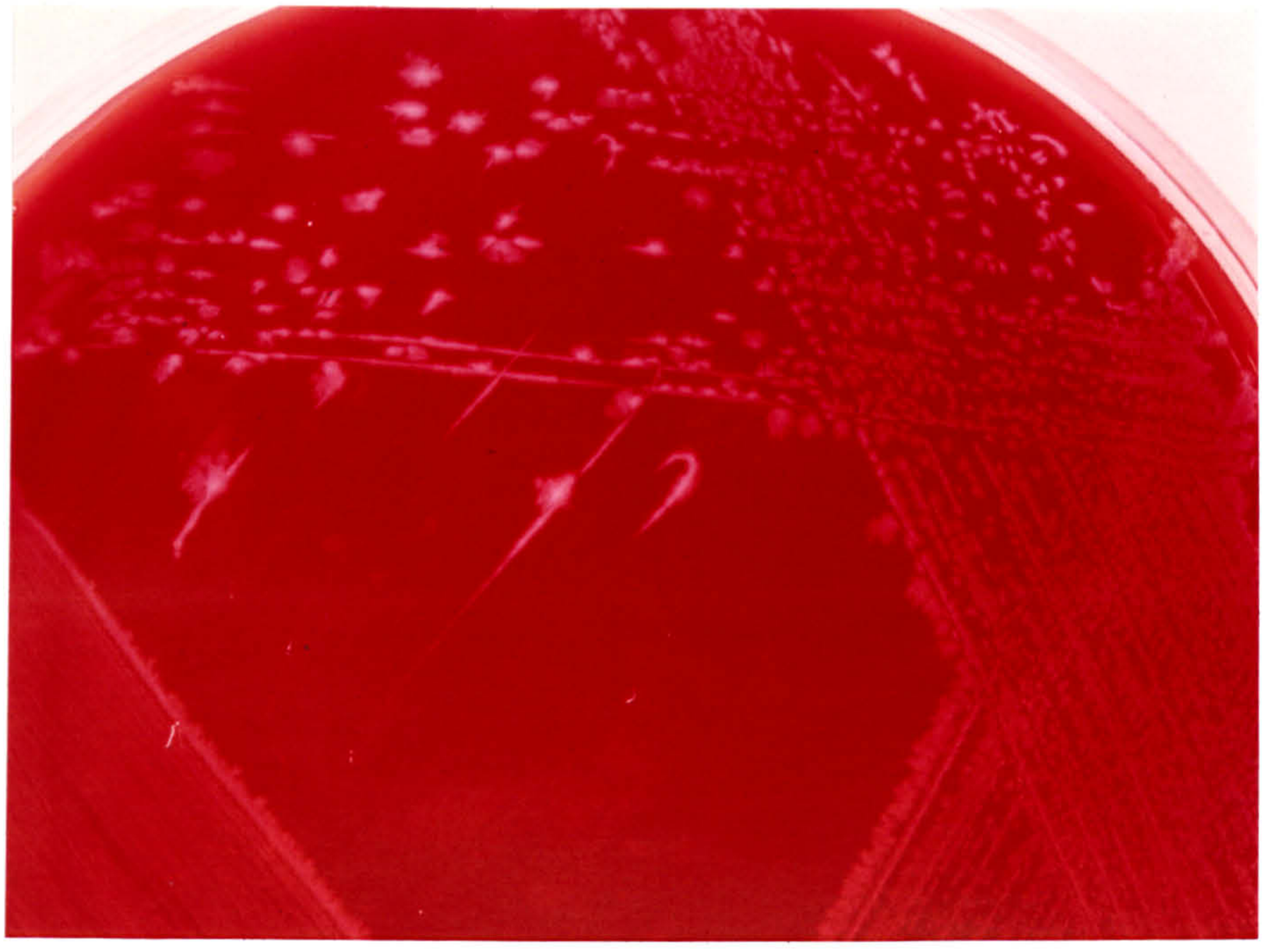


Fig. 12: Colonial morphology of Cl. sordellii isolated from cow B. 24 hours' incubation on horse blood 3% agar in anaerobic conditions at 37°C.

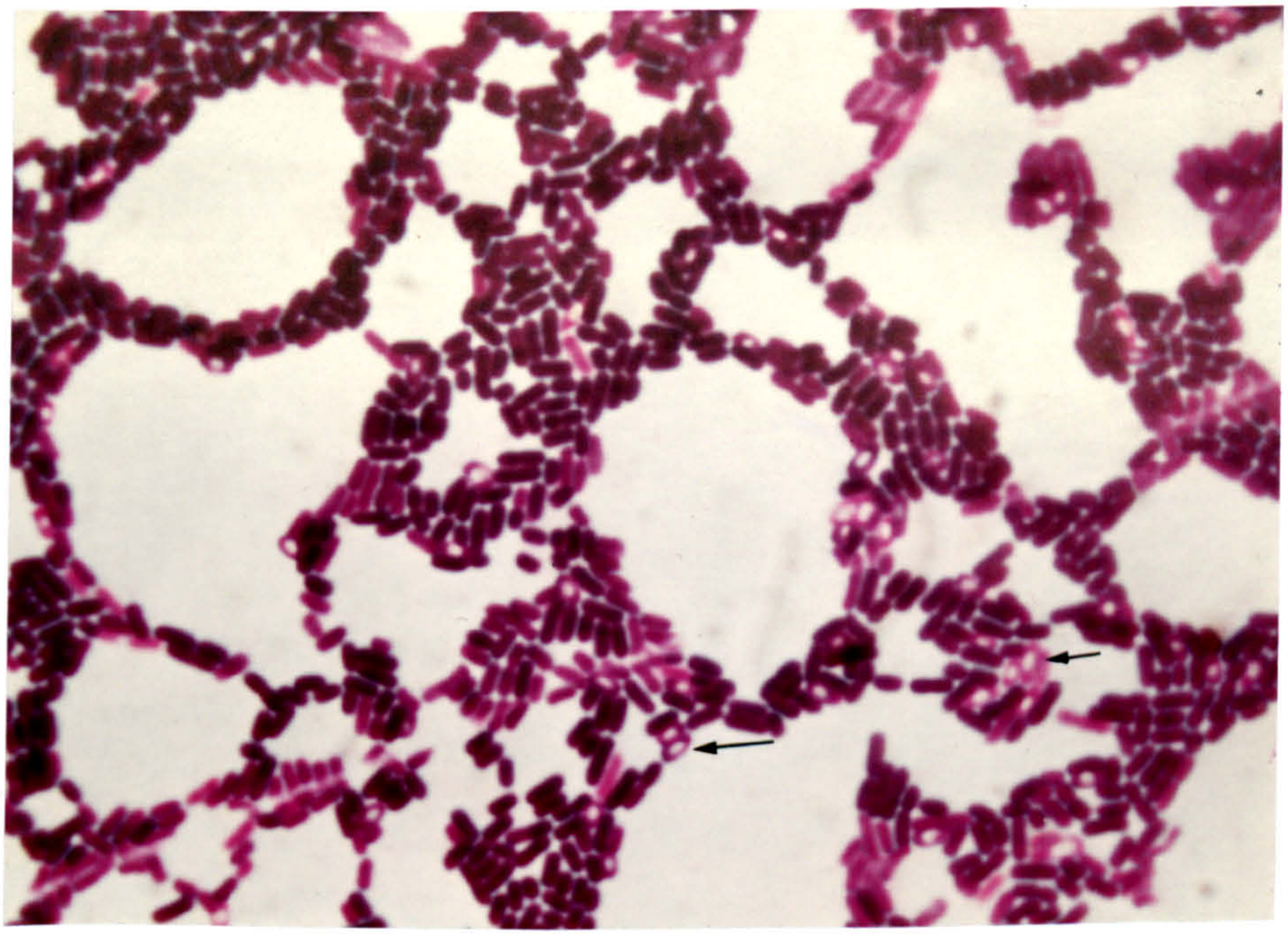


Fig. 13: Smear of a colony of Cl. sordellii from the plate shown in Figure 12.

Note the position of the spore (arrow).

Gram X 1600.

Table 6.

Sites from which Cl. perfringens Type A was isolated in 30 cases and the relative abundance of colonies.

Site of Isolation	Number of cases positive	Quantity of Cl. perfringens isolated	Case Number
Abomasum	7	scanty	22P, 31P, 34P, 42P 43P, 44P, 46P.
Small intestine	1	scanty	31
	2	moderate	83555, B
	1	profuse	83444
Large intestine	1	moderate	22
Abomasum, small and Large intestine	1	scanty	79894
	5	moderate	74331, 10, 75186 24, 45.
	7	profuse	75478, 76217, 76457, 76585, 79383, 80958, 36.
Small and Large intestine	1	scanty	75911
	1	moderate	25
	3	profuse	30, 29, 33

For key to amount of growth etc., see Table 2.

80958. Histological changes were difficult to interpret as the animals had died but included loss of the mucosal epithelium, massive dilatation of the capillaries, extravasion of blood into the lamina propria and oedema. Infiltration of lymphocytes, and neutrophilic polymorphs into

the lamina propria was seen. Massive numbers of rod-shaped bacteria were seen in the lamina propria of the mucosa and on villi. There was necrosis of the crypts. At some sites there was destruction of the full depth of the mucosa. (Fig. 14).

Straight Gram-positive rods, some with the characteristic subterminal spore were seen in direct smears from the sites of which Cl. sordellii was isolated. (Fig. 15).

Other agents such as Cl. perfringens Type A, non-haemolytic E. coli, were isolated together with Cl. sordellii in case B and in case 80958, Cl. perfringens Type A, non-haemolytic E. coli, C. fecalis, Bacteroides fragilis were also isolated.

Clostridium bifermentans

Cl. bifermentans was isolated in moderate numbers from the mucosa of the abomasum, small and large intestine of one animal (79249) which had watery mucoid diarrhoea prior to death. Abomasitis and severe congestion and erosion of both small and large intestinal mucosa were seen. The small intestinal content was watery and mucoid with thick threads of pus. Microscopic changes seen included abomasitis with necrosis, haemorrhage and neutrophilic polymorphonuclear leucocytic and mononuclear cell infiltration of the lamina propria. In the small and large intestinal mucosa, autolytic changes in the mucosal epithelium, , increased numbers of mononuclear cells, plasma cells, eosinophils and neutrophilic polymorphonuclear leucocytes were present in the lamina propria. Dilatation of mucosal capillaries and crypts was seen and the latter contained mononuclear cells. Gram-positive rods resembling Cl. bifermentans were seen in smears from the sites from which the organism was isolated.

Clostridium butyricum

Cl. butyricum was isolated from the mucosa of the abomasum and small intestinal tract of one adult (83613) with soft faeces. A number of other bacteria were isolated from the abomasum including E. coli, Strep. bovis and C. fecalis and from the small intestine E. coli and

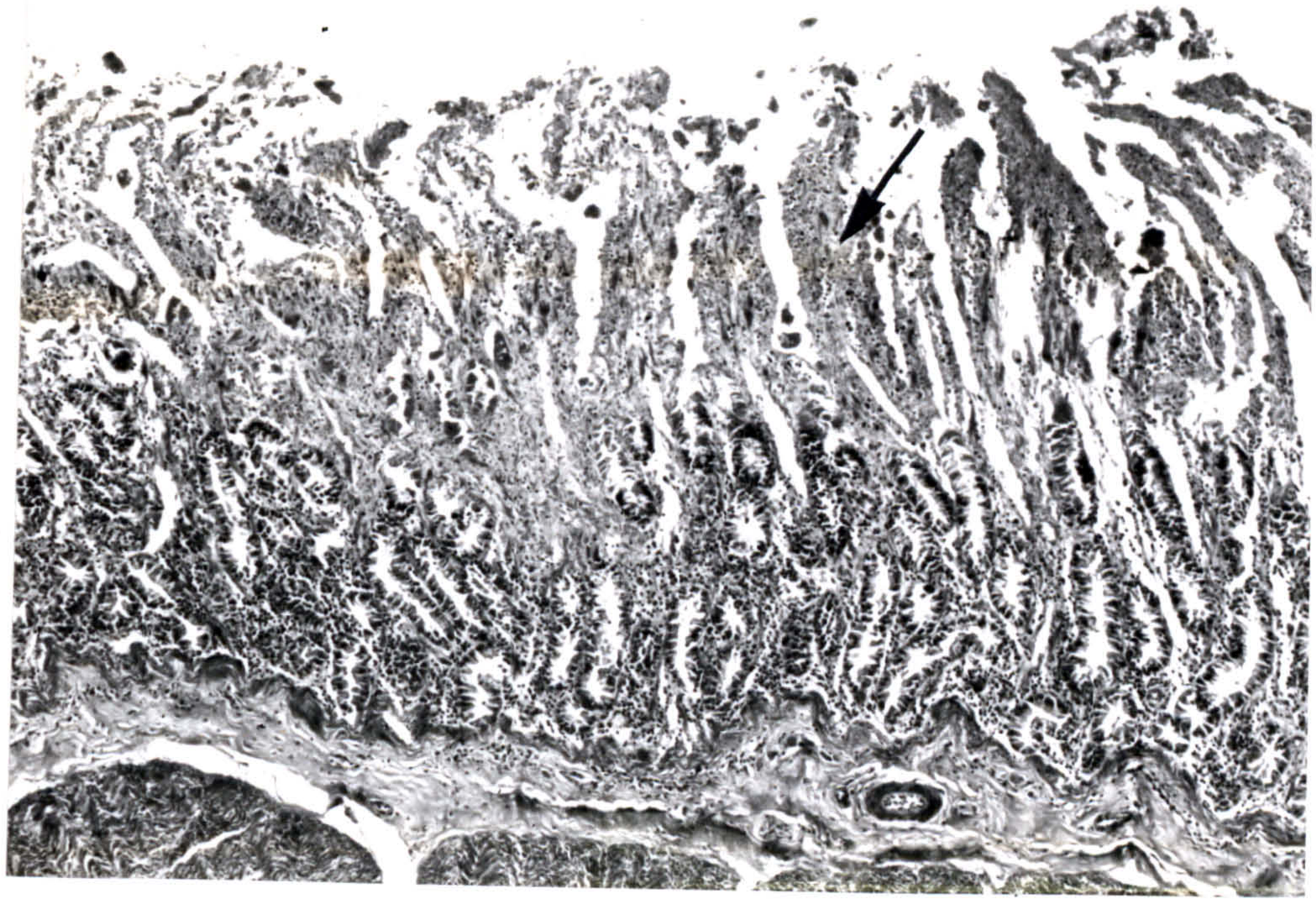


Fig. 14: Histological section of the small intestine of cow B from which Cl. sordellii was isolated.

Note the necrotic material at the lumenal surface (arrow). This animal had died but similar changes were never seen in other dead animals.

H & E X 35.

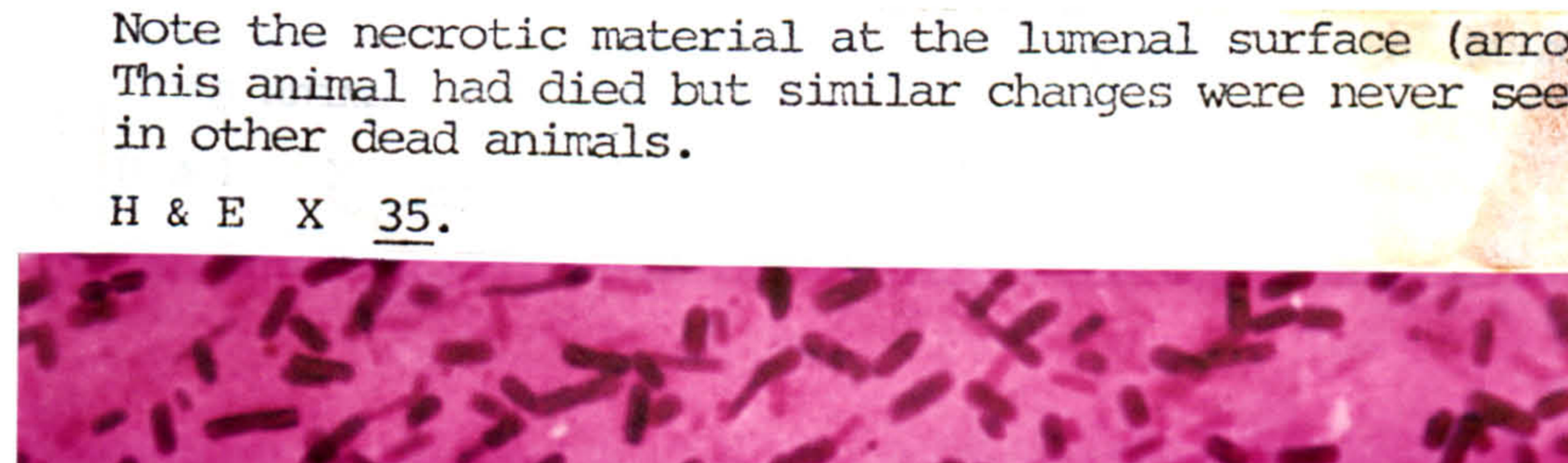


Fig. 15: Smear of the small intestinal mucosa of cow B at a site from which Cl. sordellii was isolated.

Note sporulating organism (arrow). Other bacteria were isolated (see Appendix 1).

Gram X 1600.

and C.f. ss. jejuni. Congestion and oedema of the abomasal mucosa and congestion of the small intestinal mucosa was present. The microscopical changes could not be distinguished from those of other lesions from which C.f. ss. jejuni and C. fecalis were isolated.

Unidentified Clostridium

A clostridium which could not be identified using the methods described in Chapter 2 was isolated from the mucosa of the abomasum, small and large intestine of animal 79249. Colonies were 2-4 mm in diameter with slight raised whitish to yellow in colour with circular regular less adherent and non-haemolytic. The morphology of the organism in smears resembled that of Cl. bifermentans but the cells were slightly longer with an oval central spore.

In culture it was catalase and oxidase negative, motile, fermentative, produced acid from glucose but not from lactose or salicin but a weak reaction to sucrose was recorded. It reduced nitrate and produced H_2S but did not produce indole, urease, lecithinase or a pearly layer on egg yolk agar and failed to liquefy gelatine.

It was present in moderate numbers in the sites from which it was isolated and could be distinguished from Cl. bifermentans both in culture and in smears.

Bacillus species

Bacillus species were isolated from eight of the 23 adult and 13 of the 41 calves in the survey. Twenty one isolates were identified by the methods described in Chapter 2 as Bacillus species which were identified as follows:

Twenty isolates were identified as B. licheniformis.
One isolate was considered to be B. coagulans.

Bacillus licheniformis

B. licheniformis could be identified readily in aerobic primary cultures by its characteristic colonial morphology (Fig. 16). The morphology of colonies grown under anaerobic conditions differed but

was equally distinctive. (Fig. 17). In Gram-stained smears the organism appeared as large straight or slightly curved Gram-positive rods in short and long chains. Oval and central or subterminal spores were commonly seen within the body of the cell.

B. licheniformis was isolated from the mucosa of the abomasum and small and large intestinal tract of seven adults and 13 calves. Eight animals (numbers 74589, 74331, 74989, 75799, 76245, 82773, 78530 and 49) had diarrhoea. The diarrhoeic faeces were soft, mucoid and watery. The remaining 12 animals (11, 50, 291, 3, 10, 22, 31, 54, 293, 294, 296 and 75516) had normal faeces at the time of slaughter.

Gross changes were seen in some cases at the sites from which B. licheniformis was isolated. In many cases e.g. adults 74989, 76245 and calves 3, 11, 31, it was isolated from congested, oedematous or ulcerated mucosa. In no case was it the only organism isolated and it was frequently isolated in conjunction with campylobacters (cases 11, 74589, 78530, 82773 and 49) or with Cl. perfringens Type A (cases 10, 22, 31 and 74331). It was isolated in moderate numbers from abomasal ulcers in animals which had been exposed to experimental parasitic infections (cases 11, 3, 10, 22 and 31). In other cases (294 and 296) it was isolated from grossly normal mucosa. The details of the sites from which B. licheniformis was isolated and its relative abundance are summarised in Table 7.

In all cases examined histologically, microscopic changes were seen at the sites of isolation in the gastrointestinal mucosa even when the mucosa was grossly normal. In many cases these changes were of the type attributed to the other agents present such as campylobacters and clostridia.

In the cases and sites from which clostridia and campylobacters were not isolated, the following changes were seen. In the abomasum the mucosa was disrupted locally and contained patches of oedema and a cellular infiltrate containing mononuclear cells and a few polymorphonuclear leucocytes. Some glands contained cell debris and mononuclear cells (74984, 75516, 74589, 11, 50, 291, 22, 31, 54, 293, 294, 296).

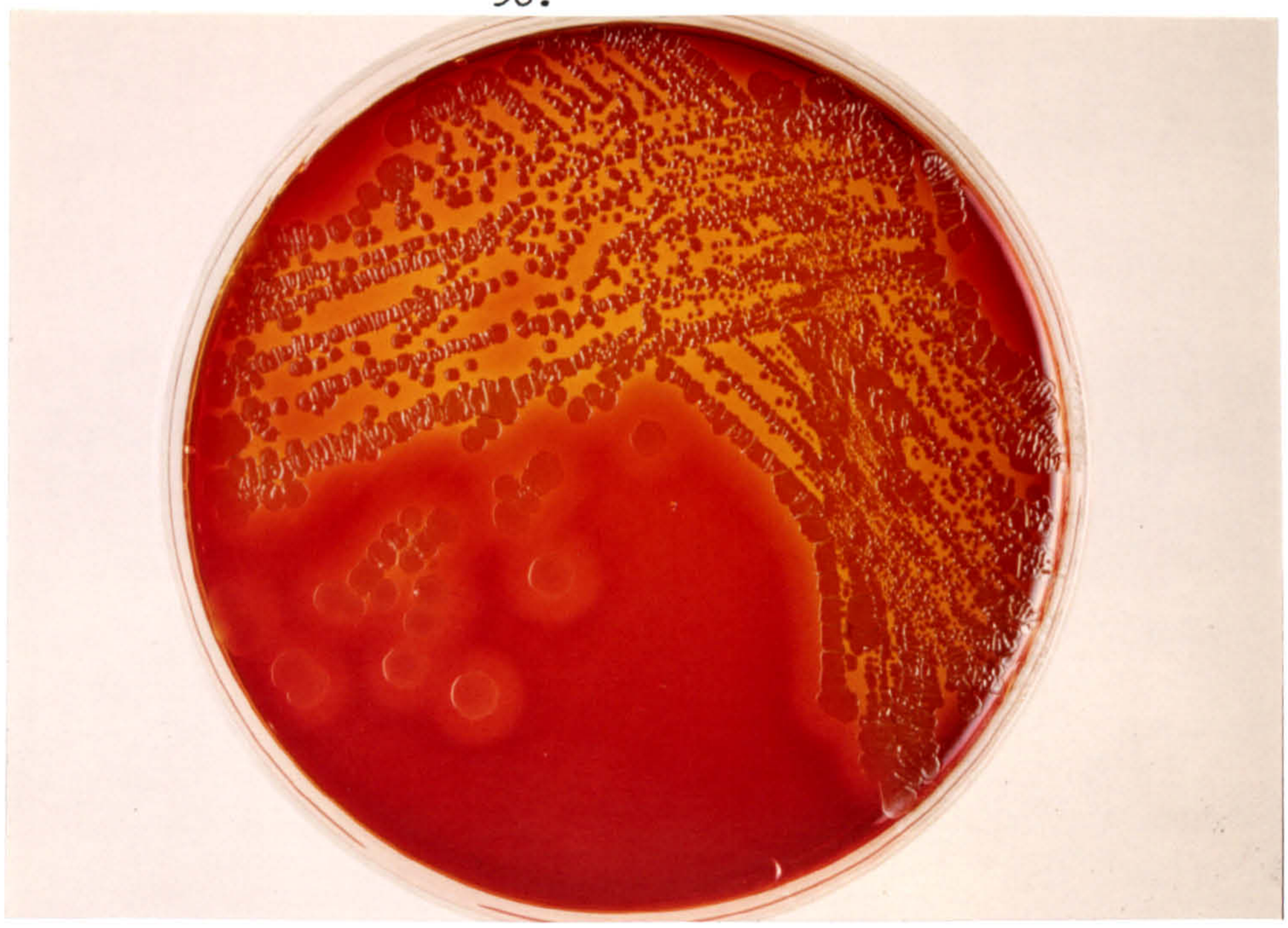


Fig. 16: Colonial morphology of B. licheniformis isolated from calf 49 and incubated aerobically.

Horse blood agar, 24 hours' culture at 37°C.

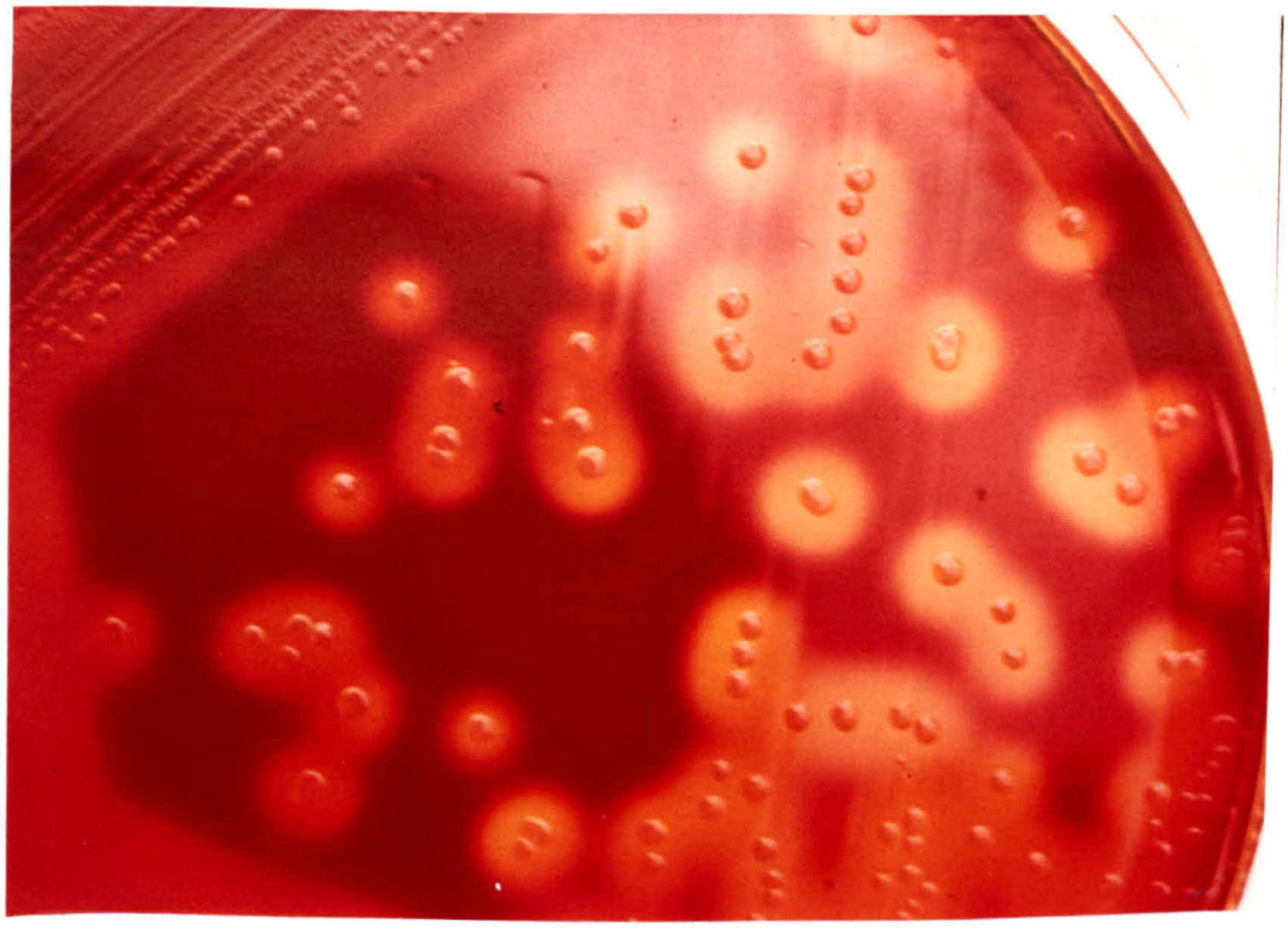


Fig. 17: Colonial morphology of B. licheniformis isolated from calf 49 and incubated anaerobically.

Horse blood agar, 24 hours' culture at 37°C.

Note the change in colonial morphology and the increased extent of haemolysis.

Table 7.

Sites from which B. licheniformis was isolated in 20 cases and the relative abundance of colonies.

Site of isolation	Number of cases positive	Quantity of B. licheniformis isolated	Case number
Abomasum	7	Moderate	293, 294, 296, 75799 50, 291, 75516
Small intestine	1	Moderate	76245
Abomasum and Small intestine	3	Moderate	54, 74989 10
Abomasum, Small and Large intestine	5	Scanty	78530, 49 74589, 74331, 82773
	4	Moderate	11, 3, 22, 31

For key to amount of growth etc., see Table 2.

No parasites were seen in sections from animals 293, 294 or 296. In the small intestinal mucosa stunting of the villi and blockage of the crypts was seen (3, 22, 54, 74989, 76245, 78530). The same type of changes were seen in the large intestinal sites (3, 31, 78530).

Straight slightly curved Gram-positive rods were seen in smears from the sites from which B. licheniformis was isolated in all cases.

Bacillus coagulans

B. coagulans was isolated in moderate numbers from the congested mucosa of the abomasum of adult 75478 together with Cl. perfringens Type A and Streptococcus bovis with soft diarrhoea.

Gram-positive rods were seen in smears from the site of which B. coagulans was isolated.

Bacteroides species

Bacteroides species were isolated from five of the 23 adult and four of the 40 calves in the survey and were identified by the methods described in Chapter 2.

Bacteroides fragilis

Colonies of B. fragilis 1-2 mm in diameter, circular, entire edge, convex, greyish-translucent (Fig. 18). The cultures had a foetid odour. B. fragilis is a straight or slightly curved Gram-negative rod with rounded ends arranged singly or in pairs end to end.

B. fragilis was isolated from four adult cattle and four calves from the abomasal, small and large intestinal mucosa. Animals number (76457, 75257, 75258, 78530, AL and 45) had diarrhoea. The remaining two cases (75067, 75516) had normal faeces at the time of slaughter. The sites from which B. fragilis was isolated and its relative abundance are given in Table 8.

In all cases other bacteria or agents were present at the site of isolation of B. fragilis. Gross changes recorded included those of Johnes disease (AL) and the appearance of the mucosa varied from normal to mildly or severely congested and eroded.

The microscopical changes found could not be distinguished from those attributed to campylobacters, clostridia and bacilli (see above) which were all present in the lesions.

Gram-negative coccobacilli resembling Bacteroides sp. were seen in smears prepared from the sites from which the organisms were isolated.

Table 8.

Sites from which B. fragilis was isolated in 8 cases and the relative abundance of colonies.

Site of isolation	Number of cases positive	Quantity of B. fragilis isolated.	Case number.
Abomasum, Small and Large intestine	1	Scanty	76457
Small and Large intestine	6	Moderate	75067, 75257 75258, AL, 75516 78530
Large intestine	1	Scanty	45

For key to amount of growth etc., see Table 2.

Bacteroides vulgatus

The colonies of B. vulgatus resembled those of B. fragilis but were surrounded by a zone of complete haemolysis (Fig. 19). The morphology of B. vulgatus in smears also resembled that of B. fragilis. B. vulgatus was isolated from the mucosa of the abomasum, small and large intestinal tracts of one adult (78530) and three calves number (49, 75257, 75258). All had diarrhoea.

The sites from which B. vulgatus was isolated and its relative abundance are summarised in Table 9. Campylobacters were present in all the sites from which B. vulgatus was isolated and the gross and histological findings were considered with those of campylobacter infection. The only sites from which B. vulgatus was recovered without the isolation of campylobacters was from congested and ulcerated small

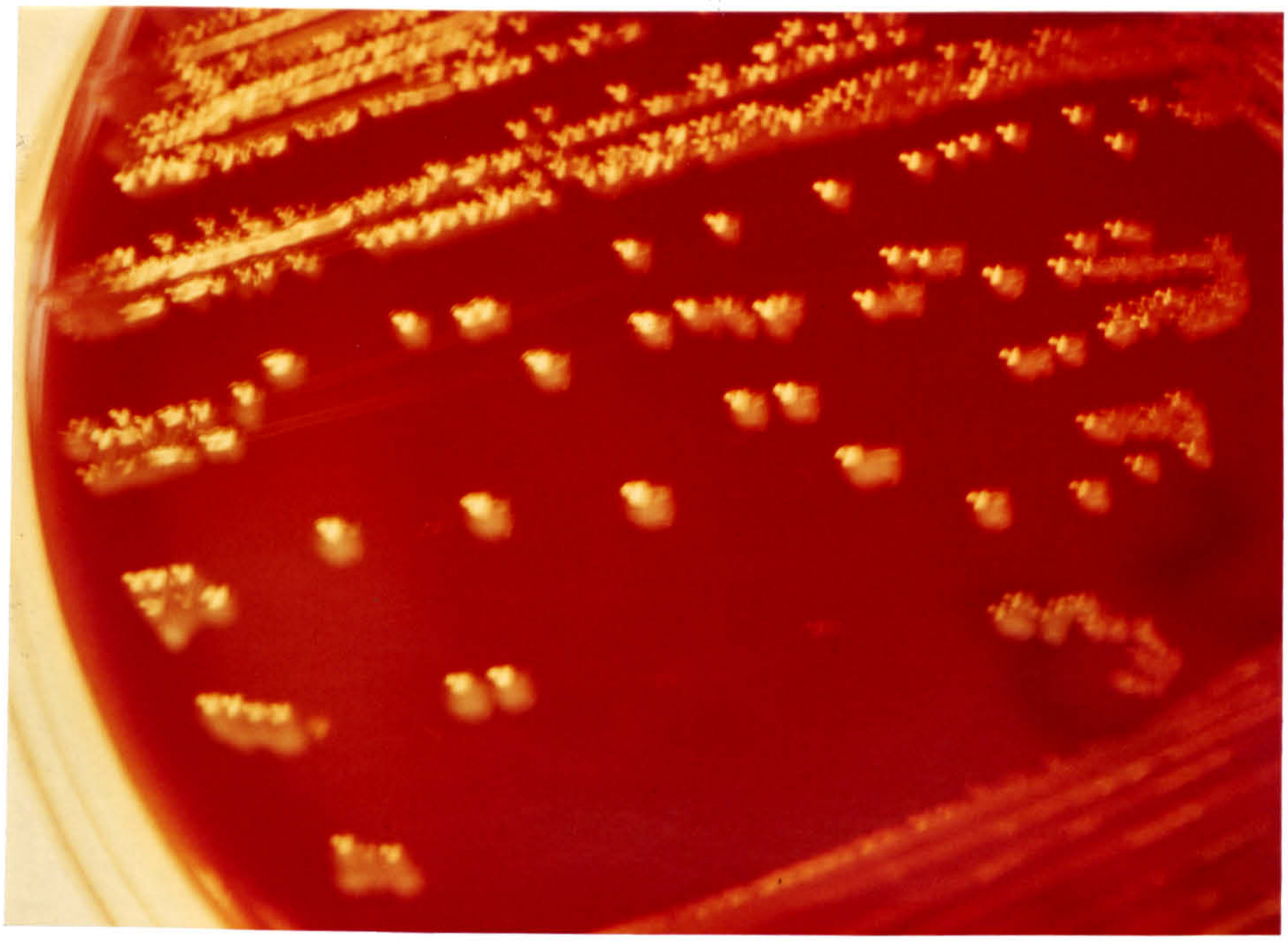


Fig. 18: Colonial morphology of B. fragilis isolated from calf 75067. 48 hours' culture on horse blood agar incubated anaerobically at 37°C.

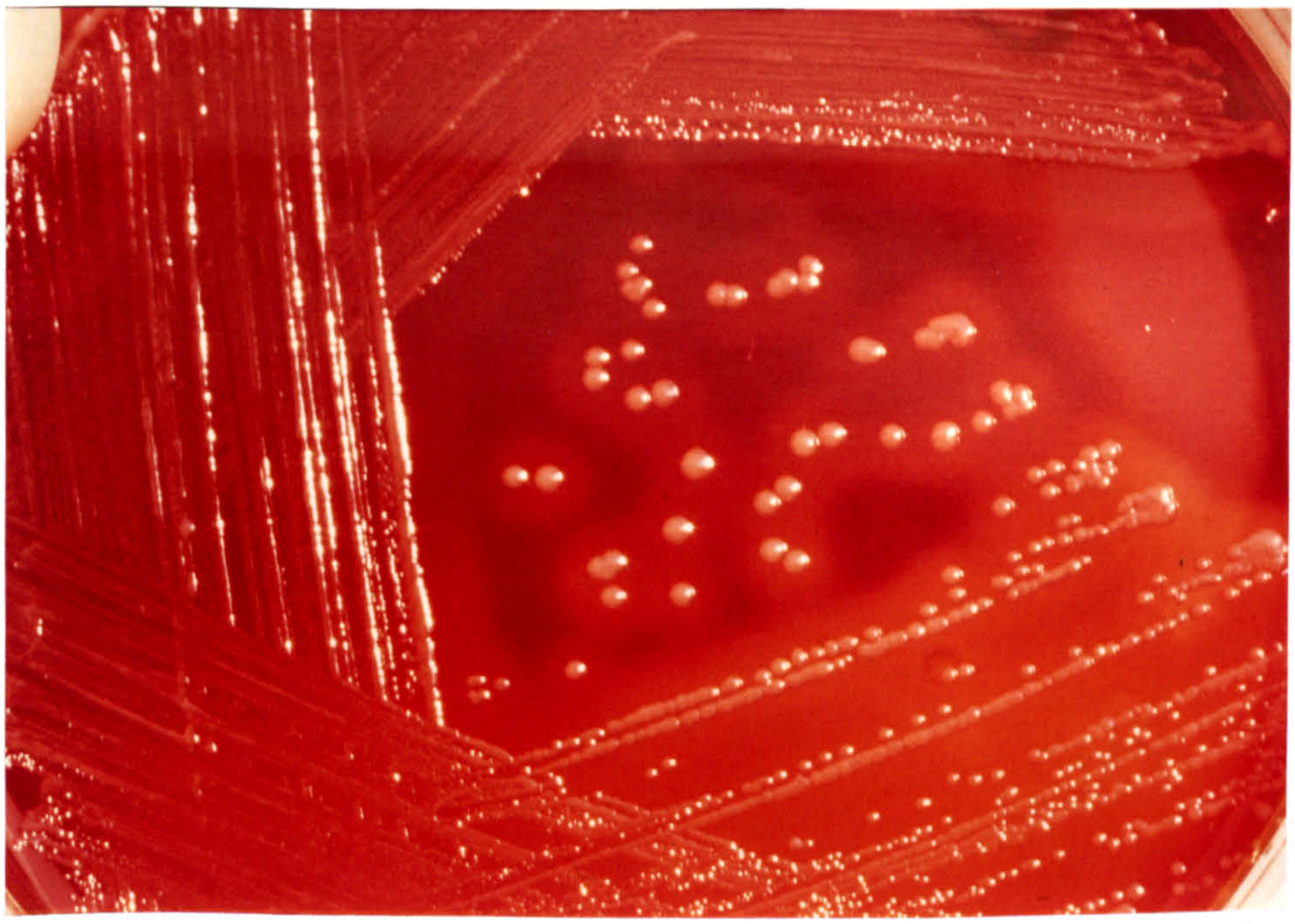


Fig. 19: Colonial morphology of B. vulgatus isolated from calf 49. 48 hours' culture on horse blood agar incubated at 37°C.

Note the haemolysis surrounding isolated colonies.

Table 9.

Sites from which B. vulgatus was isolated in 4 cases and the relative abundance of colonies.

Site of isolation	Number of cases positive	Quantity of B. vulgatus isolated	Case number
Abomasum, Small and Large intestine Lung and Liver	1	Scanty	78530
Small and Large intestine	2	Scanty	75257, 75258
Large intestine	1	Moderate	49

For key of amount of growth etc., see Table 2.

intestinal mucosa and congested large intestinal mucosa of case number 78530. Gram-negative rods were present in smears of the mucosa from all the sites from which B. vulgatus was isolated.

Bacteroides melaninogenicus

Colonies of B. melaninogenicus on blood agar are usually black or dark brown after three - four days' incubation and can be distinguished readily by their colour. Gram-stain films show small Gram-negative coccobacilli. B. melaninogenicus was isolated in moderate numbers with other bacteria from abomasitis, suppurative pneumonia and from enlarged congested liver in adult 78530 which had foul smelling, soft diarrhoeic faeces. The gross and histological findings are described above under B. vulgatus which was also isolated.

B. oralis

B. oralis was also recovered from the same animal as B. melaninogenicus and also from the small and large intestinal mucosa.

Fusobacterium necrophorum

F. necrophorum was distinguished initially by its colonial appearance (Fig. 20) and was isolated from the necrotic mucosa of the small intestine in profuse numbers of colonies in adult animal 83555 which had soft faeces. Other bacteria such as C.f. ss. intestinalis and Cl. perfringens Type A were also isolated.

The histological findings revealed a fibrinous exudate with varying amounts of eosinophilic material and containing mononuclear cells mainly lymphocytes and polymorphs, nuclear leucocytes, free red cells and desquamated epithelial cells on the lumenal surface of the mucosa. The villi were largely absent although the animal had been killed. Dilatation of capillary blood vessels was conspicuous in the remains of the lamina pripria (Fig. 21). Massive numbers of very fine pleomorphic organisms with beaded appearance resembling F. necrophorum were also seen in smears from the mucosal surface. Organisms were also seen in sections. (Fig. 22).

Fusobacterium spp.

A few colonies of another Fusobacterium with fusiform cells were isolated from abomasitis in adult 75205. No necrosis was seen.

Escherichia coli

Non-haemolytic E. coli was isolated from 19 of the 23 adult cattle and 38 of the 41 calves. It was isolated from the mucosa of abomasum, small and large intestinal tract of 57 cases in the survey.

Beta-haemolytic E. coli was isolated from two calves (52R, 53R) of the 41 calves in the survey with diarrhoeic faeces.

Neither type of E. coli was isolated from the remaining seven animals (75205, 75667, 75258, 73948, 293, 75003, 75478).

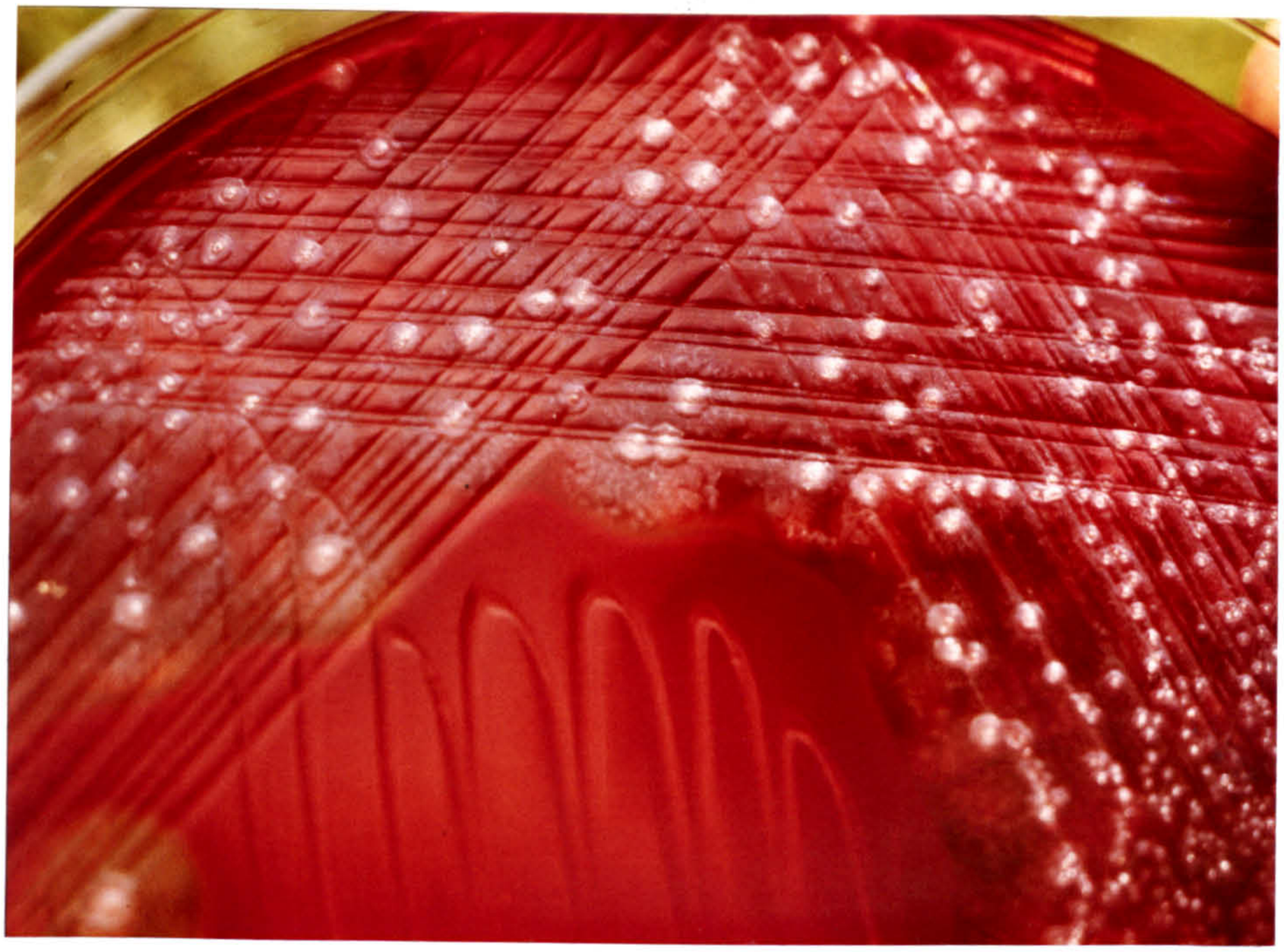


Fig. 20: Colonial morphology of F. necrophorum isolated from cow 73555. 48 hours' culture on horse blood agar incubated anaerobically at 37°C.

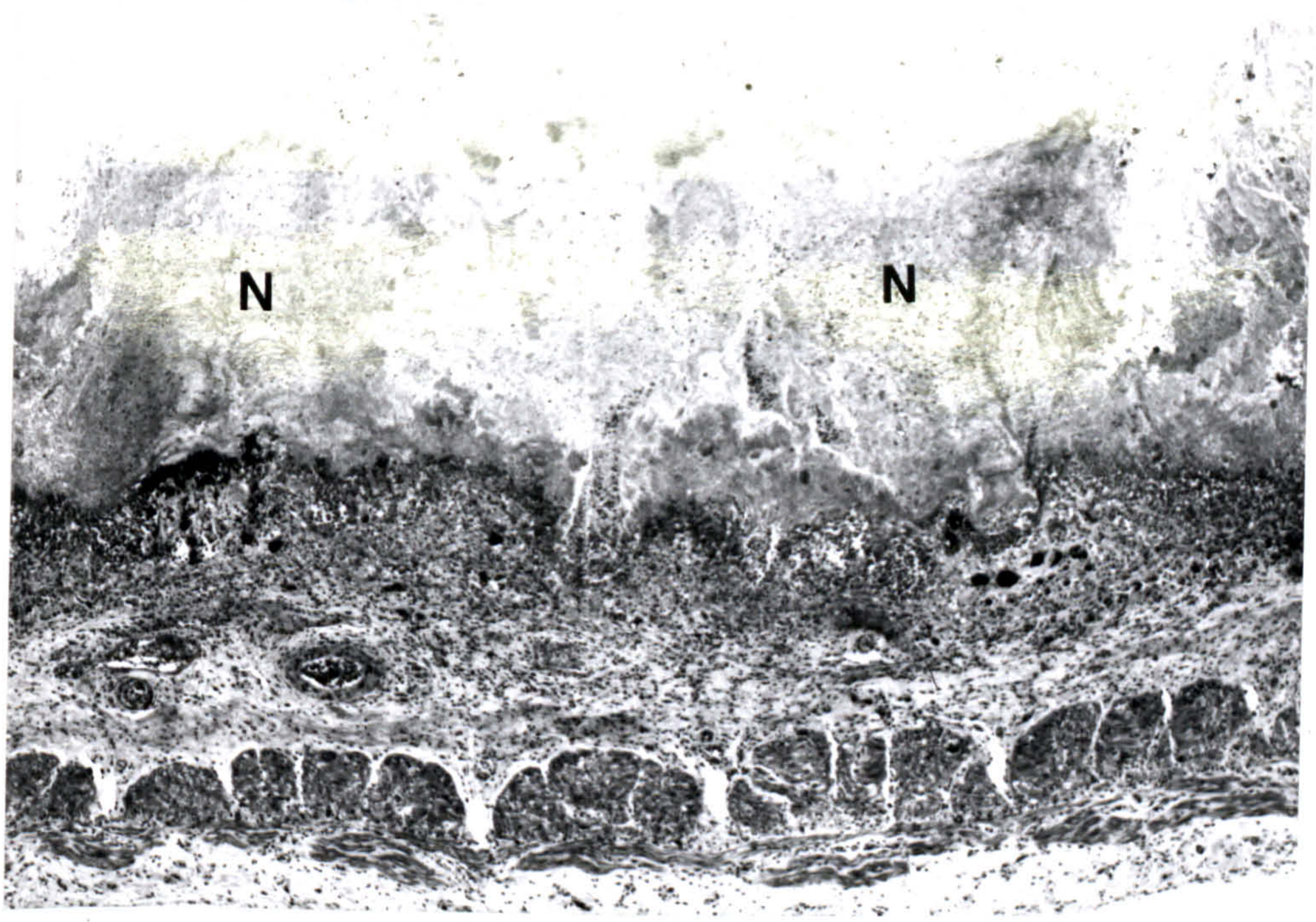


Fig. 21: Histological section of the small intestinal mucosa of cow 83555 from which F. necrophorum was isolated.

Note the massive necrosis of the lamina propria(N).

H & E X 35.

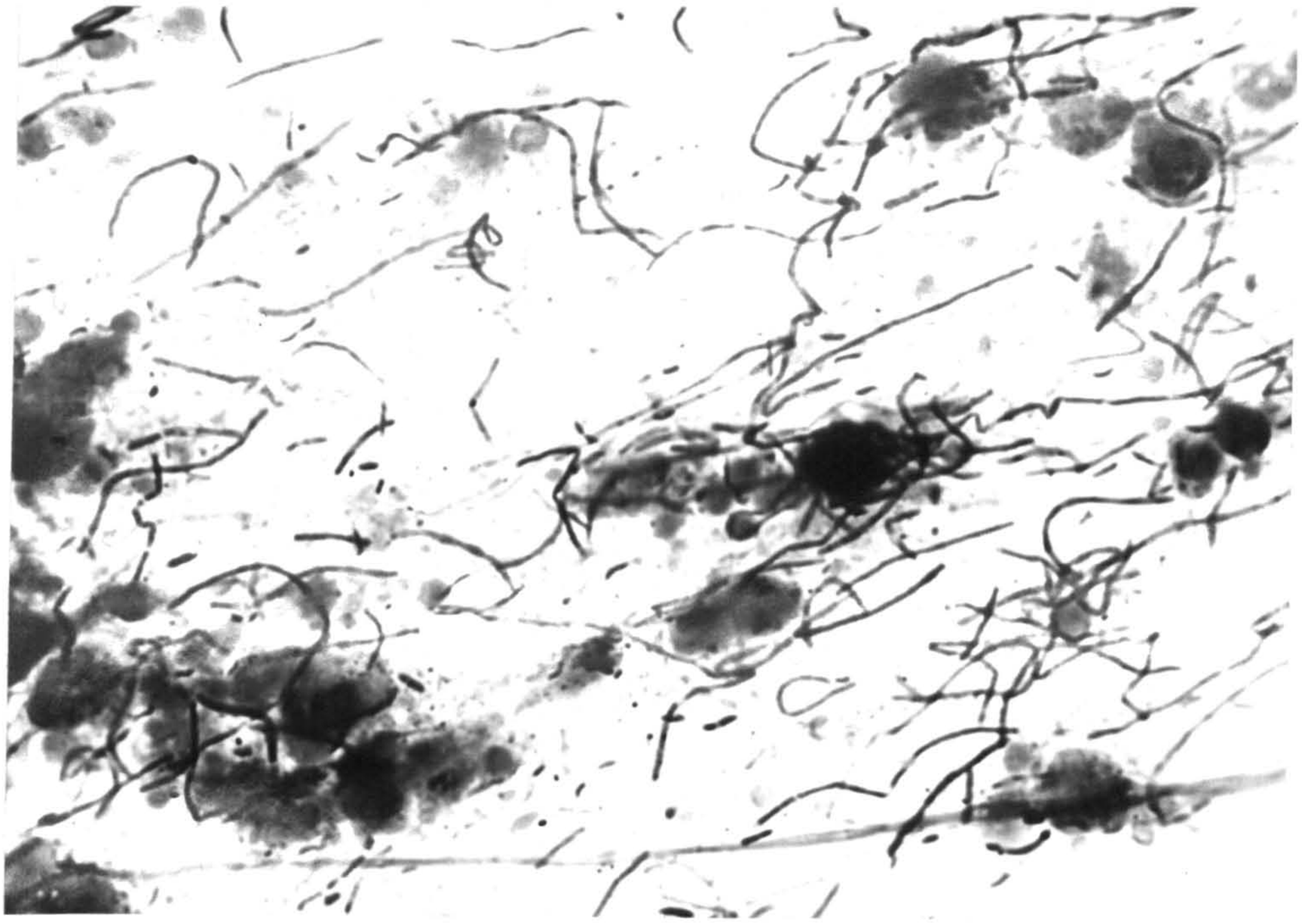


Fig. 22: Smear of the small intestinal mucosa of cow 83555 at a site from which F. necrophorum was isolated.

Note the long, filamentous beaded cells of F. necrophorum. Other bacteria were also isolated from this site (see Appendix 1).

Gram X 1200.

Gross changes recorded at the sites from which β-haemolytic E. coli was isolated included severe local congestion of the mucosa of small intestine with excess clear mucus and the presence of mucoid contents. Similar changes were also seen in the caeca of both animals. The mesenteric lymph nodes were enlarged and pale. The sites from which E. coli was isolated in the survey and its relative abundance are summarised in Table 10.

In cases 52R and 53R the lesions seen consisted of some stunting of the villi of the small intestine, capillary dilatation and presence of large numbers of mononuclear cells mainly lymphocytes, and some plasma cells in the lamina propria. The epithelial cells seen were lowered and cuboidal in shape. Slight oedema was seen in the lamina propria of the small intestine. Similar changes were also noted in the large intestine. There was a marked increase in the mitotic figures in the mesenteric lymph nodes which also contained a few neutrophils.

Gram-negative rods were seen in smears from the sites from which E. coli was isolated in all cases.

Enterobacter aerogenes

Enterobacter aerogenes was isolated from the inflamed and ulcerated mucosa of the abomasum, small and large intestinal tract of adult 75205 in moderate numbers. C. fecalis and the unidentified fusobacterium were isolated from this animal.

This animal had diarrhoea at the time of slaughter.

Pseudomonas aeruginosa

Ps. aeruginosa was isolated from the gastrointestinal tract of three animals (numbers 75911, 75205, 75478) with diarrhoea and from one (75186) without diarrhoea. In case 78530, Ps. aeruginosa was isolated from the suppurative pneumonia and from the enlarged congested liver in scanty numbers of colonies but were not isolated from the gut.

No gross or microscopic changes attributable to Ps. aeruginosa infection alone were noted as it was only one of a large number of

Table 10.

Sites from which E. coli was isolated
in 57 cases and the relative abundance of colonies.

Site of isolation	Number of cases positive	Quantity of E. coli isolated	Case number
Abomasum	9	Scanty	22P, 31P, 34P, 37P 38P, 42P, 43P, 44P 46P
	1	Moderate	A
Small intestine	4	Moderate	83555 B, 83444, 76245
Abomasum and Small intestine	1	Profuse	34
Large intestine	7	Moderate	22, 31, 54 296, 75065 294, 74989
Abomasum, Small and Large intestine	2	Scanty	75799, 49
	16	Moderate	74331, 50, 291 75516, 75186, 75257, 75911, 76217, 76457, 76585, 24, 78530 79383, 79249, 79894, 83613
	3	Profuse	33, 36, 45
Small and Large intestine	11	Moderate	74381, 52R*, 11 75715, 74589, 3, 53R 10, 82773, 52R, AL
	5	Profuse	25, 29, 30, 53R*, 80958

* Beta-haemolytic E. coli

For key of amount of growth etc., see Table 2.

bacteria found in the lesions. The sites from which Ps. aeruginosa was isolated are shown in Table 11.

Table 11.

Sites from which Ps. aeruginosa was isolated in 5 cases and the relative abundance of colonies.

Site of isolation	Number of cases positive	Quantity of Ps. aeruginosa isolated	Case number
Abomasum	1	Moderate	75911
Abomasum and Small intestine	2	Moderate	.75205, 75186
Small and Large intestine	1	Profuse	75478
Lung and Liver	1	Scanty	78530

For key to amount of growth etc., see Table 2.

Aeromonas hydrophila

A. hydrophila was isolated from the gastrointestinal mucosa of of three of the 40 calves in the survey. Two animals (75258 and 75715) had diarrhoea but the third (75065) had normal faeces. A. hydrophila was isolated in moderate numbers from the abomasum and small intestine At all sites from which it was the major organism isolated there was congestion of the mucosa and in the small intestine, excess clear mucus.

The histological changes were those of mild inflammation.

Alcaligenes odorans

A. odorans was isolated in moderate numbers from the grossly normal small and large intestinal mucosa of adult 74381 with mucosal disease and from the small intestinal mucosa of calf 73948 with malignant catarrhal fever. The site of isolation in 73948 was thickened, congested and had excess surface mucus. Both animals had normal faeces at slaughter.

Mild inflammatory changes were seen in sections of the small intestine of 74381 and similar changes including slight oedema were present in sections of the large intestine in which coccidia were also seen. Case number 73948 was not examined histologically.

Acinetobacter lwoffii

A. lwoffii was isolated in profuse culture from the congested thickened and mucoid small intestine of calf 73948 described above.

Actinobacillus lignieresii

A moderate number of colonies of A. lignieresii were isolated from the ulcerated abomasal mucosa of adult 76217 with E. coli and Clostridium perfringens Type A.

The faeces of the animal were soft, the abomasal mucosa was hyperaemic and thickened with dilated glands with shedding of the epithelial cells and an inflammatory cell exudate. Localised oedema and a chronic ulcer were present in the lamina propria (Fig. 23).

Gram-negative coccobacilli and longer rods resembling Actinobacilli were seen in smears of the lesion.

Branhamella catarrhalis

B. catarrhalis was isolated from the gastrointestinal mucosa of three adults (74589, 75003, 79894) and three calves (75067, 75257, 294) at the sites shown in Table 12. In five cases the organism was isolated from grossly abnormal mucosa but in one case (294) from normal mucosa. The changes seen included congestion and ulceration especially

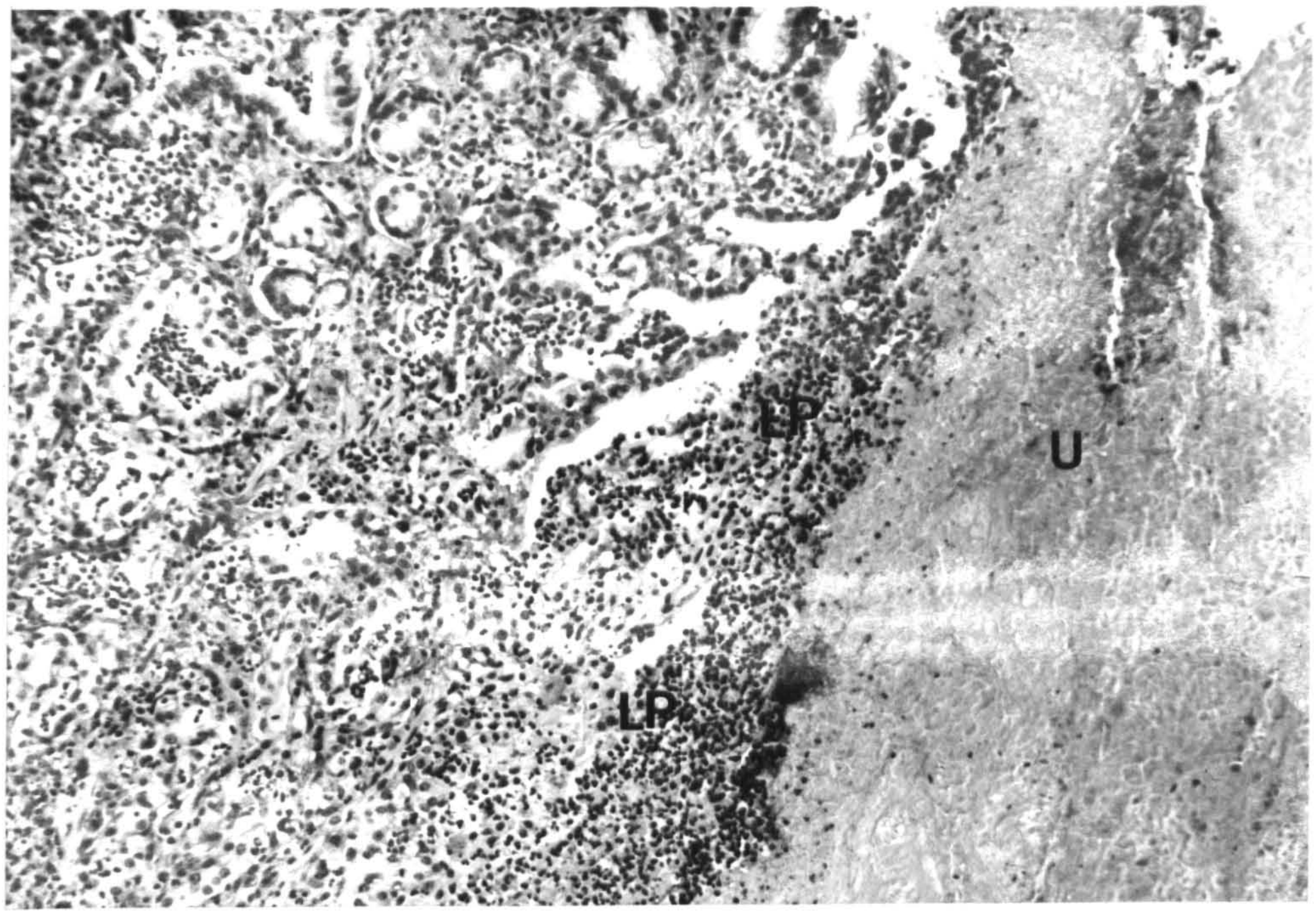


Fig. 23: Histological section of the ulcerated abomasal mucosa of cow 76217 at a site from which A. lignieresii was isolated.

Note ulcerated area (U) and the granulomatous reaction in the adjacent lamina propria (LP).

H & E X 110.

Table 12.

Sites from which Branhamella catarrhalis was isolated in 6 cases and the relative abundance of colonies.

Site of isolation	Number of cases positive	Quantity of Branhamella catarrhalis Isolated	Case number
Abomasum	2	Moderate	75067, 75257
Abomasum and Small intestine	2	Moderate	75003, 79894
Small intestine	1	Moderate	74589
Small and Large intestine	1	Moderate	294

For key to amount of growth etc., see Table 2.

in the abomasum. C. fecalis and Cl. perfringens Type A were also present in the lesion in 79894.

Inflammatory changes were present in histological sections of most sites from which the organism was isolated and it was seen with difficulty as a Gram-negative coccus in some smears made from the sites of isolation.

Moraxella non-liquefaciens

A few colonies of this small, Gram-negative plump rod were isolated from the congested and mucoid small intestinal mucosa of adult 76245. B. licheniformis and E. coli were also isolated from this site. The animal was suffering from diarrhoea of unknown cause.

Gram-negative rods were seen in smears of the lesion but

M. non-liquefaciens could not be identified by its morphology.

Pasteurella multocida

P. multocida was isolated in moderate numbers from the large intestinal mucosa of calf 75067 which had diarrhoea and pneumonia. Many other bacteria including Branhamella catarrhalis and Veillonella sp. were isolated from this site (see Appendix 1).

Veillonella species

Veillonellas were isolated in moderate numbers from areas of congestion of the small and large intestinal mucosa in calves 45, 75067 and 79383. Other agents were present in each case (see Appendix 1).

Staphylococcus epidermidis

S. epidermidis was isolated from the gastrointestinal mucosa of one adult (76585) and nine calves. The sites from which it was isolated and the relative abundance of colonies are summarised in Table 13. It was accompanied by other bacteria in each lesion (see Appendix 1) but could be identified in smears from which no other Gram-positive cocci were isolated as a large Gram-positive coccus occurring singly or in pairs.

Streptococcus species

Streptococci were isolated from six adults and five calves and 10 isolates were identified as S. bovis and one as S. zooepidemicus.

S. bovis

S. bovis was isolated from the gastrointestinal mucosa of six adults (83613, 75478, 75911, 79894, 78530 and 74331) and four calves (24, 50, 53R and 83444). Only one of these (calf 50) had normal faeces. In all cases the site of isolation was grossly abnormal, in most cases congested, sometimes severely.

The sites from which the organism was isolated and its relative abundance are summarised in Table 14.

Table 13.

Sites from which Staphylococcus epidermidis was isolated in 10 cases and the relative abundance of colonies.

Site of isolation	Number of cases positive	Quantity of Staph. epidermidis isolated	Case number
Abomasum	2	Scanty	75067, 291
	1	Moderate	80958
Abomasum and	1	Scanty	296
Small intestine	1	Moderate	34
Small and	2	Scanty	75258, 74989
Large intestine	1	Moderate	75065
Abomasum, Small	1	Scanty	76585
and Large intestine	1	Moderate	293

For key to amount of growth etc., see Table 2.

Microscopc changes were chiefly of an inflammatory nature without any specific features. Gram-positive streptococci were seen in smears made from the sites. The organism was accompanied by other bacteria in the lesions (see Appendex 1).

S. zooepidemicus

Moderate numbers of colonies of this organism were isolated from the congested abomasal and small intestinal mucosa of calf 75257 from which C.f. ss. jejuni was also recovered.

Table 14.

Site from which Streptococcus spp. was isolated in 11 cases and the relative abundance of colonies.

Site of isolation	Number of cases positive	Quantity of Streptococcus spp. isolated	Case number
Abomasum	3	Moderate (S. bovis)	75478, 78530 24
Small intestine	1	Moderate (S. bovis)	83444
Abomasum and	1	Scanty (S. zooepidemicus)	75257
Small intestine	1	Moderate (S. bovis)	75911
Abomasum, Small and Large intestine	4	Moderate (S. bovis)	50,79894, 74331, 83613
Small and Large intestine	1	Moderate (S. bovis)	53R

For key to amount of growth etc., see Table 2.

Aerococcus viridans

A. viridans was isolated from the mucosa of the abomasum, small and large intestinal tracts of five adults (A, 75516, 74381, 75205, 75186) and three calves (291, 293 3). The mucosa at the site of isolation was congested, oedematous and eroded in most cases. The sites from which the organism was isolated are summarised in Table 15. Other agents and bacteria were present with A. viridans in the lesions (see Appendix 1). The organism was seen as a small Gram-positive coccus in the smears made from the sites of isolation.

Table 15.

Site from which Aerococcus viridans was isolated in 8 cases and the relative abundance of colonies.

Site of isolation	Number of cases Positive	Quantity of Aerococcus viridans isolated	Case number
Abomasum	2	Moderate	A, 3
Abomasum, Small and Large intestine	2	Moderate	75516 74381
Small intestine	1	Scanty	75186
Large intestine	3	Moderate	291 75205, 293

For key to amount of growth etc., see Table 2.

Corynebacterium species

Corynebacteria were isolated from the gastrointestinal mucosa of three adults and four calves. Five isolates were non-fermentative and were identified presumptively as C. bovis and two were identified as C. pyogenes.

C. bovis

C. bovis was isolated from the sites shown in Table 16 in three adults (75003, 75205, 75799) and two calves (75067, 75258). The sites of isolation were either inflamed or normal (75003) and the organism was present with other bacteria (see Appendix 1). No specific histological lesions were noted.

Table 16.

Sites from which C. bovis was isolated in 5 cases and the relative abundance of colonies.

Site of isolation	Number of cases positive	Quantity of C. bovis isolated	Case number
Abomasum	2	Scanty	75258 75003
Abomasum and Small intestine	1	Scanty	75205
Abomasum, Small and Large intestine	1	Moderate	75067
Small intestine and Large intestine	1	Moderate	75799

For key to amount of growth etc., see Table 2.

C. pyogenes

C. pyogenes was isolated in moderate numbers from similar lesions in the abomasum, small intestine and large intestine in calf 75715 with other bacteria and in small numbers from the normal large intestinal mucosa of calf 75257.

Gram-positive coccobacilli and short rods resembling corynebacteria were seen in smears made from the sites of isolation.

Peptostreptococcus productus

P. productus was isolated in small numbers from congested and oedematous abomasal mucosa of adult 75186 and from the congested abomasal mucosa of calf 291. Many other bacteria were present in the

lesions (see Appendix 1).

Small Gram-positive cocci were seen in Gram-stained smears from the isolation site.

Eubacterium aerofaciens

E. aerofaciens was isolated in small numbers from the congested small and large intestinal mucosa of adult 75911 with many other bacteria (see Appendix 1).

Actinomyces bovis

A. bovis was isolated in small numbers from the large intestinal mucosa of adults AL and 75003. Both animals had Johnes disease with characteristic lesions. A. bovis could not be distinguished in smears made from the site of isolation.

Mycobacterium paratuberculosis

Acid-fast coccobacilli and rods with the morphology of M. paratuberculosis were demonstrated in smears made from the mucosal epithelium and in histological sections of intestinal mucosa in three adults (AL, 75003 and 75799). Typical lesions of Johnes disease were present in all three animals.

Results of the indirect fluorescent antibody tests using sera from the cases and bacteria isolated from lesions.

The results of this survey were completely destroyed and cannot be given in detail. Antibody to Cl. perfringens Type A, E. coli, Bacillus licheniformis and Branhamella catarrhalis was demonstrated.

DISCUSSION

The results of the survey showed that a wide variety of bacteria could be isolated from both normal mucosa and from lesions. More than one species of bacterium was often present in each site sampled. In addition other agents such as nematodes, coccidia or viruses were identified in the lesions or known to have been present in the animals concerned. In cases when agents other than bacteria had not been demonstrated it is possible that they might have been present in an adjacent piece of tissue or have been present at a time prior to the time of examination.

These factors made the interpretation of the results difficult. In some cases the bacteria isolated were known to cause enteric lesions in cattle e.g. Actinobacillus lignieresii and Campylobacter fetus subsp. jejuni but in other cases they had been considered to be normal inhabitants of the alimentary tract e.g. Bacteroides fragilis. Some of the bacteria have been described as causes of enteric disease in other species and may, by inference, be important in cattle. It was, however, possible to associate certain bacteria with particular types of lesion and the significance of these results is discussed below. An attempt had been made to incriminate bacteria from the lesions in systemic invasion by the use of indirect immunofluorescence using sera taken at slaughter on bacteria isolated from lesions but the results of these studies were destroyed.

Detailed consideration of the significance of individual bacterial species.

Salmonella spp.

It is of interest that no salmonellae were isolated from the 64 animals included in this survey, particularly in view of the clinical diagnosis of salmonellosis provided for many of the cases (see Appendix 1).

Campylobacters

Campylobacters were isolated from the intestinal mucosa of 28 of the 64 cattle examined. Twenty one of the isolates were obtained

from 41 calves aged eight months or less, and seven were isolated from adult cattle aged one year or more. It is possible that more isolates could have been obtained if the selective medium described by Skirrow (1979a) had been available earlier in the study. Gram-negative curved rods or spirals were not seen in smears made from all the sites from which campylobacters were isolated so the isolation methods used may have been a more sensitive indicator of the presence of campylobacters than direct examination.

Three species of campylobacter were distinguished by the criteria given in Chapter 2 and some isolates remained unidentified.

Eighteen isolates were identified as C.f. ss. jejuni which is incriminated in enteric disease in a number of species. C.f. ss. jejuni was isolated from lesions of the enteric tracts of cattle in this study in the absence of other obvious causes. Lesions in cases 75257, 75258 resembled those described by Jones et al. (1931) in experimental "Vibrio jejuni" infections. Gross changes associated with the presence of C.f. ss. jejuni included congestion, ulceration, acute inflammation and oedema of the small intestinal mucosa stunting of the villi in the small intestine and enlarged mesenteric lymph nodes. Microscopic changes included stunting of the villi, capillary dilatation, and the presence of large numbers of plasma cells, other mononuclear cells and eosinophils within the mucosa. In sections from the ileum (Fig. 4) and colon, cell debris or inflammatory cells were present in some crypts. Disruption of, and changes in, the lumenal epithelium of the large intestine were seen.

It seems likely, therefore that the C.f. ss. jejuni isolates were responsible for lesions of this type found in the survey.

Similar lesions were found at sites from which other campylobacters were isolated and this finding made the identification of the isolate described as "Vibrio jejuni" by Jones and Little (1931a and b) Jones et al., 1932, as the present-day C.f. ss. jejuni less certain. This uncertainty was contributed to by the isolation of more than one cam;ylobacter species from a single lesion (C. fecalis and C.f. ss. jejuni from the small intestine of 83444) or from different regions of

the gut in the same animal (C.f. ss. jejuni and C.f. ss. intestinalis from the small and large intestine of 75258) respectively. The difference in colonial morphology between C.f. ss. jejuni or intestinalis and C. fecalis made their separation at one site possible but, using the techniques of this survey, the former, with indistinguishable colonial morphology may have been present together in some lesions.

These results indicated that campylobacters were common in enteric lesions in cattle but left unknown the significance of C.f. ss. intestinalis and C. fecalis which had previously been considered not to be enteric pathogens (El Azhary, 1968 and Firehammer, 1965). The findings also made uncertain the identification of the "Vibrio jejuni" of Jones and his coworkers with the present-day C.f. ss. jejuni.

Clostridium perfringens Type A.

None of the isolates of Cl. perfringens obtained in this survey produced toxins lethal for mice under the conditions recommended for strain identification by the Anaerobe Laboratory Manual. They were therefore identified as Clostridium perfringens Type A. The tests used (mouse inoculation) would not have detected the production of entero - toxins by these strains.

Cl. perfringens Type A was isolated from the gastrointestinal mucosa of 30 cattle in the survey. This finding and the lesions found are in agreement with the results reviewed in Chapter 1, p.13) especially with those of Vance (1967) who obtained 201 isolates of Cl. perfringens Type A from the enteric tracts of beef cattle in a similar survey. Enterotoxic strains of Cl. perfringens Type A have been shown to cause disease including intestinal fluid accumulation in calves following intraduodenal inoculation (Niilo and Dorward, 1971).

The lesions associated by these authors with Cl. perfringens Type A and its toxin (Niilo, 1973) are principally those of massive congestion and hyperaemia of the mucosa of the small intestine. Cl. perfringens Type A was isolated from such lesions in this survey particularly in animals 76457 and 76585. Marked capillary dilatation and disruption of the surface epithelium appeared to be features of

lesions associated with Cl. perfringens Type A in this survey. In many of the animals from which it was isolated diarrhoea had been recorded.

These results in conjunction with the literature on the subject suggest that Cl. perfringens Type A may be a widespread cause of, or contributor to enteric disease in cattle as it is in some other species. Confirmation of this view could be obtained by the oral infection of susceptible cattle.

Clostridium sordellii

Cl. sordellii was isolated from lesions in two animals which died. In both cases faecal changes were present prior to death and in one animal haemorrhagic diarrhoea was seen. Gross changes were seen which were distinct from those seen in lesions from which Cl. perfringens Type A or campylobacters were isolated alone. Oedema and thickening of the small intestinal wall with necrotic material or blood on the surface of the mucosa and massive capillary dilatation, denudation of the lumenal epithelium and superficial necrosis were features of the lesions. Colonies of the organism were distinctive and its appearance in smears was also distinctive and it is unlikely to have been overlooked in other cases in this survey.

Brooks et al. (1956) found Cl. sordellii in enteric lesions in cases similar to those described above but failed to produce disease in experimental calves by the inoculation of pure culture. Their experimental findings suggest that the organism is not a primary pathogen although its presence in such distinctive lesions and its close relationship to Cl. difficile, a cause of pseudomembranous colitis in man, could indicate otherwise.

Clostridium bifermentans

Cl. bifermentans was isolated from the gastrointestinal mucosa in one case together with the unidentified clostridium and E. coli. No other enteric pathogens were isolated and it is quite possible that the organisms isolated were the cause of the lesion seen. It is likely

that the lesions seen were associated with the findings but some histological changes seen might be due to post mortem changes. Cl. bifermentans has been found in soil, fresh water and marine sediments, and faeces (Smith and Hobbs, 1974) but its significance as an enteric pathogen is not known.

Clostridium butyricum

Cl. butyricum was isolated from the congested and oedematous mucosa of the abomasum and small intestine of one animal. It was isolated together with C. fecalis, C.f. ss. jejuni and other bacteria.

The significance of the isolation of Cl. butyricum from the lesion is unknown especially as Wilson and Miles (1975) reported that Cl. butyricum is not pathogenic and is commonly found in rumen contents.

Bacillus licheniformis

Twenty isolates of B. licheniformis were obtained both from congested, oedematous and ulcerated mucosa and from normal mucosa. Its presence with other bacteria (especially campylobacters and clostridia), its widespread distribution in soil and food and the absence of its association with enteric disease in any species suggest that it is not a pathogen.

Evidence to the contrary comes from its isolation from pathological material such as bovine aborted foetuses (Ryan, 1970) and its presence in this survey in abomasal and other lesions in which epithelial disruption and congestion were a feature.

Similar considerations apply to Bacillus coagulans.

Bacteroides species

These were never isolated in pure culture. When the numbers normally present in the gastrointestinal contents are considered it is surprising how few isolates of these organisms were made. B. fragilis was isolated from lesions in all cases but the lesions were not characteristic. B. vulgatus was isolated from similar lesions in mixed culture as were B. melaninogenicus and B. oralis.

These species of Bacteroides have been isolated from enteric lesions in other species such as the pig with swine dysentery (Meyer et al., 1975) (B. melaninogenicus and B. vulgatus) and from the gingival crevice and infections of the oral cavity and respiratory tract B. melaninogenicus (Biberstein et al., 1968) and case 78530 in this survey) and B. oralis (Loesche et al., 1964).

The significance of these bacteria in the aetiology of the lesions in which they were found is debatable. Their presence in the gut as normal inhabitants suggests that they may be incidental although the association of all four organisms with ulcerative lesions of the gut and gingival crevice in other species suggest that they may contribute, with other bacteria to the changes seen.

Fusobacterium necrophorum

F. necrophorum was isolated from necrotic enteritis in one case (83555) together with other bacteria such as Cl. perfringens Type A, C.f. ss. intestinalis and E. coli (Appendix 1). It has already been noted (Chapter 1) that F. necrophorum is widely distributed and normally occurs as a commensal in a variety of animal species such as cattle (Kanoe et al., 1975). Under special circumstances this organism becomes associated with the development of necrotic lesions such as those seen in case 83555. It is possible that the organism isolated from that case was the cause of the lesions seen, but the evidence for its pathogenicity in the gut remains unclear. F. necrophorum could readily be demonstrated in direct smears (Fig. 22) in this case and the specific necrosis seen here (Fig. 21) probably resulted from its presence. An unidentified Fusiform organism was isolated from the site of abomasitis in case 75205 with other bacteria. Its significance is unknown.

Escherichia coli

E. coli was isolated from the mucosa of 56 of the 64 animals in the survey. Two of these isolates were β-haemolytic. As none of the isolates was typed or tested for pathogenic determinants their significance in the lesions in which they were found cannot be discussed further. It may, however, be assumed that they initiated or contributed

to some of the lesions in which they were found, particularly when isolated from the small intestinal mucosa of diarrhoeic calves.

Enterobacter aerogenes

This organism has been reported from the faeces of man and animals (Hormaeche and Edwards 1960). Its presence with other bacteria in ulcerative lesions in one animal in this series is of unknown significance.

Pseudomonas aeruginosa

Ps. aeruginosa was recovered from the lesions of the gastro-intestinal tract of four cattle in this survey. Similar findings have been reported by Matthews and Fitzsimmons (1964). It is possible that the Pseudomonas isolated in the survey was involved in the lesions seen although other bacteria were present in each case (see Appendix 1).

Aeromonas hydrophila

A. hydrophila was isolated from the mucosa of the abomasum and small intestine of three calves in this study. Two isolates were obtained together with campylobacter species and other bacteria. The third isolate of Aeromonas hydrophila was obtained from the small intestinal mucosa of animal 75065, in which no other enteric pathogens were demonstrated and it is possible that the organisms isolated were the cause of the lesion seen.

Sanyal et al. (1975) have demonstrated that some strains of A. hydrophila from human diarrhoea produce the accumulation of fluid in rabbit gut loops. Annapurna and Sanyal (1977) described histological changes such as mild infiltration of the mucosa with mononuclear cells and a reduction in the number of goblet cells in the intestinal mucosa of isolated gut loops. It is possible that the excess clear mucus noted in the small intestine of calf 75065 reflects this type of change and that the organism may have contributed to, or initiated, the lesions in which it was found. The faeces of this animal were, however, normal.

Alcaligenes odorans

A. odorans was isolated from two cases, one was from congested mucosa of small intestine and the second was from grossly normal small and large intestine. It was found in human faeces by Pedersen et al. (1970) who suggested that it might be an opportunistic pathogen.

The isolation of this bacterium from the lesions found in the survey suggests that it might contribute to them in some way.

Acinetobacter lwoffii

This organism was present only in small numbers in one case. Pedersen et al. (1970) recorded as part of the normal flora of man but said that it could be an opportunist pathogen of superficial wounds.

Actinobacillus lignieresii

The isolation of this organism from an ulcerative abomasal lesion in which granulomata were seen in histological sections (Fig. 23) suggests that it was involved in the lesion from which it was isolated as such granulomata are a feature of infection with A. lignieresii.

Branhamella catarrhalis

This organism was isolated from both congested and ulcerated mucosa and from normal mucosa in six animals. Three of these (see Appendix 1, numbers 75067, 75257 and 294) presented with pneumonia. In view of its association with other organisms in inflammatory change in mucous membranes elsewhere in the body (Catlin, 1970) it is possible that its presence in intestinal lesions is associated with concurrent pneumonia or it may be involved in the production and maintenance of intestinal lesions directly. The true distribution of the organism in intestinal lesions is difficult to establish because of the small size of both the colonies and the bacterial cells themselves.

Moraxella non-liquefaciens

The finding of this organism in small numbers in a single case is of unknown significance. In man (Pedersen et al., 1970) this organism may be a cause of lesions in the respiratory tract.

It is unlikely to have been involved in the lesions in which it was found in this survey.

Pasteruella multocida

This organism is, like B. catarrhalis and M. non-liquefaciens associated with the upper respiratory tract and its isolation together with the former from the large intestinal mucosa of a calf with pneumonia may indicate colonisation of enteric lesions by respiratory tract pathogens passing through the gut.

Veillonella species

These bacteria are common inhabitants of the gastrointestinal tract and their isolation from lesions in which campylobacters and clostridia were also present merely emphasises the difference between the microbial flora of enteric mucosal lesions and that of the lumen. Their presence in the lesions seems unlikely to be connected with their initiation.

Staphylococcus epidermidis

This organism is not usually considered pathogenic and in the five cases from which it was isolated, pneumonia was the presenting sign (see Appendix 1 and appropriate section in the results above). It is possible that its presence in lesions represents opportunist invasion or is connected with respiratory infection which may have formed the source of the organism. Amstutz (1965) and Smith (1965) both recorded the presence of staphylococci in diarrhoeic calves.

Streptococcus bovis

The isolation of this organism from lesions in 10 animals may indicate that it contributed to the lesions found. It was, however, accompanied by other bacteria. It is, however, commonly found in the faeces of calves and its significance in these lesions is not clear.

Streptococcus zooepidemicus

The presence of this organism in lesions of the abomasum and small intestine in a calf with pneumonia is also of uncertain significance.

Aerococcus viridans

A.viridans was isolated from gastrointestinal mucosa of eight of the 64 cattle. It was isolated together with other bacteria. A. viridans has been described in the literature as an inhabitant of the gastrointestinal and respiratory tracts (Williams et al., 1953) and has been isolated from infections of the urinary tract and from endocarditis in man (Evans, 1974). Moreover, Collins and Lyne (1976) described A. viridans as a common saprophyte. It is therefore difficult to interpret the role of these bacteria in lesions of the gastrointestinal tract.

Corynebacterium bovis

As no specific features were noted in the lesions in which C. bovis occurred in five animals, their significance in the lesions was not clear.

Corynebacterium pyogenes

Only two cases yielded this organism. Both had pneumonia and this organism is a frequent finding in pneumonic lesions. Its significance in the initiation and maintenance of enteric lesions is unknown.

Peptostreptococcus productus

Peptostreptococcus productus was isolated from the abomasal mucosa of two animals in association with other bacteria. Smith and Holdeman (1968) and Rogosa (1974) reported the isolation of P. productus from the intestinal tract, urine, brain abscesses, pelvic abscesses, blood and lung in man. Its involvement with the enteric lesions present in cattle remains unclear.

Eubacterium aerofaciens

E. aerofaciens was isolated with other bacteria from lesions in one animal only. Its significance in this context is therefore not clear although it has been isolated from a number of sources including the intestines of man and animals by Holdeman and Moore (1974) who also

obtained it from infections.

Actinomyces bovis

The isolation of this organism in small numbers from the lesions of Johnes disease in only two animals in this survey suggests that it plays little part as a primary cause of enteric lesions in cattle.

Mycobacterium paratuberculosis

The identification of acid-fast bacteria in lesions of Johnes disease is not surprising. It is of interest that such lesions were only found in three of the 23 adult animals in the survey - an incidence of 13 per cent.

One feature of the Johnes disease lesion is that it was not colonised either by clostridia or by campylobacters in these three cases.

The results presented and discussed above suggested that all the campylobacters, Cl. perfringens Type A and Cl. sordellii, F. necrophorum, A. hydrophila, Actinobacillus lignieresii and M. paratuberculosis were all associated with lesions which had some distinctive features and might have initiated them or contributed to their maintenance. Many of the remaining species could not be assigned any definite role in the lesions in which they were found. One finding of interest was the presence in lesions of bacteria commonly isolated from respiratory tract lesions and their frequent presence in enteric lesions in animals with pneumonia. The results of the survey of sera from cases for antibody to the bacteria isolated gave additional evidence for the involvement of some of them in the lesions but cannot be discussed further.

The campylobacters and Cl. sordellii were selected for study which are described in Chapters 4, 5,6 and 7.

Chapter 4.

Experimental infections with C.f. ss. jejuni

Introduction

C.f. ss. jejuni was isolated from 18 animals in the survey described in Chapter 3. It had, as "Vibrio jejuni" been shown to cause a characteristic enteric syndrome in cattle by Jones and Little (1931a and b) ,Jones et al. (1932) and Jones et al. (1931). Their "Vibrio jejuni" could not be identified conclusively with the present day C.f. ss. jejuni and in the survey C.f. ss. jejuni, C.f. ss. intestinalis and C. fecalis were isolated from apparently identical lesions of the enteric tract.

In order to assess the importance of C.f. ss. jejuni as a pathogen, a series of three controlled experiments were carried out in ruminating animals (Experiments 1 and 2) and in milk fed calves. The organism used in these studies was an isolate of C.f. ss. jejuni obtained from enteric lesions in the small intestine of a calf with enteritis (calf number 45). It had been cloned passaged eight times at weekly intervals and then freeze-dried using the method described in Chapter 2. Cultures for inoculation were prepared after one passage from this freeze-dried source.

These three experiments are described in Part I of this chapter.

Further studies of the relationship between the C.f. ss. jejuni and the bovine host were undertaken and these are described in Part II of this chapter.

PART I.

The inoculation of calves with pure cultures of C.f. ss. jejuni

Experiment 1.

Objective: To determine the pathogenicity of an isolate of C.f. ss. jejuni for ruminating calves.

Materials and methods

Five 6-month old Ayrshire bullocks were purchased direct from the farm where they were reared, and were divided into two groups, one of three animals (111, 114 and 119, infected) and one of two animals (100 and 115, control). Details of housing and feeding are given in Chapter 2.

Faecal samples from each animal were examined during the holding period for the presence of salmonellae, β-haemolytic E. coli, campylobacters and other bacteria, nematode eggs and coccidia by the methods described in Chapter 2.

The infected group of three calves was inoculated once with 20 ml of inoculum prepared by the method described in Chapter 2. Approximately 5×10^{10} organisms/ml were present in this inoculum.

Animals were examined daily and their appearance, appetite, respiratory rate, rectal temperature and the consistency of their faeces were noted. Faecal samples were examined daily for the presence of campylobacters and other bacteria by the methods described in Chapter 2. Negatively- stained preparations of faeces from animals 111 and 114 were examined by electron microscopy for the presence of virus particles on day 6.

Serum samples were obtained from all animals at the beginning and end of the experiment and stored according to the methods described in Chapter 2 and examined for the presence of antibody to C.f. ss. jejuni. The period of observation lasted 10 days and the animals were killed on the 11th day post-inoculation.

Post-mortem examination, histological and bacteriological examinations were carried out on all five animals by the methods described in Chapter 2 but bacteriological examination of the gall bladder and spleen were not carried out in this study.

Results

Experiment 1.

Changes in faecal consistency were noted on the second day following inoculation in all three inoculated animals. Excess clear mucus was seen on formed motions and this was accompanied by fresh blood. The faeces later became soft and fluid, dark in colour and of an even consistency containing clots of mucus (Fig. 24) some of which contained blood. These changes persisted until the animals were killed 10 days after inoculation. No faecal changes were noted in the controls. Detailed records of faecal consistency are given in Table 17, and summarised in Fig. 33. The inoculated animals appeared depressed and developed nasal discharge and coughing during the period of observation. Their rectal temperatures rose to 39.4 - 39.8°C within three - six days of inoculation and in most cases remained higher than 38.9°C throughout the remainder of the period of observation. The control animals remained normal throughout the period of observation with rectal temperatures of 38.3 - 38.9°C. The changes in rectal temperature are shown in Fig. 25.

C.f. ss. jejuni was isolated from the faeces of all the inoculated animals from the second day after inoculation but was not isolated from every sample. It could still be isolated from the faeces of two of the three inoculated animals at the end of the experiment on day 10. Organisms with the morphology of C.f. ss. jejuni were seen in Gram-stained smears of the faeces of the inoculated animals. The organism could not be isolated from the faeces of the inoculated animals prior to infection and was never isolated from the faeces of the control animals. The detailed findings are shown in Table 17. No virus particles, nematode eggs or coccidial oocysts were demonstrated in the faeces of the animals in this experiment. Salmonella spp. and β-haemolytic E. coli were not isolated.

At post-mortem examination, the two control animals were found to be macroscopically normal, with the exception of a slight excess of clear mucus in the contents of the ileum of animal 115 and slight hyperaemia of the jejunal mucosa of animal 100. A number of changes

Fig. 24: Faeces of experimentally infected calf 111 one day before slaughter.

Note the stringy mucoid nature of the faeces.

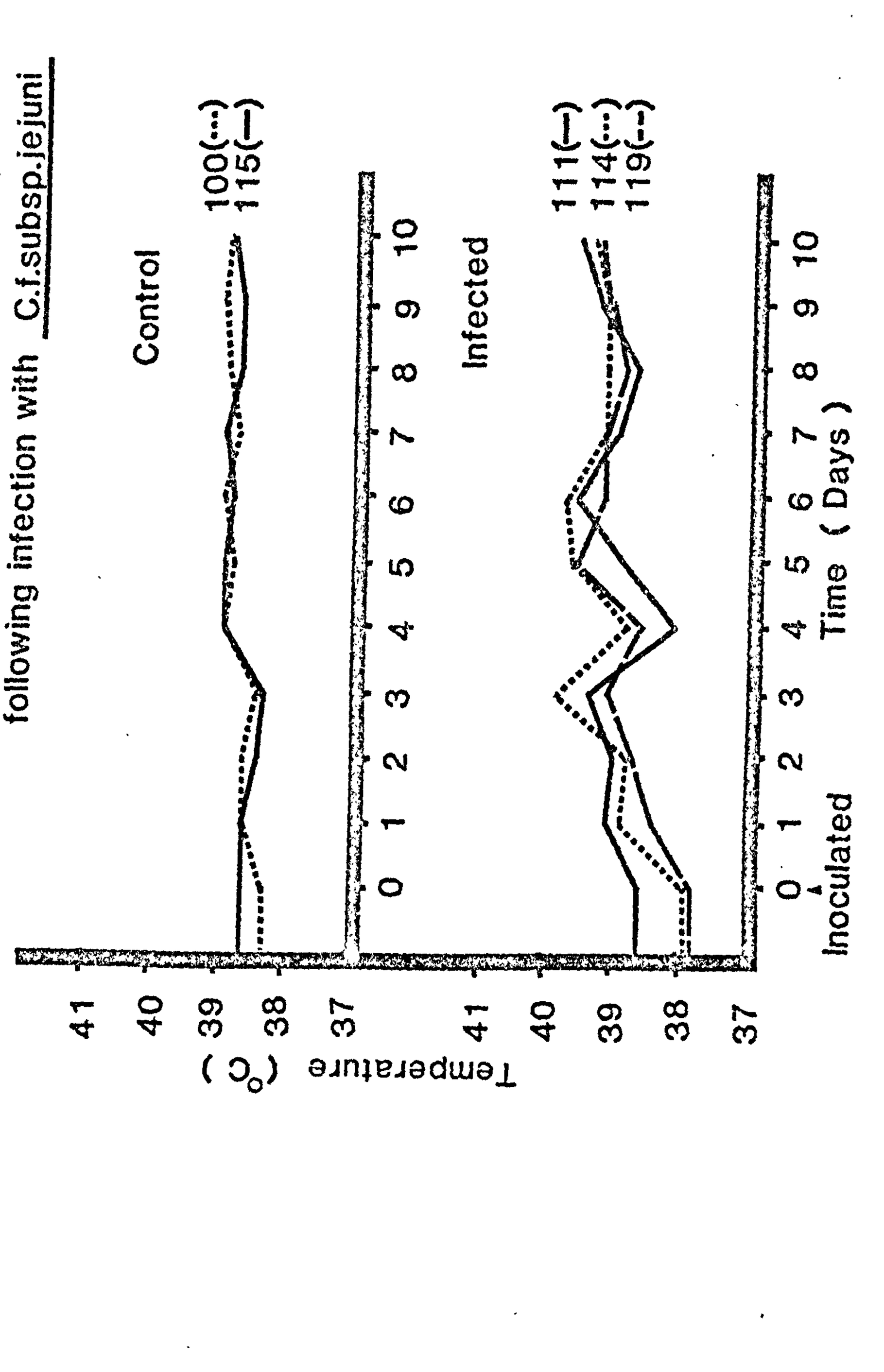


Fig (25)– Rectal Temperatures of Calves in Experiment following infection with C.f.subsp.jejuni

Table 17.

Changes in faecal consistency in Experiment 1 following the inoculation of calves with pure cultures of C.f. ss. jejuni and the isolation of the organism from their faeces

Calf No.	Inf-ected	Day of experiment 0	1	2	3	4	5	6	7	8	9	10	
111	+	F	F	FMB	SM	FM	SM	VSM	VSM	SM	SM	SM	K
		-	-	+	+	+	+	+	+	+	-	-	
114	+	F	F	FM	SM	FM	SM	SMB	SMB	SM	SM	SM	K
		-	-	+	+	+	-	+	+	-	+	+	
119	+	F	F	FM	FMB	FM	SM	SMB	SMB	SM	SM	VSMB	K
		-	-	+	+	+	-	+	+	+	-	+	
100	-	F	F	F	F	F	F	F	F	F	F	F	K
		-	-	-	-	-	-	-	-	-	-	-	
115	-	F	F	F	F	F	F	F	F	F	F	F	K
		-	-	-	-	-	-	-	-	-	-	-	

F = Firm faeces. S = Soft faeces. VS = Very soft faeces
M = Presence of mucus. B = Presence of blood.
+ = C.f. ss. jejuni isolated. - = No. C.f. ss. jejuni isolated.

were noted in the gastrointestinal tracts of the inoculated animals. Excess clear mucus was present in the jejunal contents which were dark in colour. The jejunal mucosa was slightly reddened with local raised areas (Fig.26). In all three animals the most obvious changes were seen in the ileum. The serosa of this part of the intestine appeared

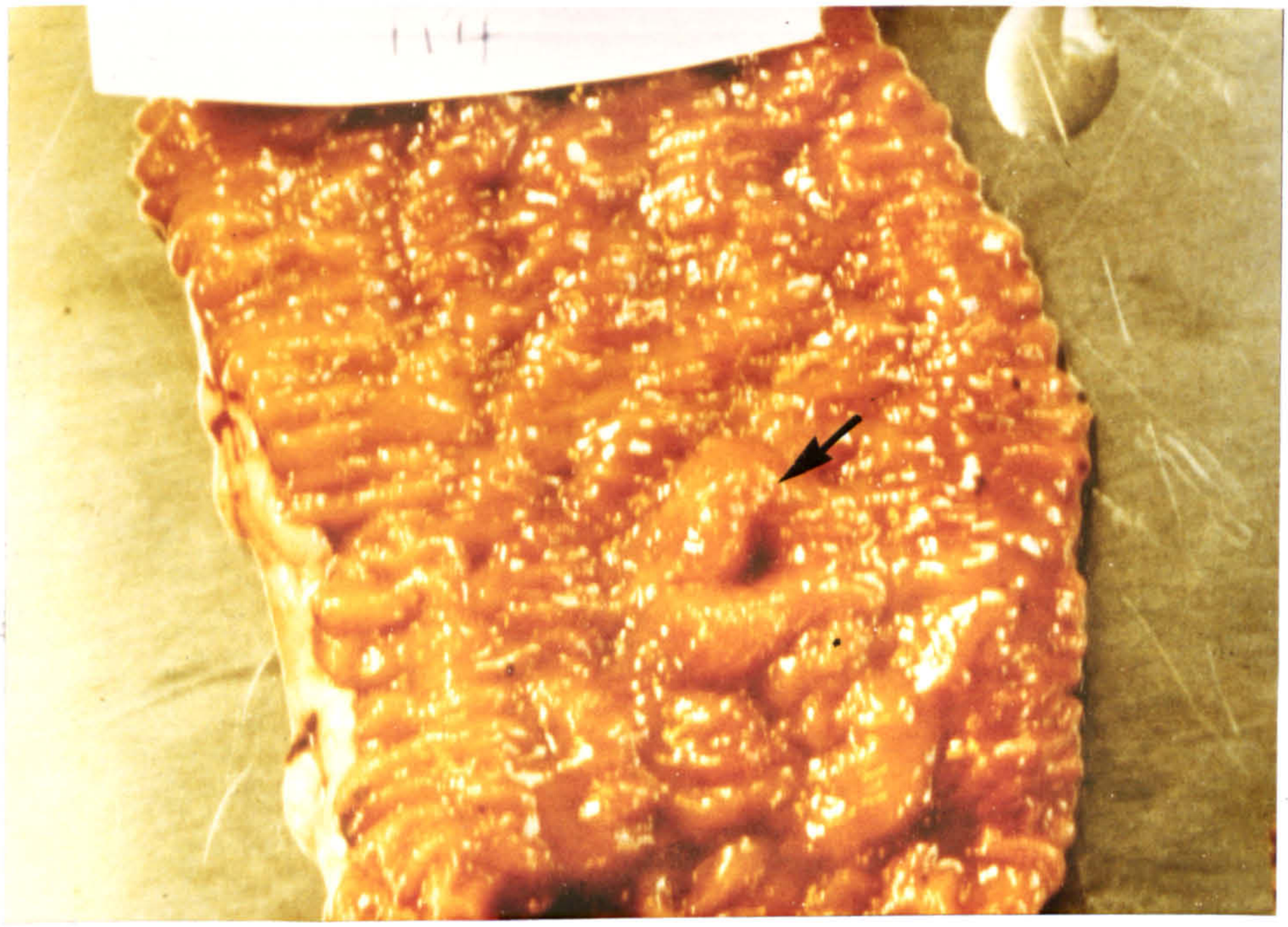


Fig. 26: Washed jejunal mucosa of experimentally infected calf 114, 10 days after infection.

Raised areas can be seen on the mucosal surface (arrow).

pale in colour and the organ was flaccid. The contents were dark in colour, fluid in consistency and contained obvious clear mucus. The wall of the ileum was thickened and fleshy (Fig. 27) and the mucosa appeared rough and granular with areas of reddening. The caecum and colon were apparently normal. The only gross changes noted were the presence of fluid mucoid contents in the proximal part of the large intestine. The mesenteric lymph nodes were enlarged, pale and oedematous when sectioned. The macroscopic appearance of the mucosa of the abomasum resembled that of the control animals.

Histological changes in the jejunal mucosa included loss of the lumenal epithelium, stunting and fusion of the villi and dilatation of the crypts. A few of the dilated crypts were filled with cellular debris. The lamina propria was thickened and contained many eosinophils, neutrophils, lymphocytes and plasma cells. Similar features were observed in the ileum in the submucosa of which lymphoid accumulations were particularly prominent. The muscular layers were increased in size. In many cases the epithelium of the crypts was cuboidal and some crypts were filled with polymorphonuclear leucocytes. Lymphoid accumulation in the ileal submucosa was noted in the controls and one crypt filled with cell debris was noted in sections of the jejunum from animal 100.

Slight dilatation of the capillaries and focal accumulation of plasma cells and macrophages were seen in caecal and colonic mucosa of the infected animals but these changes were not seen in the controls.

Changes seen in the mesenteric lymph nodes included oedema, slight congestion, the presence of active germinal centres and the presence of occasional polymorphonuclear leucocytes in the lymph nodes of infected animals. Few such changes were seen in those of the controls.

Colonies of C.f. ss. jejuni were isolated in large numbers from the mucosa of the ileum, caecum and colon in all the inoculated animals. (Table 18). No campylobacters were isolated from the liver and lung of the infected animals or from any organ of the controls. Gram-stained smears prepared from the mucosa of these organs contained curved rods with the morphology of C.f. ss. jejuni. None were seen in similar

Fig. 27: Ileum and its contents from experimentally infected calf 114, 10 days after infection.

Note the dark mucoid contents.

Table 18.

Sites from which C.f. ss. jejuni was isolated in experimental calves killed 11 days following infection with pure cultures of the organism in Experiment 1.

Site of isolation	Animal numbers				
	Infected			Control	
	111	114	119	100	115
Abomasum	-	-	-	-	-
Jejunum	-	-	-	-	-
Ileum	+	+	+	-	-
Caecum	+	+	+	-	-
Colon	+	+	+	-	-
Mesenteric L.N.	-	-	-	-	-
Liver	-	-	-	-	-
Lung	-	-	-	-	-

\+ = C.f. ss. jejuni isolated.
\- = No C.f. ss. jejuni isolated.

preparations made from the controls. The identity of the isolates was confirmed by the methods described in Chapter 2.

Agglutinating antibody to the inocular strain of C.f. ss. jejuni was present at titres of 1:640 or more in the sera of the infected animals at slaughter 11 days post-infection but could not be demonstrated in serum samples taken from the inoculated animals at the beginning of the experiment or in the sera from the control animals at slaughter. The results are shown in Table 19.

Table 19.

Levels of agglutinating antibody to the inocular strain of C.f. ss. jejuni in the sera of the animals of Experiment 1.

Animal Number	Infected	Titre present on Day 0	Day 17
111	+	0	1:1280
114	+	0	1:1280
119	+	0	1:640
100	-	0	0
115	-	0	0

Experiment 2.

Objective: To confirm the result of Experiment 1 using ruminating calves.

Materials and Methods

Six 6-month old bullocks of mixed breeds were purchased from a market. They were divided into two groups of three and numbered 8103, 8105, 8106 (inoculated) and 8101, 8104, 2674 (control) and housed separately as described above. The animals were monitored prior to infection, housed, fed, and observed by the methods described in Chapter 2 and in this chapter for Experiment 1. The inoculum was prepared by the same method as in Experiment 1 and also contained approximately 5.0 x 10^{10} organisms per ml. The period of observation lasted for 16 days post-infection and the animals were killed on the 17th day and examined post-mortem by the methods described in Chapter 2. Daily samples of unclotted blood were taken from all 3 infected animals from days 3-10 post-infection and cultured for campylobacters. The results of this study are reported in Study 1, Part II of this chapter.

Results

No salmonellae, β-haemolytic E. coli or campylobacters were isolated from the faeces of the experimental animals prior to infection and no coccidia were reported present. 50-150 strongyle eggs per gram were present in the faeces of animals 8101 and 2674.

Changes in faecal consistency began on the first day following inoculation and all three animals passed faeces of altered consistency by the third day after inoculation. The colour of the faeces varied from clay coloured to dark brown and resembled those noted in experiment 1 in consistency. Changes persisted for the entire experiment in one animal (8105). The faecal consistency in the other two animals had returned to normal by the 14th and 15th days post-infection although traces of excess mucus were still present in their faeces. The faeces of the three control animals remained normal in appearance and consistency throughout the experiment. The daily appearance of the faeces is given in Table 20 and summarised in Fig. 33.

Following infection, the animals of the infected group often appeared dull and all developed a nasal discharge and coughed from time to time. Coughing was also noted in the control group and one animal (8101) appeared depressed in the latter part of the experiment. The rectal temperatures of the inoculated group rose above 39.4°C by the fourth day after inoculation and in one case reached 41°C. Elevated rectal temperatures persisted in this group until day 13 but had dropped to normal by day 16. These changes in rectal temperature are shown in Fig. 28. No coccidial oocysts or nematode eggs were found in the faeces of infected animals during the experiment but small numbers (50 to 150 strongyle eggs per g) were present in the faeces of control animals 8101 and 2674.

C.f. ss. jejuni was isolated in profuse culture from the faeces of all the inoculated animals from the day following inoculation to the end of the experiment. Two or three colonies of C.f. ss. jejuni were isolated from the faeces of one control animal (8101) on day 9 and a similar scanty growth was obtained from the faeces of two control

Table 20.

Changes in faecal consistency in Experiment 2 following the inoculation of calves with pure cultures of C.f. ss. jejuni and the isolation of the organism from their faeces

Calf No.	Inf-ected	Day of experiment 0	1	2	3	4	5	6	7	8
8103	+	F	F	F	SM	SM	FMB	FMB	FMB	FMB
		-	+	+	+	+	+	+	+	+
8105	+	F	F	SM	SM	S	FM	VSM	SM	SM
		-	+	+	+	+	+	+	+	+
8106	+	F	S	FMB	SMB	DM	SM	SM	VSMB	FMB
		-	+	+	+	+	+	+	+	+
8101	-	F	F	F	F	F	F	F	F	F
		-	-	-	-	-	-	-	-	-
8104	-	F	F	F	F	F	F	F	F	F
		-	-	-	-	-	-	-	-	-
2674	-	F	F	F	F	F	F	F	F	F
		-	-	-	-	-	-	-	-	-

Calf No.	Inf-ected	9	10	11	12	13	14	15	16	
8103	+	FMB	FMB	DMB	SMB	FMB	F	FM	FM	K
		+	+	+	+	+	+	+	+	
8105	+	VSM	VSM	VSM	SM	SM	VSM	SMB	SM	K
		+	+	+	+	+	+	+	+	
8106	+	FM	FM	SM	F	SM	SM	F	F	K
		+	-	+	+	+	+	+	+	
8101	-	F	C	F	F	F	F	F	F	K
		-	-	-	-	+	+	-	-	
8104	-	F	F	F	F	F	F	F	F	K
		+	-	-	-	+	+	-	-	
2674	-	F	F	F	F	F	F	F	F	K
		-	-	-	-	-	-	-	-	

F = Firm faeces. S = Soft faeces. VS = Very soft faeces.
M = Presence of mucus. B = Presence of blood. D = Diarrhoea
C = Constipated faeces. + = C.f. ss. jejuni isolated.
- = No C.f. ss. jejuni isolated. K = Killed.

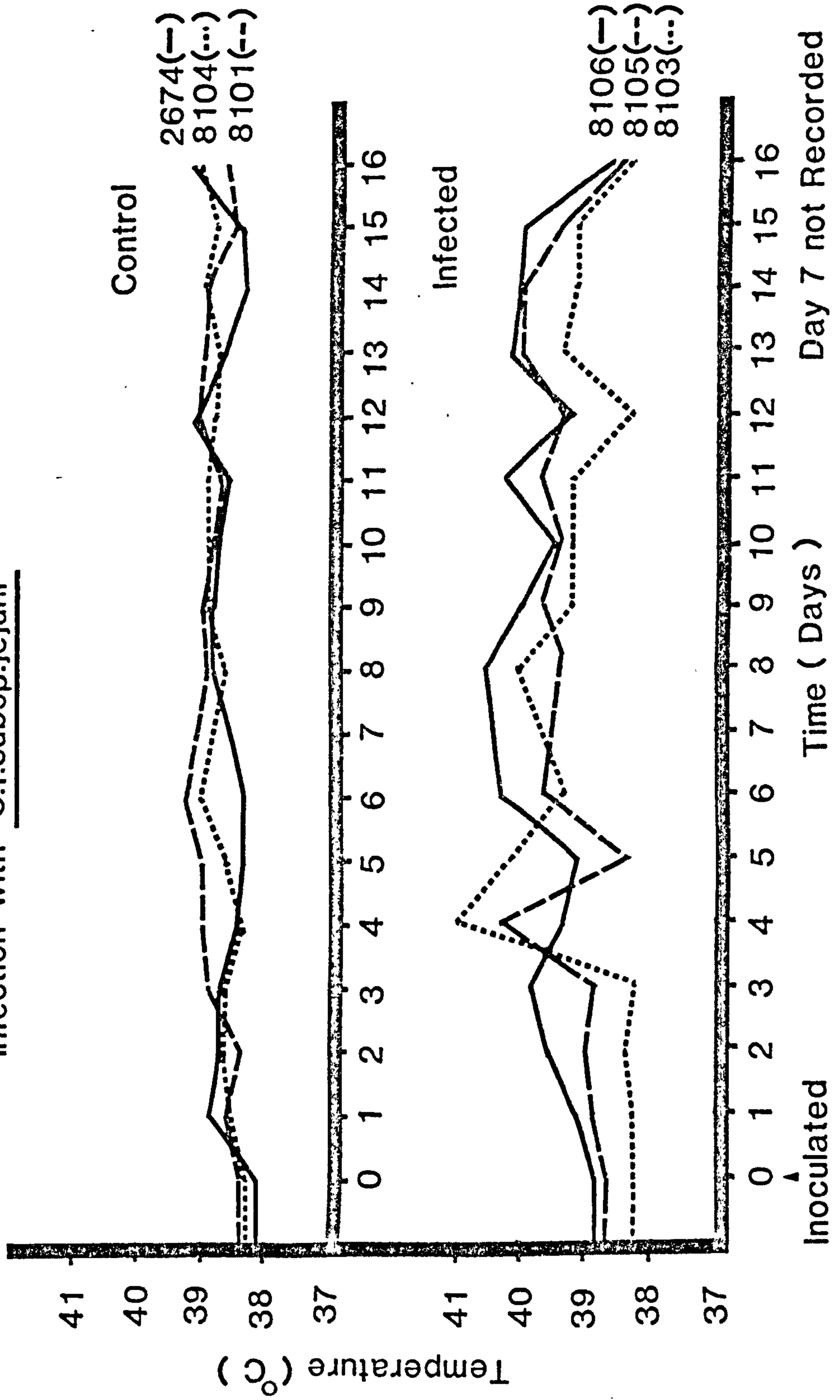


Fig (28)– Rectal Temperatures of Calves in Experiment 2 following infection with C.f.subsp.jejuni

animals (8101 and 8104) on days 13 and 14. These isolates had the same antibiotic sensitivity pattern as that of the inocular strain. These results are shown in Table 20.

Organisms with the morphology of C.f. ss. jejuni could be seen in Gram-stained smears of the faeces of the infected animals as in experiment 1 and were also seen in those of the control animals on the days on which they were isolated.

At post-mortem examination the changes found in the inoculated animals resembled those seen in the first experiment. The wall of the ileum was thickened and the serosal surface was pale and reticulated in appearance. The mesenteric lymph nodes were pale and enlarged. The contents and intestinal mucosa of all three infected animals resembled those of the infected animals in Experiment 1. Localised pneumonic areas were present in individual lung lobes in all three animals. Rumenal ulceration, rumenitis and hyperaemia and thickening of the small intestinal mucosa were found in control animal 8101 and slight thickening of the terminal ileum was noted in animal 8104. The gastrointestinal tract of the remaining control animal was normal in appearance. Pneumonic lesions were present in all three with localised abscesses in the lungs of two animals.

Microscopic changes resembling those described in Experiment 1 were present in the jejunum and ileum (Fig. 29), colon and caecum of each infected animal. Changes including some cellular infiltration in the jejunal and ileal mucosa and oedema of the abomasal wall were noted in the control animal (8101) with rumenitis and coccidial gametocytes seen in the ileal mucosa of this animal. No histological abnormalities were noted in the mucosa of the remaining control animals.

C.f. ss. jejuni colonies were isolated in large numbers from the ileum, caecum, colon in all the inoculated animals. Smaller numbers of colonies were isolated from the jejunum of one animal (8105), from the abomasum of two animals (8103, 8105) and from the gall bladders of all three. No campylobacters could be isolated from the liver or mesenteric lymph nodes.

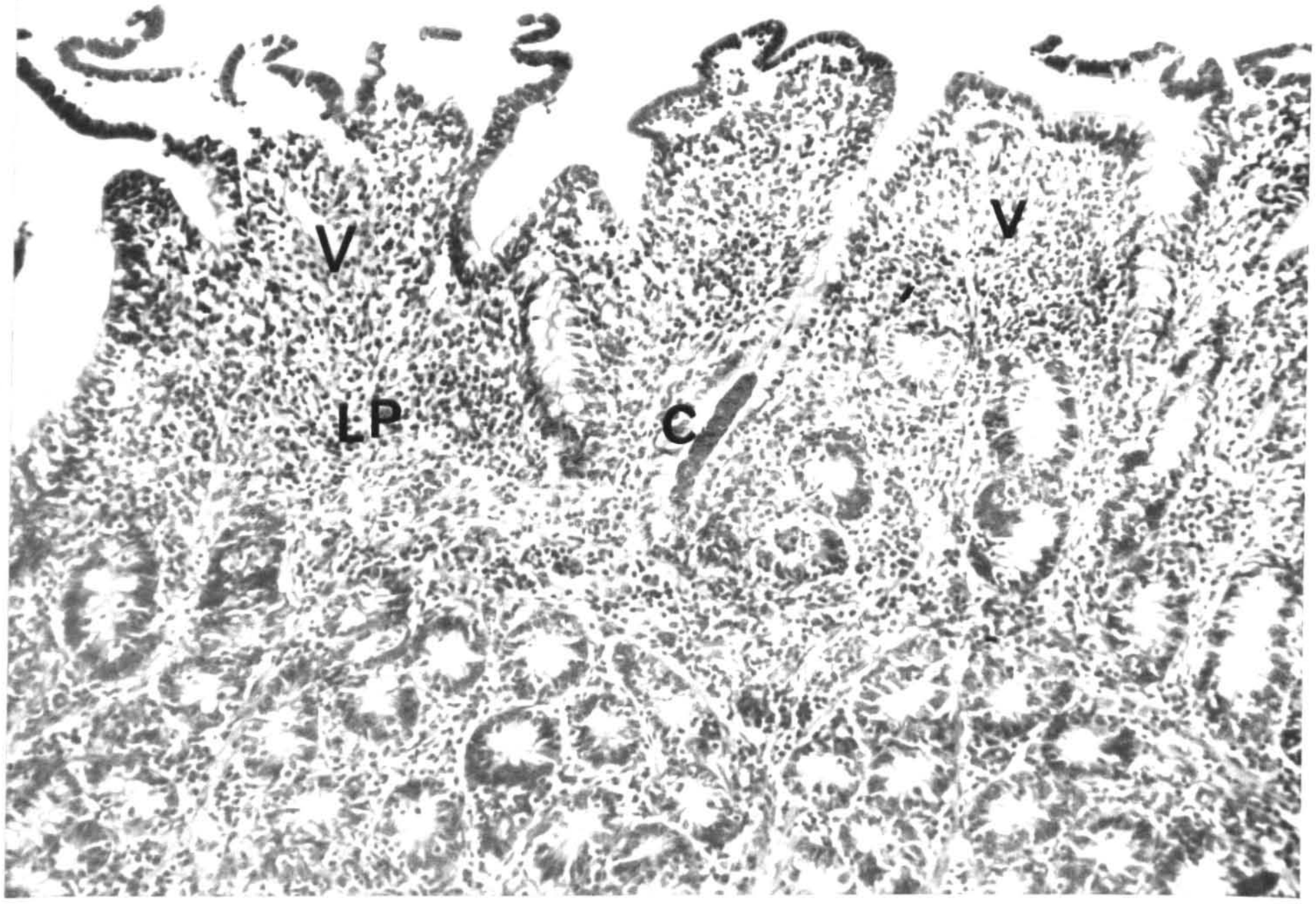


Fig. 29: Histological section of the ileal mucosa of infected calf 8105.

Note the stunted villi (V) the presence of cell debris in the crypts (C) and the hypercellularity of the lamina propria (LP).

H & E X 110.

Small numbers of colonies of C.f. ss. jejuni were isolated from the colonic mucosa of the two animals in the control groups (8101 and 8104) from which the organism had been isolated earlier. The results of the bacteriological examinations for C.f. ss. jejuni are summarised in Table 21.

Table 21.

Sites from which C.f. ss. jejuni was isolated in experimental calves killed following infection with pure cultures of the organism in Experiment 2.

Site of isolation	Animal numbers					
	Infected			Control		
	8103	8105	8106	8101	8104	2674
Abomasum	+	+	-	-	-	-
Jejunum	-	+	-	-	-	-
Ileum	+	+	+	-	-	-
Caecum	+	+	+	-	-	-
Colon	+	+	+	+	+	-
Mesenteric Lymph Node	-	-	-	-	-	-
Liver	-	-	-	-	-	-
Lung	ND	ND	ND	-	-	-
Gall bladder	+	+	+	-	-	-
Spleen	ND	ND	ND	-	ND	ND

+ = C.f. ss. jejuni isolated. - = No C.f. ss. jejuni isolated.
ND = Not done.

Antibody to the inocular strain was not found in serum samples from the controls or from the infected group at the beginning of the experiment. At slaughter, antibody to the inocular strain was present in the sera of the infected animals at titres of 1:640 or more. The details are shown in Table 22.

Table 22.

Levels of agglutinating antibody to the inocular strain of C.f. ss. jejuni in the sera of the animals of Experiment 2.

Animal Number	Infected	Titre present on Day 0	Day 17
8103	+	0	1:1280
8105	+	0	1:640
8106	+	0	1:1280
8101	-	0	0
8104	-	0	0
2674	-	0	0

Experiment 3.

Objective: To determine the pathogenicity of C.f. ss. jejuni for milk fed calves.

Materials and Methods

Sixteen Ayrshire bull calves approximately one week old were purchased from a market. An outbreak of diarrhoea occurred in the recently purchased calves and inoculation was delayed until 10 days after purchase when the animals were clinically normal.

Six 3-week old calves were used in this experiment and were divided into two equal groups which were housed separately as mentioned above and numbered 323, 345, JK995 (infected) and P268, 97, L321 (control).

The animals were monitored prior to infection, housed and observed using the methods described in the previous two experiments and in Chapter 2. The ration is described in Chapter 2. The inoculum was the same as in Experiment 1 and also contained approximately 5.0 x 10^{10} organisms per ml. The period of observation lasted for 13 days and animals were killed on the 14th day and examined post mortem by the methods described in Chapter 2. In addition the contents of the mid jejunum from infected calves (323, 345) were negatively stained and examined by electron microscopy for the presence of virus particles.

Results

No salmonellae, β -haemolytic E. coli or campylobacters were isolated from the faeces of these calves prior to infection. No coccidia or nematode eggs were reported to be present in the faeces of the calves. Reovirus particles were seen in negatively-stained preparations from the faeces of one animal in the group when diarrhoea occurred within a week of purchase. That animal was not included in the experiment described here.

The results resembled those of Experiments 1 and 2. Changes in the faeces of the infected animals began by the fourth day after infection and had resolved in two of the three animals by the end of the experiment on day 13. The faeces became softer, slightly dark in colour and contained large quantities of mucus and some blood (Figs. 30 and 31).

The faecal changes are given in detail in Table 23 and summarised in Fig. 33. The inoculated animals were dull with nasal discharge and some coughing. Rumenal movements were seen at all times. The rectal temperatures of all three inoculated animals rose to 39.4°C within two days of inoculation and the subsequent changes in rectal temperature are shown in Fig. 32.

The control animals were in poor condition at the beginning of the study. One of them (97) developed meningitis and paralysis and was destroyed on day 6 while another (L321) developed severe pneumonia

Fig. 30: Faeces from experimentally infected calf 323 two days after infection.

Note the fluid consistency and the shining area where clear mucus was present.

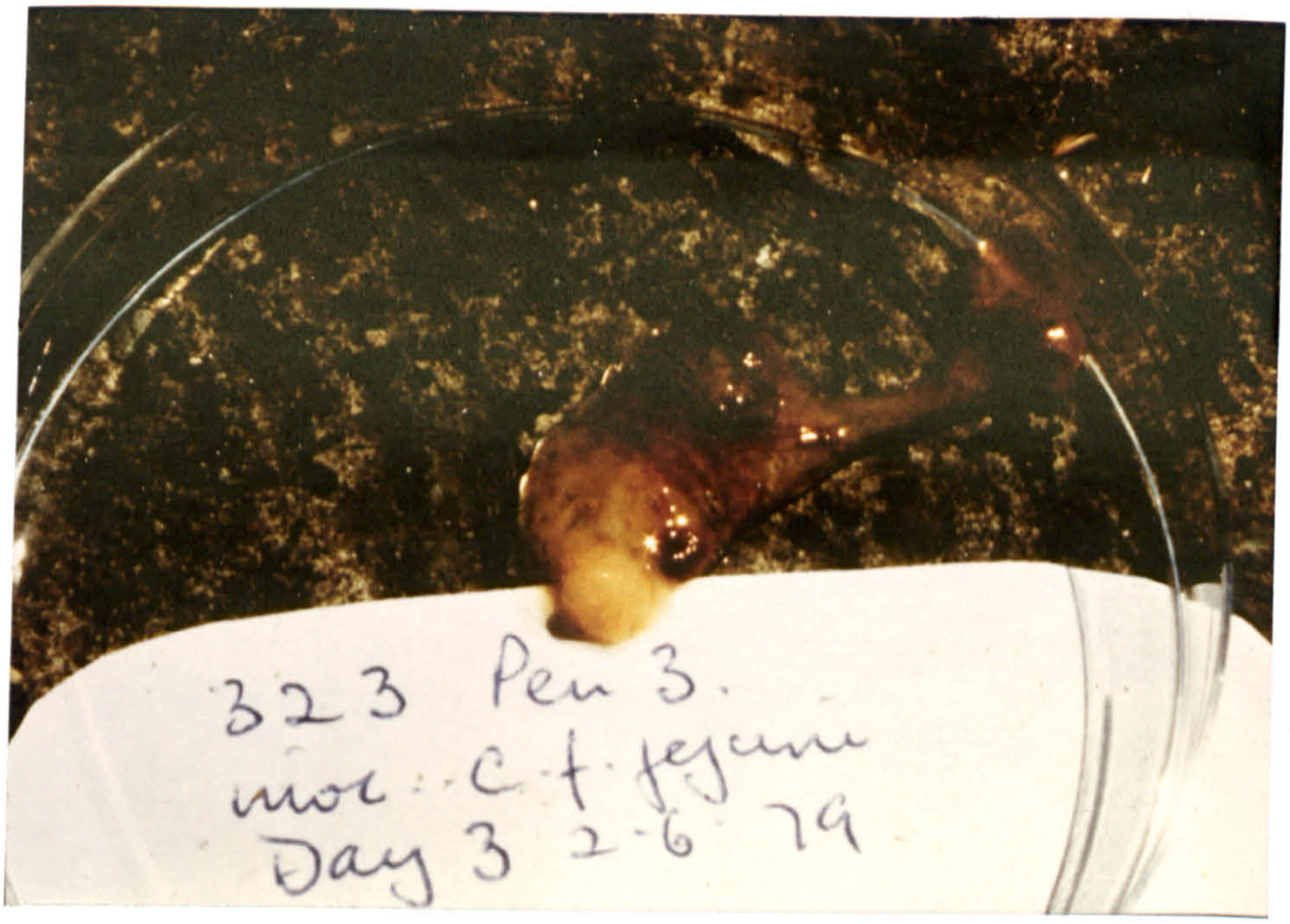


Fig. 31: Blood and mucus in rectal faeces from calf 323 three days post-infection with C.f. ss. jejuni.

Table 23.

Changes in faecal consistency in Experiment 3 following the inoculation of milk fed calves with pure cultures of C.f. ss. jejuni and the isolation of the organism from their faeces

Calf No.	Inf-ected	Day of experiment 0	1	2	3	4	5	6
323	+	F	FMB	DMB	SMB	FMB	SM	SMB
		-	+	+	+	+	+	+
345	+	F	FM	F	F	DM	SMB	CM
		-	+	+	+	+	+	+
JK995	+	F	F	F	FM	SM	SM	FM
		-	+	+	+	-	+	+
P268	-	F	F	F	F	GP	F	F
		-	-	-	-	-	-	-
97	-	F	F	F	F	F	F	F (K)
		-	-	-	-	-	-	-
L321	-	F	F	F	F	P	gP	F
		-	-	-	-	-	-	-

Calf No.	Inf-ection	7	8	9	10	11	12	13	
323	+	FM	DM	FMB	SM	VSM	SMB	SMB	K
		+	-	+	+	-	+	+	
345	+	SMB	SMB	FM	F	FM	FM	FM	K
		+	-	+	+	-	+	+	
JK995	+	FM	FM	F	FM	SM	FM	FM	K
		-	-	+	+	-	+	-	
P268	-	F	gP	F	F	F	F	F	K
		-	-	-	-	-	-	-	
L321	-	F	F	F (died)					
		-	-	-					

F = Firm faeces. S = Soft faeces. VS = Very soft faeces.
M = Presence of mucus. B = Presence of blood. D = Diarrhoea.
C = Constipated faeces. G = Greenish faeces. g = greyish faeces.
+ = C.f. ss. jejuni isolated. - = No C.f. ss. jejuni isolated.
K = Killed.
P = Pasty faeces.

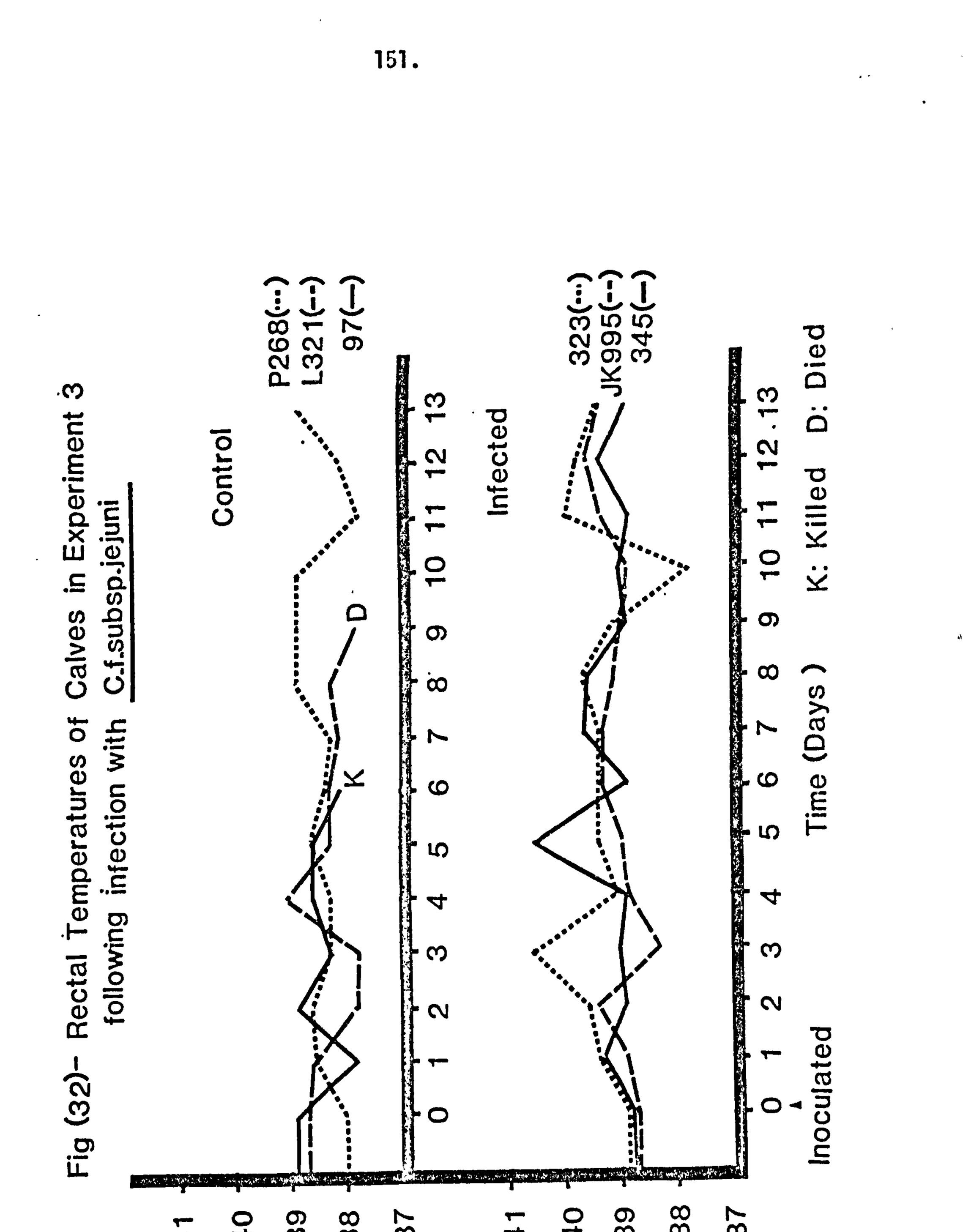


Fig (32)- Rectal Temperatures of Calves in Experiment 3 following infection with C.f.subsp.jejuni

and died on day 9. None of the control animals had any mucus or blood in their faeces although on a few occasions (Table 23) it was abnormal in consistency (pale and pasty). No virus particles, nematodes or coccidial oocysts were seen in the faeces of the experimental animals during this experiment. Salmonella spp. and β-haemolytic E. coli were not isolated.

C.f. ss. jejuni was isolated from the faeces of each infected calf from day 1 to the end of the experiment but none were isolated from the faeces of the controls (Table 23).

At post-mortem examination, the changes recorded in the inoculated calves were similar to those seen in the infected ruminating animals of Experiments 1 and 2. In all cases the liver was pale. Pneumonic lesions were present in two of the three animals (323, JK995). The alimentary tracts of all the control animals appeared grossly normal although the contents of the large intestine of one animal (P268) were fluid. Pneumonic areas were present in the lungs of all three control animals but were most severe in the lungs of the animal which had died from pneumonia (L321). Histological findings in the alimentary tract resembled closely those of Experiments 1 and 2.

C.f. ss. jejuni was isolated only from the small and large intestine of the inoculated animals and was not isolated from, or demonstrated in, the control calves. The results are summarised in Table 24.

Gram-stained smears prepared from the mucosa of these organisms contained curved rods with the morphology of C.f. ss. jejuni. None were seen in similar preparations made from the controls.

Antibody to the inocular strain of C.f. ss. jejuni was demonstrated at titres in excess of 1:1280 in samples taken from the inoculated group at the end of the experiment and was absent from the sera of the control animals and from all preinoculation sera. The detailed results are shown in Table 25.

Table 24.

Sites from which C.f. ss. jejuni was isolated in experimental calves killed following infection with pure cultures of the organism in Experiment 3.

Site of isolation	Animal numbers					
	Infected			Control		
	323	345	JK995	P268	97	L321
Abomasum	-	-	-	-	-	-
Jejunum	+	-	-	-	-	-
Ileum	+	+	+	-	-	-
Caecum	+	+	+	-	-	-
Colon	+	+	+	-	-	-
Mesenteric Lymph node	-	-	-	-	-	-
Liver	-	-	-	-	-	-
Lung	-	-	-	-	-	-
Gall bladder	-	-	-	-	-	-

+ = C.f. ss. jejuni isolated. - = No C.f. ss. jejuni isolated.

Table 25.

Levels of agglutinating antibody to the inocular strain of C.f. ss. jejuni in the sera of the animals of Experiment 3.

Animal Number	Infected	Titre present on Day 0	Day 14
323	+	0	1:1280
345	+	0	1:1280
JK995	+	0	1:1280
P268	-	0	0
97	-	0	0 (day 6)
L321	-	0	0 (day 9)

DISCUSSION

The results of the three experiments described above indicate that the isolate of C. fetus subsp. jejuni used was capable of iniating fever, diarrhoea, sporadic dysentery and enteric lesions when fed to experimental calves in pure culture. This finding is in agreement with the work of Jones and Little (1931 a and b) and Jones et al. (1932) who inoculated pure cultures of "Vibrio jejuni" into experimental calves. It suggests strongly that the organism now described as C.f. ss. jejuni has similar pathogenic properties for calves as had the "Vibrio jejuni" organism which they initially described.

The organism used had been maintained for eight weekly passages before being freeze dried and was passaged once more before being used as an inoculum. It was, however, able consistently to produce the series of changes described in the experiments. It therefore appeared to have lost little virulence as a result of its maintenance in artificial media.

The syndrome produced was essentially similar in all three experiments. Oral inoculation was followed by changes in faecal consistency in all infected animals. The time of appearance and the duration of these changes are summarised in Fig. 33. Soft faeces of uniform consistency containing varying amounts of clear mucus was passed within one and five days after inoculation. This mucus sometimes contained streaks of fresh blood. (Fig. 31). Even when mucus could not easily be seen in the faeces the insertion of a probe or pouring of rectal faeces often disclosed the presence of strands of mucus. (Fig. 24). These faecal changes lasted for 12 days (Experiments 2 and 3). They were accompanied by, and preceded by, the changes in rectal temperature recorded in Figs. 25, 28 and 32. Elevation of rectal temperature occurred within one - five days of infection and reached a maximum of 41°C in animal 8103 on day 4 of Experiment 2. The raised rectal temperatures persisted for a variable length of time but appeared to have returned to normal by day 16 in the longest study, study, Experiment 2.

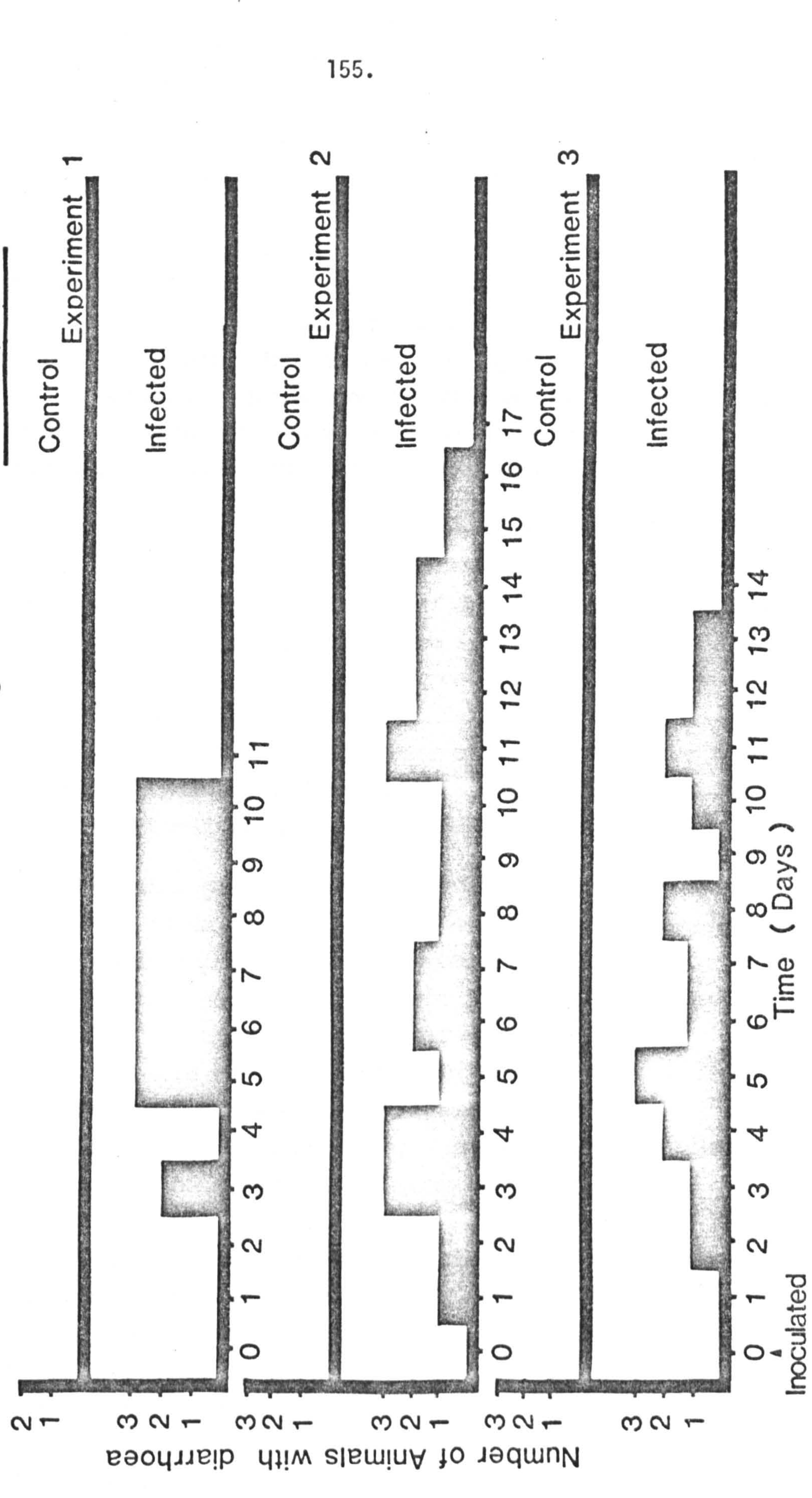


Fig (33)- The occurrence of diarrhoea in Calves following infection with C.f.subsp.jejuni

The other clinical changes noted were slight. Nasal discharge and coughing were present in both infected and control groups in Experiments 2 and 3 but only in the infected animals in Experiment 1. In all three experiments some dullness was noted in the infected animals shortly after inoculation. Dullness or depression was also noted in controls in Experiment 2 (8101) and in Experiment 3 but was attributed to other diseases. All inoculated animals appeared to eat normally and to have normal ruminal movements.

C.f. ss. jejuni was usually recovered from the faeces 24 hours after inoculation (Tables 17, 20 and 23) and the isolation of profuse cultures was possible for a number of days following inoculation. Failures to isolate the organism from faeces occurred in all three experiments as early as the 4th day post inoculation (JK995, Experiment 3) but it was not clear whether this failure was due to technical reasons, to a reduction in the number of organisms present in the faeces or to destruction of the organism in transit. Transit times were short and unlikely to have been responsible.

The gross post-mortem findings were similar in each case. The serosa of the ileum was pale, flaccid and its surface was reticulated. The mesenteric lymph nodes were enlarged, pale and oedematous. The contents of the ileum were fluid, dark in colour and appeared to contain mucus and the large intestinal contents resembled the faeces. Gross lesions were seen in the small intestinal mucosa in inoculated animals in each experiment and consisted of thickening of the ileal wall, mild inflammation and increased granularity of the ileal mucosa. They resembled those described by Jones and Little (1931 a and b). These changes were less commonly seen in the jejunum (calf 114, Experiment 1). No gross changes were seen in the large intestinal mucosa when compared with controls. In general, the changes summarised here were absent from the control animals although some elements of them were seen in individual control animals from which C.f. ss. jejuni was not isolated (115 and 100, Experiment 1; 8101 and 8104, Experiment 2). The histological changes in the enteric tract of experimental animals resembled those seen in the survey in animals from which C.f. ss. jejuni was isolated. They consisted of lowering of the

villus height, inflammatory cell infiltration into the lamina propria and thickening of the whole mucosa. In the ileum, reactive changes were prominent in the lymphoid tissue and crypt abscesses were present. In the majority of sections examined, no coccidia or crytpsporidia could be seen. Coccidia were, however, found in the epithelium of a control animal in Experiment 2 and their possible presence in the remaining animals of that experiment must therefore be inferred.

Individual control animals were found to have some or all of the above changes (animal 100, Experiment 1 and animal 8101, Experiment 2).

C.f. ss. jejuni was isolated both from the small and large intestinal mucosa in all inoculated animals and from the gall bladders of inoculated animals in Experiment 2 (Tables 18, 21 and 24). It was most common in the ileum and large intestine but was recovered from the jejunum in animal 8105 (Experiment 2) and 323 (Experiment 3). It was also recovered from the abomasum of the former.

Agglutinating antibody to the inocular strain could be demonstrated in the sera of all inoculated animals at slaughter. (Tables 19, 22 and 25).

The changes summarised above appeared to result from inoculation with C.f. ss. jejuni. The evidence in support of this comes from the results of the experiments themselves in that the clinical changes recorded were confined to the inoculated animals and the organism could be isolated both from the faeces of the inoculated animals and from their gastrointestinal tracts at slaughter. The presence of serum antibody in the inoculated animals after infection provided added confirmation.

In some respects the results were not completely unequivocal. Other enteric pathogens were present in the experimental animals (nematodes and coccidia in Experiment 2 and reovirus in Experiment 3) and their presence may have affected the lesions seen at slaughter. They do not appear to have affected the clinical features of the syndrome produced. Intercurrent disease was also present. In particular

pneumonia and rhinitis appeared to affect both control and inoculated groups (Experiments 2 and 3). Only in animal 8101 (Experiment 2) did intercurrent disease (rumenitis) appear to affect the lesions found in the enteric tract. The gross and histological changes in the inoculated animals were not marked and could represent the response to a number of agents. This view is strengthened by the occurrence of crypt abscesses and ileal mucosal thickening in control animals but the findings in all three studies were so consistent that it appears that inoculation results in the gross and histological changes are as described above. However, the consistent isolation of the organism in large numbers from the sites of these lesions and its identification in smears from the mucosa suggest that the lesions seen in inoculated animals were caused by C.f. ss. jejuni.

The failure to isolate the organisms on every occasion from the faeces of experimentally-infected calves is of interest. It is not clear whether this reflects a failure to identify the organism when present or whether it represents irregular shedding of the organism in the faeces. In view of the presence of intestinal infection in all inoculated animals at slaughter, the former view seems most likely. It does, however, demonstrate that faecal culture is of value as a means of assessing the infected status of animals but that it is not infallible. This is of particular relevance in Experiment 2 in which C.f. ss. jejuni was recovered in small numbers from the faeces of two control animals (8101 and 8104) and later from their colonic mucosa. It may be that this infection represents cross infection between inoculated and control groups or the inapparent infection of the animals with a strain other than the inocular one. This latter view is supported by the absence of any antibody to the inocular strain in the control animals concerned.

The development of clinical signs was complete within four-six days of inoculation and as post-mortem examination was not carried out until 11-17 days post-inoculation the lesions seen may have been those of chronic disease or early recovery. Accordingly the studies described in Part II of this chapter were carried out in order to study the early development of the lesions.

PART II.

Studies of the pathogenesis of C.f. ss. jejuni infections in calves.

The results of the studies described above in Part I showed that the isolate of C.f. ss. jejuni used produced an enteric syndrome in calves with an incubation period of one - five days following experimental infection.

The possibility that pathological changes seen at slaughter some days after the development of clinical signs might not be the same as those present at the beginning of the infection was considered. There was also some uncertainty as to whether the organism was restricted to the gut and its exact relationship to the tissue at various stages in the development of the disease.

Accordingly, three studies were carried out to examine these relationships.

Study 1.

Blood culture from the experimentally-infected calves of Experiment 2.

Study 2.

Experimental infection and sequential killing of calves with subsequent examination of the developing lesions by gross examination, histology and electron microscopy (Experiment 4).

Study 3.

Adhesion studies with bacterial cultures and isolated intestinal brush borders.

Study 1.

Blood culture from calves experimentally infected with C.f. ss. jejuni in Experiment 2.

In this study an attempt was made to determine the presence or

absence of C.f. ss. jejuni in the blood of experimentally-infected calves during the early part of the clinical disease.

Materials and methods

Unclotted blood was collected from the jugular vein of each infected animal in Experiment 2 on seven days. On days 3-4 and 6-10, 5 ml samples were taken aseptically into sterile plastic containers coated with potassium EDTA (Brunswick) using the syringes and needles described in Chapter 2.

The blood samples were kept cool and examined by culture for the presence of C.f. ss. jejuni within 30 minutes of sampling. Each sample was examined for the presence of C.f. ss. jejuni directly by inoculating a horse blood agar plate with 0.1 ml of blood which was then streaked and incubated microaerophilically. The remainder of the sample was inoculated into 50 ml of Trypticase Soy Broth (BBL-Becton Dickinson Ltd.) containing 1.2 g/l trisodium citrate and incubated microaerophilically for 48 hours. 0.1 ml amounts were then inoculated onto horse blood agar plates and incubated microaerophilically to examine for the presence of C.f. ss. jejuni.

Results

No campylobacters were isolated from any of the calves sampled on the days noted above.

Study 2.

Experimental infection and sequential killing of calves with subsequent examination of the gross, microscopic and ultrastructural features of the lesions

Experiment 4.

Objective : To study the pathogenesis of C.f. ss. jejuni using ruminating calves.

Materials and methods

Nine 4-month old calves were obtained from a single source and used in this study. They comprised four Ayrshires, three Herefords and

two Friesians. The infected group consisted of six animals (numbers 94, 95, 97J, 98, 99J and 100J) and the uninoculated control group consisted of animals numbers 91, 92 and 93.

The two groups were monitored prior to infection, housed, fed and observed by the methods described in Chapter 2 and for Experiment 1. Faecal samples from each animal were examined for the presence of salmonellae, β-haemolytic E. coli, campylobacters and other bacteria, nematode eggs and coccidia during the holding period by the methods described in Chapter 2. Each member of the inoculated group was inoculated orally with 20 ml of suspension of a pure culture of C.f. ss. jejuni containing approximately 5.2×10^9 organisms/ml. All animals were examined daily and their appetite, appearance, respiratory rate, rectal temperature, the presence or absence of ruminal movement, and the consistency of their faeces were recorded.

Faecal samples from each animal were examined daily for C.f. ss. jejuni and other bacteria by the methods described in Chapter 2. Colonies resembling those of Campylobacter were confirmed as such by the methods described in Chapter 2.

Serum samples were taken from each animal at slaughter and the sera stored by methods described in Chapter 2 and examined for the presence of agglutinating antibody to C.f. ss. jejuni. Animals were killed at daily intervals and controls were killed at appropriate intervals and examined post mortem.

Post-mortem examination was carried out as described in Chapter 2. No examination for virus particles was carried out in this study, but material from five sites in the gastrointestinal tract was taken and processed for electron microscopical examination.

Results

No salmonellae or β-haemolytic E. coli were isolated from the faeces of these calves prior to infection. No coccidia or nematode eggs were reported present.

The incubation period before the onset of diarrhoea in the calves of this experiment varied from one - three days post inoculation. Changes in faecal consistency began on the first day following inoculation in four inoculated calves and most inoculated calves passed diarrhoeic faeces which varied from very soft to soft with excess clear mucus and streaks of blood before slaughter. The clinical findings are summarised in Table 26.

The rectal temperature of most of the inoculated calves rose to between 39.4°C - 40°C and in one infected animal (95) reached 40.3°C (Fig. 34).

The uninoculated animals remained clinically normal throughout the time of the experiment (Table 26 and Fig. 34). No coccidial oocysts or nematode eggs were found in the faeces of any of the animals nor were Salmonella spp., β-haemolytic E. coli or other bacteria isolated.

C.f. ss. jejuni was isolated from the faeces of all inoculated animals but never from the faeces of the controls. The findings are given in Table 26.

No campylobacters were isolated from the faeces of control animals.

Daily summary of gross pathological changes - Infected animals.

Day 1 - Calf 98.

No gross changes were seen in any part of the gastrointestinal tract with the exception of the jejunal and ileal contents which were slightly mucoid. Pneumonic lesions were present in both lungs.

Day 2 - Calf 95.

The abomasal and jejunal mucosa was grossly normal but the jejunal content was watery and mucoid. The terminal ileum appeared slightly thickened with soft contents and excess clear mucus. The large intestinal mucosa was grossly normal although the content of caecum was slightly mucoid. The mesenteric lymph nodes were enlarged

Table 26.

Changes in faecal consistency in Experiment 4 following the inoculation of calves with pure cultures of C.f.ss. jejuni and the isolation of the organism from their faeces.

Calf number	Inf-ected	Day of experiment 0	1	2	3	4	5	6
98	+	F	F					
		-	+	K				
92	-	F	F					
		-	-					
95	+	F	F	FM				
		-	+	+	K			
94	+	F	DM	SM	SM			
		-	+	+	+	K		
93	-	F	F	F	F			
		-	-	-	-			
100J	+	F	FMB	SM	FM	SM		
		-	+	+	+	+	K	
99J	+	F	FMB	FM	SM	DM	SM	
		-	+	+	+	+	+	K
97J	+	F	FMB	SM	FM	SMB	SMB	SM
		-	+	+	+	+	+	+
91	-	F	F	F	F	F	F	F
		-	-	-	-	-	-	-

F = Firm faeces.
M = Presence of mucus.
B = Presence of blood .
K = Killed.
D = Diarrhoea
S = Soft faeces
+ = C.f.ss. jejuni isolated.
- = No C.f.ss. jejuni isolated.

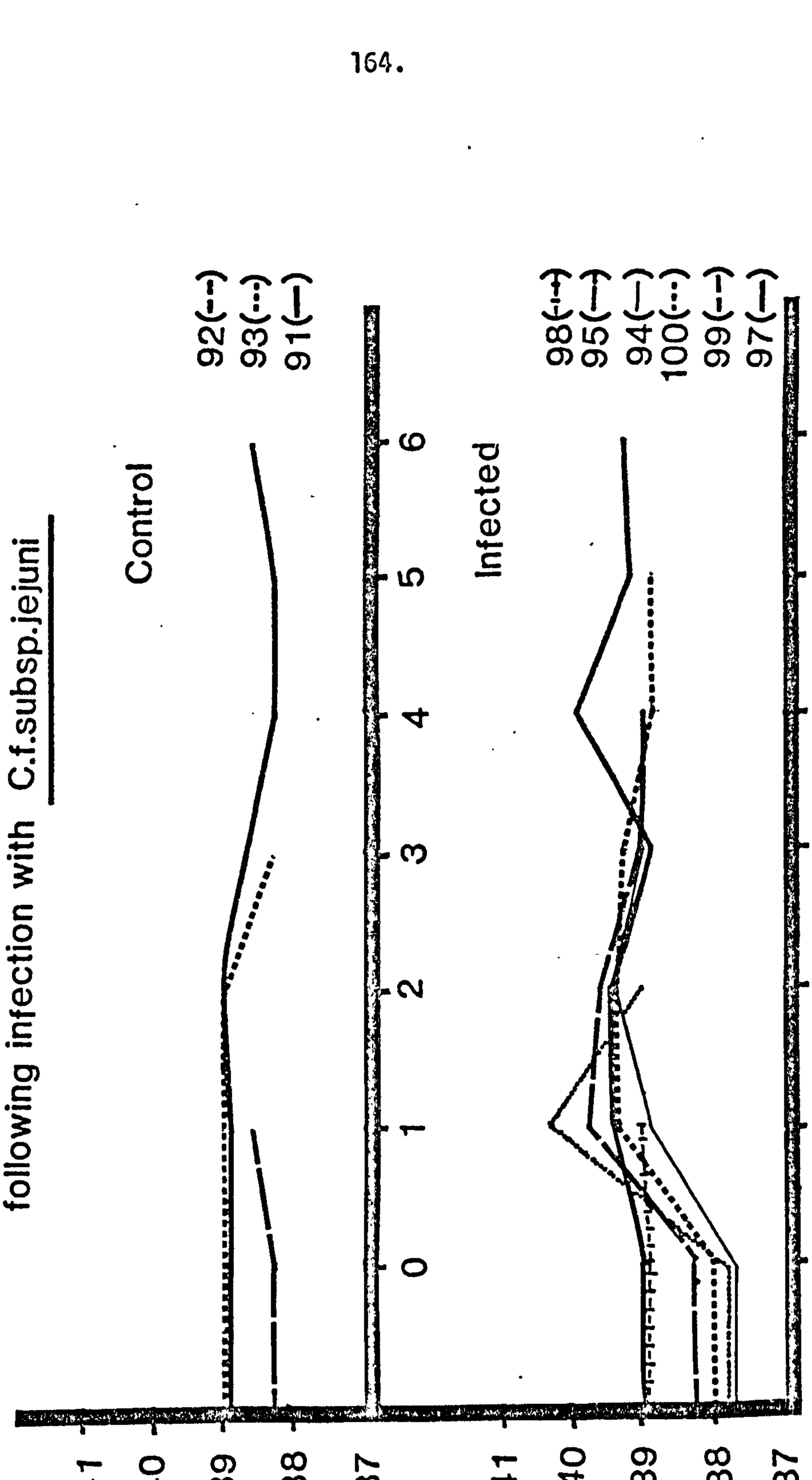


Fig (34)– Rectal Temperatures of Calves in Experiment 4 following infection with C.f.subsp.jejuni

Day 3 - Calf 94.

The abomasal mucosa of infected calf (94) was normal and the contents appeared normal with excess mucus. The small intestine was flaccid. The jejunal mucosa was thickened and hyperaemic. The jejunal contents were watery and mucoid with thin cloudy mucus. The most obvious changes were seen in the ileum. The mucosa appeared flattened, thickened and was severely hyperaemic. The contents were dark greenish, fluid and mucoid in consistency.

The caecal contents were soft, watery and contained excess clear mucus and the mucosa appeared normal. The mucosa of the proximal end of the colon appeared slightly congested although the contents were firm with excess thick clear mucus on the surface of the contents. The mesenteric lymph nodes appeared enlarged. The gall bladder was distended with bile.

Day 4 -Calf 100 J

The abomasal mucosa and its contents resembled those of infected calf 94. The jejunal contents were watery, contained excess clear mucus and were khaki in colour (Fig. 35). The mucosa was hyperaemic with a few localised raised inflamed areas. The appearance of the ileum was similar to that of inoculated animal 94. The contents of both the caecum and the colon were soft and watery with excess clear mucus but the mucosa was normal in appearance. The mesenteric lymph nodes were enlarged and the gall bladder was distended with bile.

Day 5 - Calf 99 J

The abomasum and its contents resembled those of calf 100J. The small intestine was flaccid and the changes seen in the jejunum and ileum resembled those seen in calf 100J as did the contents of the large intestine. The mucosa of the caecum was slightly congested but the mucosa of the colon appeared grossly normal. The mesenteric lymph nodes and gall bladder were enlarged.

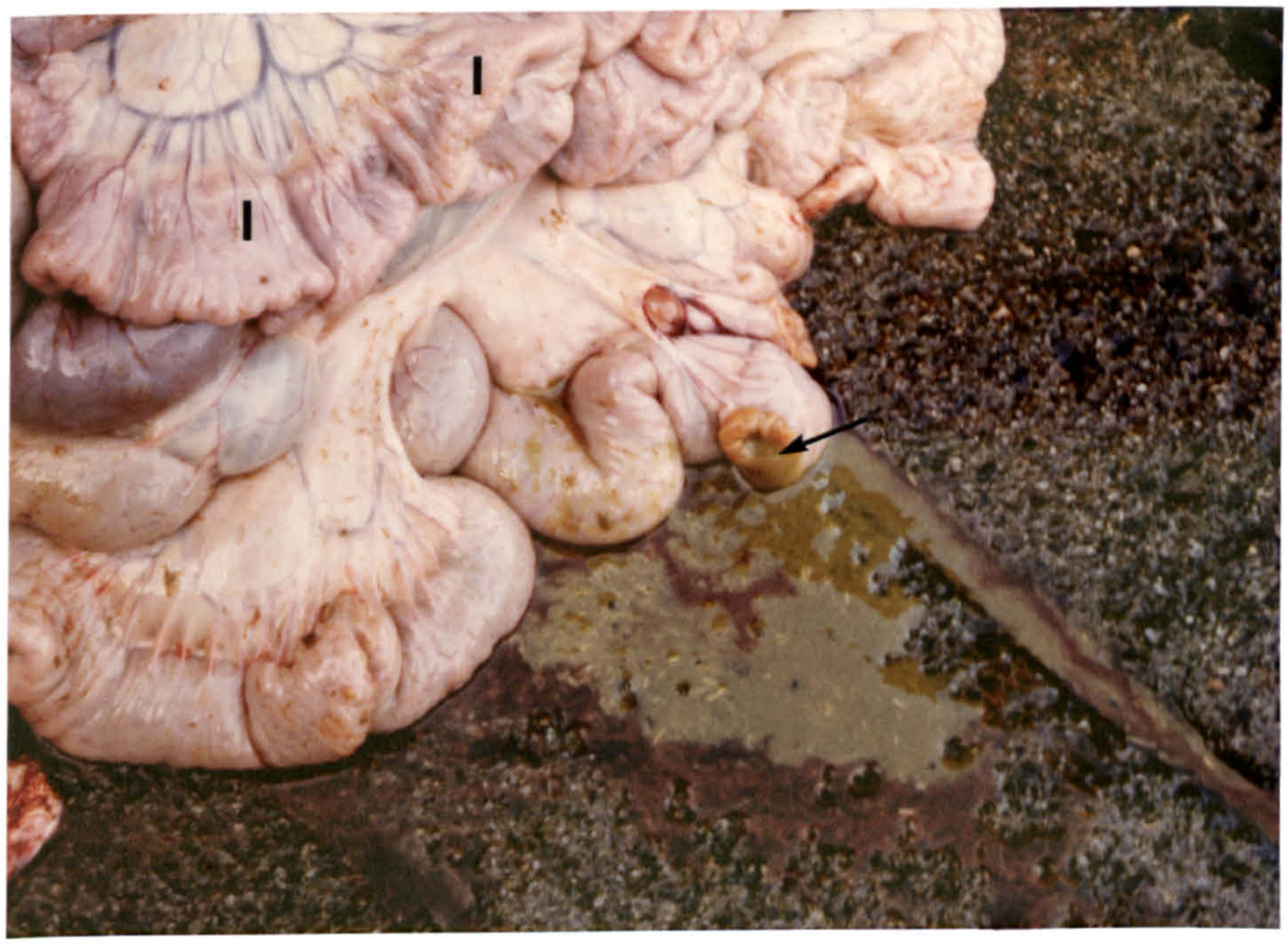


Fig. 35: Small intestine and contents of calf 100J at slaughter four days after infection with C.f. ss. jejuni.

Note the flaccid appearance of the ileum (I) and its whitish wall. The contents are mucoid and of even consistency (arrow).

Day 6 - Calf 97 J

The abomasal mucosa and its contents were grossly normal. The mucosa of the jejunum and ileum were congested and flaccid. The ileum was thickened, its wall was fleshy and the mucosal surface was flattened. Small and large intestinal contents were watery and mucoid. The mucosa of the large intestine was grossly normal. The mesenteric lymph nodes and the gall bladder were enlarged.

Control animals

There were no marked differences in the gross post mortem findings of the control animals killed on days 1, 3 and 6. Gross changes were few. There was slight thickening of the terminal ileum in calves 92 (day 1) and 93 (day 3) and the mesenteric lymph nodes were slightly enlarged in calf 91, (day 6). Pneumonic lesions were present in the lungs of all three control animals.

Daily summary of the microscopical changes

Microscopical changes are described here on a daily basis. In all cases the H and E sections are reviewed but in some cases the sections available could not be stained by Young's method and for technical reasons descriptions of the silver-stained sections therefore cannot be given.

Infected calves

Day 1 - Calf 98.

The changes seen in the abomasal mucosa consisted of mild capillary dilatation and the presence of masses of cellular debris and mononuclear cells plugging the abomasal glands. In thick araldite sections stained by methylene blue / AZURII bacteria were seen in large numbers at the mouths of the glands. Stunted villi with subepithelial spaces were seen in the jejunum, in the lamina propria of which a few neutrophilic polymorphonuclear leucocytes were seen. The ileal lacteals were dilated, the lymphatic tissue was prominent and changes resembling those in the jejunum were seen (Fig. 36). Silver-stained material containing bacterial forms were seen closely adjacent to the mucosal epithelium in the crypts in lymphoid areas of the ileum (Fig. 37).

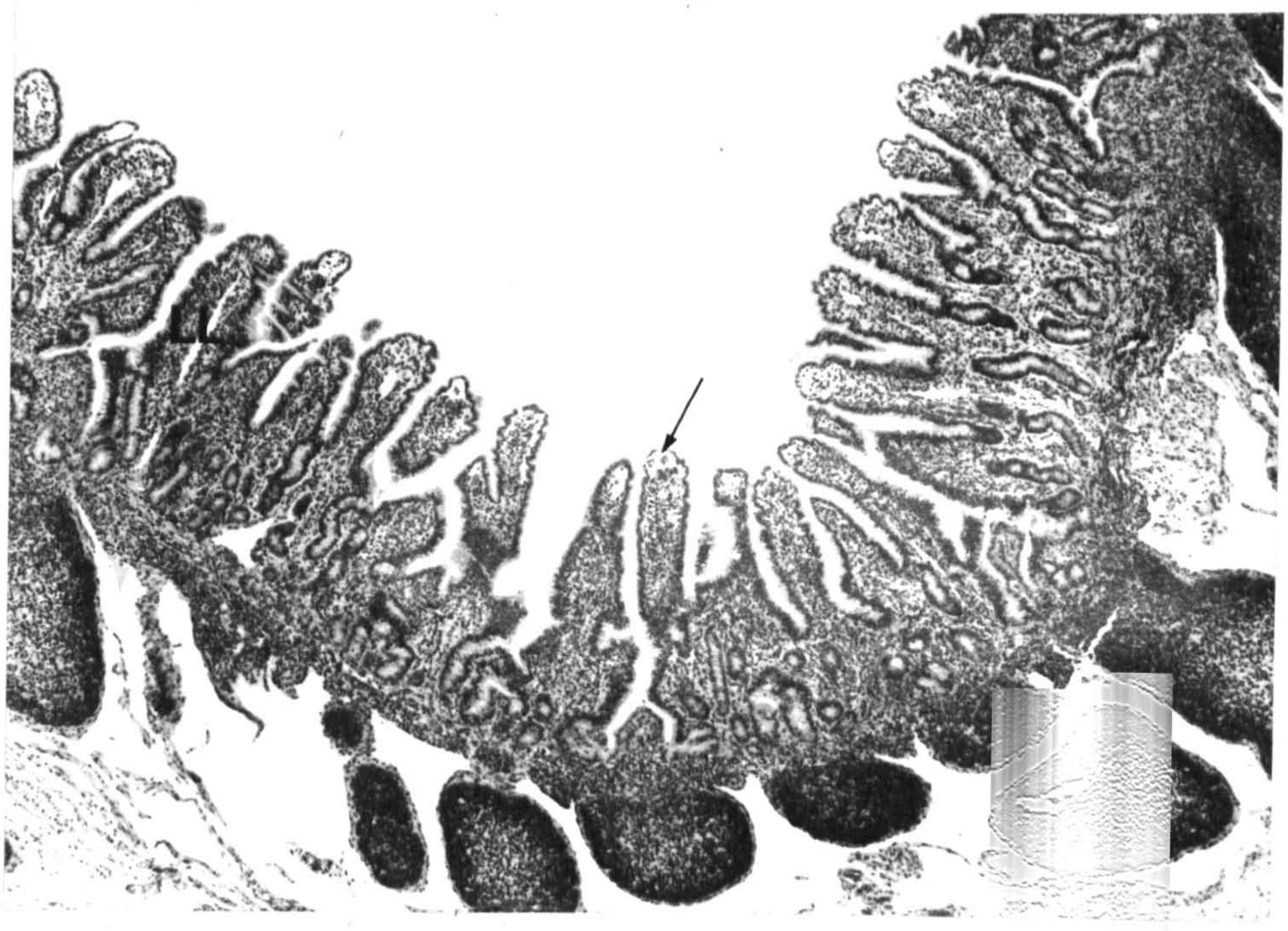


Fig. 36: Histological section of the ileal mucosa of calf 98. Killed 24 hours after infection with C.f. ss. jejuni.

Note the separation of the epithelium at the tips of the villi (arrow) and the lacteals (LL).

H & E X 35.

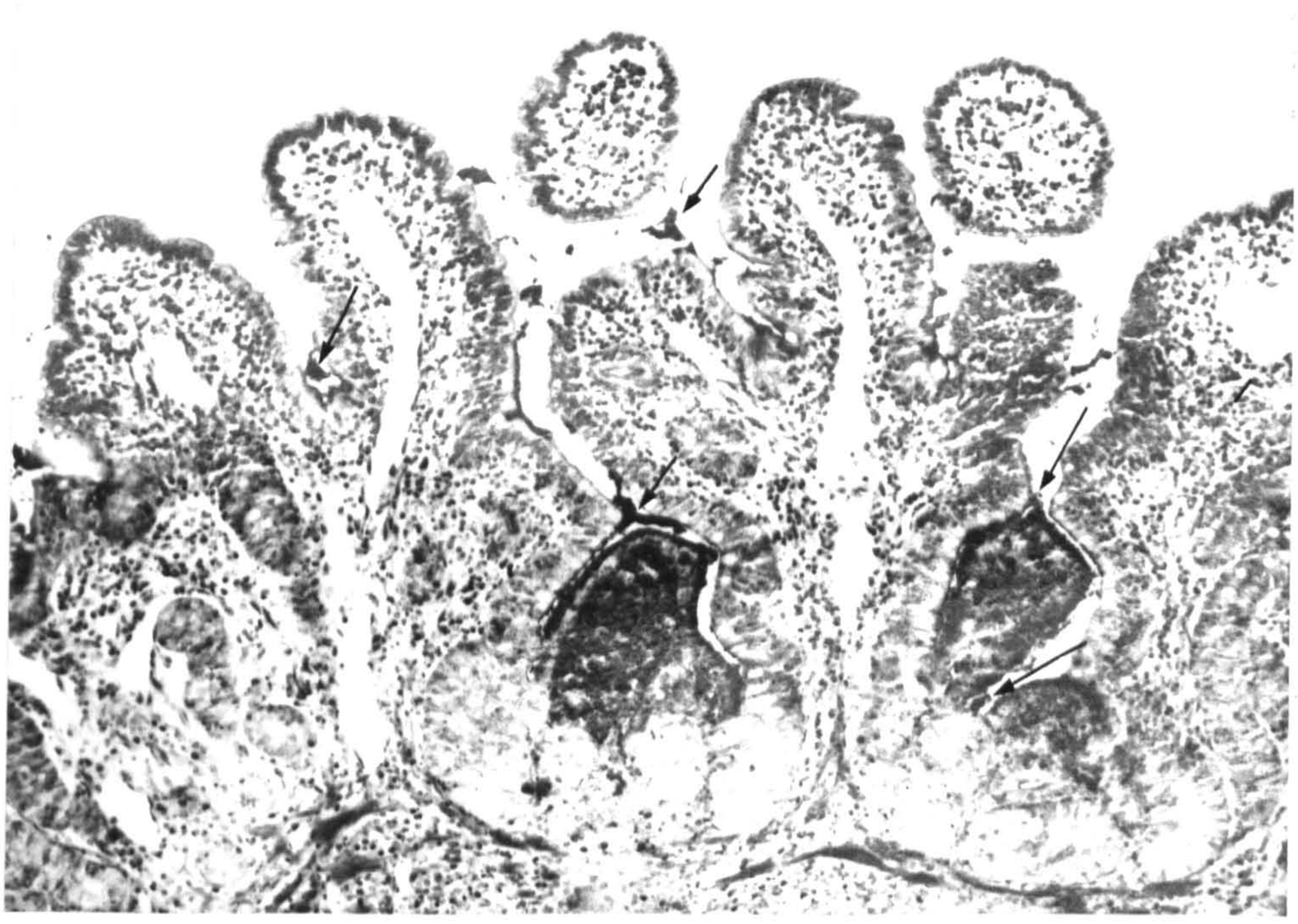


Fig. 37: Silver-stained histological section of the ileal mucosa of calf 98.

Note the distribution of silver-stained material in the crypts. (arrows).

Young's X 100.

Cellular infiltration of the lamina propria by a few polymorphonuclear leucocytes and patches of oedema was seen in the mucosa of the caecum and colon. The mesenteric lymph nodes were oedematous and contained some polymorphonuclear leucocytes.

Day 2 - Calf 95.

The abomasal changes resembled those in calf 98 (Fig. 38) (day 1) and were accompanied by very localised patches of oedema. Silver-stained bacteria were seen in large numbers on the mucosal surface and plugging the abomasal glands (Fig. 39). Changes in the jejunum and ileum were more severe than those in calf 98 (day 1) (Fig. 40). Cuboidal cells were present on the lumenal epithelium (Fig. 41) and crypts containing inflammatory cells were present in both the jejunum and ileum (Fig. 42). Silver-stained curved rods and cocci were seen in very small numbers closely adjacent to the mucosal epithelium and in some of the crypts.

Histological appearance of the caecum and colon resembled that of calf 98 (day 1) but patches of oedema were present and many more polymorphonuclear leucocytes were present in the mucosa of the caecum (Fig. 43). Silver-stained bacteria were seen closely adjacent to mucosal surface, at the mouths of the crypts and within the crypts (Fig. 44) and possibly in the lamina propria in some places. The mesenteric lymph nodes were oedematous and inflammatory cells were prominent.

Day 3 - Calf 94.

The changes seen in the abomasal mucosa resembled those seen in calf 95 (day 2). There was more cell debris on the mucosal surface but similar numbers of silver-stained bacteria were present at the mouths of glands, within them and, possibly, in the lamina propria. In the jejunum stunted and fused villi were seen. There was loss of the lumenal surface epithelium and an increase in cellular infiltration of the lamina propria. Silver-stained curved rods and cocci were seen closely adjacent to mucosal surface and in very small numbers on the epithelial cell surfaces in most of the crypts. The ileum, caecum and colon resembled those in calf 95 (day 2) with some cell debris on the mucosal surface. In 1µ araldite sections massive discharge of goblet cells was seen in the caecal and colonic crypts. Large numbers of polymorphonuclear

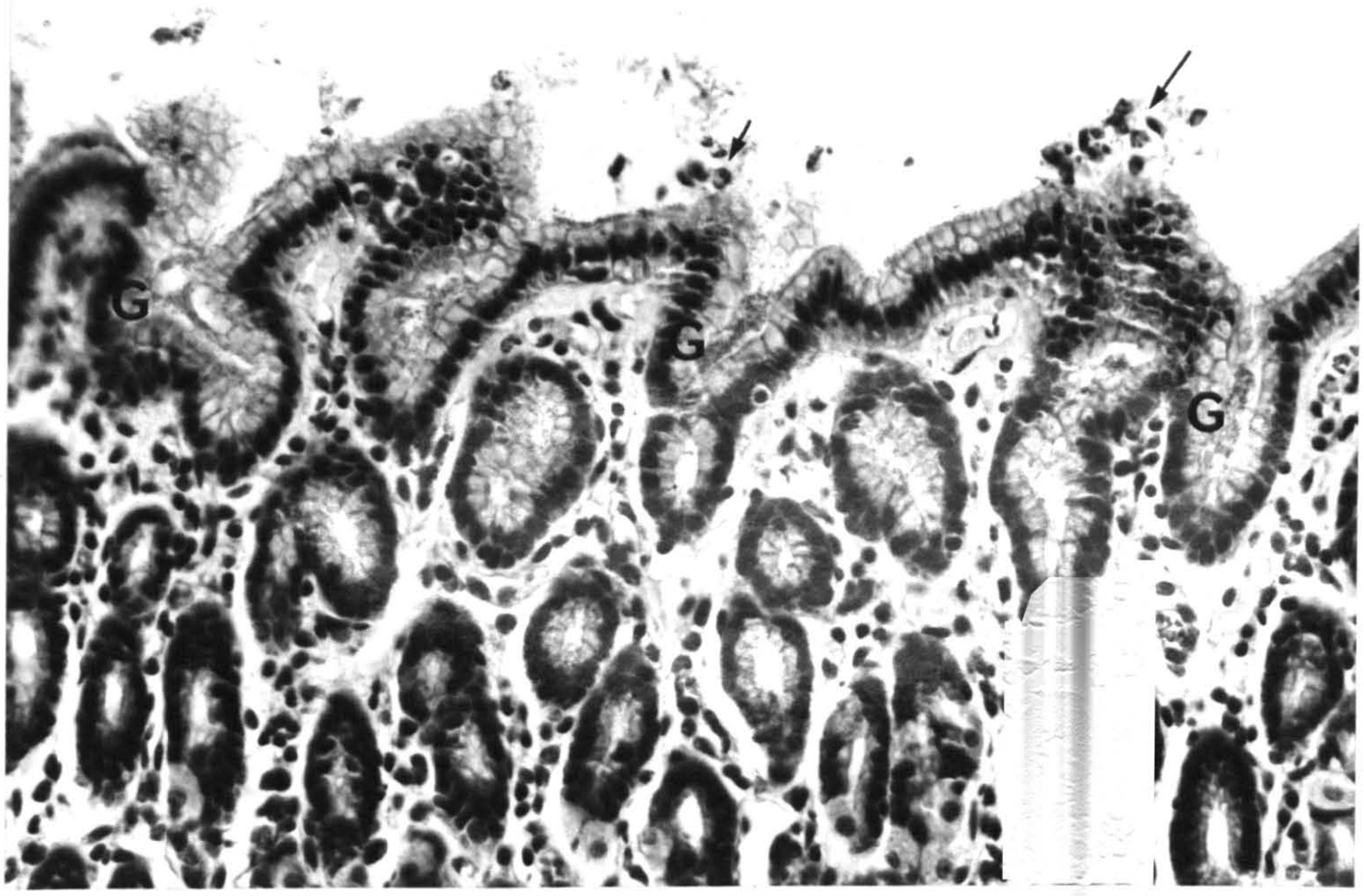


Fig. 38: Histological section of the abomasal mucosa of calf 95 two days post-infection.

Note the presence of cell shedding at the lumenal surface (arrows) and the bacterial material plugging the mouth of the glands (G).

H & E X 250.

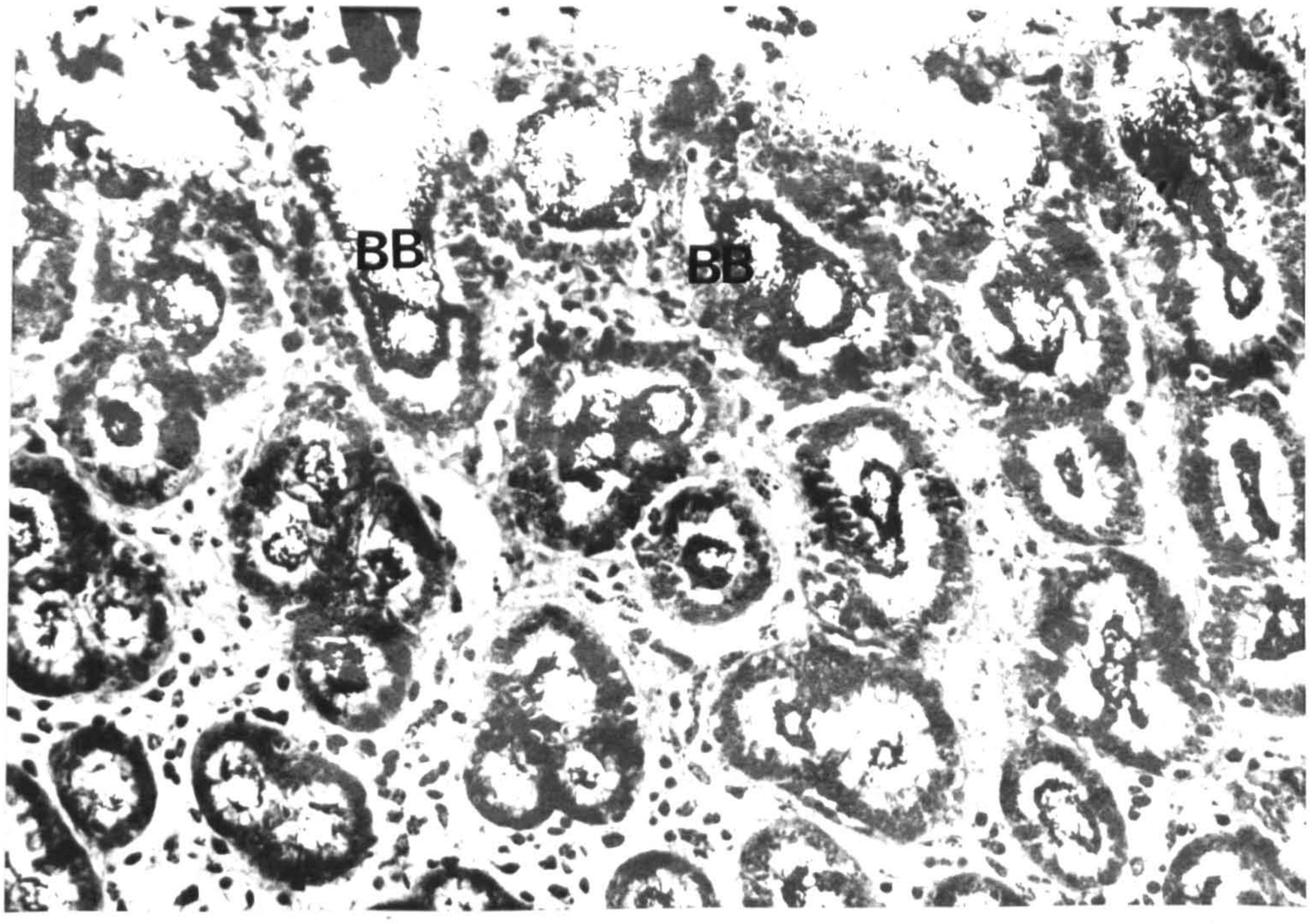


Fig. 39: Silver-stained histological section of the abomasal mucosa of calf 95.

Note the presence of silver-stained bacteria plugging the mouth of the glands (BB).

Young's X 250.

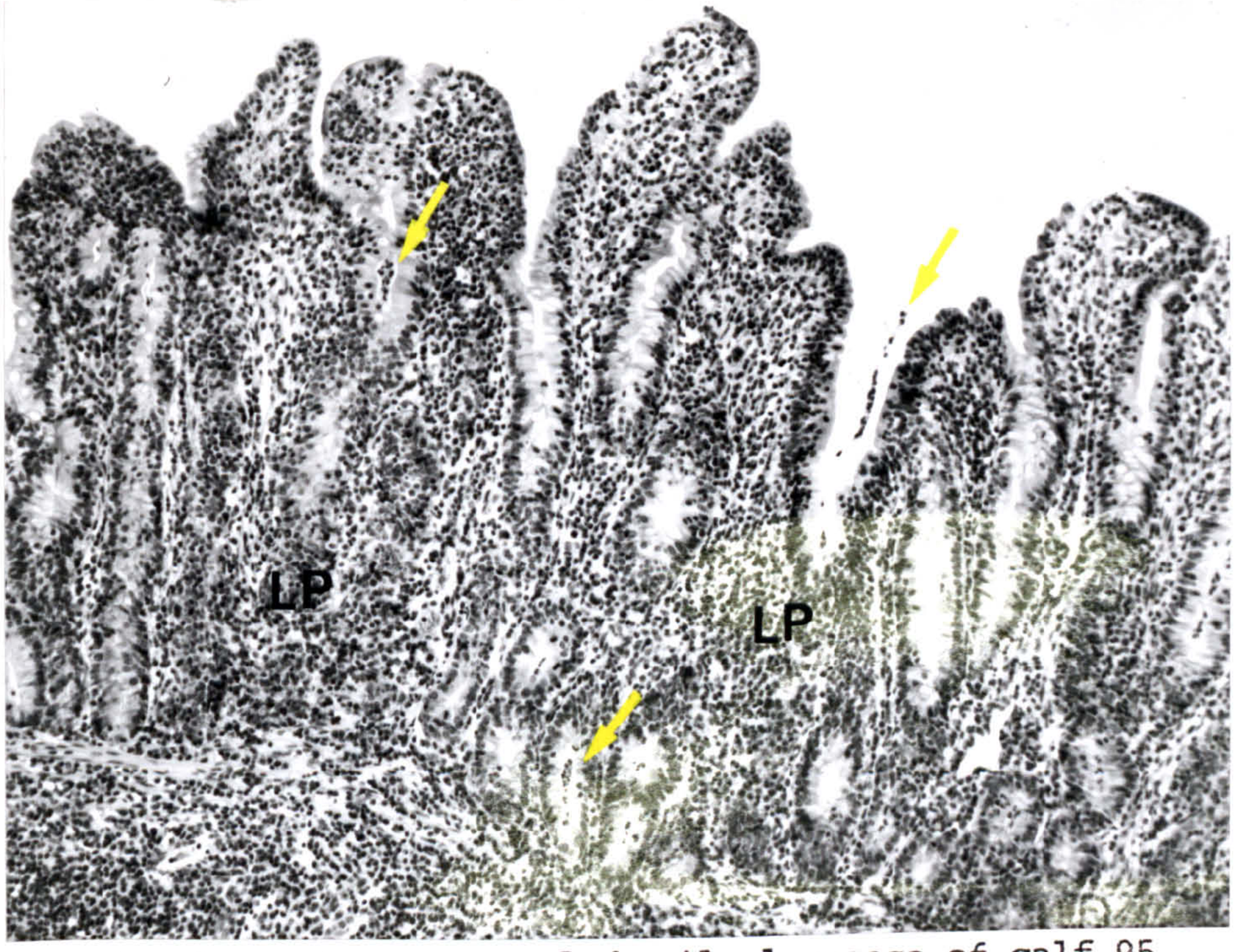


Fig. 40: Histological section of the ileal mucosa of calf 95.

Note the presence of inflammatory cells in the crypts of the mucosa (arrows) and the increased cellularity of the lamina propria (LP).

H & E X 110.

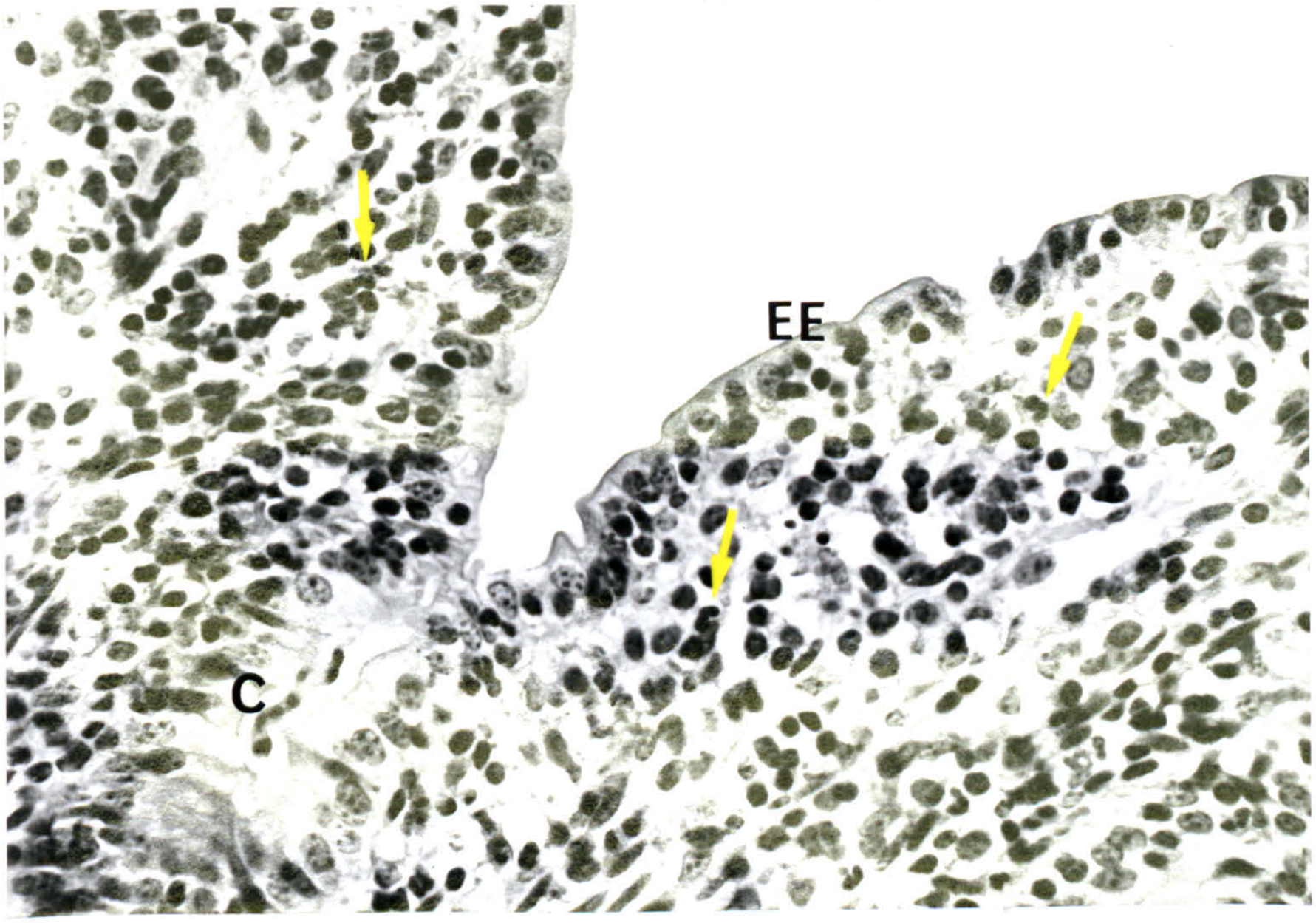


Fig. 41: Lumenal epithelium of the ileal mucosa of calf 95.

Note the cuboidal epithelial cells on the lumenal surface (EE), the presence of cells in the crypt (C) and of polymorphs in the lamina propria (arrows).

H & E X 400.

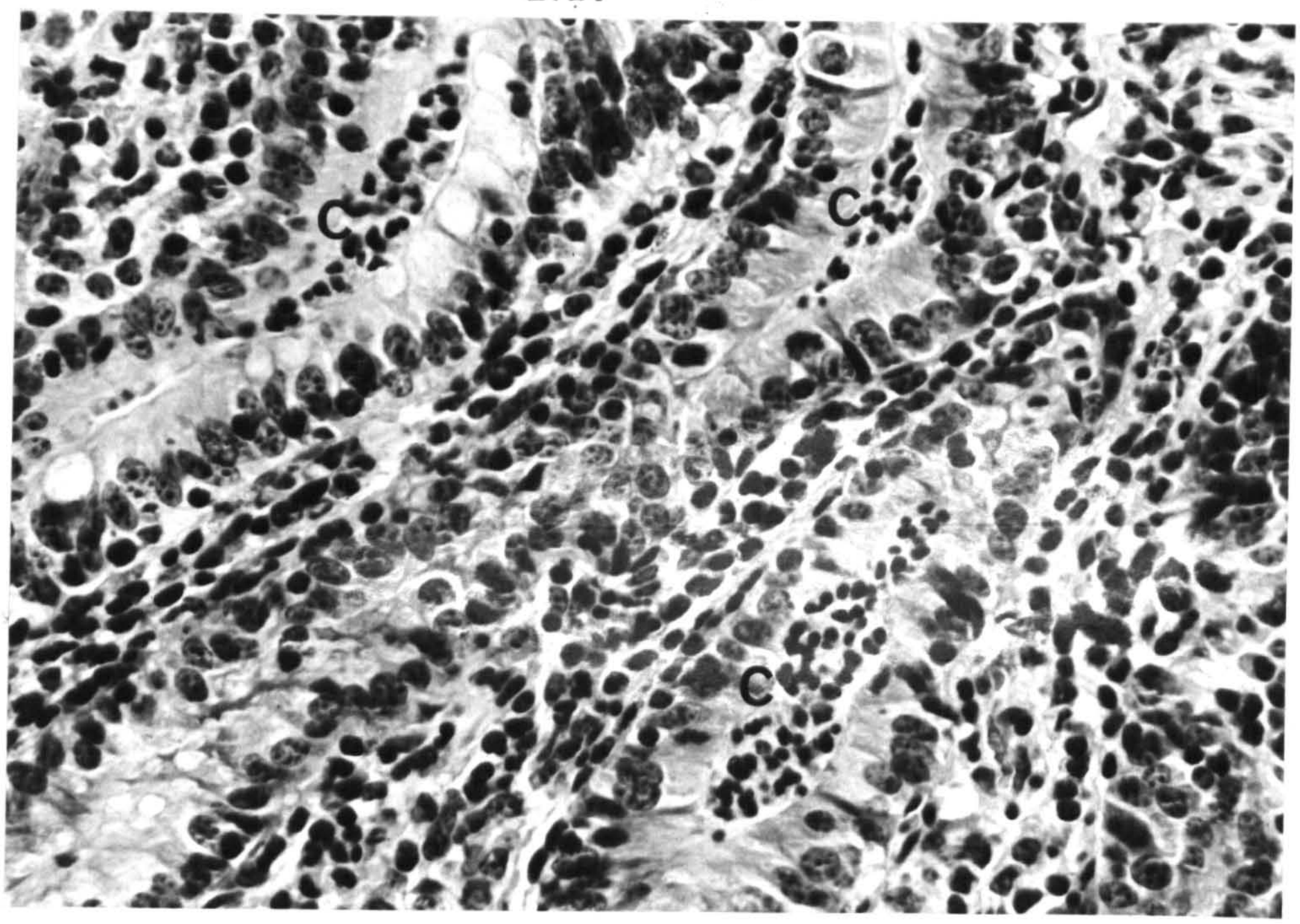


Fig. 42: Crypts in the ileal mucosa of calf 95.
Note the inflammatory cells (C) in the lumens of the crypts.
H & E X 400.

Fig. 43: Histological section of the caecal mucosa of calf 95.
Note the hypercellularity of the lamina propria (LP) and the large number of goblet cells in the crypts (C). Between the crypts are areas of lesser cellularity which contained eosinophilic oedema fluid.
H & E X 110.

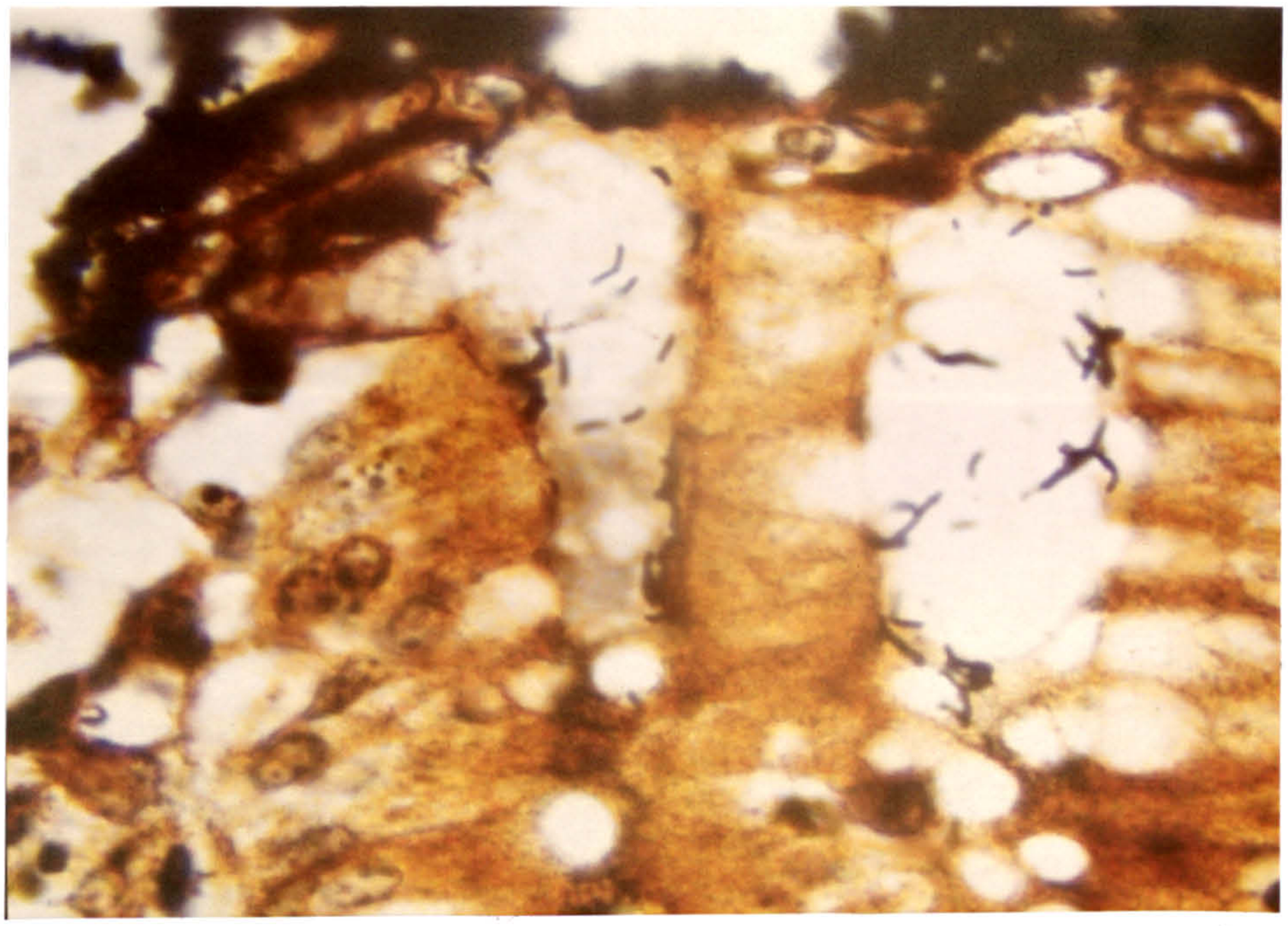


Fig. 44: Silver-stained histological section of the lumenal border and crypts of the colonic mucosa of calf 95.

Note the curved silver-stained rods present in the mouth of the crypts.

Young's X 1600.

neutrophilic and eosinophilic leucocytes were present in the lamina propria. Silver-stained bacteria were seen in these sections particularly in crypts (Figs. 45 and 46). The mesenteric lymph nodes resembled those in calf 95 (day 2).

Day 4 - Calf 100J

The changes seen resembled those in calf 94 (day 3) but the abomasal mucosa was eroded in many places. Silver-stained bacteria were found in the same locations as in calf 94 (day 3). The jejunal mucosa resembled that of calf 94 (day 3) but with erosion of epithelial surface and masses of cellular debris on the lumenal surface. The mucosa of the ileum resembled that of calf 94 (day 3) but with crypts containing inflammatory cells. Silver-stained bacteria were found in small numbers in the lamina propria where surface disruption had occurred.

The mucosa of the caecum and colon resembled those of calf 94 (day 3) but mild capillary dilatation was seen. Silver-stained bacteria were present in the same sites as in calf 94 (day 3). The mesenteric lymph nodes resembled those of calf 95 (day 2).

Day 5 - Calf 99J

The appearance of the abomasal mucosa resembled that of calf 94 (day 3) as did the mucosa of the jejunum and ileum but with the addition of crypts containing inflammatory cells. Silver-stained bacteria were found in the same locations as in calf 94 (day 3).

The mucosa of the caecum and colon resembled that of calf 100J (day 4). The mesenteric lymph nodes resembled those of calf 94 (day 3).

Day 6 - Calf 97J

The abomasal mucosa resembled that of calf 99J (day 5) but there was more cellular infiltration in the lamina propria. Silver-stained bacteria were found in the same locations as in calf 94 (day 3). The jejunal and ileal mucosa resembled that of calf 99J (day 5) but with more cellular infiltration in the lamina propria. Coccidial gametocytes were found in the ileal mucosa. Thin, spiral silver-stained bacteria were seen clearly (Fig. 47) in the brush border area of the mucosal

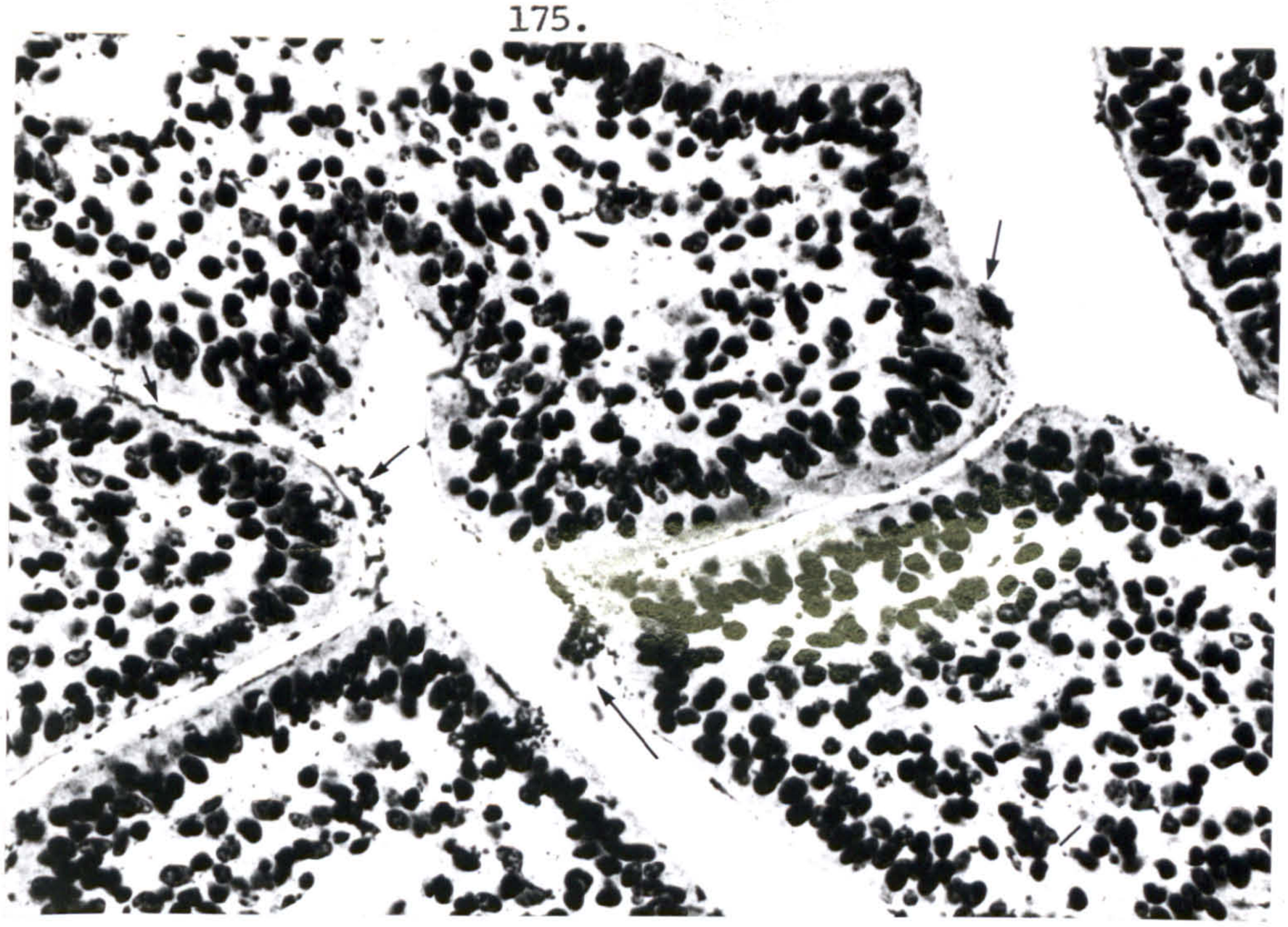


Fig. 45: Silver-stained histological section of the lumenal surface of the ileal mucosa of calf 94 three days post-infection.

Note the silver-stained material (arrows) closely adherent to the lumenal epithelium.

Young's X 400.

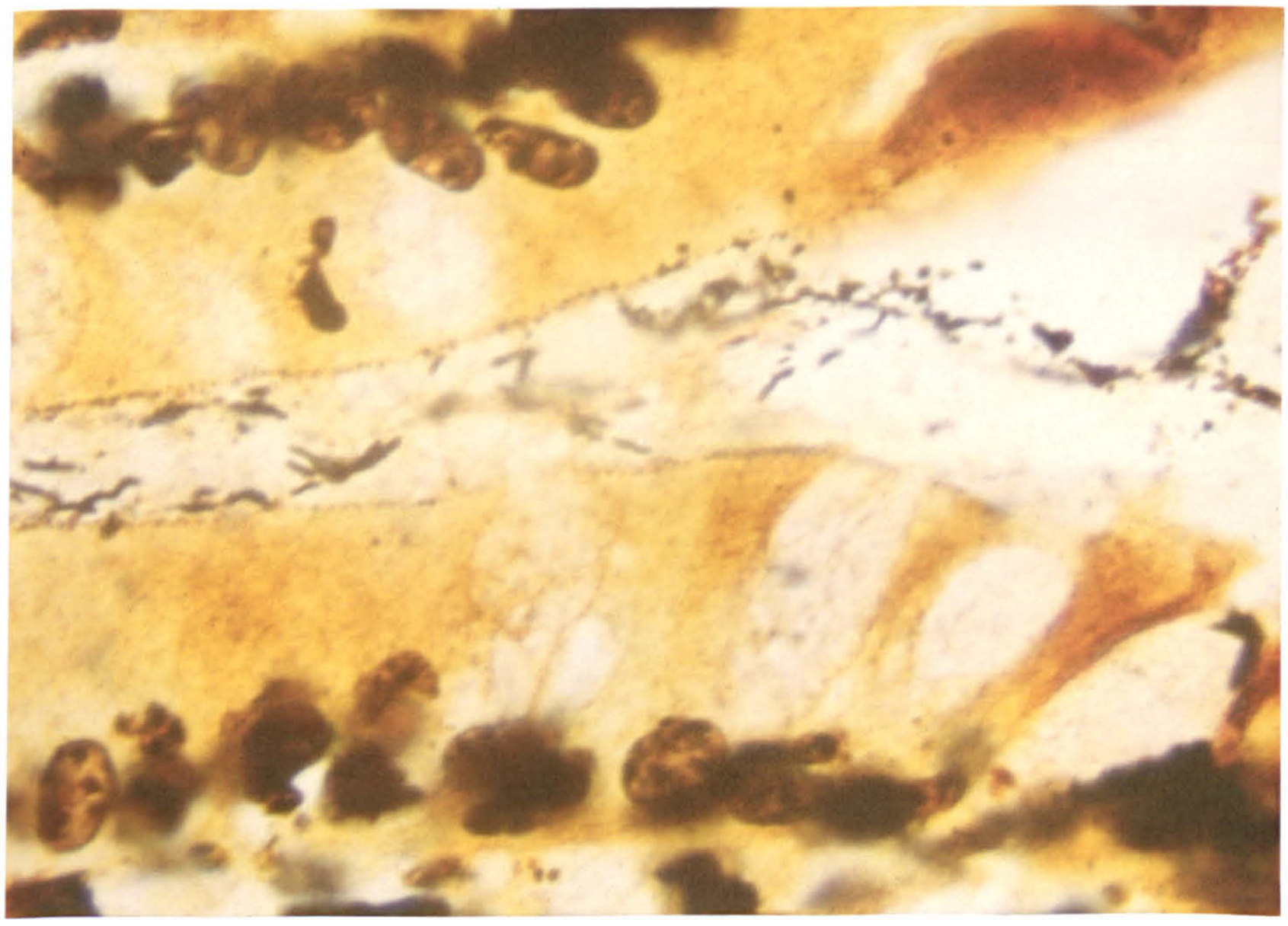


Fig. 46: Silver-stained histological section of the caecal mucosal surface of calf 94.

Note the presence of curved silver-stained microorganisms in the crypts.

Young's X 1600.

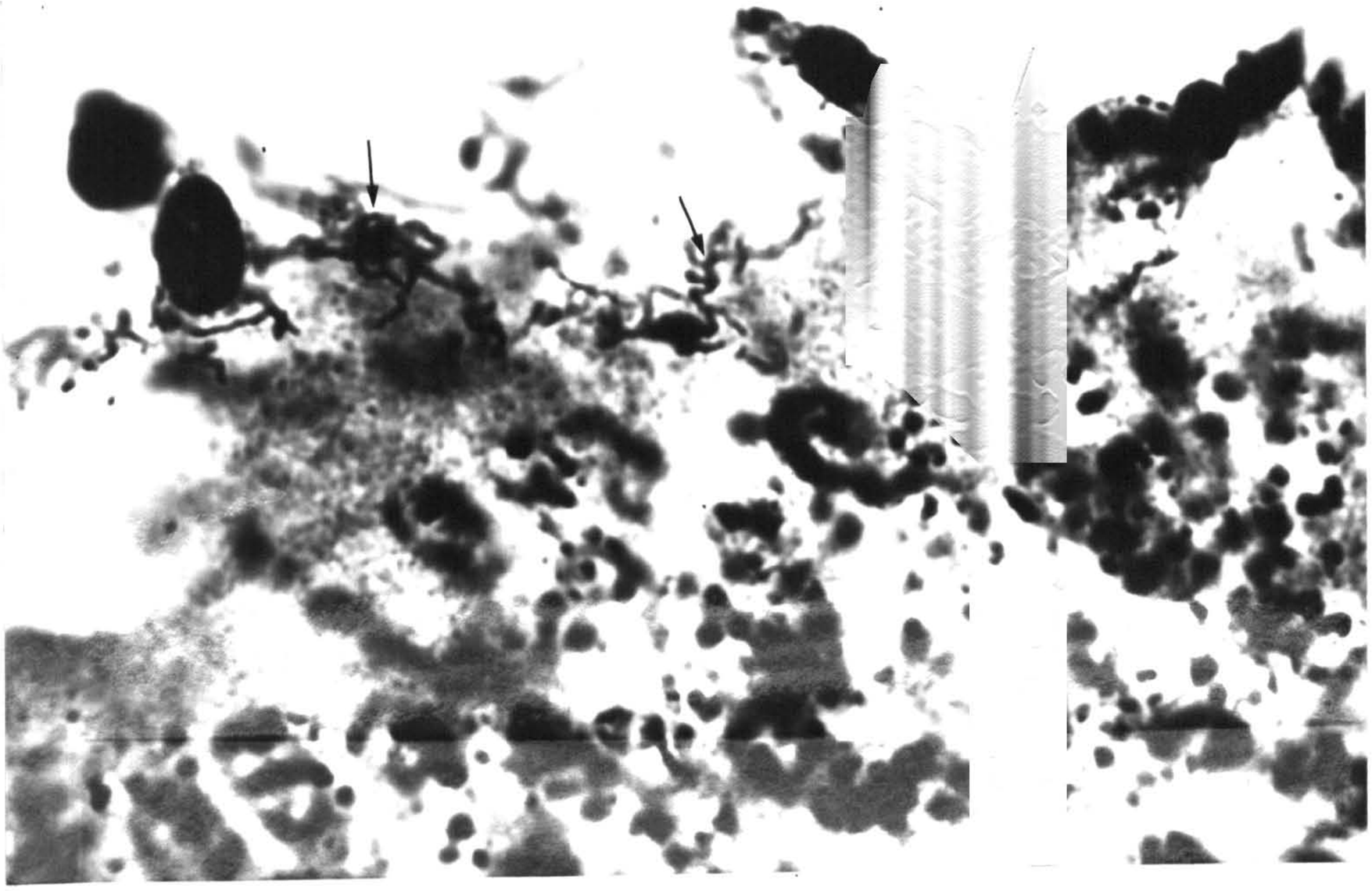


Fig. 47: Silver-stained histological section of the jejunal mucosa of calf 97J six days post-infection.

Note the presence of silver-stained spiral microorganisms on the mucosal epithelium (arrows).

Young's X 1500.

epithelial cells and in some crypts. They did not appear to penetrate the lamina propria.

The mucosa of the caecum and colon resembled that of calf 99J (day 5) but crypt abscesses were also present in the colonic mucosa. The mesenteric lymph nodes resembled those in calf 94 (day 3).

Control calves

Few histological changes were found in the controls. There was mild capillary dilatation in the abomasal mucosa of control animals (calf 92, day 1; calf 91, day 6). There was mild cellular infiltration of the remainder of the gastrointestinal tract and stunting of the jejunal villi in calf 92 (day 1). Coccidial gametocytes were found in the lamina propria of the jejunum and ileum in calf 91 (day 6).

No silver-stained curved rods or cocci could be seen in any parts of section of the jejunum and ileum of calf 92 (day 1); the abomasum, the jejunum and ileum of calf 93 (day 3), the abomasum, jejunum and the caecum of calf 91 (day 6) but a few silver-stained large cocci were seen closely adjacent to mucosal surface of ileum of calf 92 (day 1). Sections of the other areas could not be stained satisfactorily by Young's method.

Ultrastructural Studies

Electron microscopy was carried out to provide more information about the bacteria seen in the early stages of the study and their relationships with the tissues.

The thick (1μ) araldite sections confirmed the findings of the histological studies described above and thin sections from animals 98, 95, 94 and 100J were examined with the following results:

Bacteria were present in large numbers in the glands of the abomasal mucosa of animals 98, 95 and 94 (Fig. 48). Their morphology and dimensions did not suggest those of C.f.ss. jejuni. They were lying adjacent to epithelial cells but did not appear closely involved with them or to be within them.

In sections of the jejunum and ileum of animals 98, 95, 94 and 100J bacteria were very sparse in the thick sections and in thin sections

Fig. 48: Electron micrograph of bacteria in an abomasal gland of calf 95 two days following infection.

Note the many rod-shaped bacteria (B) and the filamentous flagellar material between them (F). Microvilli are sparse on the lumenal surface of the epithelial cells (M).

X 12,600

could not be found within epithelial cells or in contact with the brush border, even after extensive search. Many epithelial cells in sections from 95, 94 and 100J were abnormal with shortened microvilli and degenerating mitochondria (Fig. 49). In sections from the ileum of 94 neutrophils could be seen in the crypt lumens and in some cases contained bacteria with the approximate dimensions of C.f. ss. jejuni (Fig. 50).

In sections from the mucosa of the caecum and colon of 98 a few curved or spiral organisms were seen in the lumens of the crypts and on the lumenal surface of the mucosa (Fig. 51). In sections from the caecum and colon of 95, spiral bacteria were seen within the lumens of the crypts (Fig. 52) and changes in the epithelial cells of those crypts were obvious. These included discharge of goblet cells. These changes were more prominent in the caecal and colonic mucosa of 94 (Fig. 53) but organisms were considerably less common .

Colonies of C.f. ss. jejuni were isolated from the gastro-intestinal tract throughout the study but not from organs outside i.e. (lung, gall bladder, mesenteric lymph nodes and spleen) only in one animal killed one day post infection. The sites of isolation are given in Table 27.

Other bacteria such as E. coli and Streptococcus spp. were isolated from the large intestine of all animals. These bacteria were not isolated from the abomasum and small intestine particularly in the sites examined by electron microscopy and those which were silver-stained.

Gram-stained smears prepared from the mucosa of all levels of the gut from which C.f. ss. jejuni was isolated contained curved rods with the morphology of C.f. ss. jejuni. None were seen in similar preparations made from the controls. Gram-negative rods and Gram-positive cocci were also seen in smears from lesions made from the large intestine.

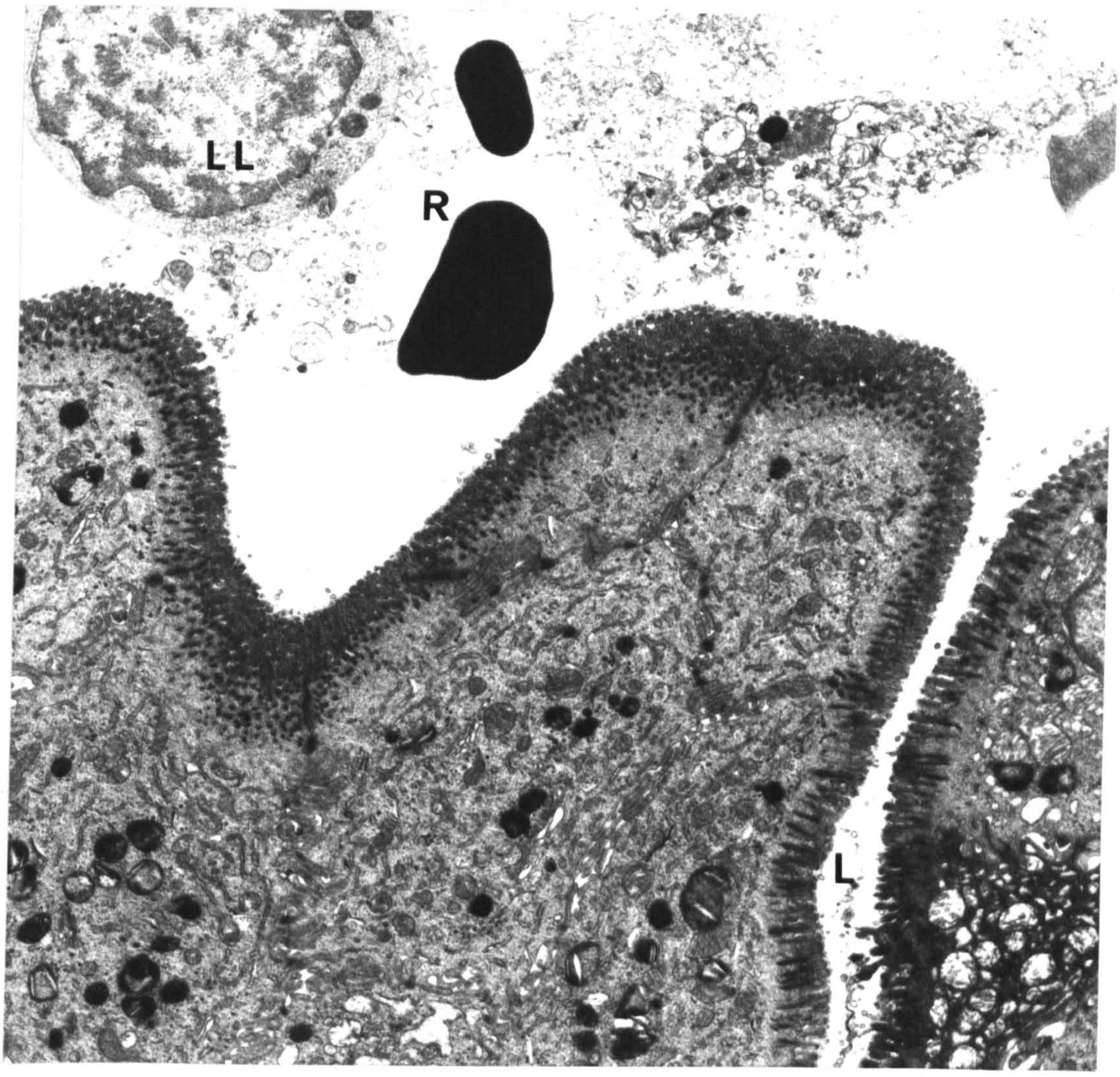


Fig. 49: Electron micrograph of the crypt epithelial cells of the ileal mucosa of calf 94 three days post-infection.

Note the presence of a small lymphocyte (LL) and red blood corpuscles (R). A damaged epithelial cell may be seen (bottom right). Cell debris is present in the crypt lumen (L).

X 8,000.

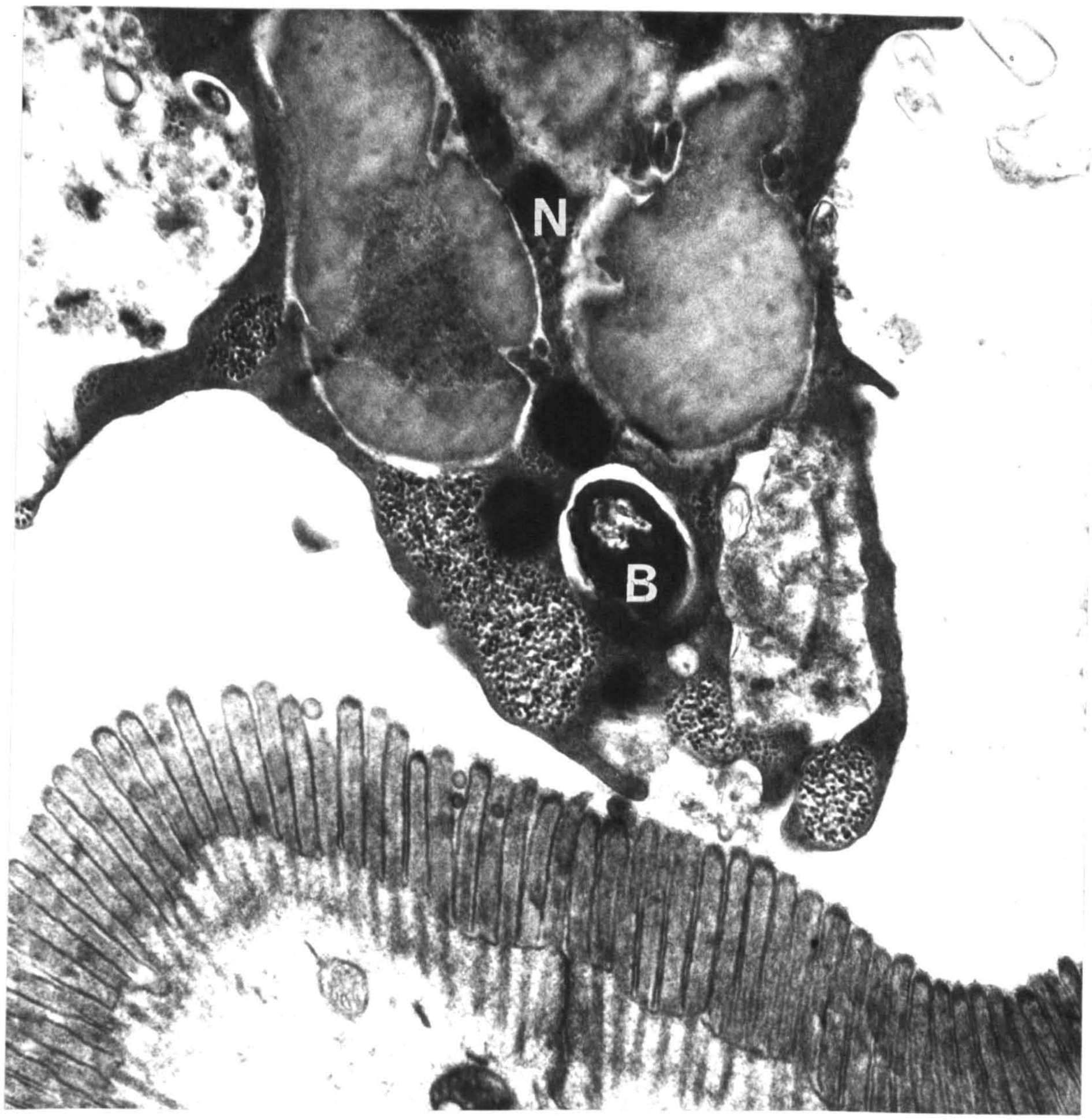


Fig. 50: Electron micrograph of a neutrophil containing a bacterium with the dimensions of C.f. ss. jejuni from a crypt in the ileal mucosa of calf 94.
Note bacterium (B) in a vacuole of the neutrophil (N).
X 30,000.

Fig. 51: Electron micrograph of spiral bacterium in a crypt of the caecal mucosa of calf 95.

Note the damaged microvilli of the epithelial cells (M) and the spiral bacterium (B) which is enlarged and inset below.

Figure X 12,6000, Inset X 50,000.

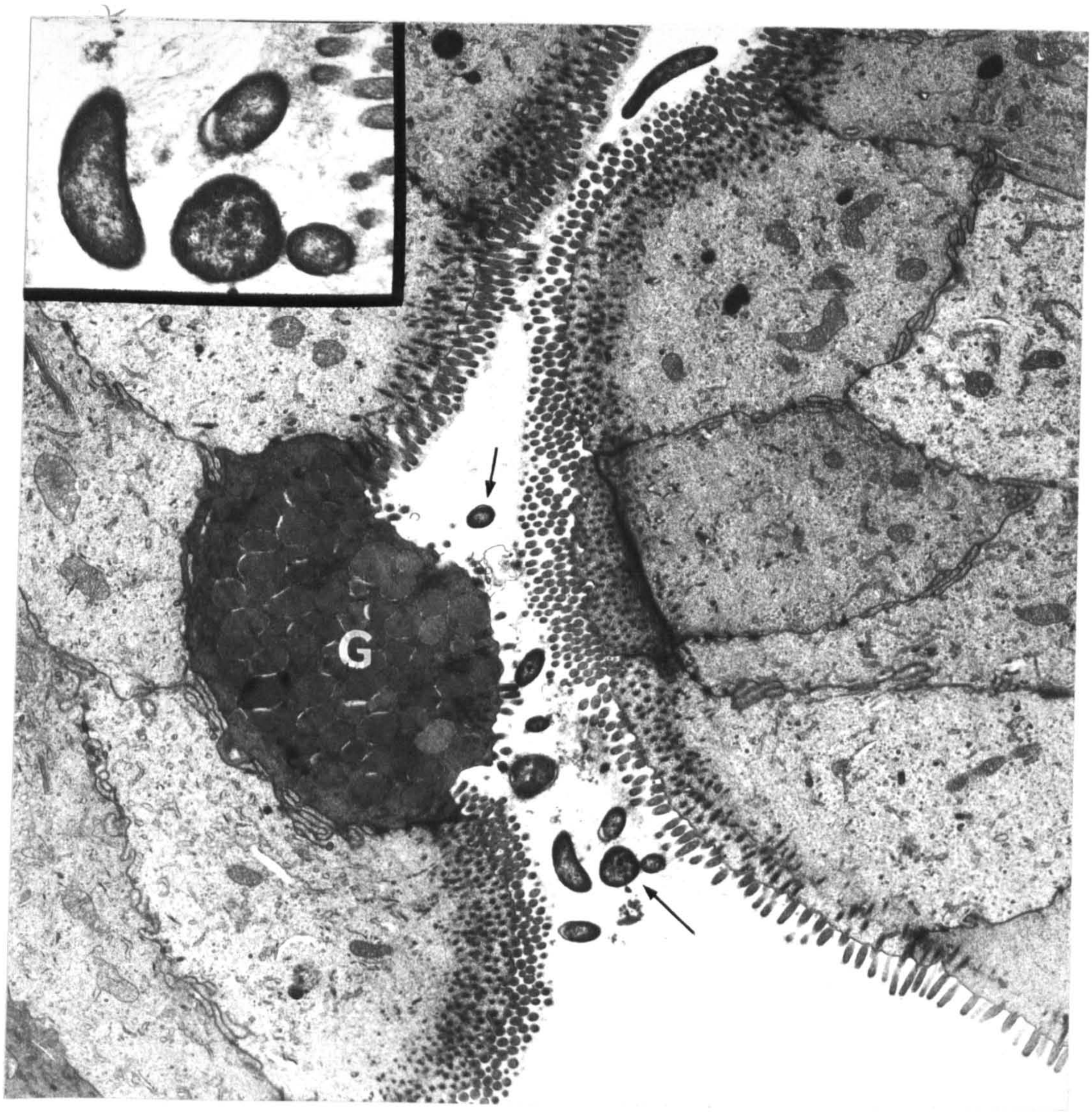


Fig. 52: Electron micrograph of bacteria in a crypt of the caecal mucosa of calf 95.

The bacteria (arrows) have a diameter of 0.3 µm. That of C.f. ss. jejuni is 0.2 - 0.5 µm.

A goblet cell (G) may be seen.

Figure X 8,000. Inset X 32,000.

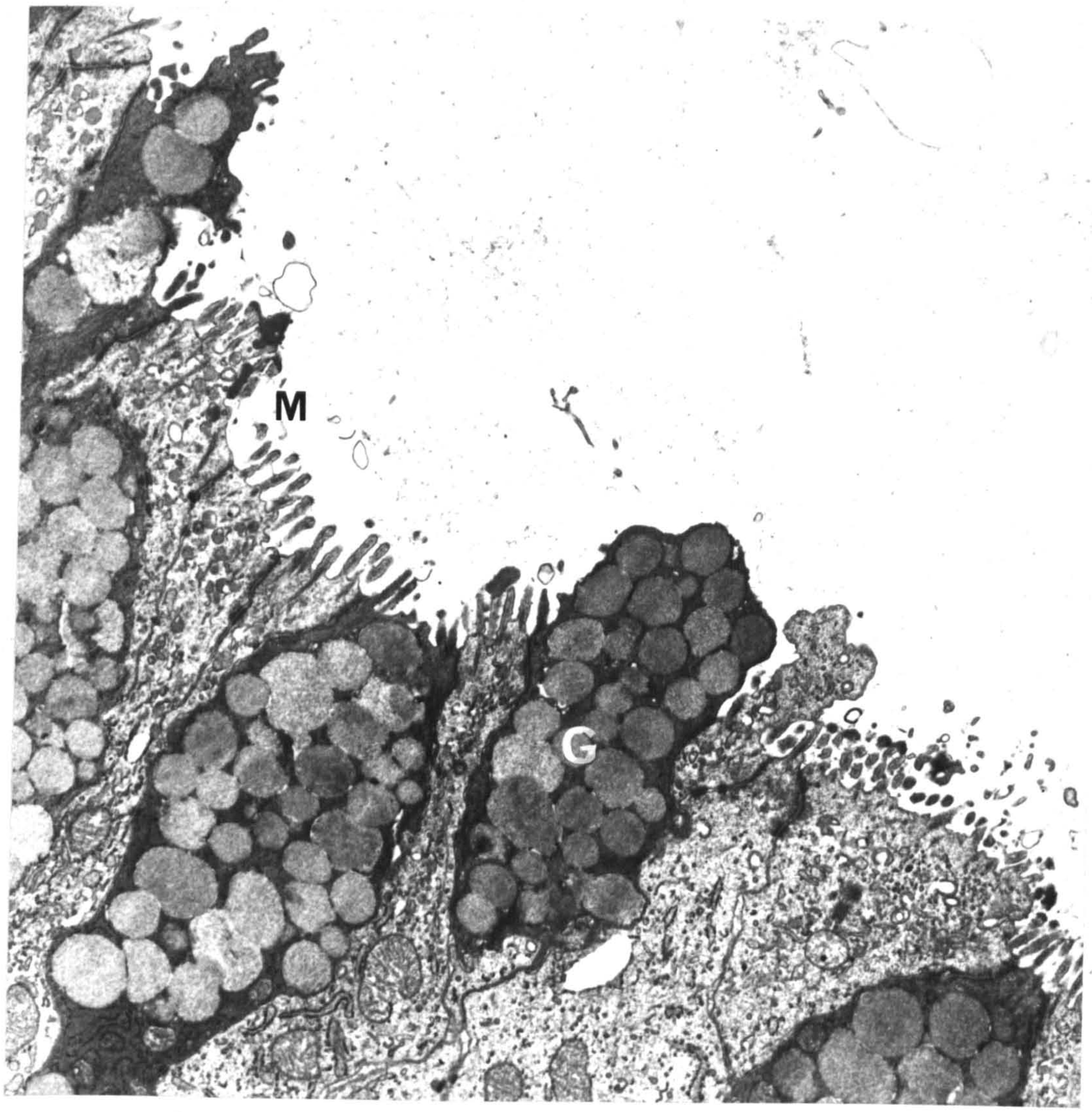


Fig. 53: Electron micrograph of cells lining crypts in the colonic mucosa of calf 94.

Note the sparse microvilli (M), the discharging goblet cell (G).

X 12,600.

Table 27.

Sites from which C.f.ss. jejuni was isolated from calves killed at daily intervals following infection with pure cultures of the organism in Experiment 4.

Site of isolation	Day of experiment 1		2	3		4	5	6	
	I(98)	C(92)	I(95)	I(94)	C(93)	I(100J)	I(99J)	I(97J)	C(91)
Abomasum	+++	-	+++	++	-	-	-	-	-
Jejunum	++	-	++	++	-	-	-	+	-
Ileum	++	-	++	++	-	++	++	+	-
Caecum	++	-	++	++	-	++	++	++	-
Colon	++	-	++	++	-	++	++	++	-
Mesenteric Lymph Node	+	-	+	-	-	-	-	-	-
Liver	-	-	-	-	-	-	-	-	-
Lung	+	-	-	-	-	-	-	-	-
Gall Bladder	++	-	-	-	-	-	-	-	-
Spleen	+	-	+	-	-	-	-	-	-
Kidney	-	-	-	-	-	-	-	-	-

I = Infected C = Control.

\+ = C.f.ss. jejuni isolated

\- = No C.f.ss. jejuni isolated

± = Less than 5 colonies seen

++ = 5-10 colonies seen

+++ = More than 10 colonies seen.

Antibody to C.f. ss. jejuni of the inocular strain could not be demonstrated in control animals. Agglutinating antibody to the inocular strain of C.f. ss. jejuni was present at titres of 1:160 in the sera of infected animals at slaughter three days post infection and at 1:640 in the other infected animals. The titres reached are shown in Table 28.

Table 28.

Levels of agglutinating antibody to the inocular strain of C.f. ss. jejuni in the sera of the animals in in Experiment 4.

Day of experiment	Infected						Control		
	1	2	3	4	5	6	1	3	6
Animal number	(98)	(95)	(94)	(100J)	(99J)	(97J)	(92)	(93)	(91)
Level of antibody	0	0	1:160	1:640	1:640	1:640	0	0	0

Study 3.

Adhesion studies with bacterial cultures and isolated intestinal brush borders

In this study an attempt was made to investigate the interaction between the host mucosal surface and C.f. ss. jejuni. C.f. ss. intestinalis, C. fecalis and Cl. sordellii were also studied for their ability to adhere to isolated brush borders in two studies.

Materials and methods

The isolates of the campylobacter species and of Cl. sordellii used in this study were those obtained from enteric lesions in the survey and used in the transmission studies described in Chapters 4, 5, 6 and 7.

β-haemolytic E. coli was used as positive control for this study. It was isolated from the diarrhoeic faeces of calf 61 which

formed one of the control groups in Experiment 2, Chapter 5. The bacteria under test were maintained as freeze-dried isolates at the passage numbers described in the appropriate chapters, but the E. coli was stored on slopes. Cultures were prepared for this test within one subculture of the stored state. Brush borders were obtained from two calves aged three - four months. In both studies, the calf was part of a respiratory disease experiment, had no enteric disease or lesions and was negative on culture for any of the organisms tested here. Lengths of ileum were used.

The method used was that of Sellwood et al. (1975) with minor differences in Study 2, in which the concentrations of brush borders and organisms used differed from the published method and are described below.

Horse blood agar plate cultures were inoculated overnight or for 48 hours at 37°C. The growth was suspended in Krebs-Henseleit buffer and washed twice with the same solution and finally resuspended in the buffer. These suspensions were standardised at tube 8 using Wellcome opacity tubes (Wellcome Reagents Ltd).

Brush borders were resuspended in the same buffer and also standardised at tube 8.

Results

The results of the first study were of interest. Bacteria were seen adhering to brush borders in preparations containing campylobacters and E. coli. The numbers of both bacteria and brush borders was very low and few bacteria were seen adhering to brush borders in the preparations made using the concentrations suggested by Sellwood et al. (1975).

The results of the second study using higher concentrations of both brush borders and bacteria were more clear cut. All three types of campylobacter readily adhered to the isolated brush border preparations when the test was performed at room temperature and 37°C.

Cl. sordellii did not show any adherence.

E. coli adhered to isolated brush borders in the numbers and with the appearance shown in the figures published by Sellwood et al. (1975). The campylobacters were adherent to but arranged more loosely around the brush borders than the E. coli. There was a marked contrast between these brush borders and those exposed to Cl. sordellii on which no organisms were seen. The latter resembled the negative control preparations.

DISCUSSION

The negative results of the cultural study of blood samples may have been due to technical factors but appeared to indicate that C.f. ss. jejuni was not present or present only in small numbers in the blood of animals after the development of clinical signs. This appeared to be confirmed in the pathogenesis study.

C.f. ss. jejuni was isolated from organs other than the gut only in animals killed within 48 hours of inoculation (98 and 95). This finding suggests that bacteraemia occurred early in the disease and was rare thereafter. Organs in which the organisms were found included the spleen and mesenteric lymph node, the gall bladder (from which the organism had been recovered in Experiment 2) and the lung. The presence of C.f. ss. jejuni in a lung lesion in animal 98 and its absence from similar lesions in other animals in the series was of interest. Systemic distribution was more marked in animal 98 than in animal 95 suggesting that any bacteraemia occurred within 24 hours of infection. Further studies including blood culture at regular intervals within the first 48 hours after infection might confirm this supposition. The source of these bacteria was probably the gut as inoculation was carried out by stomach tube and the inoculum rinsed out with a large volume of saline. Unless the organism was regurgitated with cud the exposure of the tonsils and pharynx to the inoculum was unlikely.

The clinical signs which followed infection in Experiment 4 resembled those seen in Experiments 1, 2 and 3.

C.f. ss. jejuni was isolated from the faeces of the inoculated animals at the same time as in the previous experiments (Table 26). Gross lesions were not seen in calf 98 killed 24 hours after infection but in calf 95 killed 48 hours post infection, mild changes in the consistency of the intestinal contents was seen. They were mucoid. The mesenteric lymph nodes were visibly enlarged and this enlargement was seen in all subsequent calves but not in the controls. Enteric lesions were visible on gross inspection in animal 94 killed on the third day after infection and were seen in all infected animals killed later in the

study. They were similar in most cases and are summarised below.

The abomasal mucosa and its content were normal with excess clear mucus, and the small intestine was flaccid with watery mucoid contents. The jejunal mucosa was thickened and hyperaemic. The ileal mucosa was flattened, thickened and hyperaemic with dark, greenish, fluid, mucoid contents. The caecal contents were soft and watery with excess clear mucus. The mucosa of the proximal end of the colon was slightly congested. The gall bladder of calf 94 and all subsequent animals was distended with bile. Hyperaemia was more prominent in the jejunal and ileal mucosa of calves 100J, 99J, 97J killed on days 4, 5 and 6 but the abomasal content was normal in the last calf 97J killed at 6 days. Ileal thickening became more prominent with time.

Although no gross lesions were seen in calf 98 (day 1) a few microscopical changes were seen. Mild capillary dilatation was seen at all levels of the gut and mononuclear cells plugged the abomasal glands. Stunted villi was seen in the jejunum. An inflammatory response was present in the lamina propria and a few neutrophilic polymorphonuclear leucocytes were present.

Similar but more severe changes were seen in the infected animals killed later in the study. Crypt abscesses were seen in both jejunum and ileum in calf 95 (day 2), in the ileum alone in calf 100J (day 4), in calf 99J (day 5) and in calf 97J. There was some erosion of the lumenal surface epithelium in all regions of the gut with an increase in cellular infiltration of the lamina propria from day 3 after infection until the end of the experiment. Silver-stained curved rods and cocci were prominent in the crypts of the abomasal mucosauntil the fourth day post infection. Silver-stained organisms appeared to lie within the small intestinal lamina propria and were also prominent at the mouths of crypts and on the surface of the lumenal epithelium in all infected animals. They appeared to be present inside epithelial cells but this may have been due to the thickness of the sections. They were particularly prominent in the jejunal mucosa of animal 97J (Fig. 47) although their presence there may have been related to coccidial infection which was also present. No lumenal epithelial erosion was seen in controls.

Electron microscopical examination showed that bacteria with dimensions resembling those of C.f. ss. jejuni were present adjacent to the brush border in some crypts particularly in the jejunum, ileum and large intestine and within neutrophils (Fig. 50). It was difficult to find evidence of penetration of the epithelium by organisms and the invasion seen in the silver-stained sections was not confirmed. These results differ from those of Butzler and Skirrow (1979) who noted invasion of cells in the caecal wall of experimentally infected chicks. The location of the organism largely within the lumen differs markedly from the relationship between the porcine intestinal epithelium and C.s. ssp. mucosalis described by Rowland and Lawson, (1974 and 1975b) Lawson et al., 1976. The identity of the bacteria was not confirmed as C.f. ss. jejuni, although the isolation of C.f. ss. jejuni from the sites at which bacteria were seen and their morphology suggests that they were indeed C.f. ss. jejuni. Their identity could be confirmed in future studies by immunofluorescence or immunoperoxidase. Cells at all levels of the crypt epithelium of infected animals appeared to be damaged although invasion was not seen. In particular, massive discharge of mucus from goblet cells was seen at day 3 (94) (Fig. 53) and in later cases. These findings suggest that the earliest pathological and clinical changes following infection with C.f. ss. jejuni are not associated with wide-spread invasion of the mucosa by the organism in cattle.

In contrast to the evidence for and against invasion discussed above, the results of the attempts to demonstrate the adhesion of C.f. ss. jejuni and other campylobacters to isolated brush border preparation suggested that adherence noted in sections could also occur in vitro but that it was looser than that associated with E. coli.

C.f. ss. jejuni was isolated from the gastrointestinal tract at all levels (Table 27). The distribution of the organism in the gut was of interest. As in Experiments 1, 2 and 3 described above, the organism was isolated in large numbers from the mucosa of the ileum, caecum and colon in every case even in animal 98 killed within 24 hours of infection. A striking feature of the distribution in this study was, however, its isolation in large numbers from the anterior gastrointestinal tract in the animals killed within three days of infection. In Experiments 1, 2

and 3 it had been isolated in small numbers and infrequently from these sites. This finding suggested that changes in the anterior part of the gastrointestinal tract might be just as important as those in the lower portion in the early part of the disease. This supposition was given some weight by the numbers of curved, spiral and rod-shaped organisms seen in Youngs stained and thick Araldite sections of the abomasum even though it was not possible to identify them unequivocally as C.f. ss. jejuni by electron microscopy.

Further evidence for the involvement of C.f. ss. jejuni in these changes is provided by the presence of agglutinating antibody to the inocular strain of C.f. ss. jejuni which was detected in the serum of infected animal 94 killed on the third day post infection and in all animals killed subsequently. The appearance of serum antibody followed the development of the inflammatory reaction in the crypts seen in day 2 (calf 95) and fully developed by day 3 (calf 94). The sequence of changes observed in this study and discussed are probably due to infection with C.f. ss. jejuni but coccidial gametocytes were present in the intestinal mucosa of infected animal 97J and control 91C killed on the sixth day. The presence of coccidial infection in both infected and control groups may have affected the pathological changes in this study but did not appear to have affected the clinical changes recorded (Table 26).

The results of this study provided further evidence that C.f. ss. jejuni is a primary pathogen of cattle and that it initiated the changes described above by close association with the mucosal epithelium but not, apparently, by extensive invasion of the mucosa. An element of doubt about this conclusion must remain as invasion may have been circumscribed in terms of time or location and may have been missed in the samples studied in detail.

Chapter 5.

Experimental infections with C.f. ss. intestinalis

Introduction

C.f. ss. intestinalis was isolated from six animals in the survey described in Chapter 3. It was isolated from lesions which resembled those from which C.f. ss. jejuni was isolated and, because this finding conflicted with the opinion of El Azhary (1968), who had considered it as a non-pathogen for the enteric tract, it was decided to test its pathogenicity for milk-fed and ruminating calves when given by the oral route in two experiments.

The strain of C.f. ss. intestinalis used was that isolated from the severely congested small intestinal mucosa of a 2-week old calf, number 75715. The isolate was cloned six times and was then freeze dried by the method described in Chapter 2. Cultures for inoculation were prepared from this freeze dried source after one further passage.

Experiment 5.

Objective: to study the pathogenicity of an isolate of C.f. ss. intestinalis for milk fed calves.

Materials and methods

Six milk-fed calves were used in this study. They formed part of the group used in Experiment 3 in Chapter 4. The three control animals P268, 97 and L321 were the same animals used as controls for the experimental infection with C.f. ss. jejuni in that experiment. The infected group of three animals (numbers 301, 322 and S26) were of similar age (3 weeks) to those used in Experiment 3, Chapter 4 and were also Ayrshire bull calves housed as described above. The examinations carried out in the pre-infection period on the faeces of the other calves (Chapter 4) were carried out on the faeces of this group with the exception of electron microscopical examination of negatively-stained faecal samples. Each calf was inoculated once with 20 ml of inoculum prepared by the method described above (Chapter 2)

and containing approximately 4.4×10^9 organisms per ml. All animals were examined daily and their appearance, appetite, respiratory rate, the rectal temperature and the consistency of their faeces were recorded.

Faecal samples from each animal were examined daily for the presence of C.f. ss. intestinalis and other bacteria by the methods described in Chapter 2. Colonies considered on morphological grounds to be those of campylobacters were confirmed as C.f. ss. intestinalis by the methods described in Chapter 2.

Serum samples were taken from each animal at the beginning and end of the experiment and stored according to the methods described in Chapter 2.

Serum samples were examined for the presence of agglutinating antibodies to the inocular strain of C.f. ss. intestinalis and to C.f. ss. jejuni using the methods described in Chapter 2.

The period of observation lasted 13 days and the animals were killed on the 14th day post-inoculation. Post-mortem examination was carried out as described for Experiment 3, Chapter 4. No examination for virus particles was carried out in the inoculated group in this study.

Results

No salmonellae, β-haemolytic E. coli or campylobacters were isolated from the faeces of these calves prior to infection. No coccidia or nematode eggs were reported as being present. Slight changes in the faecal consistency were noted on the second day post-infection in two animals (numbers 301, S26). The faeces became soft with excess clear mucus and some fresh blood within a few days of inoculation. The results of the faecal examinations are summarised in Table 29. Those of the control group are also given in Experiment 3, Chapter 4 (Table 22).

Rectal temperatures rose to 39.7°C on day 1 (animal number 322) and to 39.4°C on day 6 (animal number 301). Fever persisted at a higher level (40°C) in this animal on both day 7 and 8. In animal S26 the

Table 29.

Changes in faecal consistency in Experiment 5 following the inoculation of milk fed calves with pure cultures of C.f. ss. intestinalis and the isolation of the organism from their faeces.

Calf No.	Inf-ected	Day of experiment 0	1	2	3	4	5	6
301	+	F	F	FM	SM	FgM	FM	Fg
		-	+	+	+	+	+	+
322	+	F	F	F	F	F	F	FMB
		-	+	+	+	+	+	+
S26	+	F	F	FM	SM	FMB	DM	FM
		-	+	+	+	+	+	+
P268	-	F	F	F	F	GP	F	F
		-	-	-	-	-	-	-
97	-	F	F	F	F	F	F	F (K)
		-	-	-	-	-	-	-
L321	-	F	F	F	F	F	gP	F
		-	-	-	-	-	-	-

Calf No.	Inf-ected	7	8	9	10	11	12	13	14
301	+	SMG	DMB	F	F	SM	DY	DY	K
		+	+	-	+	+	+	-	
322	+	FM	F	F	F	FM	F	FMB	K
		-	+	-	+	-	+	-	
S26	+	F	FM	FM	F	FM	FM	FM	K
		+	+	-	+	-	+	-	
P268	-	F	gP	F	F	F	F	F	K
		-	-	-	-	-	-	-	
L321	-	F	F	F (Died)					
		-	-	-					

F = Firm faeces
M = Presence of mucus
g = Greyish faeces
S = Soft faeces
D = Diarrhoea
P = Pasty faeces.

B = Presence of blood
Y = Yellowish faeces
G = Greenish faeces
K = Killed
\+ = C.f. ss. intestinalis isolated
\- = No C.f. ss. intestinalis isolated.

rectal temperature was 39.4°C only on day 5 post-inoculation. The results are shown in Figure 54. Dullness was noted in the inoculated animals on days 10 (animals 322 and S26) and on day 12 (animal 301).

The condition of the control animals has been described in detail under Experiment 3, Chapter 4 above.

No nematode eggs or coccidial oocysts were seen in the faeces of the experimental animals during the experiment. Salmonella spp. and β-haemolytic E. coli were not isolated.

C.f. ss. intestinalis was isolated from the faeces of all the infected animals from the first day after inoculation but was not isolated from the faeces of every calf each day. The results are recorded in Table 29. No campylobacters were isolated from the control animals

At post-mortem examination, gross changes were noted only in the gastrointestinal tracts of the infected animals. Localised congestion of the abomasal mucosa of infected animal (322) was seen. Congested areas 1 cm x 1 cm and pyloric ulcers were seen in the mucosal abomasum of infected animal (301).

Hyperaemic areas 1 cm x 1 cm were present in the jejunal mucosa of infected animal 301. The jejunum was flaccid in animals 322 and S26 but the mucosa of jejunum of both animals appeared normal. The wall of the jejunum of calf S26 was thickened. The contents of the jejunum of all three infected animals was watery and mucoid. The ileum of 322 and S26 was flaccid and the mucosa of the ileum was slightly congested and had a thickened wall (animals 322 and S26) with watery, mucoid contents. Patches of congestion were seen in the ileal mucosa of 301.

The contents of large intestines of animals 322 and S26 were slightly mucoid and the mucosa was slightly congested in both animals. The gall bladder was enlarged and distended with bile in animal 301. The mesenteric lymph nodes were enlarged, pale and oedematous when sectioned in all three animals.

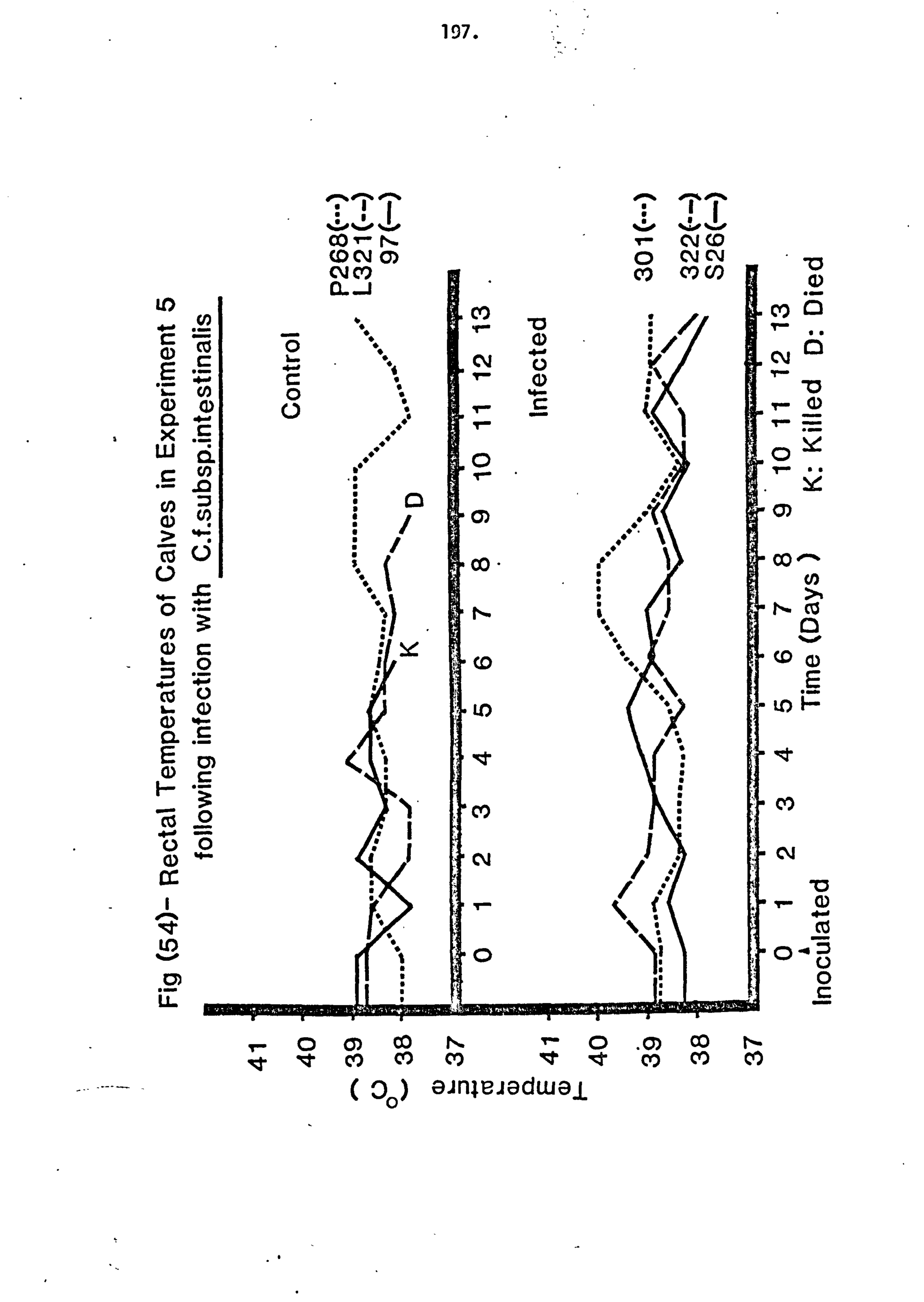


Fig (54)- Rectal Temperatures of Calves in Experiment 5 following infection with C.f.subsp.intestinalis
Control
Infected
P268(...)
L321(-.-)
97(—)
301(...)
322(-.-)
S26(—)
K
D
K: Killed D: Died
Time (Days)
Inoculated
Temperature (°C)
41
40
39
38
37
0 1 2 3 4 5 6 7 8 9 10 11 12 13

The post-mortem findings in the control group are given in detail in Experiment 3, Chapter 4 above. The histological changes in the jejunum, ileum, caecum and colon of the infected group resembled those seen in the group infected with C.f. ss. jejuni (Experiment 3, Chapter 4). Changes included cellular infiltration of the jejunal lamina propria. The most obvious changes were seen in the ileal mucosa and included stunted villi, disruption of the lumenal epithelial surface and crypt abscesses (Fig. 55). The lamina propria was thickened and contained many eosinophilic cells and lymphocytes. A few plasma cells and neutrophil polymorphonuclear leucocytes were seen. Lymphoid accumulation was prominent in the submucosa of all the infected animals. Dilatation of the capillaries and localised accumulations of lymphocytes and plasma cells and a few neutrophil polymorphonuclear leucocytes were seen in the caecal and colonic mucosa of the infected animals. Changes such as oedema, slight congestion, active germinal centres and the presence of neutrophilic polymorphonuclear leucocytes were seen in the mesenteric lymph nodes of the infected group.

Colonies of C.f. ss. intestinalis were isolated from the mucosa of the abomasum, jejunum, ileum, caecum, colon, mesenteric lymph node, liver and gall bladder of the inoculated animals. Theresults are described in Table 30. Gram-stained smears prepared from the mucosa of the organs contained curved rods with the morphology of campylobacters.

Antibody to C.f. ss. intestinalis of the inocular strain could not be demonstrated in serum samples taken from the inoculated animals at the beginning of the experiment or in the serum from the control animals at the beginning of the experiment or at slaughter.

Agglutinating antibody to the inocular strain of C.f. ss. intestinalis was present at titres of up to 1:320 in the sera of the infected animals at slaughter 14 days after infection. The results are shown in Table 31. No antibody to the isolate of C.f. ss. jejuni used in the C.f. ss. jejuni infected group was found in any of the sera of animals used in this experiment.

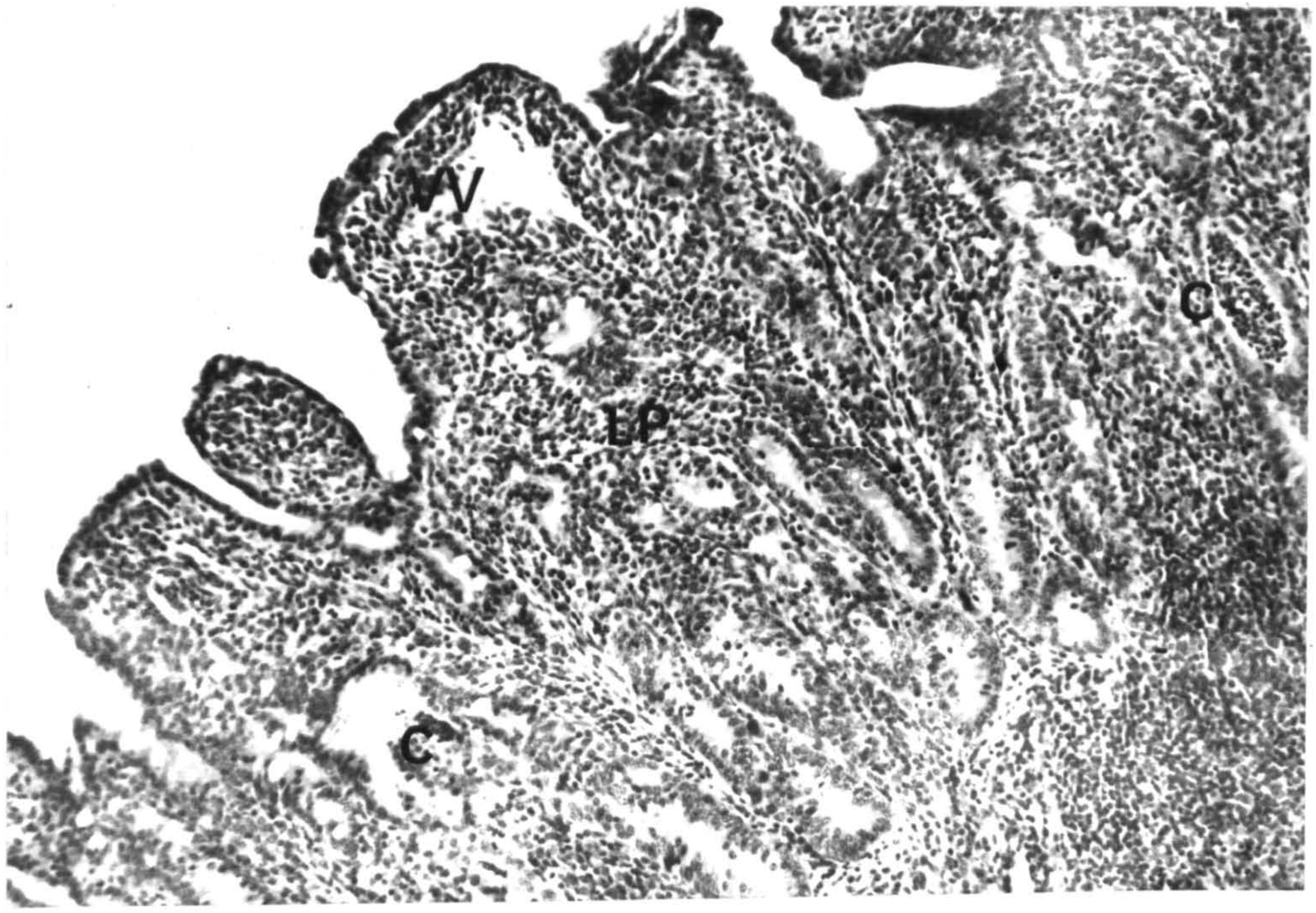


Fig. 55: Histological section of the ileal mucosa of calf 301 killed at 14 days following infection with C.f.ss. intestinalis.

Note the lowered villi (VV), the presence of a crypt abscess (C) and the hyper-cellularity of the lamina propria (LP).

H & E X 110.

Table 30.

Sites from which C.f. ss. intestinalis was isolated in experimental calves killed following infection with pure cultures of the organism in Experiment 5.

Site of isolation	Animal number					
	Infected			Control		
	301	322	S26	P268	97	L32
Abomasum	-	-	+	-	-	-
Jejunum	+	-	+	-	-	-
Ileum	+	+	+	-	-	-
Caecum	+	-	+	-	-	-
Colon	+	+	+	-	-	-
Mesenteric Lymph Node	+	-	-	-	-	-
Liver	+	-	-	-	-	-
Lung	-	-	-	-	-	-
Gall Bladder	+	+	+	-	-	-

+ = C.f. ss. intestinalis isolated. - = No C.f. ss.intestinalis isolated.

Table 31.

Levels of agglutinating antibody to the inocular strain of C.f. ss. intestinalis in the sera of the animals in Experiment 5.

Animal Number	Infected	Titre present on Day 0	Day 14
301	+	0	1:640
322	+	0	1:320
S26	+	0	1:320
P268	-	0	0
97	-	0	0 (day 6)
L321	-	0	0 (day 9)

Experiment 6.

Objective: to confirm that the isolate of C.f. ss. intestinalis used in Experiment 5 was pathogenic to ruminating calves.

Materials and methods

A group of five 4-month old Ayrshire calves was purchased from the market and divided into two groups, one of three calves numbered 63, 64 and 65 (inoculated) and one of two calves numbered 61 and 62 (control). Each group was monitored prior to infection, housed separately, maintained and treated in the same way as the ruminating animals used in the previous experiments.

Prior to infection the faeces of the animals in this experiment were examined for the presence of campylobacters, β-haemolytic E. coli, salmonellae, other bacteria and parasites by the methods described in Chapter 2. No virological examination was carried out.

The inoculum was prepared by the same method as in Experiment 5 and contained approximately 5.2×10^9 organisms per ml. All animals were examined daily and their appearance, appetite, respiratory rate, rectal temperature and the consistency of their faeces were noted. Faecal samples were examined daily for the presence of C.f. ss. intestinalis and other bacteria by the methods described in Chapter 2. Colonies resembling those of campylobacters were confirmed as C.f. ss. intestinalis by the methods described in Chapter 2.

Negatively-stained preparations of faeces from animals 61 and 62,were examined by electron microscopy for the presence of virus particles on day 18.

Serum samples were taken from each animal at the beginning and end of the experiment and stored as described in Chapter 2. Serum samples were examined for the presence of agglutinating antibodies to the inocular strain of C.f. ss. intestinalis and to C.f. ss. jejuni using the methods described in Chapter 2.

The period of observation lasted 20 days and the animals were killed on the twenty-first day post-inoculation. Post-mortem

examination was carried out as described in Chapter 2.

Results

No salmonella, β-haemolytic E. coli or campylobacters were isolated from the faeces of these calves prior to infection. No coccidia or nematode eggs were reported as being present.

Changes in faecal consistency were noted on the first day following inoculation in all three inoculated animals. All infected calves passed soft faeces with excess clear mucus in which fresh blood could be seen. The faeces later became very soft, and dark in colour. These changes persisted throughout the experiment. (Table 32).

Rectal temperatures were elevated in all three inoculated calves and reached 40°C in animal number 63 on the second day post-inoculation. Rectal temperatures remained raised in the inoculated group for the majority of the study (Fig. 56) and a transient rise in body temperature also occurred in control animal 61 during the period of diarrhoea associated with the isolation of β-haemolytic E. coli.

The faeces of the two control animals became soft with some traces of mucus on day 16 (animal 61) and on day 18 (animal 62). The faeces later became watery, pale yellow in colour with excess clear mucus and on day 20 became firm again with some mucus.

The inoculated animals appeared dull for three days post-inoculation. Nasal discharge and coughing was noted in all infected and control calves.

β-haemolytic E. coli was isolated from both of the control animals on days 16 and 18 and was isolated from these animals daily until the end of the experiment. Rotavirus particles were seen in the faeces of control animal 61 on day 18. No nematode eggs or coccidial oocysts were seen in the faeces of the experimental animals during the entire period of the experiment. Salmonella spp. was not isolated. C.f. ss. intestinalis was isolated from the faeces of all the infected animals following inoculation (Table 32).

Table 32.

Changes in faecal consistency in Experiment 6 following the inoculation of calves with pure cultures of C.f. ss. intestinalis and the isolation of the organism from their faeces.

Calf No.	Inf-ected	Day of experiment 0	1	2	3	4	5	6	7	8	9	10
63	+	F	SM	SM	DM	SM	SM	F	FMB	F	F	FM
		-	+	+	+	+	+	+	+	+	+	+
64	+	F	SMB	SMB	DM	DM	DM	DM	SM	SM	SM	SM
		-	+	+	+	+	+	+	+	+	+	+
65	+	F	SM	SM	FM	SM	DM	F	DM	FM	F	FM
		-	+	+	+	+	+	+	+	+	+	+
61	-	F	F	F	F	F	F	F	F	F	F	F
		-	-	-	-	-	-	-	-	-	-	-
62	-	F	F	F	F	F	F	F	F	F	F	F
		-	-	-	-	-	-	-	-	-	-	-

Calf No.	Inf-ected	11	12	13	14	15	16	17	18	19	20	21
63	+	PM	DM	FM	FM	FM	DM	SM	F	FM	F	K
		+	+	+	-	-	+	+	-	+	+	
64	+	PM	SM	FM	F	F	SM	SM	SM	F	F	K
		+	+	+	-	+	+	+	+	+	+	
65	+	SM	DM	SM	SM	FM	PM	FM	FM	PM	F	K
		+	+	+	+	+	+	+	-	+	+	
61	-	F	FM	F	F	F	SM	F	DM	DM	FM	K
		-	-	-	-	-	-*	-*	-*	-*	-*	
62	-	F	FM	F	F	F	F	F	SM	F	FM	K
		-	-	-	-	-	-	-	-*	-*	-*	

F = Firm faeces
S = Soft faeces
D = Diarrhoea
B = Presence of blood
M = Presence of mucus

P = Pasty faeces
\+ = C.f. ss. intestinalis isolated
\- = No C.f. ss. intestinalis isolated
* = β-haemolytic E. coli isolated.

Fig (56)– Rectal Temperatures of Calves in Experiment 6 following infection with C.f.subsp.intestinalis

Control

Infected

62(—)
61(···)

65(—)
64(--)
63(···)

Temperature (^{o}C)

41 40 39 38 37

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Inoculated

Time (Days)

Organisms with the morphology of campylobacters could be seen in Gram-stained smears of faeces of the infected animals but not in those from the controls. At post-mortem examination, the two control animals were found to be macroscopically normal with the exception of a slight excess of clear mucus in the contents of the ileum of animal 61 and slight congestion of its ileal mucosa. The contents of its jejunum were pale, watery and yellowish in colour, as were those of the ileum of animal 62.

A number of changes were noted in the gastrointestinal tracts of the inoculated animals. Slight congestion of the abomasal mucosa was noted in animal 65. Thickening of the small intestinal mucosa was found in infected animals 63 and 65. The jejunal contents were dark in colour, fluid and mucoid in consistency (with the exception of animal number 64 in which they were khakhi in colour) and the jejunal mucosa was slightly congested in animal 65. In all animals of the infected group the most obvious changes were seen in the ileum. It was flaccid with dark, fluid mucoid contents and a thickened and fleshy wall. The mucosa appeared mildly congested. The mucosa of the caecum of animal 65 was slightly congested as was the colonic mucosa of animals 63 and 64. The contents of the large intestines of the three infected animals were fluid and mucoid in consistency. The colonic contents of control animal 62 were similar in appearance. The mesenteric lymph nodes were enlarged, pale and oedematous when sectioned in animals 63, 64,65 (infected) and 61 (control). Haemal lymph nodes were prominent in infected animals 63 and 65.

Localised pneumonic areas were present in individual lung lobes in all control and infected groups.

The histological changes found in this experiment resembled those described in Experiment 1. The abomasal mucosa was slightly thickened with localised accumulation of eosinophils and mononuclear cells, mainly lymphocytes. The jejunal mucosa was also thickened and contained large numbers of mononuclear cells, mainly lymphocytes and macrophages with a few plasma cells and some neutrophilic polymorphonuclear leucocytes. The villi were stunted. Slight dilatation

of the capillaries and prominence of the lacteals was seen in infected animals 64 and 65. The villous epithelium was disrupted in a few places. Coccidial gametocytes were prominent in all infected animals. Similar changes were seen in the ileal mucosa in which some crypt abscesses were seen and in which the submucosal lymphoid tissue was prominent. There was slight hyperaemia and erosion of epithelial surfaces in some places in the colonic mucosa of all the inoculated animals. Milder changes were seen resembling those described above in both the small and large intestinal mucosa of the controls. Coccidial oocysts were also prominent in the jejunal mucosa of these animals.

Histological changes similar to those described in Experiment 5 were found in the mesenteric lymph nodes of all animals. Colonies of C.f. ss. intestinalis were isolated from the mucosa of the jejunum, ileum, caecum, colon and gall bladder of the inoculated group. The results are summarised in Table 33.

Gram-stained smears prepared from the mucosa of these animals contained curved rods with the morphology of campylobacters.

β-haemolytic E. coli was isolated from the jejunum of calf 61 and from the ileum, caecum and colon of animals 61 and 62 (controls). None were recovered from the inoculated group.

Antibody to C.f. ss. intestinalis of the inocular strain could not be demonstrated in serum samples taken from the inoculated animals at the beginning of the experiment or in the sera from the control animals at slaughter. Agglutinating antibody to the inocular strain of C.f. ss. intestinalis was present in the sera of infected animals taken at slaughter on 21 days post infection. The results are shown in Table 34. No antibody to C.f. ss. jejuni of the strain used in Chapter 4 could be detected in any of the sera.

Table 33.

Sites from which C.f. ss. intestinalis was isolated in experimental calves killed following infection with pure cultures of the organism in Experiment 6.

Site of Isolation	Animal number				
	Infected			Control	
	63	64	65	61	62
Abomasum	+	+	-	-	-
Jejunum	+	+	+	-	-
Ileum	+	+	+	-	-
Caecum	+	+	-	-	-
Colon	+	+	+	-	-
Mesenteric Lymph Nodes	-	-	-	-	-
Liver	-	-	-	-	-
Lung	-	-	-	-	-
Gall Bladder	-	+	-	-	-

+ = C.f.ss. intestinalis isolated. - = No C.f.ss. intestinalis isolated

Table 34.

Levels of agglutinating antibody to the inocular strain of C.f. ss. intestinalis in the sera of the animals in Experiment 6.

Animal Number	Infected	Titre present on Day 0	Day 21
63	+	0	1:320
64	+	0	1:320
65	+	0	1:160
61	-	0	0
62	-	0	0

DISCUSSION

The results of these two experiments indicated that the milk-fed and ruminated calves were susceptible to infection with C.f. ss. intestinalis. It was capable of initiating fever and faecal changes which included softening and the presence of excess clear mucus and streaks of blood.

The syndrome produced resembled closely that which followed the inoculation of milk-fed and ruminant calves with pure cultures of C.f. ss. jejuni. The faecal changes were similar but the fever noted was lower and was later in onset.

The incubation period for this syndrome varied from one - six days in these two studies. The raised rectal temperature (up to 39.6°C) persisted for a number of days in most of the inoculated animals. (Figs. 54 and 56) and the faecal changes for the duration of the experiment in Experiment 5 (13 days) and in a milder form until day 19 in Experiment 6). These faecal changes are summarised in Fig. 57.

C.f. ss. intestinalis was isolated from the faeces of the inoculated animals in these two studies. Its isolation followed inoculation and it was isolated frequently until the end of each experiment. It was never isolated from the faeces of animals before inoculation or from the controls but it was not cultured consistently from every animal on every day of the experiment (Tables 29 and 32). As in the experiments described in Chapter 4, it was not clear whether this failure to isolate the organism on every occasion was for technical reasons or whether it was absent on the occasions on which it was not recovered.

The macroscopic changes seen at post-mortem examination were confined to the gastrointestinal tract and the associated lymph nodes with the exception of incidental pneumonic changes.

Hyperaemic areas of the jejunal and ileal mucosa were associated with the isolation of C.f. ss. intestinalis and campylobacters were seen in smears. The most obvious finding was thickening of the ileal

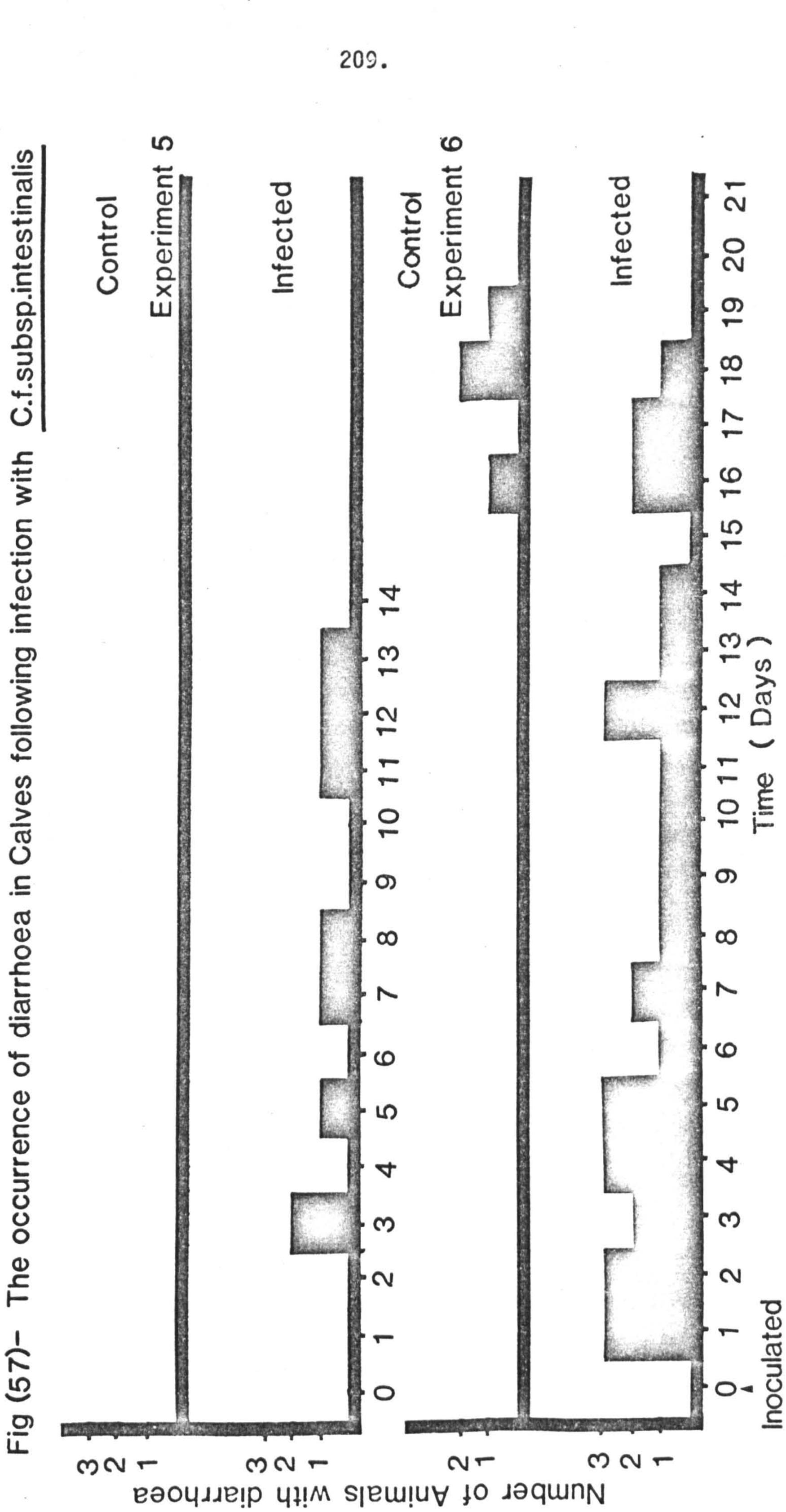


Fig (57)– The occurrence of diarrhoea in Calves following infection with C.f.subsp.intestinalis
Experiment 5
Control
Infected
Experiment 6
Control
Infected
Number of Animals with diarrhoea
Time (Days)
Inoculated

mucosa. These changes were not seen in the control animals with the exception of mild congestion of the ileal mucosa in animal 61 (Experiment 6), and P268 (Experiment 5). In most cases, the large intestinal contents were slightly mucoid.

In all cases the abomasal mucosa was normal in appearance with the exception of slight congestion of the mucosa in animals 322 (Experiment 1) and 65 (Experiment 2).

The histological findings in all inoculated animals resembled these described in Chapter 3 in C.f. ss. jejuni infection. C.f. ss. intestinalis was recovered from the gastrointestinal tract and from the gall bladder in the inoculated animals (Experiment 5) and from inoculated animal 64 (Experiment 6) as in the C.f. ss. jejuni Experiment 2 (Chapter 4). It was also isolated from the liver and mesenteric lymph nodes of one inoculated animal (301, Experiment 5) in contrast to the findings with C.f. ss. jejuni in Experiments 1 - 3. The presence of the organism in the gall bladder, mesenteric lymph nodes and liver in these two experiments may suggest that systemic spread of C.f. ss. intestinalis occurs at some stage in the pathogenesis of the infection.

These findings and their similarity to those resulting from infection with C.f. ss. jejuni suggest strongly that C.f. ss. intestinalis is capable of initiating the changes described. In confirmation of this, agglutinating antibody to the inocular strain of C.f. ss. intestinalis was only demonstrated in the sera of infected animals after inoculation. The failure to demonstrate agglutinating antibody to C.f. ss. jejuni indicates that infection with that organism or, at least, the strain used as antigen was not responsible for the changes seen.

Other agents were present in the experimental animals. In Experiment 5, the calves used formed part of a group in which reovirus infection had occurred. No clinical evidence for the presence of this virus was found in the infected group in Experiment 5 although the changes seen in the controls (Experiment 3, Chapter 4) may have been associated with such infection. No other possible causes of the syndrome seen were detected in Experiment 5.

In Experiment 6, nematode eggs and coccidial oocysts were not reported as present in the faeces prior to inoculation but at post-mortem examination histological evidence for coccidial infection was found in the jejunum of all five animals. The gross and histological changes observed in the small intestines of these animals may, therefore, have been partly due to coccidial infection. The changes, however, were more severe in the infected group and this coccidial infection did not appear to result in clinical signs in the control group (Table 32).

E. coli and rotavirus infection also occurred in the control animals (61 and 62) in this experiment as an identifiable incident beginning on day 16 and not affecting the inoculated group. These infections may have contributed to the alteration in their intestines and contents described above.

It seems, therefore, that infection with C.f. ss. intestinalis can initiate changes resembling those produced by C.f. ss. jejuni in both milk-fed and ruminating calves and should be regarded as a possible cause of the presence of blood and mucus in the faeces of both milk-fed and ruminating calves. It is also possible that it is responsible for the elevation of body temperature seen in the infected calves and that it initiated post-mortem changes similar to those caused by C.f. ss. jejuni and described in Chapter 4.

The results of the study are therefore at variance with those of El Azhary (1968) and cast further doubt upon the exact identity of "Vibrio jejuni" originally described by Jones and his co-workers. They underline the findings of the survey (Chapter 3) and Allsup and Hunter (1973) that C.f. ss. intestinalis can be isolated from lesions in the bovine enteric tract and clarify the problems of the pathogenicity of this sub-species. Some element of doubt about their pathogenicity may, however, remain, because of the possible role of predisposing factors such as coccidial or virus infections.

Chapter 6.

Experimental infections with C. fecalis

Introduction

C. fecalis was isolated from six animals in the survey described in Chapter 3. The lesions from which it was isolated resembled those from which C.f. ss. jejuni and C.f. ss. intestinalis were isolated in the survey. C. fecalis was considered by Firehammer (1965) and Smibert (1978) to be a non-pathogen, but in view of its isolation in the survey, it was decided to evaluate its pathogenicity for milk-fed calves and ruminant calves in two experiments.

The isolate used was from abomasal lesions in an 18-month old heifer (No. 75205, Chapter 3). It was cloned eight times at weekly intervals and then freeze-dried using the method described in Chapter 2. Cultures for inoculation were prepared after one passage from this freeze-dried source.

Experiment 7.

Objective: to determine the pathogenicity of an isolate of C. fecalis for milk-fed calves.

Materials and methods

Six milk-fed calves were used. They formed part of the group of calves used in Experiment 3 (Chapter 4). The three control animals, P268, 97 and L321 were the same animals used as controls for the experimental infection with C.f. ss. jejuni described above. The infected group of three animals (numbers 719, B91 and C192) were of similar age (3 weeks) and were also Ayrshire bull calves. The animals were monitored prior to infection, housed, fed and observed using the methods in the previous experiments and in Chapter 2.

Each calf was inoculated once with 20 ml of inoculum prepared by the method described above (Chapter 2) and containing approximately 8.0×10^{10} organisms per ml.

The animals were examined daily and their appearance, appetite, respiratory rate, the appearance of their faeces, the presence or absence of ruminal movements and their rectal temperature were recorded. Faecal samples from each animal were examined daily for the presence of bacteria by the methods, described in Chapter 2. Colonies considered on morphological grounds to be those of campylobacter were confirmed as C. fecalis by the methods described in Chapters 2 and 3.

Negatively-stained preparations of faeces from animal 719 were examined by electron microscopy for the presence of virus particles on the day of inoculation and six days post-infection.

Serum samples were taken from all animals at the beginning and end of the experiment. The sera were stored according to the methods described in Chapter 2, and examined for the presence of agglutinating antibody to C. fecalis and C.f. ss. jejuni by the methods described in Chapter 2. The period of observation lasted 13 days, the animals were killed on the 14th day and examined post-mortem by the methods described in Chapter 2.

Results

No salmonellae, β-haemolytic E. coli or campylobacters were isolated from the faeces of these calves prior to infection. No coccidia or nematode eggs were reported as being present in the faeces of the calves. Reovirus was present in the faeces of calf 719 during an episode of diarrhoea in the pre-infection observation period but was not seen in the samples taken on the day of inoculation.

Minor changes in faecal consistency were noted in the faeces of both inoculated and control animals. The faeces became pasty and pale in colour. The appearance of the faeces is summarised in Table 35. In one animal (719) the rectal temperature was 39.4°C on day 3 but in all the other animals, both inoculated and control, rectal temperatures failed to rise above 39°C. The details are shown in Fig. 58.

One animal (719) from the inoculated group developed ataxia and paralysis four days post-infection and was killed on day 6. The condition of the control animals has been described in detail in

Table 35.

Changes in faecal consistency in Experiment 7 following the inoculation of milk-fed calves with pure cultures of C. fecalis and the isolation of the organism from their faeces.

Calf No.	Inf-ected	Day of experiment 0	1	2	3	4	5	6	7
719	+	F	P	P	P	FB	F	PM (K)	
		-	+	+	+	+	+	-	
B91	+	F	F	P	F	F	F	P	F
		-	+	+	+	+	+	-	-
C192	+	F	P	F	P	F	P	F	P
		-	+	+	+	+	+	-	-
P268	-	F	F	F	F	GP	F	F	F
		-	-	-	-	-	-	-	-
97	-	F	F	F	F	F	F	F (K)	
		-	-	-	-	-	-	-	
L321	-	F	F	F	F	F	gP	F	F
		-	-	-	-	-	-	-	-

Calf No.	Inf-ected	8	9	10	11	12	13	14
B91	+	F	F	F	F	F	F	K
		-	+	-	+	+	-	
C192	+	P	F	P	PM	P	P	K
		-	+	-	-	+	-	
P268	-	gP	F	F	F	F	F	K
		-	-	-	-	-	-	
L321	-	F	F (Died)					
		-	-					

F = Firm faeces
P = Pasty faeces
M = Presence of mucus
G = Greenish faeces
g = greyish faeces
B = Presence of blood
\+ = C. fecalis isolated
\- = No C. fecalis isolated

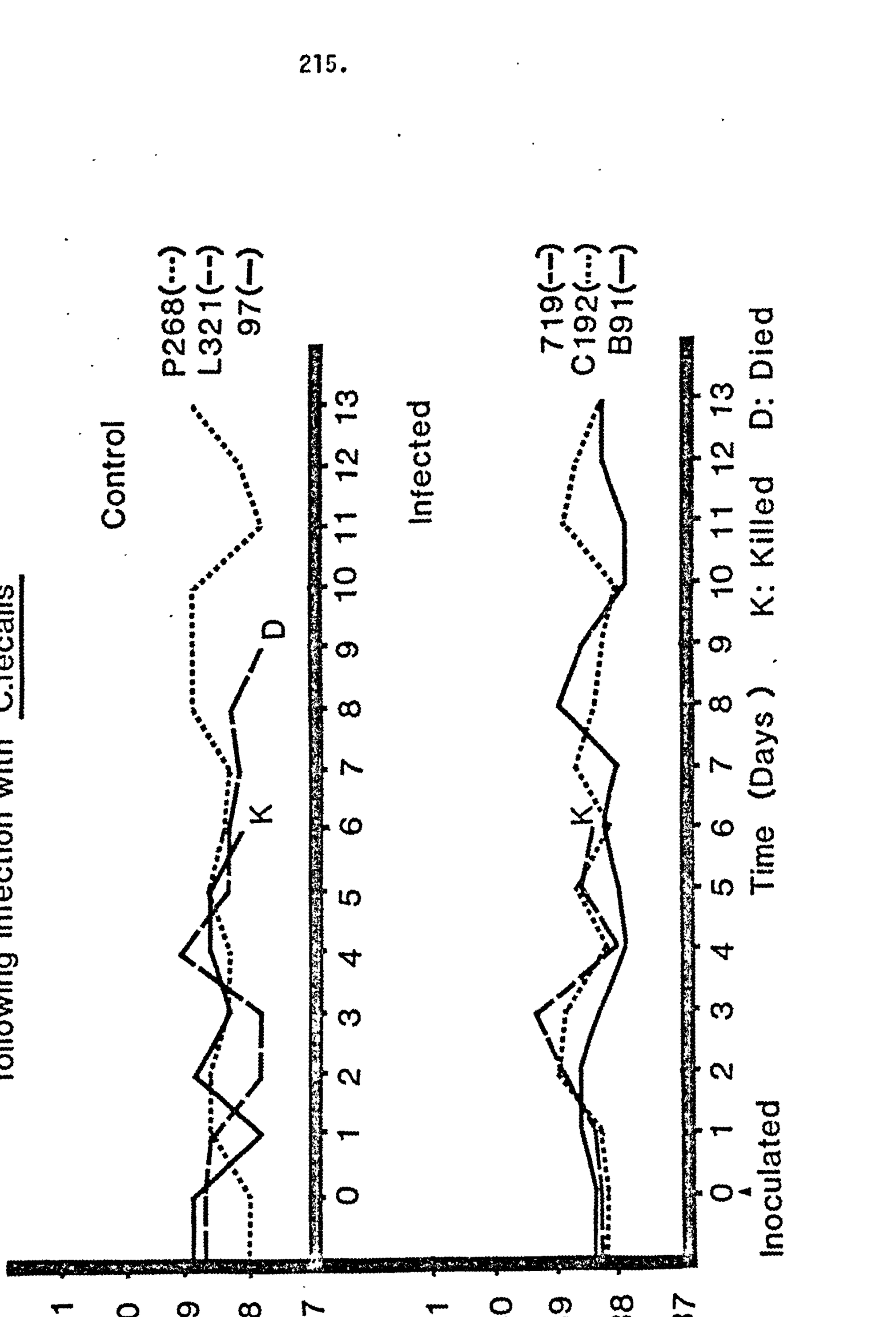


Fig (58)– Rectal Temperatures of Calves in Experiment 7 following infection with C.fecalis

Experiment 3, Chapter 4. No virus particles were seen in the faeces of calf 719 at slaughter on day 6. No nematode eggs or coccidial oocysts were reported present in the faeces of the experimental animals during the experiment. Salmonellae, β-haemolytic E. coli and other campylobacters were not isolated.

C. fecalis was isolated from the faeces of the inoculated animals within 24 hours of inoculation and could still be isolated from the faeces up to 12 days post-infection. The details of the isolation are given in Table 35. C. fecalis was not isolated from the control animals.

At post-mortem examination gross changes were noted only in the gastrointestinal tracts of the infected animals. The abomasal mucosa of B91 was slightly reddened. The jejunal contents were fluid and bile stained in two animals (B91 and C192) and the jejunal mucosa was reddened in two animals (719 and B91). The ileum of calf 719 was flaccid and the ileal mucosa was slightly reddened in calves 719 and B91. The contents of the large intestine were pale and pasty in 719 and C192 but the mucosa of this organ was normal in appearance in all three animals. The mesenteric lymph nodes of all three animals were enlarged, pale and oedematous when sectioned.

Some histological changes were seen in the alimentary tracts of the infected group. The abomasal mucosa was thickened with local accumulations of eosinophil and neutrophil polymorphonuclear leucocytes. The jejunal mucosa was also slightly thickened with stunting of the villi, slight dilatation of the capillaries and prominent lacteals. The villous epithelium was disrupted in calf B91 and calf 719 inflammatory cells were present in the crypts. Local accumulations of inflammatory cells and mononuclear cells were seen. Similar changes were seen in the ileal mucosa in which the submucosal lymphoid tissue was prominent. The large intestinal mucosa was also mildly inflamed in all three animals. The changes seen were most severe in calf B91 in which coccidial gametocytes were prominent. Lymphadenitis was seen in the mesenteric lymph nodes.

The gastrointestinal tracts of the controls were normal with the exception of calf P268 in which changes resembling those described above were seen in the ileal and large intestinal mucosa.

C. fecalis colonies were isolated in large numbers from the mucosa of all regions of the gut but not from that of the controls. The findings are summarised in Table 36.

Gram-stained smears were prepared from the mucosa of all five regions of the intestinal tract and curved Gram-negative rods with the coarse spiral appearance of C. fecalis were seen in smears made from all sites in the infected animals but not in those made from the mucosa of the controls.

Table 36.

Sites from which C. fecalis was isolated in experimental calves killed following infection with pure cultures in Experiment 7.

Site of isolation	Animal number					
	Infected			Control		
	719	B91	C192	P268	97	L321
Abomasum	+	+	+	-	-	-
Jejunum	+	+	+	-	-	-
Ileum	+	+	+	-	-	-
Caecum	+	+	+	-	-	-
Colon	+	+	+	-	-	-
Mesenteric Lymph Nodes	-	-	-	-	-	-
Liver	-	+	+	-	-	-
Lung	-	-	-	-	-	-
Gall bladder	+	+	+	-	-	-

+ = C. fecalis isolated

- = No C. fecalis isolated.

Antibody to C. fecalis of the inocular strain could not be demonstrated in serum samples taken from the inoculated animals at the beginning of the experiment or in the serum from the control animals at the beginning of the experiment or at slaughter. Agglutinating antibody to the inocular strain of C. fecalis was present at a titre of 1:640 in the sera of the infected animals at slaughter (Table 37). No antibody could be demonstrated to the strain of C.f. ss. jejuni used in the previously described experiments.

Table 37.

Levels of agglutinating antibody to the inocular strain of C. fecalis in the sera of the animals in Experiment 7.

Animal Number	Infected	Titre present Day 0	at slaughter (day)	
719	+	0	1:640	(6)
B91	+	0	1:640	(14)
C192	+	0	1:640	(14)
P268	-	0	0	(14)
97	-	0	0	(6)
L321	-	0	0	(9)

Experiment 8.

Objective: to confirm that the isolate of C. fecalis used in Experiment 7 was pathogenic for ruminating calves.

Materials and methods

A group of five 4-month old castrated Friesian cross calves were purchased from a market.

Three calves (numbers 421, 422 and 423) were infected and two (numbers 424 and 425) were kept as uninoculated controls. Each group was monitored prior to infection, housed separately, maintained and treated in the same way as the ruminating animals used in the previous

experiments. Each of the inoculated group was inoculated once with 20 ml of the suspension of the isolate of C. fecalis used in Experiment 7 and prepared by the methods described above (Chapter 2). The inoculum contained approximately 8.8×10^{10} organisms per ml.

All animals were examined daily and their appearance, appetite, respiratory rate, the appearance of their faeces, the presence or absence of ruminal movements, and their rectal temperature were recorded. Faecal samples were examined daily for the presence of C. fecalis and other bacteria by the methods described in Chapter 2. Colonies resembling those of campylobacters were confirmed as C. fecalis by the methods described in Chapter 2. Serum samples were taken from each animal at the beginning and end of the experiment and stored according to the methods described in Chapter 2.

These serum samples were examined for the presence of agglutinating antibodies to the inocular strain of C. fecalis and to C.f. ss. jejuni using the methods described in Chapter 2.

The period of observation lasted 19 days and the animals were killed on the 20th day post-inoculation. Post-mortem examination was carried out as described in previous experiments. No examination for virus particles was carried out in this study.

Results

No salmonellae, β-haemolytic E. coli or campylobacters were isolated from the faeces of these calves prior to infection. No coccidia or nematode eggs were reported as being present.

The results of the clinical observations are summarised in Table 38. All infected calves passed soft faeces containing blood and large quantities of mucus (Fig. 59). In some cases (Fig. 60) mucus was seen on formed motions. The faeces of the control animals remained normal. Rectal temperatures remained normal in both infected and control animals with the exception of calf 422 in which a rectal temperature of 40.6°C was recorded within 24 hours of infection. This declined to normal by day 4. The details of the rectal temperatures recorded are given in Fig. 61.

Table 38.

Changes in faecal consistency in Experiment 8 following inoculation of calves with pure cultures of C. fecalis and the isolation of the organism from their faeces.

Calf No.	Inf-ected	Day of experiment 0	1	2	3	4	5	6	7	8	9	10
421	+	F	FM	FM	FM	SM	FM	F	F	FMB	F	SMB
		-	+	+	+	+	+	-	-	+	-	+
422	+	F	FMB	SMB	SM	SM	SM	F	SMB	SMB	SMB	SM
		-	+	+	+	+	+	-	+	+	-	+
423	+	F	SM	FMB	SM	FM	FM	SM	FM	FM	FM	SM
		-	+	+	+	+	+	+	-	+	-	+
424	-	F	F	F	F	F	F	F	F	F	F	F
		-	-	-	-	-	-	-	-	-	-	-
425	-	F	F	F	F	F	F	F	F	F	F	FM
		-	-	-	-	-	-	-	-	-	-	-

Calf No.	Inf-ected	11	12	13	14	15	16	17	18	19	20
421	+	SM	SMB	FMB	FMB	FM	FMB	FM	F	F	K
		+	-	-	+	-	-	-	-	-	
422	+	SMB	FM	F	FM	F	FMB	FM	F	F	K
		+	+	-	+	+	-	-	-	-	
423	+	SMB	FMB	FMB	FM	FMB	FMB	FM	F	F	K
		-	-	-	-	+	+	-	-	-	
424	-	F	F	F	F	F	F	F	F	F	K
		-	-	-	-	-	-	-	-	-	
425	-	F	SM	F	F	F	F	F	F	F	K
		-	-	-	-	-	-	-	-	-	

F = Firm faeces
S = Soft faeces
M = Presence of mucus
B = Presence of blood

\+ = C. fecalis isolated
\- = No C. fecalis isolated
K = Killed.

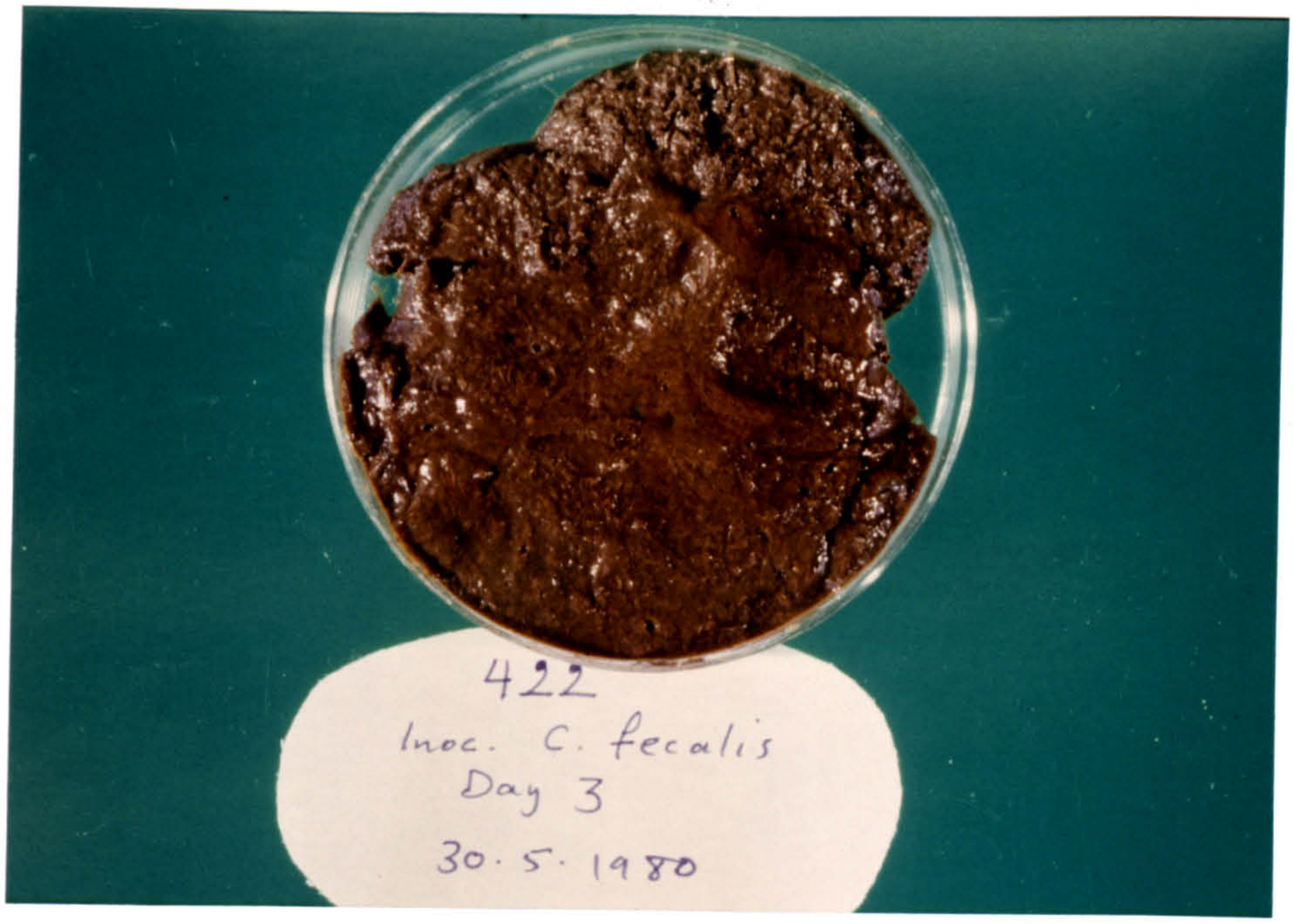


Fig. 59: Faeces of calf 422 three days following infection with C. fecalis.

Note the mucus on the surface.

Fig. 60: Formed motion passed by calf 422 two days following infection.
Note the quantity of mucus (arrow).

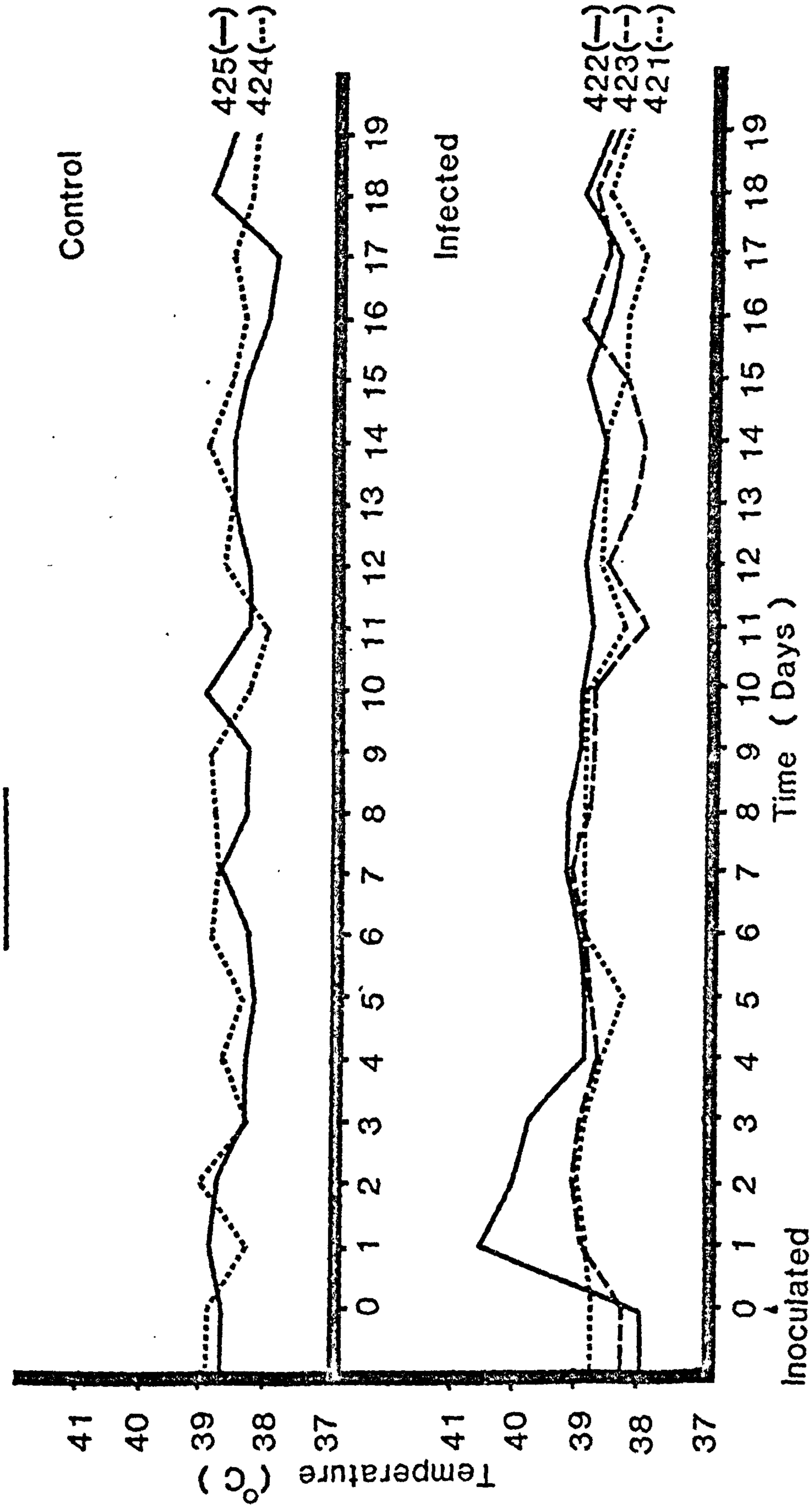


Fig (61)– Rectal Temperatures of Calves in Experiment 8 following infection with C.fecalis

No salmonellae, β- haemolytic E. coli or other campylobacter species were isolated from the faeces of the animals used in this study. No coccidial oocysts or nematode eggs were seen. C. fecalis was isolated from the faeces of all infected animals daily from day 1 to day 5 and was subsequently isolated from time to time until day 16 after which it could not be recovered, as described in Table 38. C. fecalis was never isolated from the faeces of the controls.

At post-mortem examination gross changes were most prominent in the gastrointestinal tract. Local pneumonic lesions were seen in animal 422 but other thoracic and abdominal organs appeared grossly normal. Slight hyperaemia of the abomasal mucosa was seen in animals 421 and 422. The small intestines appeared flaccid in all three infected animals and the ileum was thickened and its serosa was pale and reticulated in each case particularly in the terminal portion. The mesenteric lymph nodes were enlarged, pale and oedematous on section in all three animals (Fig. 62). The jejunal contents were mucoid and watery in consistency and the mucosa was mildly congested in calves 421 and 422. The ileal contents were similar but the mucosa was hyperaemic and thickened in all three. The contents of the large intestines of all three calves were soft and contained shreds of mucus in which blood could be seen. The mucosa of this part of the intestine was grossly normal in appearance.

The histological changes found in the infected group resembled those seen in the infected animals in Experiment 7. Abomasal changes were seen and included localised cellular accumulation, capillary dilatation and local erosion of mucosal epithelium (Fig. 63). The additional changes seen in the jejunum in this group include some disruption of the villous epithelium and sub-epithelial spaces in calf 422 in the lamina propria of which coccidial gametocytes and accumulations of eosinophils were seen. Dilated capillaries were not a feature of the jejunal mucosa in this group. The submucosal lymphoid tissue was particularly prominent in the ileum (Fig. 64) but otherwise the changes seen resembled those in Experiment 7. Coccidial gametocytes were seen in the ileal mucosa of animal 422. The changes seen in the large intestinal mucosa were slight and resembled those seen in

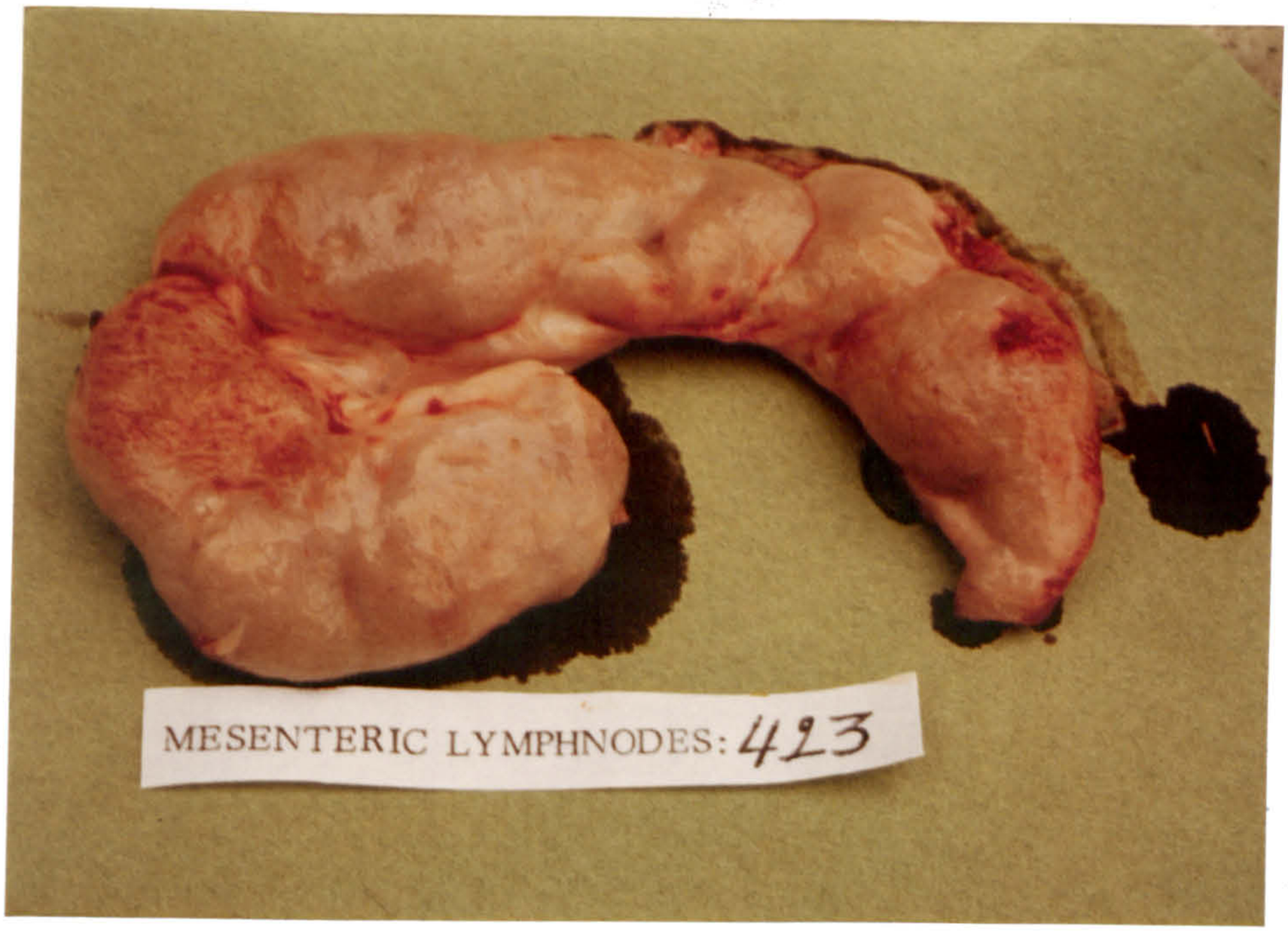


Fig. 62: Swollen and oedematous mesenteric lymph nodes from calf 423 twenty days following infection with C. fecalis.

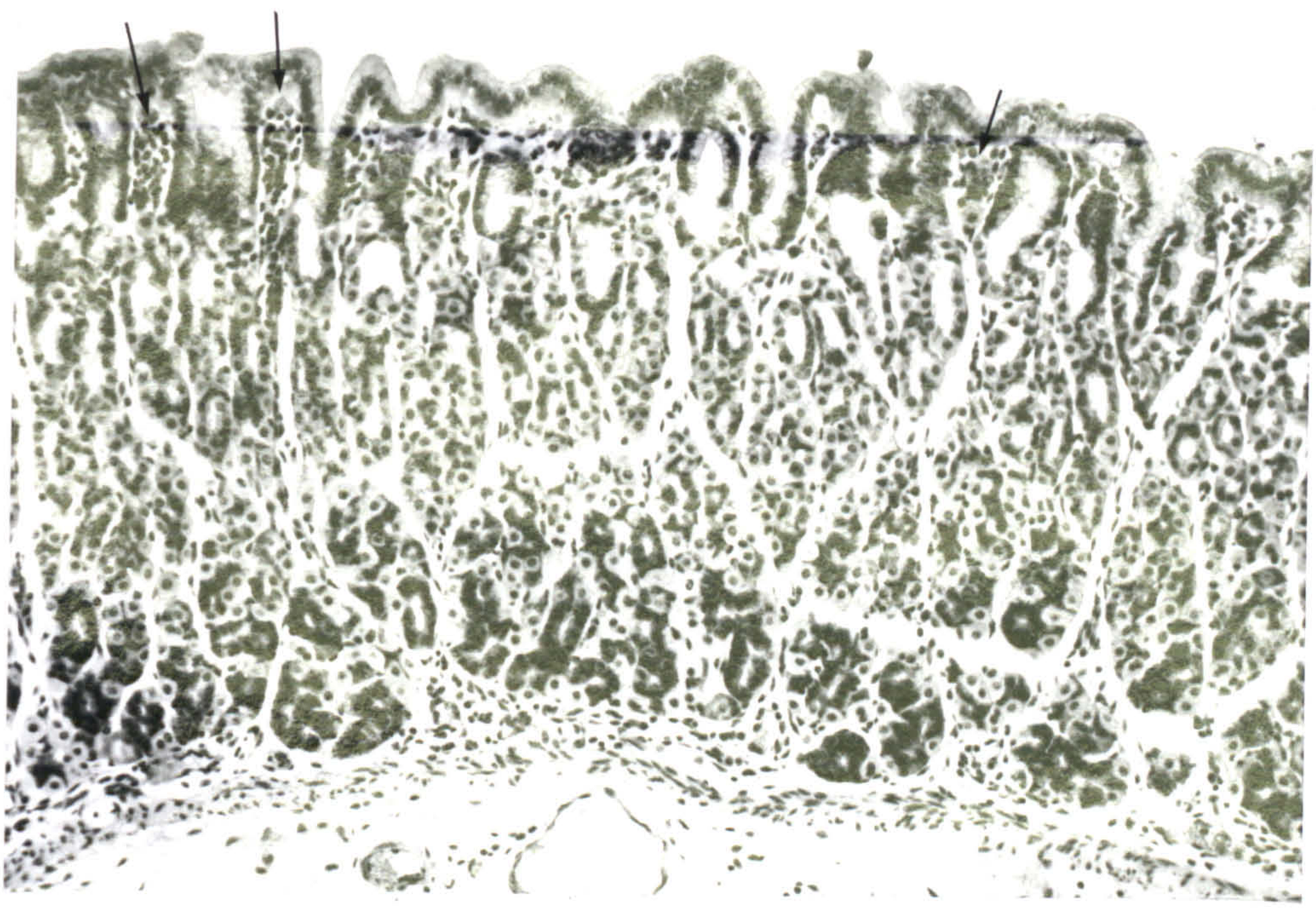


Fig. 63: Histological section of the abomasal mucosa of calf 423.

Note the accumulation of cells in the lamina propria immediately beneath the epithelium (arrows).

H & E X 110.

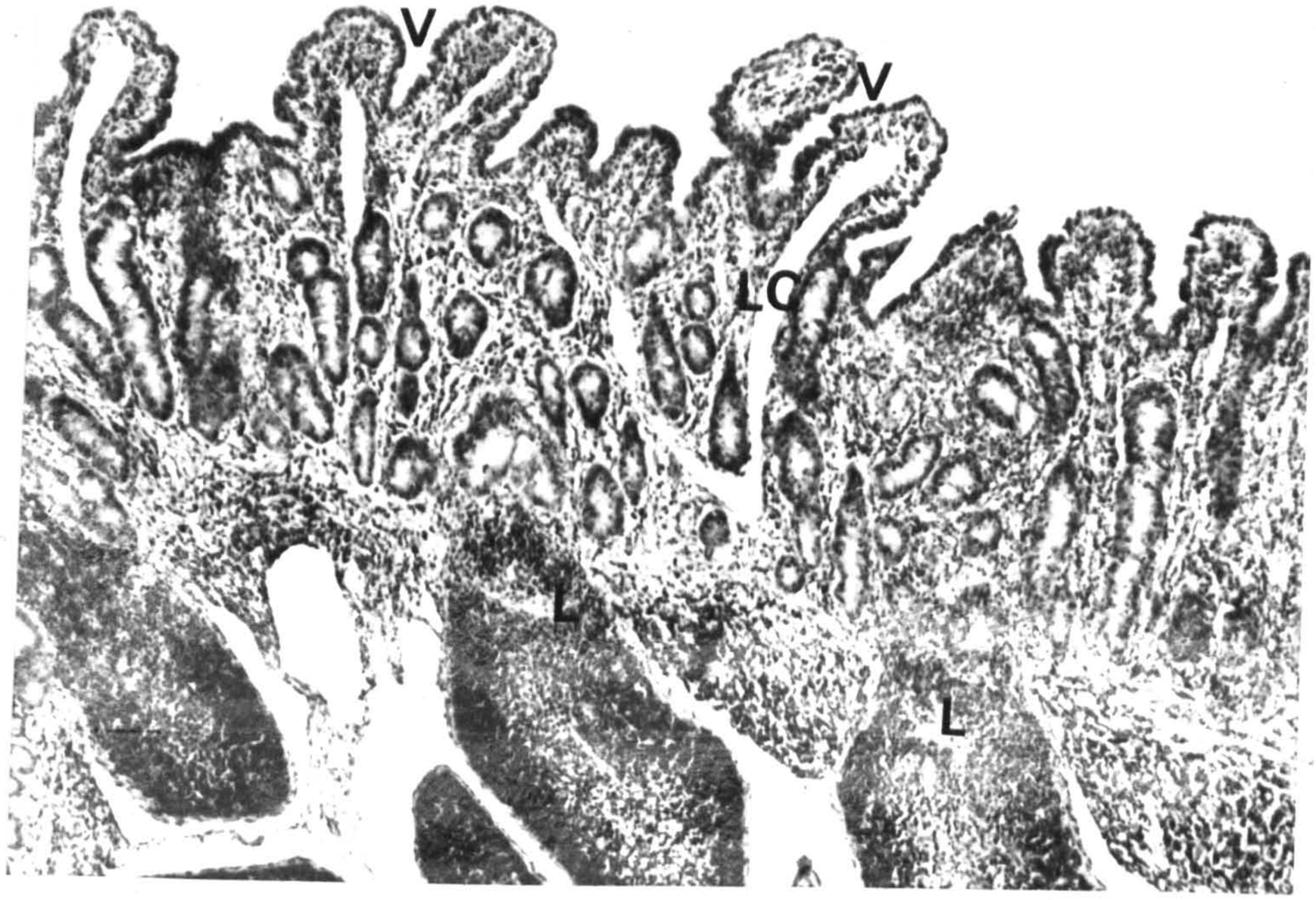


Fig. 64: Histological section of the ileal mucosa of calf 423.

Note the large amount of submucosal lymphoid tissue (L), stunting of the villi (V) and the dilated lacteals (LC).

H & E X 35.

the infected animals of Experiment 7. The mesenteric lymph nodes were oedematous and contained inflammatory cells.

Few histological changes were seen in similar sections from the control animals. Local accumulations of mononuclear cells were seen in all regions of the intestine, coccidial infection was noted in the ileum of calf 424 and a crypt blocked with inflammatory cells was seen in the ileal mucosa of calf 425. The mesenteric lymph nodes were histologically normal.

Colonies of C. fecalis were isolated from the mucosa of all the sites sampled in the gastrointestinal tract of the infected animals. The gall bladder also yielded C. fecalis as did the liver in one case (422). These results are given in Table 39.

Table 39.

Sites from which C. fecalis was isolated in experimental calves killed following infection with pure cultures of the organism in Experiment 8.

Site of isolation	Animal number				
	Infected			Control	
	421	422	423	424	425
Abomasum	-	+	+	-	-
Jejunum	+	-	+	-	-
Ileum	+	+	+	-	-
Caecum	+	-	+	-	-
Colon	+	+	+	-	-
Mesenteric Lymph Nodes	-	-	-	-	-
Liver	-	+	-	-	-
Lung	ND	-	ND	ND	ND
Gall bladder	+	+	+	-	-

+ = C. fecalis isolated. - = No C. fecalis isolated.

ND = Not Done.

Curved Gram-negative rods with the morphology of C. fecalis were seen in Gram-stained smears prepared from the gastrointestinal mucosa in all the sites sampled (Fig. 65). They were not seen in smears prepared from similar sites in the control animals.

Antibody to C. fecalis of the inocular strain could not be demonstrated in serum samples taken from the inoculated animals at the beginning of the experiment or in the serum from the control animals at the beginning of the experiment or at slaughter.

Agglutinating antibodies to the inocular strain of C. fecalis were present at a titre of 1:640 in sera taken from the infected animals at slaughter 20 days after infection. The results are shown in Table 40.

No antibody to C.f. ss. jejuni could be detected.

Table 40.

Levels of agglutinating antibody to the inocular strain of C. fecalis in the sera of the animals in Experiment 8.

Animal Number	Infected	Titre present on Day 0	Titre present on Day 20
421	+	0	1:640
422	+	0	1:640
423	+	0	1:640
424	-	0	0
425	-	0	0

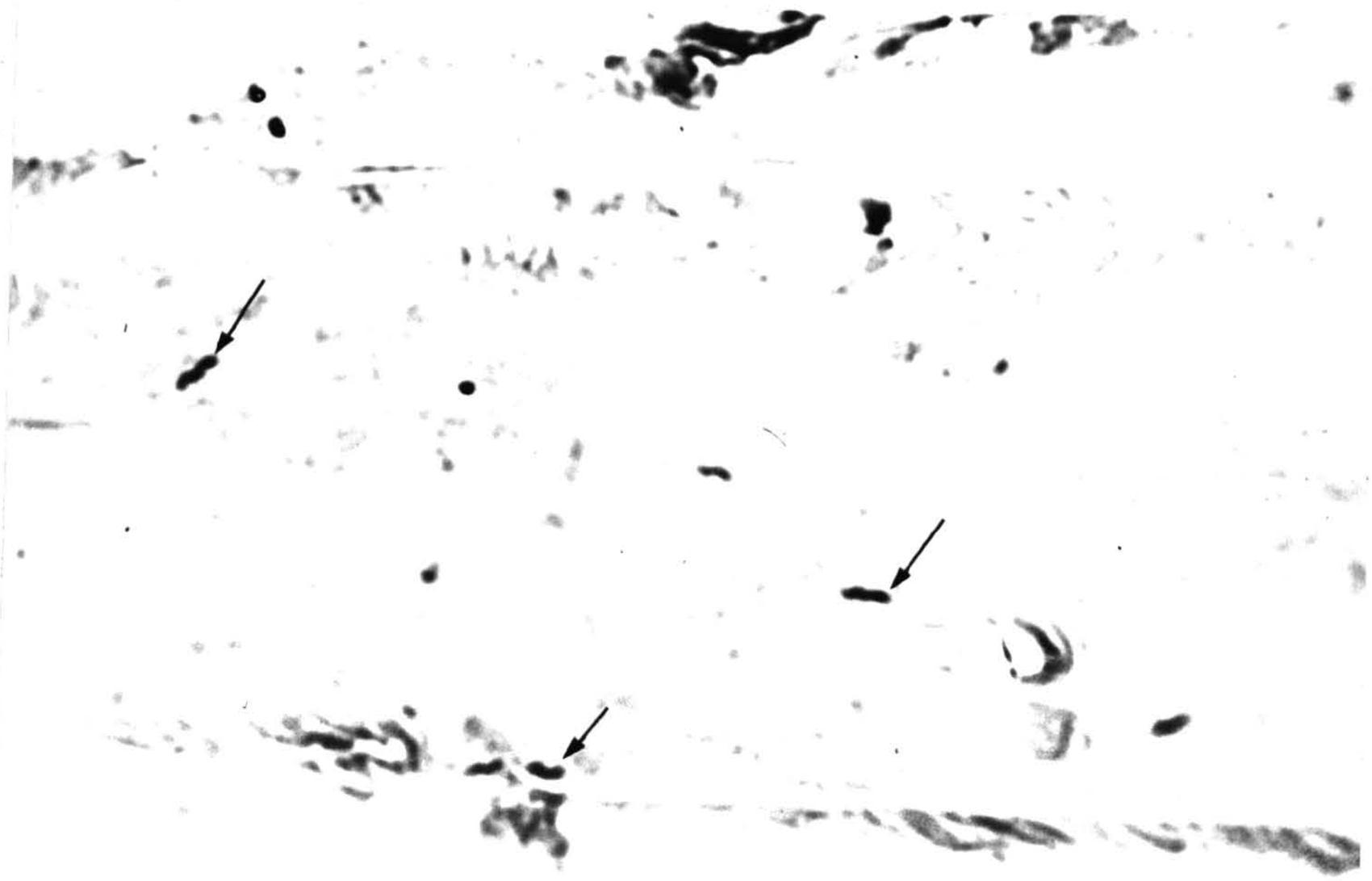


Fig. 65: Smear of the ileal mucosa of calf 423.

Note the coarse curved rods with the morphology of C.fecalis (arrow).

Gram X 1500.

DISCUSSION

The results of these two experiments indicated that C. fecalis, like C.f. ss. jejuni and C.f. ss. intestinalis was capable of initiating clinical and pathological changes when fed to calves.

The ruminating calves appeared more susceptible than the milk-fed calves to the isolate of C. fecalis used. C. fecalis initiated clinical changes such as the presence of blood, mucus and soft faeces in inoculated experimental ruminating calves in Experiment 8 but only minor clinical signs were noted in the inoculated milk-fed calves (Experiment 7). The incubation period for this disease varied from one to three days in these two studies. It was found that rectal temperatures were not raised consistently in either infected group in this study (Figs. 58 and 61). The changes in faecal consistency varied from animal to animal but at some time during the course of the disease the faeces became pasty to soft (Fig. 66) and in Experiment 8 (Table 38). excess clear mucus and streaks of blood were frequently seen.

C. fecalis was recovered only from the faeces of the infected animals. In both experiments it was recovered consistently during the first five days following inoculation and only irregularly thereafter (Tables 35 and 38). Recovery of the organism was not clearly associated with changes in faecal consistency. Other campylobacter species were not isolated from the faeces of infected groups or from any of the control animals in these studies.

Enlarged mesenteric lymph nodes, mild abomasitis and flaccidity, thickening and mucosal hyperaemia of the ileum were present in all inoculated animals at post-mortem examination. The large intestinal mucosa appeared grossly normal in all infected animals although changes in the large intestinal contents were noted particularly in the ruminating animals in Experiment 8 in which blood and mucus was present.

The histological findings resembled those described in C.f. ss. jejuni (Chapter 4) and C.f. ss. intestinalis (Chapter 5) infections but were less severe.

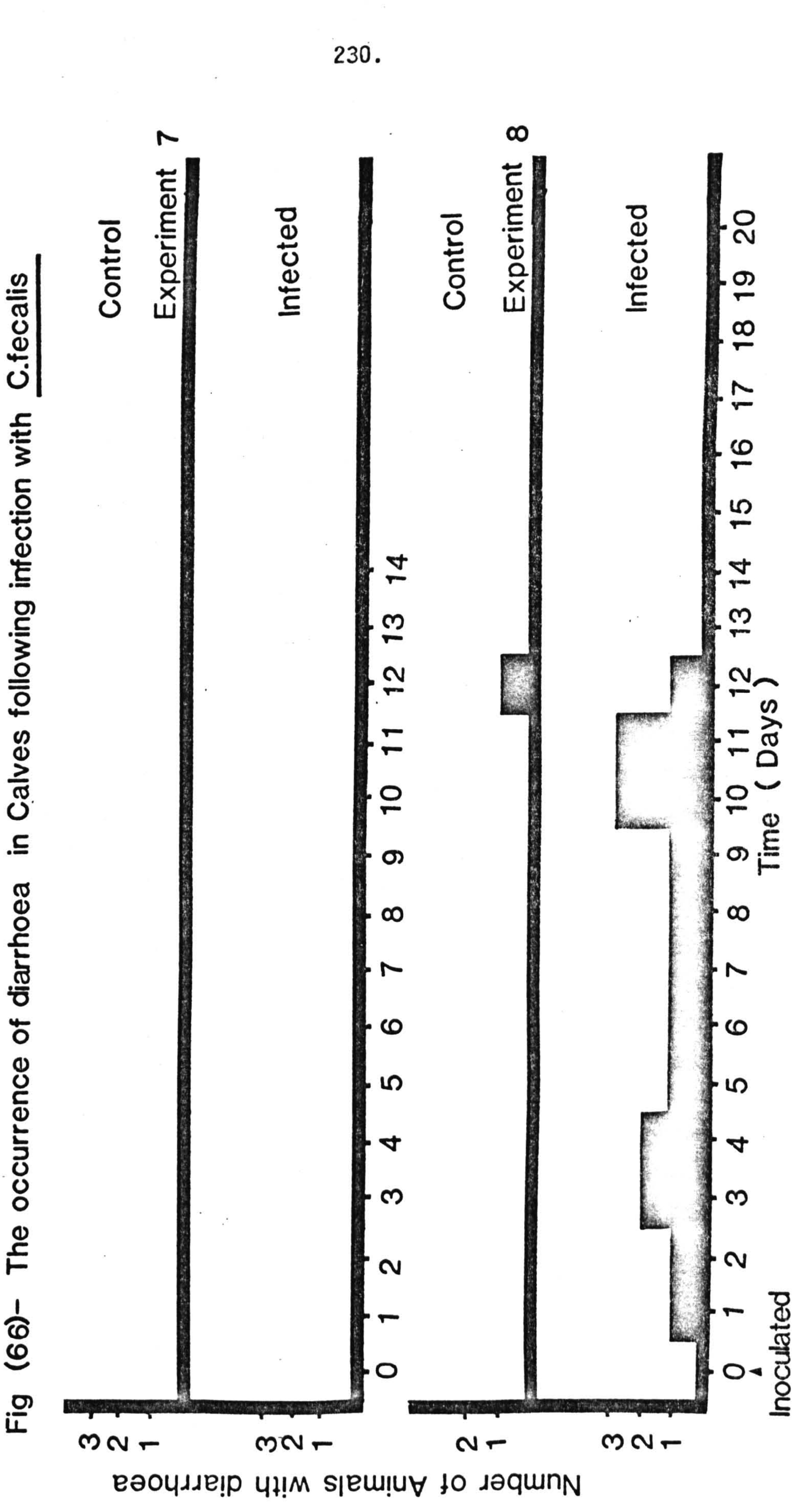


Fig (66)– The occurrence of diarrhoea in Calves following infection with C.fecalis

The isolation of C. fecalis from all sites in the gastro-intestinal tracts of the infected animals suggests that the abomasitis and ileitis seen histologically could have been associated with the organism.

The presence of the organism in the gall bladder in each infected animal and in the livers of three infected animals may suggest that systemic spread of the organism may take place at some stage in the pathogenesis of the disease. The presence of abomasitis and the isolation of the organism from the abomasum may indicate that this campylobacter can survive better in the abomasum or is more likely to cause changes in that organ than the isolates of C.f. ss. jejuni used in Chapter 4 and C.f. ss. intestinalis used in Chapter 5.

The involvement of C. fecalis in these changes is supported by the presence of agglutinating antibody to the inocular strain of C. fecalis which was only detected in the sera of infected groups of animals after inoculation.

The involvement of other agents in the clinical signs and the lesions found in these two experiments is probable but felt to be of limited importance. In Experiment 7 the cause of the ataxia suffered by calf 719 was not established and the part played by reovirus infection in the enteric lesions in any animal of this group is not clear. In Experiment 8, coccidial gametocytes were present in the intestinal mucosa of calf 422 (infected) and 424 (control). The presence of coccidial infection in both infected and control groups may have affected the pathological changes in this study but did not appear to have affected clinical changes recorded (Table 38 and Fig. 61) unless it predisposed to infection.

The results of this study suggest that C. fecalis is a primary pathogen of cattle and can initiate the changes described above in ruminating animals. Its failure to produce similar changes in milk-fed calves may be due to age susceptibility or possibly to the persistence of some degree of colostral immunity which was not detectable as serum agglutination titres to the organisms used.

A marked difference from C.f. ss. jejuni infection was that the isolate of C. fecalis did not appear to be responsible for prolonged elevation of body temperature and was more frequently isolated from the abomasum and liver. The opinion of Firehammer (1965) and Smibert (1978) that C. fecalis is non-pathogenic appears to be contradicted by the findings described above.

Chapter 7.

Experimental infection with Cl. sordellii.

Introduction

Clostridium sordellii was recovered from two animals in the survey, an adult with haemorrhagic diarrhoea and a calf with soft faeces. Congestion and necrosis of the small intestine was a feature of both cases. The other bacteria present in the affected small intestines were E. coli, Clostridium perfringens Type A and C. fecalis but none of them had been associated with this type of change in other cases. Brooks et al. (1956) had isolated Cl. sordellii from similar cases and the organism is closely related to Cl. difficile which is associated with pseudomembranous colitis in man.

These factors suggested that Cl. sordellii might be an enteric pathogen in cattle although Brooks et al. (1956) had failed to reproduce enteric disease by the oral inoculation of calves in their experiments. The organism used in these studies was an isolate of Cl. sordellii obtained from haemorrhagic enteritis of the small intestine of a dead cow (case B, Chapter 3). It had been cloned, passaged three times and then freeze-dried using the method described in Chapter 2. Cultures for inoculation were prepared after one passage from this freeze-dried source.

Experiment 9.

Objective: to determine the pathogenicity of an isolate of Cl. sordellii for ruminating calves.

Materials and methods

Five 6-month old Ayrshire calves were purchased direct from the farm on which they were reared. They formed part of the group of calves used in Experiment 1 in Chapter 4. The two control animals, 100 and 115 were the same animals used as controls for the experimental infection with C.f. ss. jejuni. The infected group of three animals used in this study were numbered 1R, 2R and 3R. Details of housing and feeding are given in Chapter 2 and Chapter 4. Faecal samples from each animal were

examined for the presence of salmonellae, β -haemolytic E. coli, campylobacters, other bacteria, nematode eggs and coccidia during the holding period by the methods described in Chapter 2. Each calf was inoculated orally with 20 ml of inoculum prepared as described above (Chapter 2) and containing approximately 8.4×10^8 organism per ml. Inoculation was carried out once only.

Animals were examined daily and their appearance, appetite, respiratory rate, the presence or absence of rumen movement, rectal temperature and the consistency of their faeces were recorded.

Faecal samples from each animal were examined daily for Cl. sordellii and other bacteria by the methods described in Chapter 2. Colonies resembling those of Cl. sordellii were confirmed as such by the methods described in Chapter 2. Negatively-stained preparations of faeces from calves 2R and 3R were examined by electron microscopy for the presence of virus particles on day 5. Serum samples were obtained from all animals at the beginning and end of experiment and stored according to the methods described in Chapter 2 and examined for the presence of antibody to Cl. sordellii. The period of observation lasted 11 days and the animals were killed on the 11th day post-inoculation. Post-mortem examination was carried out as described in Chapter 2.

Results

No salmonellae, β -haemolytic E. coli, campylobacters or colonies of Cl. sordellii were isolated from the faeces of these calves prior to infection. No coccidia or nematode eggs were reported as being present.

Few changes in faecal consistency were seen in the faeces of inoculated groups. In one animal (2R), the faeces became very soft with excess mucus on day 5 and 6 post-infection and remained soft until day 8. The other inoculated animals passed slightly soft faeces with mucus. The results of the faecal changes are summarised in Table 41. Raised rectal temperatures were also noted in the inoculated animals. They reached 39.4°C on day 5 (1R and 2R). The changes in rectal temperature are summarised in Fig. 67. Uninoculated calves 100 and 115 remained normal as described in Experiment 1, Chapter 4. No virus particles were seen in the faeces of calves 2R and 3R.

Table 41.

Changes in faecal consistency in Experiment 9 following the inoculation of calves with pure cultures of Cl. sordellii and the isolation of the organism from their faeces.

Calf No.	Inf-ected	Day of Experiment 0	1	2	3	4	5	6	7	8	9	10	11	
1R	+	F	F	F	FM	FM	FM	F	FM	SM	SM	F	F	K
		-	+	+	+	+	-	-	-	-	-	-	-	
2R	+	F	F	F	FM	FM	VSM	VSMB	SM	SM	FM	F	F	K
		-	+	+	+	+	+	-	-	-	-	-	-	
3R	+	F	F	FM	F	F	SM	F	FM	SM	FM	F	F	K
		-	+	+	+	+	+	-	-	-	-	-	-	
100	-	F	F	F	F	F	F	F	F	F	F	F	K	
		-	-	-	-	-	-	-	-	-	-	-		
115	-	F	F	F	F	F	F	F	F	F	F	F	K	
		-	-	-	-	-	-	-	-	-	-	-		

F = Firm faeces
M = Presence of Mucus
B = Presence of blood
S = Soft faeces
VS = Very soft faeces
K = Killed
\+ = Cl. sordellii isolated
\- = No Cl. sordellii isolated.

No salmonellae, β-haemolytic E. coli, or campylobacters were isolated from the faeces of either group. Cl. sordellii was isolated from the faeces of the inoculated animals within 24 hours of inoculation and could still be isolated from the faeces up to five days post-infection. The details of the isolations are given in Table 41.

At post-mortem examination only minor gross changes were noted in the gastrointestinal tracts of the control animals (Experiment 1, Chapter 4). The abomasal mucosa of all inoculated animals was grossly

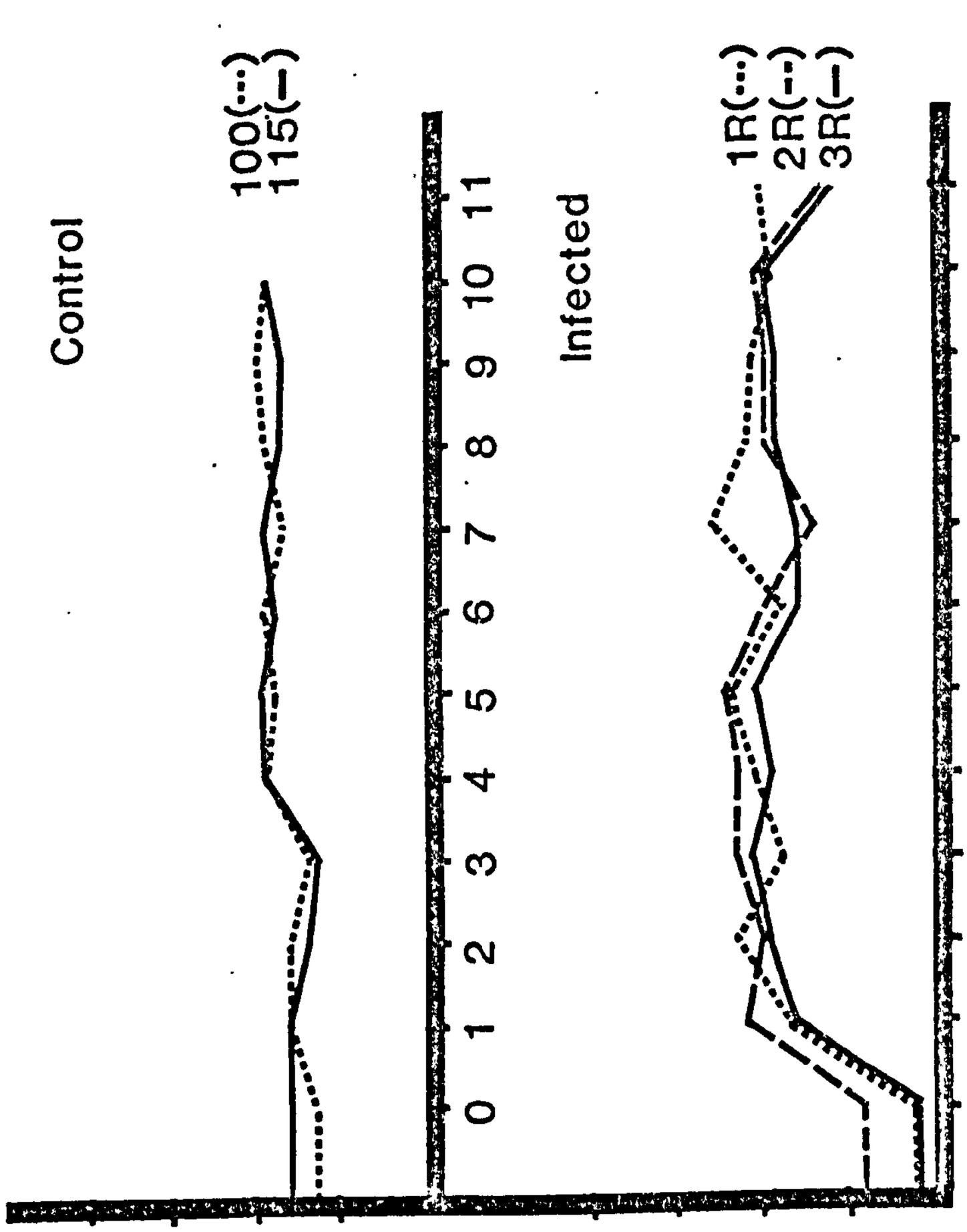


Fig (67)– Rectal Temperatures of Calves in Experiment following infection with Cl.sordellii

normal. The abomasum contained fluid and grit in animals 2R and 3R. The jejunal mucosa was hyperaemic in two (1R and 2R) and the jejunal content in all three infected animals were fluid and mucoid. The ileal mucosa was congested and thickened in calf 3R, although it was grossly normal in the other two calves (1R and 2R). The ileal contents of all three animals were fluid and mucoid. The contents of the large intestine were mucoid in all three infected animals but the mucosa of this organ was normal in appearance. The mesenteric lymph nodes of animals 2R and 3R were enlarged, pale and oedematous when sectioned.

Hilstological changes were seen in the alimentary tract of the infected group. The abomasal mucosa was disrupted in a few places and slightly thickened with cellular infiltrations of eosinophil and mononuclear cells. Some glands were plugged with mucus and groups of rod-shaped bacteria were seen at the mouth of the glands. Neutrophil polymorphonuclear leucocytes were present in the abomasal mucosa. The jejunal villous epithelium was disrupted in calves 2R and 3R and inflammatory cells were present in the crypts. The jejunal mucosa was slightly thickened and local accumulations of inflammatory cells and mononuclear cells were seen. Similar changes were also seen in the ileal mucosa in which the submucosal lymphoid tissue was prominent. The large intestinal mucosa was also disrupted and inflamed in all three inoculated animals. Lymphadenitis were seen in the mesenteric lymph nodes. The histological findings of the control animals were described in Experiment 1, Chapter 4.

Colonies of Cl. sordellii were isolated from the organs shown in Table 42. Gram-stained smears prepared from the mucosa of these organs contained Gram-ppositive rods with the characteristic spores together with vegetative organisms. The identity of the isolates was confirmed by the methods described in Chapter 2. C.f. ss. intestinalis were also isolated from the small and large intestines of all infected animals.

No agglutinating antibody to Cl. sordellii could be demonstrated by the method used in the sera of either inoculated or control group.

Table 42.

Sites from which Cl. sordellii was isolated in experimental calves 11 days following infection with pure cultures of the organism in Experiment 9.

Site of isolation	Animal number				
	Infected			Control	
	1R	2R	3R	100	115
Abomasum	Cont.	-	Cont.	-	-
Jejunum	Cont.	-	Cont.	-	-
Ileum	Cont.	-	-	-	-
Caecum	Cont.	+	+	-	-
Colon	Cont.	-	-	-	-
Mesenteric Lymph Node	-	-	-	-	-

Cont. = contamination

\+ = Cl. sordellii isolated

\- = No Cl. sordellii isolated.

Experiment 10

Objective: to confirm the results of Experiment 9 using ruminating calves.

Materials and methods

Five 4-month old calves of Friesian breeds were purchased from a market. They were divided into one group of two animals (10 and 11) (control) and one of three animals numbered 12, 14 and 16 (infected) and housed separately as described in Chapter 2. The animals were monitored prior to infection, housed, fed, and observed by the methods described in Chapter 2 and for Experiment 9. The inoculum was prepared by the same method as in Experiment 9 and also contained approximately 8.0×10^8 organisms per ml. The period of observation lasted for 21

days post-infection and animals were killed on the 22nd day post-infection and were examined post-mortem by the methods described in Chapter 2.

No examination for virus particles was carried out in this study.

Results

No salmonellae, β-haemolytic E. coli, campylobacters or Cl. sordellii were isolated from the faeces of these calves prior to infection. No coccidia or nematode eggs were reported as being present.

Faecal changes began on the first day following inoculation in all three inoculated animals and had disappeared by day 13 (Table 43). The charges seen varied from soft faeces with excess clear mucus to faeces streaked with blood. The faecal changes are summarised in Table 43. The faeces of the two control animals remained normal in appearance and consistency throughout the experiment. During the period on the experiment the two control animals developed nasal discharge and coughed from time to time. The rectal temperatures of the inoculated group rose to 39.3°C and in one case reached 39.6°C on day 16 and 40°C on day 17. The changes in rectal temperature are summarised in Fig. 68.

No salmonellae, β- haemolytic E. coli or campylobacters were isolated from the faeces of the animals used in this study. No coccidial oocysts or nematode eggs were reported.

Cl. sordellii was isolated from the faeces of all the inoculated animals from the day following inoculation to the 6th day post-infection The results of daily isolation are summarised in Table 43.

At post-mortem examination the changes found in the inoculated animals resembled those seen in the first experiment. The jejunal mucosa of all infected animals was inflamed and the jejunal contents were fluid and mucoid. The ileal mucosa was mildly hyperaemic in animals 14 and 16 and the walls were flaccid. The ileal contents of those two animals were fluid, mucoid and frothy in animal 14. The

Table 43.

Changes in faecal consistency in Experiment 10 following inoculation of calves with pure cultures of Cl. sordellii and the isolation of the organism from their faeces.

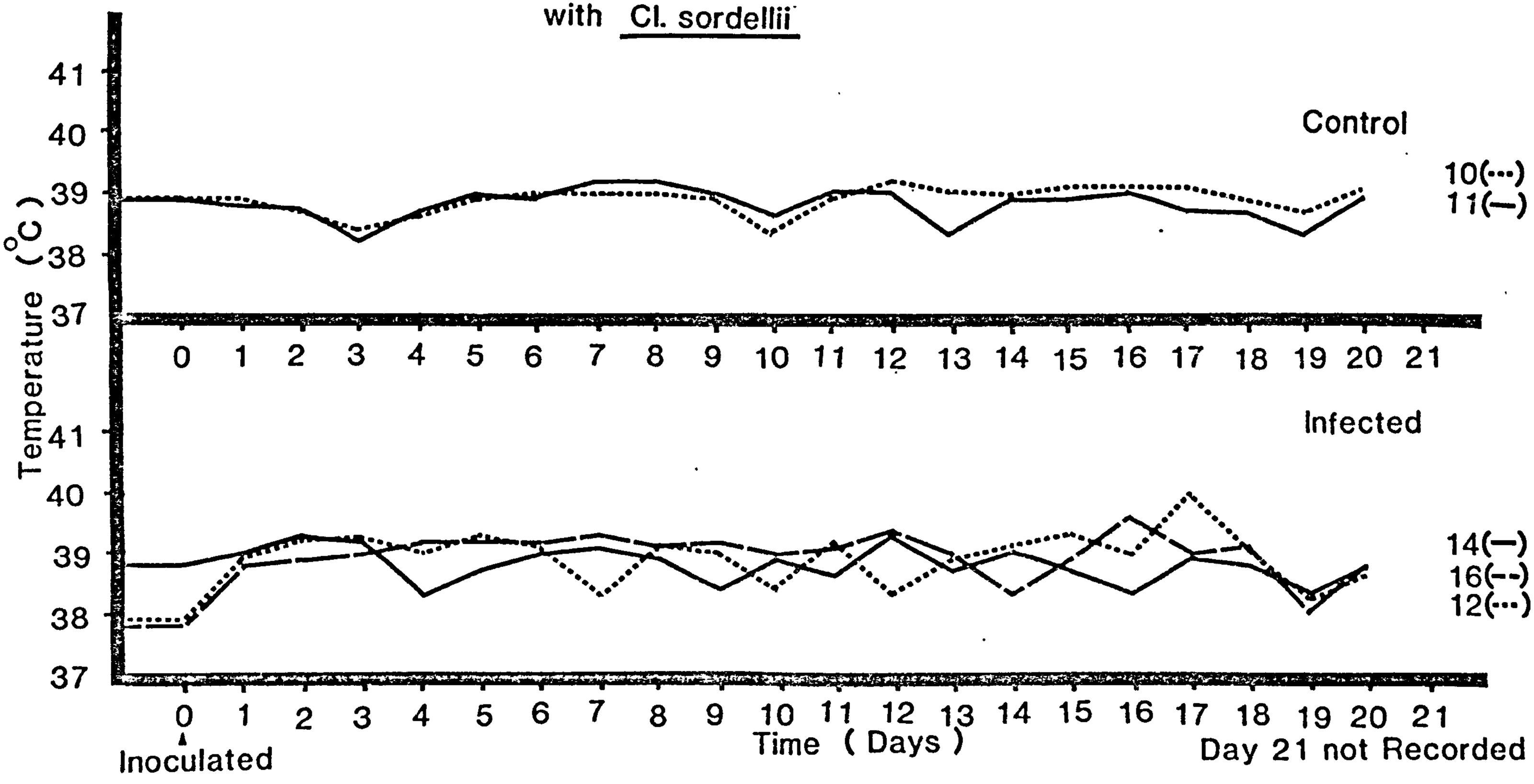
Calf No.	Inf-ected	Day of experiment 0	1	2	3	4	5	6	7	8	9	10	11
12	+	F	SMB	SM	FM	F	FM	F	F	SM	F	FM	F
		-	+	+	+	+	+	-	-	-	-	-	-
14	+	F	SM	FM	FM	F	F	SM	FM	F	F	FM	F
		-	+	+	+	+	+	+	-	-	-	-	-
16	+	F	FMB	SM	SM	FM	SM	SM	SM	SM	F	SM	FM
		-	+	+	+	+	+	+	-	-	-	-	-
10	-	F	F	F	F	F	F	F	F	F	F	F	F
		-	-	-	-	-	-	-	-	-	-	-	-
11	-	F	F	F	F	F	F	F	F	F	F	F	F
		-	-	-	-	-	-	-	-	-	-	-	-

Calf No.	Inf-ected	12	13	14	15	16	17	18	19	20	21	22
12	+	F	F	F	F	F	F	F	F	F	ND	K
		-	-	-	-	-	-	-	-	-		
14	+	FM	F	F	F	F	F	F	F	F	ND	K
		-	-	-	-	-	-	-	-	-		
16	+	FM	F	F	F	SM	F	F	F	F	ND	K
		-	-	-	-	-	-	-	-	-		
10	-	FM	F	F	F	F	F	F	F	F	ND	K
		-	-	-	-	-	-	-	-	-		
11	-	FM	F	F	F	F	F	F	F	F	ND	K
		-	-	-	-	-	-	-	-	-		

F = Firm faeces
S = Soft faeces
M = Presence of mucus
B = Presence of blood

ND = Not done
\+ = Cl. sordellii isolated
\- = No Cl. sordellii isolated
K = Killed

Fig (68)- Rectal Temperatures of Calves in Experiment 10 following infection with Cl. sordellii



contents of the large intestine were soft with excess clear mucus in animal 14 and there was patchy hyperaemia of the mucosa. The mesenteric lymph nodes of all the infected animals were enlarged, pale and oedematous when sectioned. The two control animals were found to be grossly normal, with the exception of a mild congestion of the jejunal mucosa of animal 11. Pneumonic lesions were present only in the two control animals.

Histological changes were present in the gastrointestinal tract of the inoculated animals. The abomasal mucosa was thickened with patchy infiltration with polymorphonuclear leucocytes and plasma cells and lymphocytes. Patches of oedema and capillary dilatation were also seen. In animal 12 and 14 cell debris was present in the glands and in animal 14 bacteria were seen in large numbers in this debris. In all three animals the jejunal villi were stunted with dilated lacteals filled with proteinaceous fluid and there was local oedema of the lamina propria and cellular infiltration. Crypt abscesses were present. Similar findings were made in the ileum in which oedema of the lamina propria, cellular infiltration and prominence of the lymphoid tissue were seen. There was local erosion of the surface. In the caecum the surface epithelium was eroded in animals 12 and 16 and this erosion was accompanied by many rods in animal 16 (Fig. 69). There was cellular infiltration with polymorphonuclear leucocytes and some oedema and inflammation of the lamina propria. Similar changes were seen in the colonic mucosa but without erosion. The mesenteric lymph nodes of all three animals were reactive.

Slight different changes were seen in the controls in which abomasal changes resembling those noted above were accompanied by the presence of fungal hyphae. Fusion and stunting of the villi and cellular infiltration were present in the small intestines. Coccidial gametocytes were seen in the jejunum (animal 10) and ileum (animal 11). The caecum and colonic mucosa appeared relatively unaffected.

Colonies of Cl. sordellii were isolated from the caecum in all animals and from the ileum in one animal as summarised in

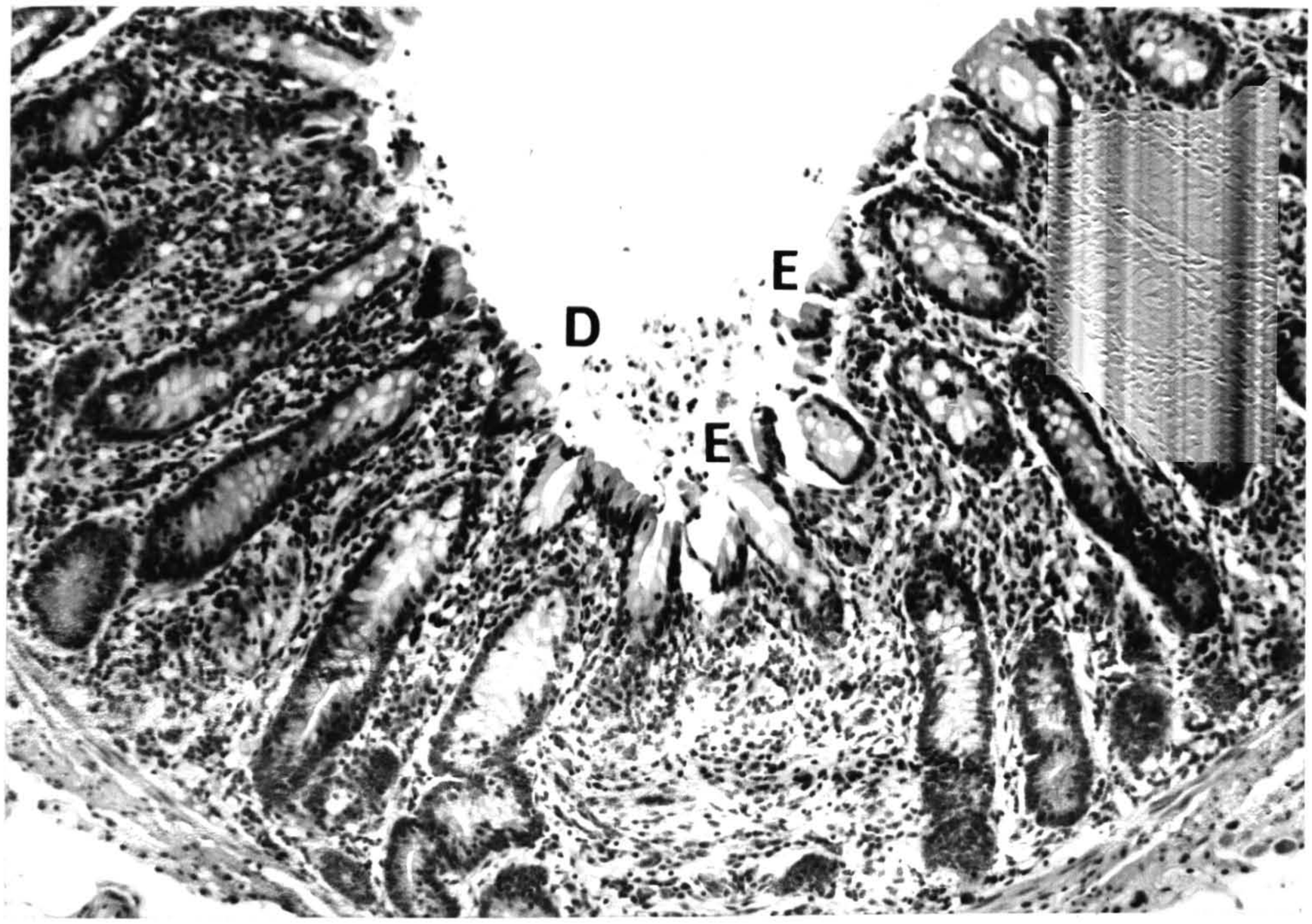


Fig. 69: Histological section of caecal mucosa of calf 16 twenty-one days following infection.

Note the patchy erosion of the lumenal epithelium (E) and the presence of cell debris in the lumen (D).

H & E X 110.

Table 44. Gram-stained smears prepared from the mucosa of these organs were seen to contain organisms with the morphology of Cl. sordellii as described in Experiment 9 (Fig. 70).

No agglutinating antibody to Cl. sordellii could be demonstrated by the method used in the sera of either the inoculated or the control group.

Table 44.

The sites from which Cl. sordellii was isolated in experimental calves 21 days following infection with pure cultures of the organism in Experiment 10.

Site of Isolation	Animal number				
	Infected			Control	
	12	14	16	10	11
Abomasum	+	-	+	-	-
Jejunum	-	-	-	-	-
Ileum	-	+	-	-	-
Caecum	+	+	+	-	-
Colon	+	-	+	-	-
Mesenteric Lymph Nodes	-	-	-	-	-
Liver	-	-	-	-	-
Lung	-	-	-	-	-
Gall bladder	-	-	-	-	-
Spleen	-	-	-	-	-

\+ = Cl. sordellii isolated

\- = No Cl. sordellii isolated

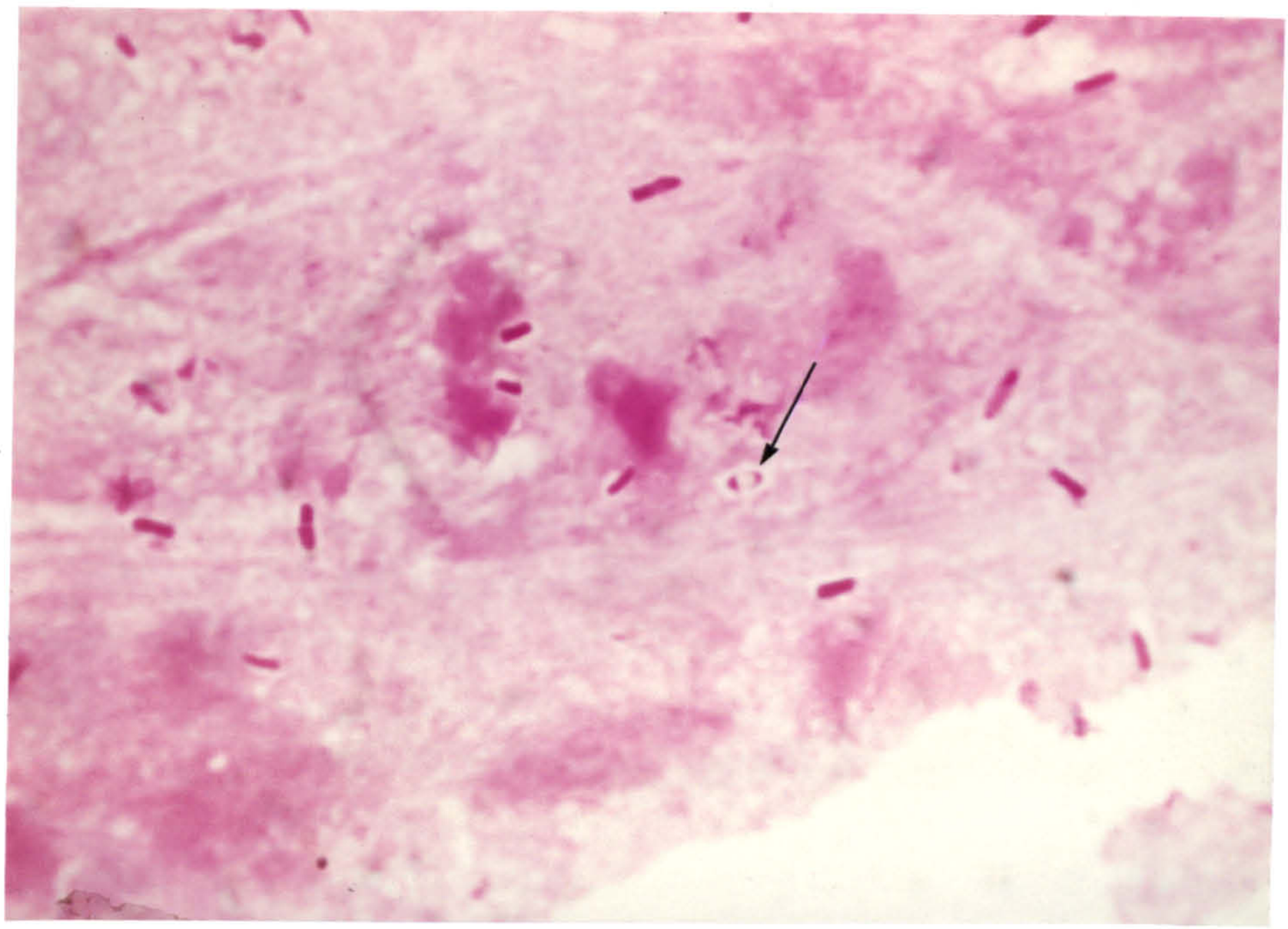


Fig. 70: Smear of the ileal mucosa of calf 14 twenty-one days following infection.

Note the presence of an organism with the morphology of Cl. sordellii (arrow).

Gram X 1600.

Experiment 11.

Objective : to study the pathogenesis of Cl. sordellii using ruminating calves.

Materials and methods

Ten 4-month old calves were used in this study and were purchased direct from the farm where they were reared. There were three Ayrshire, two Hereford, three Friesian and two cross bred calves. The animals were monitored prior to infection, housed, fed, and observed by the methods described in Chapter 2 and for Experiment 9.

Infection was carried out by inoculation with living culture (five calves numbered A, B, C, 99S and 100S in a single group), prepared by the methods described in Experiment 10 and containing approximately 8.0×10^8 organisms per ml. A second group of two animals (numbers 2 and 5) were each inoculated with 200 ml of the culture filtrate described in Chapter 2. These animals were housed with the uninoculated controls (animals 1, 3 and 4).

Serum samples were taken from each animal at slaughter and stored according to the methods described in Chapter 2 and examined for the presence of antibody to Cl. sordellii by the methods described in Chapter 2. Animals were killed at daily intervals with appropriate controls and examined post-mortem according to the schedule set out below. (Table 45).

Samples were treated in the way described in Chapter 2. No examination for virus particles was carried out on the inoculated group in this study.

Results

No salmonellae, β-haemolytic E. coli, campylobacters or colonies of Cl. sordellii were isolated from the faeces of these calves prior to infection. No coccidia or nematode eggs were reported present.

The faecal changes seen in this experiment are summarised in Table 45. Faecal changes were noted in all four calves (C, A, 99S and 100S) alive on day 2 and inoculated with living cultures of Cl. sordellii. Only slight changes such as the softening and the presence of mucus were seen. The two calves (2 and 5) inoculated with Cl. sordellii toxin, passed firm or pasty faeces with excess clear mucus containing streaks of fresh blood.

During the period of experiment one of the calves infected with culture (calf C) appeared very dull and stopped ruminating. Coughing was noted from time to time in both groups of animals. The rectal temperature of inoculated animals (calf 99S) rose to 39.4°C within 24 hours of infection and reached 39.7°C (animal 5 inoculated with toxin). The results are given in Fig. 71. The uninoculated calves (number 1, 3 and 4) remained normal.

No coccidial oocysts or nematode eggs were found in the faeces of any of the animals nor were Salmonella spp., β-haemolytic E. coli or Campylobacter spp. isolated.

Cl. sordellii was isolated from the faeces of all animals inoculated with live culture from the day following inoculation to the end of the experiment but not from the faeces of controls or animals inoculated with toxin. The results of isolation are shown in Table 45.

The post-mortem findings in the inoculated animals are described according to the day of death.

Day 1. Calf B (living inoculum)

No gross lesions were found in any part of gastrointestinal tract. The jejunal contents were slightly abnormal in that there were bubbles on the mucosa. The ileal contents were dark and soft with excess clear mucus and the contents of the large intestine were soft and mucoid. The mesenteric lymph nodes were enlarged and pale.

Day 2. Calf C (living inoculum)

The abomasal mucosa was slightly congested. The jejunum was flaccid, the mucosa was locally congested in several areas and the

Table 45.

Changes in faecal consistency in Experiment 11 following the inoculation of calves with pure cultures of Cl. sordellii or its toxin and the isolation of the organism from their faeces.

Calf No.	Infected	Day of observation 0	1	2	3	4	5
B	+	F	F				
		-	+ K				
1	-	F	F				
		-	+				
C	+	F	FM	FM			
		-	+	+ K			
A	+	F	F	PM	FM		
		-	+	+	+ K.		
3	-	F	F	F	F		
		-	-	-	-		
99S	+	F	FM	PM	FD	F	
		-	+	+	+	+ K	
2	+(T)	F	FM	PMB	FM	FM	
		-	-	-	-	-	
100S	+	F	F	FM	FD	F	F
		-	+	+	+	+	- K
5	+(T)	F	FM	PM	PM	FMB	FM
		-	-	-	-	-	-
4	-	F	F	F	F	F	F
		-	-	-	-	-	-

F = Firm faeces
B = Presence of blood
M = Presence of mucus
D = Dry faeces
P = Pasty faeces
(T) = Toxin inoculation.

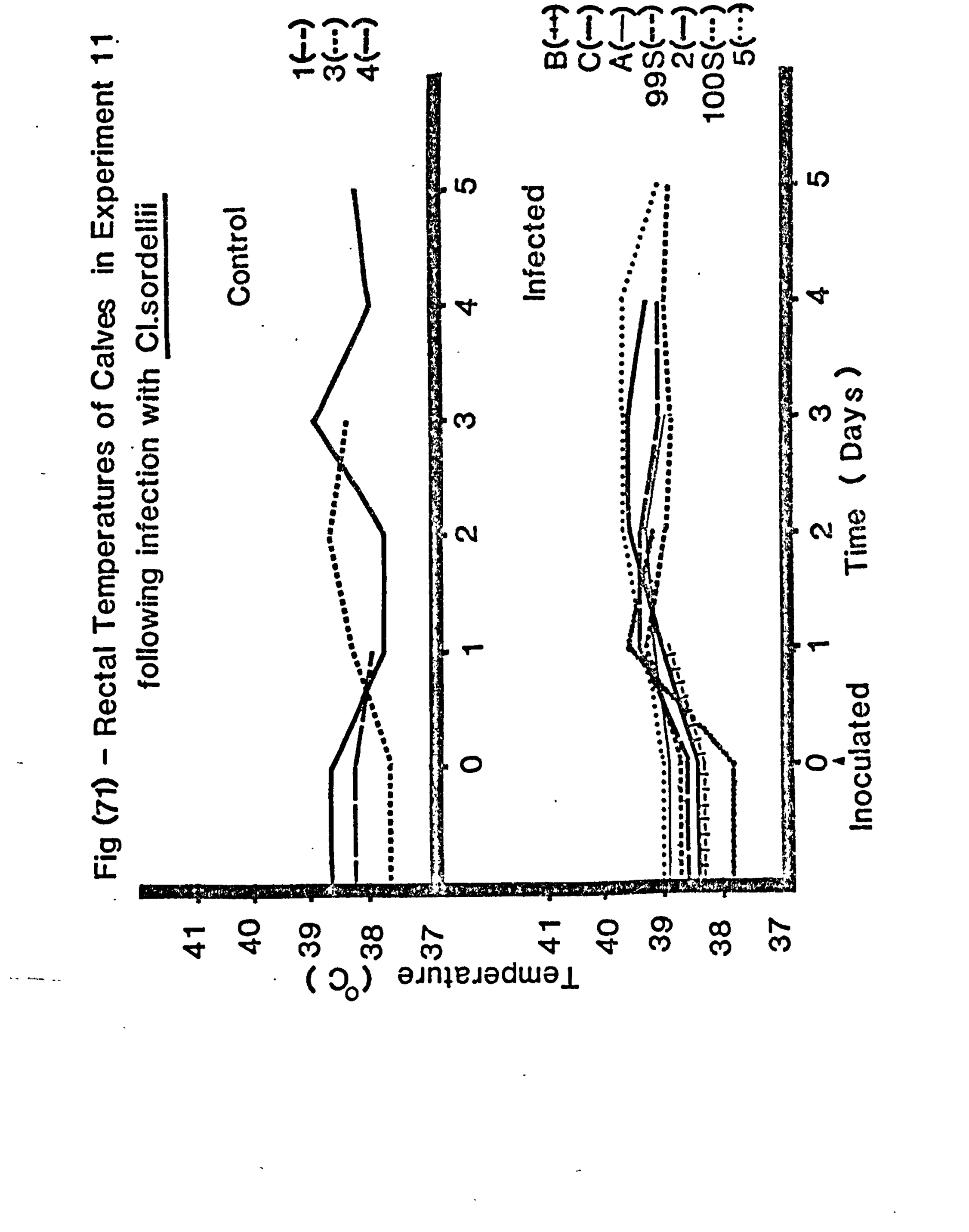


Fig (71) – Rectal Temperatures of Calves in Experiment 11 following infection with Cl.sordellii

contents were watery and mucoid. The mucosa of the terminal ileum appeared fleshy and thickened and the contents were dark and mucoid. The large intestinal mucosa was grossly normal although the contents were slightly mucoid. The mesenteric lymph nodes were enlarged.

Day 3. Calf A (living inoculum)

The abomasal mucosa of calf A was similar to that of calf C. The small intestinal mucosa and the contents also resembled those of calf C. The caecal mucosa was congested with mucoid contents. The mesenteric lymph nodes were enlarged.

Day 4. Calf 99S (living inoculum) and Calf 2 (toxin)

The jejunal contents of calf 99S were pinkish in colour but the remainder of the findings in the abomasum and small intestine resembled those found in calf C (day 2). The large intestine and its contents resembled those of calf A (day 3). In calf 2, the small intestinal contents were yellowish-green and mucoid. The mucosa appeared normal. In the large intestine mucoid material was adherent to the mucosal epithelium. The mesenteric lymph nodes were normal.

Day 5. Calf 100S (living culture) and Calf 5 (toxin)

The findings in calf 100S resembled those in calf A and those in calf 5 resembled those in calf C. The jejunal mucosa of this animal is shown in Fig. 72.

Control animals

The control animals were grossly normal (calf 1 killed on day 1), with the exception of the ileal contents which were watery and slightly mucoid (calf 3, day 3) and normal with the exception of slight thickening of the jejunal mucosa (calf 4 day 5).

Few histological changes were recorded in the controls. In all animals there was mild capillary dilatation in the abomasal mucosa and the presence of cell debris on the epithelial surface.

The was mild cellular infiltration of the remainder of the gastro-intestinal tract and stunting of the jejunal villi in calf 4

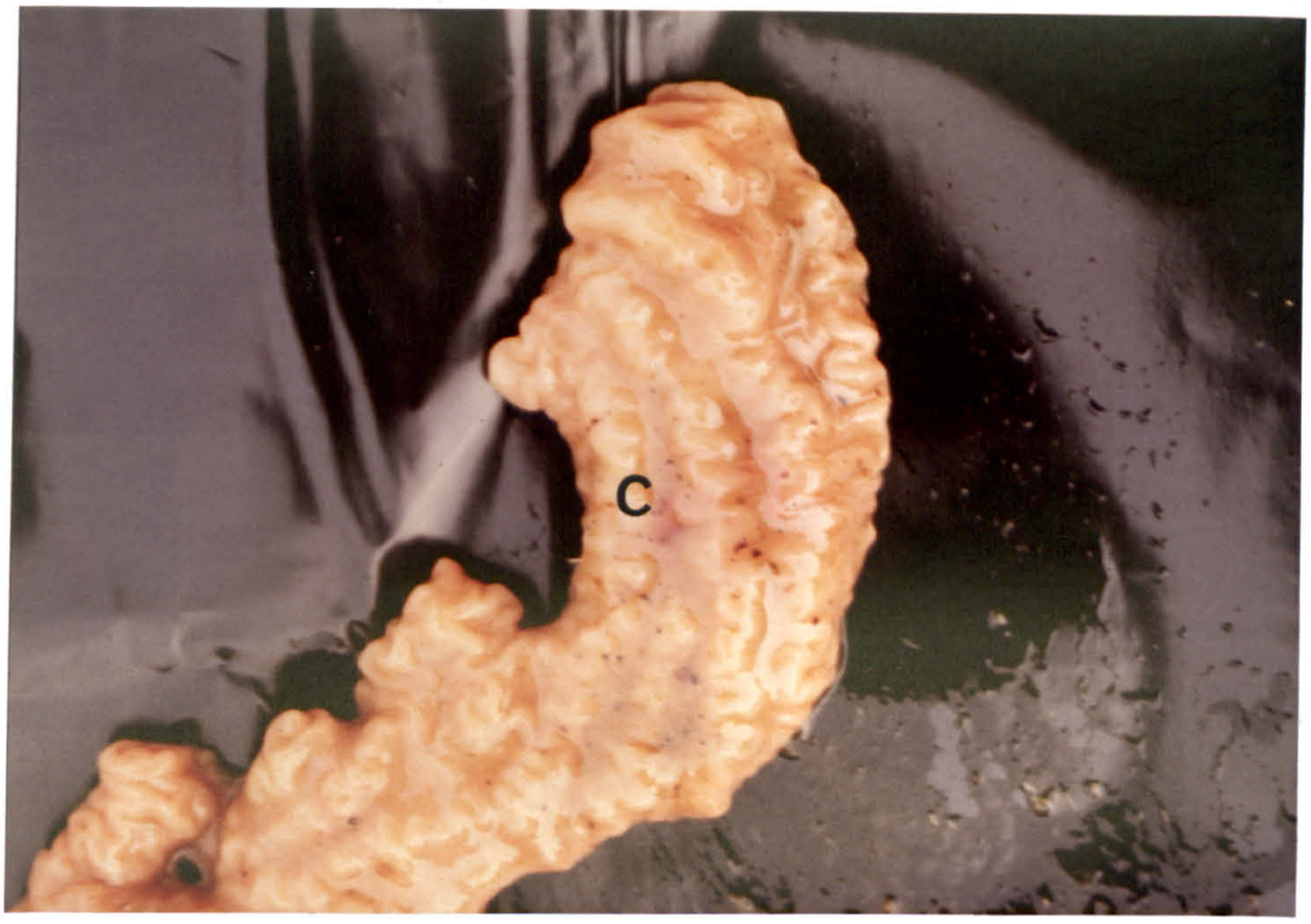


Fig. 72: Jejunal mucosa of calf 5 five days following inoculation with Cl. sordellii culture supernatant.

Note the mucoid appearance of the mucosa and the area of mild congestion (C).

(day 5). The histological changes in the animals infected with whole culture were as follows:-

Day 1. Calf B

The appearance of the mucosa resembled that of the controls. In addition there were masses of bacteria and cell debris plugging the abomasal glands, stunted villi with prominent subepithelial spaces and dilated lacteals in the jejunum in the lamina propria of which large numbers of neutrophilic polymorphonuclear leucocytes were seen. In the ileum the goblet cells were prominent and the changes seen resembled those in the jejunum. Cell debris was present on the surface of the caecum and colon and the mesenteric lymph nodes were oedematous.

Day 2. Calf C.

Changes resembled those in calf B but the abomasal mucosa was oedematous and cuboidal cells were present on the lumenal epithelium of the ileum. Hyperaemia and crypt abscesses were seen in the lamina propria of that organ. Crypt abscesses were also present in the caecum.

Day 3. Calf A.

The changes seen resembled those seen in calf C. There was more dilatation of the abomasal capillaries and more cell debris on the mucosal surface (Fig. 73). Dilated capillaries and eosinophilic material were seen in the crypts of the jejunum and stunted villi and an increase in cellular infiltration were seen in the ileum where lymphoid tissue was prominent. The caecal mucosa is shown in Fig. 74.

Day 4. Calf 99S

The abomasum resembled that of calf B (day 1). The ileum resembled that of calf A (day 3) and in the caecal and colonic mucosa large numbers of neutrophil and eosinophil polymorphonuclear leucocytes were seen.

Day 5. Calf 100S

The abomasum resembled that of calf B (day 1) except that free red blood cells were present in the debris. The jejunal mucosal

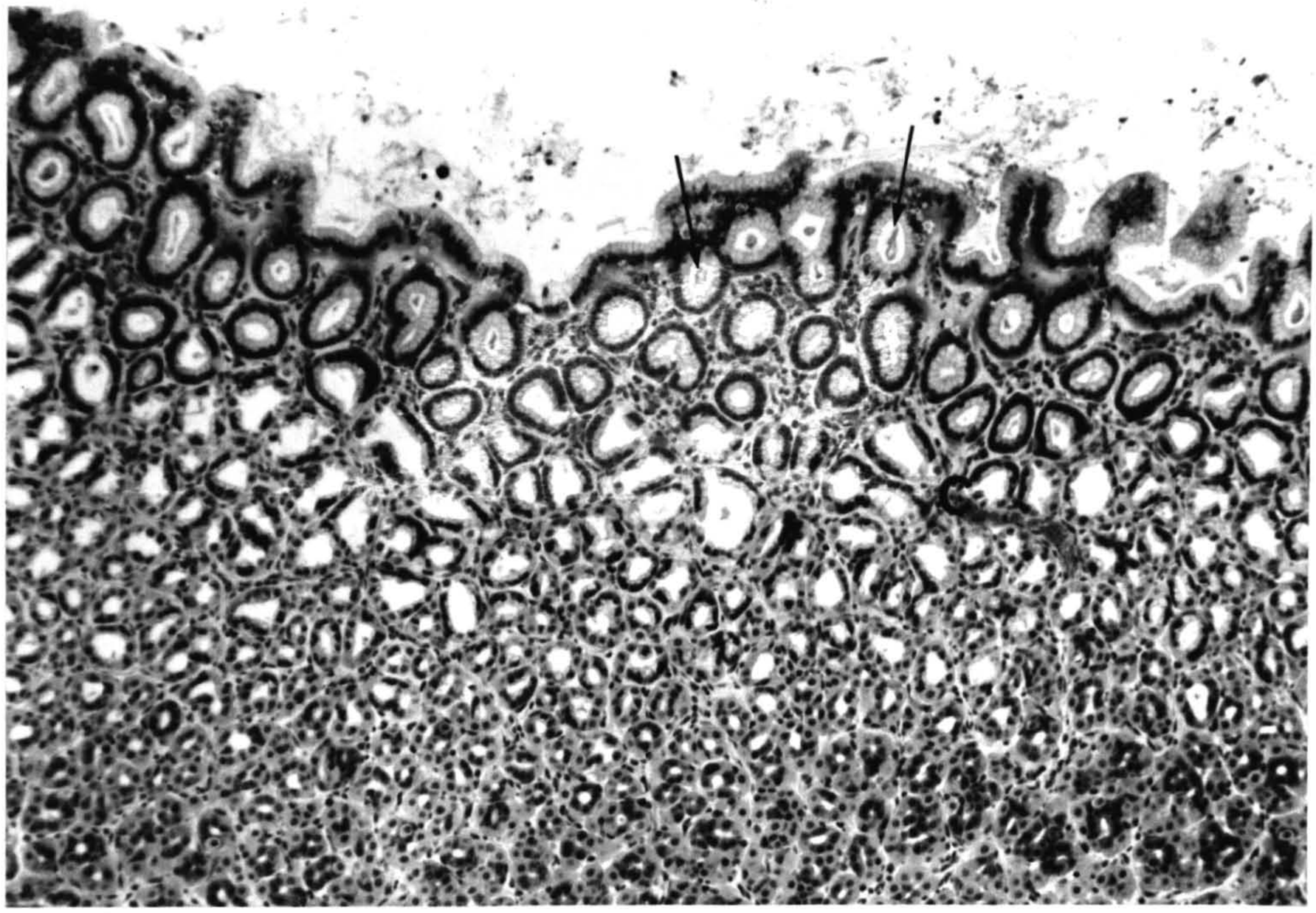


Fig. 73: Histological section of the abomasum of calf A three days post-infection with Cl. sordellii.

Note the presence of bacteria and debris in the glands (arrows) and the dilated capillary (C).

H & E X 110.

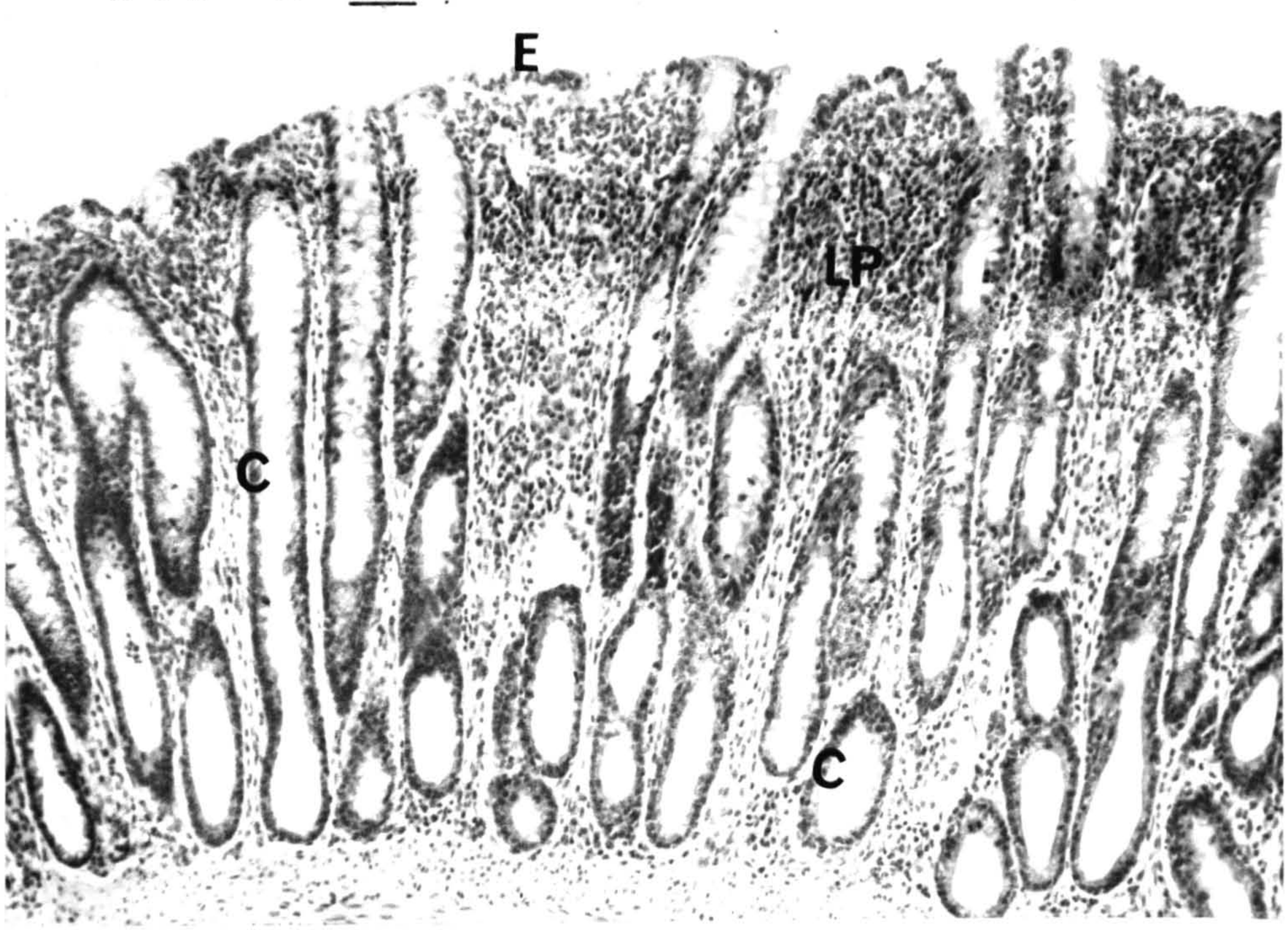


Fig. 74: Histological section of the caecal mucosa of calf A.

Note the dilated crypts (C) the disorganised lumenal epithelium (E) and the hypercellularity of the lumenal part of the lamina propria (LP).

H & E X 35.

epithelium was eroded locally and there was massive cellular infiltration. The ileal findings resembled those in calf A (day 3) and the caecum and colon resembled those of calf C (day 2).

Toxin-inoculated calves

The histological changes in the toxin-inoculated calves (2 and 5) resembled those in the other inoculated animals to a certain extent.

Day 4. Calf 2.

Inflammatory cells were present in the abomasal glands, and there was erosion of the jejunal epithelium and massive cellular infiltration. The ileum resembled that of calf A (day 3) (Fig. 75) and the caecum and colon resembled those of the controls with slightly more capillary dilatation and cellular infiltration.

Day 5. Calf 5.

Abomasal changes resembling those in calf A were seen and there was stunting of villi in the jejunum. Coccidial gametocytes were common in the ileal mucosa. The remaining findings resembled those in calf A.

Colonies of Cl. sordellii were only isolated from the gastro-intestinal tracts of the animals of the group infected with live culture. None were isolated from the toxin-inoculated or control animals. The regions of the gut from which they were isolated are shown in Table 46. They could be seen in Gram-stained smears of infected mucosa.

No agglutinating antibody to Cl. sordellii was demonstrated in any of the sera in this study.

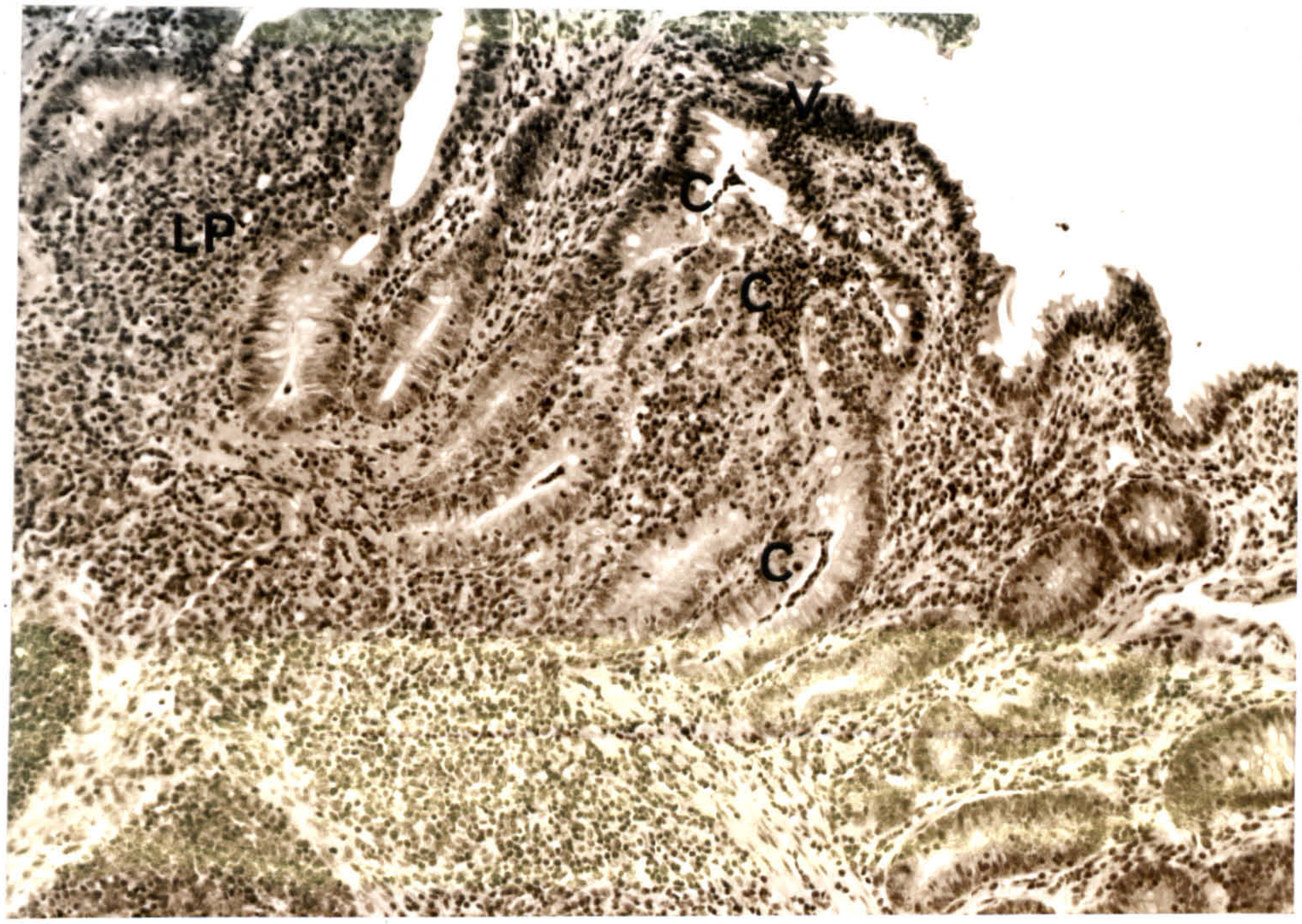


Fig. 75: Histological section of the ileal mucosa of calf 2 four days following infection with Cl. sordellii culture filtrate.

Note probable fusion of villi (V), the presence of crypt abscesses (C) and the hypercellularity of the lamina propria (LP).

H & E X 110.

Table 46.

Sites from which Cl. sordellii was isolated from animals killed at daily intervals following infection with pure cultures and of the organism and its toxin in Experiment 11.

Site of isolation	Day of observation									
	1st		2nd	3rd		4th		5th		
	B(I)	1(C)	C(I)	A(I)	3(C)	99S(I)	2(T)	100S(I)	5(T)	4(C)
Abomasum	+	-	+	+	-	+	-	+	-	-
Jejunum	+	-	-	-	-	-	-	-	-	-
Ileum	+	-	+	+	-	+	-	-	-	-
Caecum	+	-	+	+	-	+	-	+	-	-
Colon	+	-	+	+	-	+	-	+	-	-
Mesenteric Lymph Nodes	-	-	-	-	-	-	-	-	-	-
Liver	-	-	-	-	-	-	-	-	-	-
Gall Bladder	-	-	-	-	-	-	-	-	-	-
Spleen	-	-	-	-	-	-	-	-	-	-
Faeces	+	-	+	+	-	+	-	+	-	-

\+ = Cl. sordellii isolated

\- = No Cl. sordellii isolated

I = Infected

C = Control

T = Toxin

DISCUSSION

The results of Experiments 9 and 10 indicated that Cl. sordellii was capable of iniating clinical and pathological changes when pure cultures were used to inoculate calves by the oral route when the results were compared with controls.

The clinical signs and pathological findings were similar in both experiments. Clinical signs were first seen 1-8 days after infection. In Experiment 9 softening of the faeces and the passage of blood were first seen on days 3-7 but in Experiment 10 they occurred on day 1 within 24 hours of inoculation (Fig. 76). The reason for this difference is not clear as both groups were inoculated with similar numbers of organisms and both groups of animals were of similar breed, age and size. The changes in faecal consistency varied from animal to animal but at some time during the course of the disease the faeces became soft to firm with excess clear mucus. The presence of fresh blood was seen on occasions (Tables 41 and 43). Rectal temperatures were very slightly raised, or not raised at all in the syndrome produced (Figs. 67, 68 and 71).

Cl. sordellii was isolated only from the faeces of the infected animals. In these two experiments it was recovered consistently during the first five days following inoculation. (Tables 41 and 43). Recovery of the organism was not clearly associated with changes in faecal consistency.

Mild changes were seen in the gastrointestinal tract and its associated lymph nodes in the infected animals. These mild changes may have resulted from infection with Cl. sordellii but the organism was only recovered from the large intestine in Experiment 9, considerable contamination occurred in this study and the selective medium was not yet employed. In Experiment 10 when selective medium was used, the organism was recovered from the abomasum and ileum in addition to the large intestine. It is therefore probable that lesions seen in the jejunum and ileum in both experiments owed little to the active presence of Cl. sordellii but could have represented healing lesions.

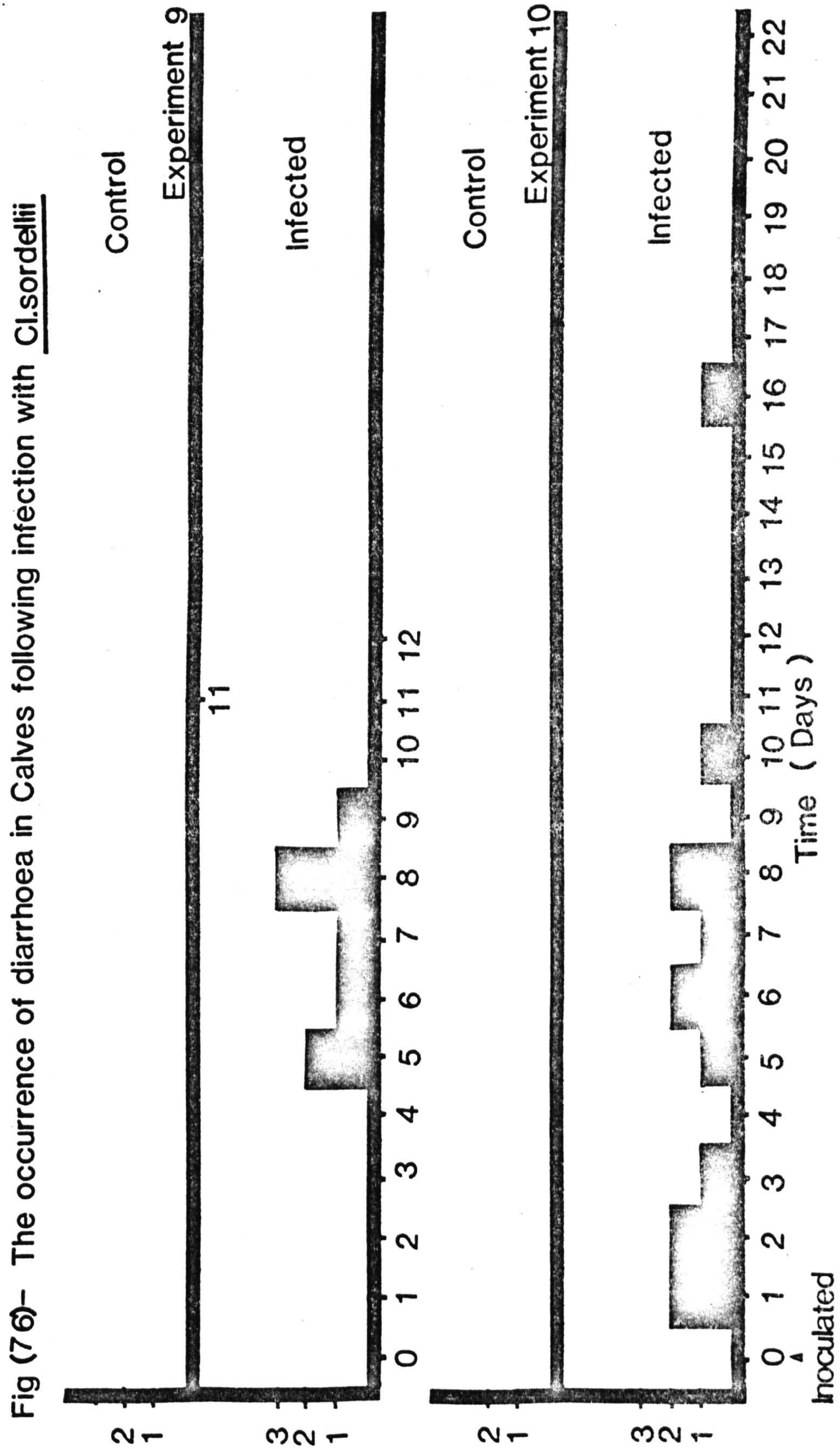


Fig (76)– The occurrence of diarrhoea in Calves following infection with Cl.sordellii

The mild congestion of the jejunal mucosa and hyperaemic thickening of the terminal ileal mucosa seen in the inoculated calves may have been associated with the inoculation of Cl. sordellii. These changes were not seen in the control animals with the exception of a slight excess of clear mucus in the contents of the ileum of animal 115 and slight hyperaemia of the jejunal mucosa of animals 100 (Experiment 9) and 11 (Experiment 10). In most cases the large intestinal content was mucoid and the mucosa was normal with the exception of infected animal 14 (Experiment 10) in which patchy areas of hyperaemia were seen.

In all cases the abomasal mucosa was normal in appearance although the contents were fluid in infected calves 2R and 3R (Experiment 9). Enlargement of the mesenteric lymph nodes was seen in all infected animals. The main feature of the histological findings was disruption of the mucosa of gastrointestinal tract and the presence of slight patches of oedema. This was most prominent in the caecum and colon of infected animals in both experiments and Cl. sordellii was isolated from these lesions.

Other possible pathogens were present in the experiment animals. C.f. ss. intestinalis was isolated in small numbers from the ileum and colon of the three infected animals in Experiment 9. They may have affected the pathological findings but their presence probably did not affect the course of the experiment as they were never detected in the faeces and probably represent an old infection. No other enteric bacterial pathogens were identified, but in Experiment 10 coccidial gametocytes were seen in the small intestinal mucosa of the two control animals and the changes seen in the infected animals may have been affected by the presence of coccidial infection.

The pathological findings were, of course, made after partial or complete clinical recovery and as such may have been less obvious than might have been expected at the height of the disease.

The results of the two studies did, however, contradict those of Brooks et al. (1956) who failed to produce disease by the oral inoculation of calves with pure cultures of Cl. sordellii. The reasons for this difference are not clear.

The results of Experiment 11 (in which animals were killed at daily intervals) confirmed the results of Experiments 9 and 10 in which it was shown that infection with pure cultures initiated only a mild clinical syndrome. The pathological changes of the developing disease were restricted to the gastrointestinal tract. Microscopic changes appeared on the first day post-inoculation and consisted of an inflammatory response in the lamina propria of all levels of the gastrointestinal tract. Gross lesions did not appear until the second and third day post-inoculation when the intestine became flaccid with dark contents and local congestion of the mucosa of the small intestine was observed. Crypt abscesses, oedema and hyperaemia were the major histological features.

On the fourth day post-infection the small intestinal contents of animal 99S were pinkish in colour in addition to the changes noted previously. Histological changes of inflammation were most pronounced in the caecum. Similar changes were seen on day 5.

These changes appeared to be due to the toxin as animals inoculated with toxin alone and from which no bacteria were isolated developed clinical signs resembling those seen in the infected animals In calf 2 changes resembling those in calves infected with cultures were seen but they were not evident in calf 5.

Coccidial infection was present in the calves and may have affected the pathological changes recorded although it was not noted in any of the animals inoculated with live cultures of Cl. sordellii.

The results presented above suggest that there is a marked difference between natural and the experimental infection with Cl. sordellii. This may be due to factors which were not identified in the naturally-affected animals or due to the presence of some level of protective immunity in the experimental animals. The destruction of serum samples and serological records prevented any full examination of the specific immune status of the calves used.

CHAPTER 8.

General Discussion

Introduction

The results of individual studies have been discussed in some detail in Chapters 3, 4, 5, 6 and 7.

In this chapter an attempt has been made to put some results into the context of enteric bacteriology and enteric disease in general.

A large number of bacterial species were isolated from animals studied in the survey. The sites from which they were isolated varied from apparently normal mucosa to severe ulcerative or haemorrhagic lesions. In many cases mucosa which was grossly normal was abnormal when examined histologically. In addition, animals with normal faecal consistency were found to have localised inflammatory lesions in some part of their gastrointestinal tract.

Many of the lesions were attributable to non-bacterial causes as a result of clinical examination, clinical pathology, gross or microscopic pathology or parasitological examination. In many cases, however, the bacteria isolated from the lesions may have been responsible for them or have contributed to their development. The relationship of individual bacteria to the lesions in which they were found and some consideration of their relation to disease in general has been discussed in Chapter 3. The results of that chapter are considered below in the context of the results of Chapters 4, 5, 6 and 7 and of the literature generally. The significance of the remaining results is also discussed.

Results of the survey and their interpretation in terms of bovine enteric disease

A large number of bacterial species was isolated from the bovine enteric mucosa. The bacteria isolated differ in many respects from those isolated from the lumenal flora of the bovine enteric tract. The reasons for this may be twofold:

Firstly, the techniques used for isolation may have been inadequate for the recovery of strict anaerobes such as Butyrovibrio spp. or Bacteroides melaninogenicus unless present in large numbers. The use of prereduced media rather than blood agar and the use of totally anaerobic systems such as those described in the Anaerobe Laboratory Manual might have resulted in the isolation of many more organisms from the lesions. As an example, no lactobacilli were recovered and it is probable that the atmospheric and nutritional conditions under which the majority of Lactobacillis grow were not present in this study.

Secondly, there may be a different bacterial flora in the mucosa and its crypts from that present in the lumen. The evidence for this is circumstantial in view of the technical reasons discussed briefly above but the rarity of the isolation of Cl. butyricum, B. fragilis, Eubacterium spp., Veillonella spp. and Peptostreptococcus spp. suggests that, since these species could be maintained with ease under the conditions of growth used, they may have been absent from the mucosa although known to be present in large numbers in the lumen of the gut of many species of animals (Moore et al., 1969; Schulze and Günther, 1978).

It is possible that the organisms isolated from the mucosa in this study reflected the population actually present there. The evidence for this view is that organisms seen in direct smears taken from the same sites as the inoculum for cultures resembled those actually isolated. This was particularly so with morphologically distinctive organisms such as C. fecalis, F. necrophorum and Cl. sordellii.

The isolation of these bacteria was of limited value in establishing their relationship to disease. In many cases organisms were isolated from grossly normal mucosa, although histological lesions were present. More than one species was often present in a lesion although certain types of lesion were associated with the presence of bacteria such as campylobacters, Cl. perfringens Type A, Cl. sordellii and A. lignieresii as discussed in Chapter 3. The disadvantages of any

single disciplinary approach to a multifactorial disease are evident in the interpretation of the results of the survey.

In some cases history and clinical, pathological or specialist examinations had suggested the presence of an underlying cause to the lesions found. In those cases, the lesions primarily associated with the agent concerned could be discounted when considering the appearance of the site from which the bacterium in question was isolated. A prime example of this type of interpretation is that of the small intestinal lesions in calves 45, 25 and 33 from which campylobacters were isolated but in which their presence was discounted and the lesions were considered largely due to rotavirus infection as this had been demonstrated in the group. Similar interpretations were made in the case of calves 11, 22P, 31P, 34P, 37P, 38P, 44P and 46P which were being used in parasitological infection studies.

In many of the animals, the history of enteric disease was insufficiently complete and the information about viral, chamydial and parasitic agents inadequate to say with certainty that one or more of these types of agents were or had been present in the enteric tracts of the animals concerned. For this reason conclusions about the involvement of such agents in the lesions found at the site of isolation and the role of bacteria in them must of necessity be tentative.

A technical difficulty also increases this uncertainty as the portions of tissue examined histologically differ from those examined bacteriologically. In addition, infectious agents such as coccidia, cryptosporidia or virus may have been present in the tissue a short time prior to death but could not be demonstrated in the material at the time of examination. The absence of a full history of antimicrobial therapy may also have affected the interpretation of the results obtained and may, for example, account for the failure to isolate Salmonella spp.

In spite of these difficulties in interpretation a number of points emerged. They were:

1. Enteric bacteria described as pathogens in the literature were not found in this study.

A number of species of microorganisms whichmight, from the literature reviewed in Chapter 1, be expected in enteric lesions were not isolated from, or demonstrated in, the enteric tracts studied in the survey. The most surprising of these was Salmonella spp. This failure is unlikely to have been for technical reasons as cultures of small intestinal contents were prepared in tetrathionate broth from each animal and the intestinal tracts, lymph nodes and, in some cases, gall bladders were cultured on selective medium. If salmonellae had been present in large numbers they would have been detected. Their absence is therefore likely to reflect the rarity of the organism in the populations from which animals in the survey were obtained. These were experimental animals kept at the Veterinary School and clinical cases admitted for study. In most cases the animals in this series had been ill for some time and may often have been treated on the farm of origin. Many were older animals and may have recovered from any salmonella infection.

A number of other agents were not demonstrated. These included the enterotoxigenic types B, C, D and E of Cl. perfringens. These were rarely found in the extensive surveys of Niilo and Avery (1963) and Vance (1967) and the results obtained in this survey confirmed that their North American results also apply in Britain. Yersinia entero-colitica was not detected, neither were the less commonly found genera of the Enterobacteriaceae, (Providencia, Aerobacter and Klebsiella). In view of the presence of spirochaetes in diarrhoea and colonic lesions in other species such as the pig (Taylor, 1980) and the dog (Zymet, 1969) and their frequent presence in the contents of the bovine alimentary tract (Bryant, 1952), it was of interest that none were seen in smears isolated from the mucosa of the colon or caecum in this study. It is possible that the use of selective media or filtration techniques would have revealed their presence but they were clearly not present in large numbers. They were, however, present in the colonic and caecal mucosa of animal 95 in Experiment 4 and some of the silver-stained organisms seen in sections might also have been spirochaetes. Candida albicans

and other yeasts were not seen in this series, perhaps for similar reasons.

2. A number of bacterial species were associated with particular lesions.

This point has been discussed in Chapter 3 in detail for each bacterial species isolated. Lesions were associated with the presence of campylobacters, Cl. perfringens Type A, Cl. sordellii, F. necrophorum, A. hydrophila, Actinobacillus lignieresii and M. paratuberculosis. Their presence in lesions of the particular type found had been recorded in the literature (C.f. ss. jejuni, Cl. perfringens Type A, F. necrophorum, A. hydrophila, A. lignieresii, and M. paratuberculosis) but the presence of campylobacters other than C.f. ss. jejuni in lesions identical to those in which jejuni was found was of interest. In addition, the presence of Cl. sordellii in distinctive lesions had been recorded in the literature (Brooks et al., 1956) but the organism had been considered non-pathogenic in experiments. These two findings were selected for further study and these studies and their findings are given in Chapters 4, 5, 6 and 7. The results of these studies suggested that all four bacterial isolates tested could initiate disease and reproduce lesions resembling in some way those from which the organisms were isolated. Two inferences can be drawn from this work, firstly that the other bacteria listed above may all be involved in enteric disease and may be primary pathogens in the same way as organisms such as M. paratuberculosis and secondly that many of the other bacteria found in lesions may be pathogens even though the lesions were not grossly or microscopically distinctive.

3. Other bacteria and their causal relationship to the lesions in which they were found.

The remaining bacterial isolates could not be associated specifically with any particular lesion. This was the case with E. coli which was isolated from a wide variety of mucosal sites in the majority of animals in this survey. As no serotyping or pathogenicity testing was carried out, it was not possible to relate E. coli to any particular type of lesion. The only exception was the recovery of two β-haemolytic isolates in which the changes seen may

have been associated with their presence. A deliberate decision was taken at the beginning of this study not to spend time on identifying serotypes of E. coli in view of the extensive literature on the association of enteropathogenic E. coli with intestinal lesions (Morin et al., 1978; Moon, 1974 and Blood et al., 1979). This decision has, however, meant that the significance of some of the other bacterial isolates was more difficult to assess.

Of the remaining organisms, B. licheniformis was most commonly found, sometimes in association with severe lesions and sometimes from only mildly altered mucosa. Most Bacillus species are considered as non-pathogenic with the exception of B. anthracis although enteric syndromes have been associated with food contaminated with haemolytic strains of B. cereus in man. In this study, B. licheniformis may have been common in the food and have been an incidental contaminant of the mucosa and any lesion. It has been found (Jayne-Williams, 1979) in the rumenal contents of a healthy calf at levels of $10^{6.5}$ per ml and it is possibl that multiplication of B. licheniformis in the rumen may act as a source of the organism for the colonisation of the mucosa of the lower alimentary tract. The organism was present most commonly in the upper alimentary tract but was recovered from all sites sampled. It does produce an obvious, marked haemolysin, particularly when grown in anaerobic conditions and it is possible that it may play a part in the lesions in which it is found. Some evidence for its involvement in pathogenic processes has been reviewed in the discussion in Chapter 3 and its involvement with enteric changes in man has also been reported by Sugar and McCloskey (1977).

In view of its close association with the mucosa in so many cases, the difficulty of distinguishing any specific lesion associated with it and the success of the experimental infections with campylobacters and Cl. sordellii, it is a prime candidate for inoculation into non-immune experimental calves. This might help resolve its role in the enteric tract.

The criteria discussed above for B. licheniformis also apply to many of the other bacteria isolated, especially to Bacteroides

vulgatus, B. melaninogenicus and Cl. bifermentans. A further series of cases might provide more evidence for their involvement in enteric lesions but experimental study would probably resolve their role more rapidly.

4. Bacteria present in large numbers in the gut lumen of normal animals were sometimes present in the mucosa.

E. coli is the prime example of these organisms but Streptococcus bovis, B. fragilis, Veillonella spp., Eubacterium aerofaciens and Peptostreptococcus productus were also found. They may be incidental in the mucosa or individual strains of serotypes may, as in E. coli, be associated with disease.

5. Bacteria commonly present in the respiratory tract were found in enteric lesions.

A large group of bacteria normally associated with disease in the respiratory tract was found in the mucosa of the enteric tract. In many cases these bacteria were present in enteric lesions. The bacteria included P. multocida, C. pyogenes, Branhamella catarrhalis, Streptococcus zooepidemicus, Aerococcus viridans and Acinetobacter lwoffii. Once again it is not clear whether these organisms were found in these sites because they are present in large numbers in the lumenal contents, whether they had colonised existing lesions by bacteraemic spread or whether they actually initiated or perpetuated enteric lesions. The association between respiratory and subsequent enteric infections is however, well known in the case of organisms such as Mycobacterium tuberculosis, Mycobacterium bovis and Chlamydia. In these infections the organisms may infect both the respiratory and enteric tracts.

The association between respiratory and enteric lesions is worthy of further study.

Experimental studies with Campylobacter spp.

The isolation of C.f. ss. jejuni, C.f. ss. intestinalis and C. fecalis and unidentified campylobacters from inflammatory lesions

of the bovine enteric tract has been discussed in Chapter 3. The conclusion reached was that all the organisms appeared to be associated with the same type of lesion although only C.f. ss. jejuni was considered in the literature to be an enteric pathogen. The experimental studies described in Chapters 4, 5 and 6 and discussed briefly in those chapters, showed that all three isolates tested for their pathogenicity could initiate clinical and pathological changes which differed only in their severity. Some of these studies have been published (Al-Mashat and Taylor, 1980a and b ; Al-Mashat and Taylor, 1981), (See Appendix 2.).

Most attention in the recent literature has been paid to C.f. ss. jejuni because of its prominent role in food poisoning and enteritis in man. The biochemical distinctions between C.f. ss. jejuni and other campylobacters have been extensively studied on the assumption that only C.f. ss. jejuni is a pathogen. In view of the results obtained in Chapters 4, 5 and 6 this cannot be the case in cattle. Confirmation of the pathogenicity of C.f. ss. jejuni for both calves and sheep has been produced by Firehammer and Myers (1981) who produced a clinical syndrome in calves resembling that described in Chapter 4. Detailed pathological findings were not given. Little further attention has been given to C.f. ss. intestinalis and C. fecalis as causes of enteric disease in cattle or in man, and these studies form the first evidence for the pathogenicity of C.f. ss. intestinalis and C. fecalis for the enteric tract of any animal other than the brief description of the isolation of C.f. ss. intestinalis from lesions in calves by Allsup and Hunter (1973).

The identification of the isolates used in this study as C.f. ss. jejuni and C.f. ss. intestinalis is in little doubt as they conform to published criteria for these subspecies (Smibert, 1974 and 1978; Veron and Chatelain, 1973) but the evidence for the identification of the C. fecalis isolates as such is less well documented. The isolate used here has been confirmed as C. fecalis by Skirrow (1979b) and fulfils the criteria for C. fecalis described by Smibert (1974 and 1978). There seems, therefore, to be little doubt that the

organism identified as C.f. ss. jejuni, C.f. ss. intestinalis and C. fecalis really do correspond to the species described in the literature.

In view of this identification and the results of the experiments cited above it is of interest to speculate about the scarcity of the attention paid to campylobacters other than C.f. ss. jejuni by workers in the medical field. The frequent use of an incubation temperature of 43°C by the medical microbiologist as suggested by Skirrow (1977) makes the isolation of C.f. ss. intestinalis extremely unlikely. In addition the difference in colonial morphology between C. fecalis and C.f. ss. jejuni may make the selection of colonies of the former less likely by medical laboratory technicians. There may also be an epidemiological factor. C.f. ss. jejuni may be the organism most widespread in human food and in the environment. It has now been recorded from horses (Atherton and Ricketts, 1980); cat and dogs (Bruce et al., 1980; Fleming, 1980; Skirrow, 1981); poultry (Cruikshank, 1981 from mastitis in cattle (Lander and Gill, 1980) and from seagulls and other wild birds (Skirrow, 1981). Many animal species are capable of harbouring campylobacters, of serotypes which are identical to those causing disease in man (Lauwers, et al., 1981). Many human outbreaks have been associated with contact with animals (Svedhem and Norkrans, 1980).

It is also clear from epidemiological evidence that campylobacters are present in water particularly in summer (Pearson et al., 1977). The disease caused by C.f. ss. jejuni in man and other species may be more severe than that associated with the other two organisms as in the limited studies described in Chapters 4, 5 and 6. However, infections in man with C.f. ss. intestinalis have been reported. They are usually identified as blood-borne infections often associated with meningitis but this syndrome is often preceded by blood-stained diarrhoea about which there seems to be little information (Bokkenheuser, 1981).

There is little direct evidence in the medical literature to contradict the findings described and discussed above that C.f. ss.

intestinalis and C. fecalis may be enteric pathogens in the same way as C.f. ss. jejuni. The evidence for their consideration as non-pathogens in animals was reviewed in Chapter 1 and discussed in Chapters 3, 4, 5 and 6.

The studies described in Chapter 4 provided some information about the pathogenesis of C.f. ss. jejuni infections in cattle. No previous studies of this type have been recorded in cattle although some information about the relationships between C.f. ss. jejuni and the host has been published for species such as chickens, dogs, monkeys and man. Some of this information comes from clinical observations, some from experimental studies with whole animals and some from in vitro studies.

Infection appears to be oral in all cases. The source of infection is usually contaminated food or drinking water or fomites which have been in contact with infective faeces. Following infection, fever and some abdominal discomfort occur. These are accompanied one to five days after infection by diarrhoea which in all species appears to contain varying amounts of blood and mucus. In a few cases in man, the organism can be recovered from the blood during the course of the disease (Butzler and Skirrow, 1979). In the monkey, the organism can be recovered from the blood for one to three days after infection (Fitzgeorge et al., 1981).

In the studies described in Chapter 4 little evidence was found for the presence of C.f. ss. jejuni outside the gut. Blood cultures from clinically affected animals were negative in Study 1, Part 2 and the organism was isolated from sites outside the gut only within the first 48 hours after infection in Experiment 4 (Table 27). It was, however, recovered from the gall bladder in Experiment 2 (Table 21) and the inference is that in some cases bacteraemia occurs early in the disease, but that it is not a major feature of the pathogenesis. The demonstration of serum agglutinating antibody to C.f. ss. jejuni within 72 hours of infection (Experiment 4) may account for the brief period of the bacteraemia.

The organism appears to colonise the whole gastrointestinal tract and may be found in the abomasum and jejunum in large numbers within the first 48 hours of infection but later becomes limited to the ileum, caecum and colon.

Jones and Little (1931a and b) recorded the organism in the jejunum but this early colonisation of the abomasum does not appear to have been described previously and may account for its recovery from abomasal lesions in cases 22P, 31P, 34P, 37P, 38P, 44P, 46P and 82773 in the survey. Its presence in the colon and caecum has parallels in the pig with C.f. ss. coli (Vibrio coli), Doyle, 1944, 1948), in man (Lambert et al., 1979) and in gnotobiotic dog (Prescott and Barker, 1980) with C.f. ss. jejuni.

Within an infected portion of the gut the organisms are present both in the contents and in close association with the mucosa. They may be isolated from the thickness of the mucosa and silver-stained organisms resembling them were seen in the silver-stained sections prepared in Experiment 4. These organisms appeared to be within the crypts and at the mouth of the crypts and in some cases to lie within the lamina propria. In the electron microscopical studies of tissue from the animals of Experiment 4, organisms with dimensions and ultra-structure resembling that of C.f. jejuni were seen in the sites mentioned above with the exception of the lamina propria.

A number of experiments have suggested that C.f. ss. jejuni can attach to cells and penetrate them. Butzler and Skirrow (1979) describe penetration in the caecum of the chicken and in in vitro studies with HeLa cells, Newell and Pearson (1981) also suggested that this type of relationship could occur. The failure to observe invasion in this series has been discussed briefly in Chapter 4. Additional factors may include the possible presence of immunity in the calves used. No agglutinating antibody to the strain used was detected and no evidence was obtained by culture of faeces or of the mucosa of the controls that C.f. ss. jejuni was present. There may, however, have been some local immunity which prevented invasion. In addition, the rapid rise in serum agglutinating antibody noted may have prevented invasion of the

lamina propria in these calves.

The effects noted in the mucosa may have been caused by a toxic product of the organism. There is considerable discussion on this point at the moment. Some, e.g., Gubina et al. (1981) have suggested that an enterotoxin is produced. Others such as Butzler and Skirrow (1979) agree, but suggest that it is not a heat labile enterotoxin. The most likely type of toxin is an endotoxin as demonstrated by Fumarola et al. (1981) and it may be the Lipid A described by Naess and Hofstad (1981) in biochemical studies. The outpouring of neutrophilic polymorphonuclear leucocytes and lymphocytes into the crypts and their presence in the lamina propria would suggest that a mechanism of this type could be involved.

Organisms with the morphology and ultrastructure of C.f. ss. jejuni were adjacent to, but not closely adherent to the microvilli of the lumenal epithelial and crypt cells in the acute early stage of the disease (Figs. 51 and 52). This loose association was similar to that noted in the adhesion studies carried out in vitro and described in the final part of Chapter 4.

Invasion of the epithelial cells was not seen and was not considered to be the cause of the lesions seen in the calves killed later in the pathogenesis study.

C.f. ss. jejuni is a microaerophilic organism and its requirement for conditions of this type may govern its location in the body and the numbers present. The studies described above are experimental infections with C.f. ss. jejuni alone but in many cases the organism is present with other agents or in lesions initiated by them. Their presence in the ileum, caecum and colon in infected animals may reflect their microaerophilic requirements and their absence from the crypts of inflamed mucosa in the ileum and presence in the lumen of affected organs may reflect a local increase in oxygen tension. In the caecum and colon where conditions are more anaerobic, the optimum site for

growth may be the crypts of the mucosa and in particular the region at the mouth of the crypt. Tissue debris, serum and blood produced by C.f. ss. jejuni or other agents may enhance their ability to tolerate anaerobic conditions and effect their distribution and numbers within the gut.

The organism appeared to persist in the mucosa of infected animals long after reliable recovery from faecal samples was possible (Experiments 1 and 3) and all experimental animals were positive upon mucosal culture even when difficulty had been experienced in isolating the organism from the faeces. This suggests that the detection of carriers by faecal sampling may be difficult as is bourne out by the finding of infection with C.f. ss. jejuni in the controls of Experiment 2 and finding C.f. ss. intestinalis in the animals infected with Cl. sordellii in Experiment 9.

Finally, C.f. ss. jejuni can clearly cause an enteric syndrome which can be detected on clinical and pathological grounds. In enteric disease of cattle, infection with this organism may contribute to the features described above - low fever, the presence of blood and mucus in the faeces, thickening of the terminal ileum and oedema of the mesenteric lymph nodes. In many cases C.f. ss. jejuni is probably present in the inoculum which initiates the disease but in others it may be present locally in the mucosa of carrier animals and multiply when conditions favour its growth. Its association with 'winter dysentery' or 'winter diarrhoea' has not been demonstrated in the experiments described here. Some features of that disease - the short incuabation period, presence of blood and mucus in the faeces and the uniform texture of that faeces are present in both winter dysentery and the experimental syndrome. In no case, however, was the diarrhoea as profuse as that commonly seen in outbreaks of winter dysentery. This may be due to the age of the animals affected (adults in many outbreaks of winter dysentery) or to the fact that all experimental animals had a high roughage diet and not the low roughage one commonly available to affected stock in field outbreaks.

Some experimental studies in which calves were infected with

C.f. intestinalis and C. fecalis have been discussed in Chapters 5 and 6. It is of interest that both organisms could induce changes resembling those caused by C.f. ss. jejuni, thus confirming the findings of the survey that all three were present in similar lesions. The syndromes produced were less marked than with C.f. ss. jejuni but they may well contribute to enteric disease in cattle and should not be ignored or regarded as normal inhabitants of the gastrointestinal tract as was the case before this study was carried out.

Experimental studies with Clostridium sordellii

Cl. sordellii was isolated from distinctive lesions of the intestinal tract of two cases in the survey and in the experiments described in Chapter 7, an isolate of the organism was used to infect calves. The experimental disease was mild when compared with the pathological findings in the animals from which the organism was isolated in the survey but shared some essential features. The relationship of the changes seen to toxin production has been discussed briefly in Chapter 7. Additional evidence for the importance of toxin in the pathogenesis comes from the in vitro study of adhesion of organisms to isolated brush borders described in the last part of Chapter 4 Part 2. Cl. sordellii did not adhere to isolated brush borders and in the serial killing carried out in Experiment 11 no evidence for ulceration was seen in sites from which Cl. sordellii was isolated.

Studies of the relationship of Cl. sordellii and its relations Cl. bifermentans and Cl. difficile to enteric disease is still developing and the association of the latter with pseudomembranous colitis in man has been described by George et al.(1978); Burdon (1981) and Bolton et al. (1980). These three authors have stressed the role of toxin production by Cl. difficile in the disease in man. Faecal examination for the toxin is not uncommonly carried out in cases of pseudomembranous colitis for the detection of infection with Cl. difficile. The toxins of all members of this group are antigenically related and the methods used for toxin analysis in human faeces could be employed in further work. In addition the role of the toxin in the syndrome produced in

Experiments 9, 10 and 11 could be confirmed by the use of specific antisera to it and by vaccination with a toxoid.

The lesions seen in the natural cases described in the survey resemble to some extent those of pseudomembranous colitis in man but only in the chronic stage of infection (Experiments 9 and 10) was Cl. sordellii restricted to the large intestine in the acute studies described in Experiment 11, the organism was also present in the small intestines where some changes were seen. A report of the isolation of Cl. difficile from the small intestine has recently been published (Taylor et al., 1981) suggesting that this organism can also inhabit the small intestine.

The studies described in Chapter 7 and discussed briefly here have drawn attention to the potential enteropathogenicity of Cl. sordellii in cattle and suggest that further studies of its frequency in the cattle population and especially in animals with haemorrhagic enteritis would be of value. These might take the form of cultural studies, studies of the frequency of serum antitoxic antibody or studies of the presence of toxin in the faeces.

Conclusion

In conclusion, this study has shown that a number of bacteria are to be found in the mucosa of the bovine enteric tract and that many of them are associated with gross or microscopic lesions. The bacteria isolated from the lesions were in many cases not considered to be pathogens of the bovine enteric tract but the evidence provided by the experiments described in Chapters 4, 5, 6 and 7 suggests that at least some of them may be able to initiate changes. The studies described here provide a starting point for a radical reassessment of bacteria and their relationship to the bovine enteric tract and suggest that bacteria may be as important in enteric disease in cattle as viruses and protozoa are currently thought to be. The studies also provide information of importance in the understanding of enteric disease in other animal species, both in terms of the approach used here and in terms of the species shown to be involved in enteric lesions.

References

Allsup, T.N. and Hunter, D. (1973).
Vet. Rec. 93, 389-392.

Allsup, T.N., Matthews, K.P., Hogg, S.D. and Hunter, D. (1972).
Vet. Rec. 90, 14-15.

Al-Mashat, R.R. and Taylor, D.J. (1980a).
Vet. Rec. 107, 31-34.

Al-Mashat, R.R. and Taylor, D.J. (1980b).
Vet. Rec. 107, 459-464.

Al-Mashat, R.R. and Taylor, D.J. (1981).
Vet. Rec. 109, 97-101.

Amstutz, H.E. (1965).
J.A.V.M.A. 147, 1360-1363.

Anaerobe Laboratory Manual (1977).
4th ed. Eds. L.V. Holdeman, E.P. Cato and W.E.C. Moore.
Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Anderson, N.V. (1980).
VETERINARY GASTROENTEROLOGY. Lea and Febiger, Philadelphia.

Anderson, N., Armour, J., Jarrett, W.F.H., Jennings, F.W., Ritchie, J.S.D. and Urquhart, G.M. (1965).
Vet. Rec. 77, 1196-1204.

Annapurna, E. and Sanyal, S. (1977).
J. MED. MICROBIOL. 10, 317-323.

Anon (1971).
Nutrient requirements of dairy cattle, Washington, D.C.
National Academy of Science.

Armour, J. (1970).
Vet. Rec. 86, 184-190.

Armour, J. (1974).
Vet. Rec. 95, 391-395.

Atherton, J.G. and Ricketts, S.W. (1980).
Vet. Rec. 107, 264-265.

Barr, F.S., Carman, P.E. and Clarkson, T.B. (1955).
Am. J. Vet. Res. 16, 515-516.

Bergey's Manual of determinative bacteriology (1974).
8th ed. Eds. R.E. Buchanan and N.E. Gibbons. Williams and Wilkins, Baltimore.

Biberstein, E.L., Knight, H.D. and England, K. (1968).
J.A.V.M.A. 153, 1045-1049.

Bibrack, B. and McKercher, D.G. (1971).
Am. J. Vet. Res. 32, 805-807.

Blood, D.C. and Helwig, D.M. (1957).
Aust. Vet. J. 33, 144-146.

Blood, D.C., Henderson, J.A., Radostits, O.M., (1979).
Veterinary Medicine, a textbook of the diseases of cattle, sheep, pigs and horses. 5th ed. Balliere Tindall, London.

Bokkenheuser, V.D. (1981).
Abstract - Proc. Inter. Workshop on campylobacter infection - University of Reading. 24-26th May.

Bolton, R.P., Sherriff, R.J. and Read, A.E. (1980).
Lancet, 1, 383-384.

Bosworth, T.J. (1943).
J. COMP. PATH. 53, 245-255.

Bridger, J.C. and Woode, G.N. (1975).
Br. vet. J. 131, 528-535.

Brooks, M.E. Sterne, M. and Betty, R.W. (1956).
Vet. Rec. 68, 121-122.

Brown, D.D., Ross, J.G. and Smith, A.F.G. (1976).
Res. vet. Sci. 21, 335-340.

Bruce, D., Zochowski, W. and Fleming, G.A. (1980).
Vet. Rec. 107, 200-201.

Bryant, M.D. (1952).
J. Bacteriol. 64, 325-335.

Bryner, J.H., Frank, A.H. and O'Berry, P.A. (1962).
Am. J. Vet. Res. 23, 32-41.

Bulmer, W.S., Tsai, K.S. and Little, P.B. (1975).
J.A.V.M.A. 166, 233-238.

Burdon, D.W. (1981).
Med. Lab. Science. 38, 253-258

Burrows, M.R., Sellwood, R. and Gibbons, R.A. (1976).
J. Gen. Microbiol. 96, 269-275.

Butzler, J.P. and Skirrow, M.B. (1979).
Clinics in Gastroenterology. 8, 737-765.

Bywater, R.J. (1976).
Jl S. Afr. vet. Ass. 47, 193-195.

Catlin, B.W. (1970).
INTER. J. SYST. BACTERIOL. 20, 155-159.

Clarke, E.G.C. and Clarke, M.L. (1975).
Veterinary Toxicology. First edition. Bailliere Tindall, London.

Collins, C.H. and Lyne, P.M. (1976).
Microbiological Methods. 4th ed. Butterworths, London. p.416.

Cowan, S.T. and Steel, K.J. (1974).
Manual for the identification of medical bacteria. 2nd ed.
Revised by S.T. Cowan, Cambridge University Press, Cambridge.

Cruickshank, J.G. (1981).
Abstract - Proc. Inter. workshop on campylobacter infection - 24 - 26h May University of Reading.

Davis, A.B., Robert, T.A., Smart, J.L. and Bradshaw, P.R. (1974).
Vet. Rec. 94, 412-414.

De Jong, H. and Ekdahl, M.O. (1965).
N.Z. vet J. 13, 59-64.

Doughri, A.M., Young, S. and Storz, J. (1974).
Am. J. Vet. Res. 35, 939-944.

Dow, C., Jarrett, W.F.H. and McIntyre, W.I.M. (1956).
Vet. Rec. 68, 620-623.

Doyle, L.P. (1944).
Am. J. Vet. Res. 5, 3-5.

Doyle, L.P. (1948).
Am. J. Vet. Res. 9, 50-51.

Doyle, T.M. and Spears, H.N. (1951).
Vet. Rec. 63, 355-359.

Doyle, T.M. (1953).
Vet. Rec. 65, 363-365.

Drury, R.A.B. and Wallington, E.A. (1967).
Carleton's Histological Technique. 4th ed. Oxford University Press, Ely House, London W.I. p. 331.

Dunne, H.W., Huang, C.M. and Lin, W.J. (1974).
J.A.V.M.A. 164, 290-294.

Du Plessis, J.L., Cameron, C.M. and Langen, E. (1967).
J.S. Afr. vet. med. Ass. 38, 121-128.

Durham, P.J.K. and Burgess, G.W. (1977).
N.Z. vet. J. 25, 233-235.

Durham, P.J.K., Stevenson, B.J. and Farquharson, B.C. (1979).
N.Z. vet. J. 27, 30-32.

El Azhary, M.A.S.Y. (1968).
Mededelingen der Veeartsenijschool van de Rijksuniversiteit te Gent. 12, 1-75.

El Nasir, M. (1959).
Bull. epiz. Dis. Afr. 7, 223-225.

Eugster, A.K. and Storz, J. (1971).
J. INFEC. DIS. 123, 41-50.

Evans, J.B. (1974).
Bergey's Manual of Determinative Bacteriology.
8th ed. Eds. R.E. Buchanan and N.E. Gibbons. Baltimore,
Williams and Wilkins. p. 515-516.

Fincher, M.G. and Fox, F.H. (1963).
DISEASES OF CATTLE . Edited by Gibbons, W.J.
AMERICAN VET. PUBLICATION pp. 207-210.

Firehammer, B.D. (1965).
Cornell Vet. 55, 482-494.

Firehammer, B.D. and Myers, L.L. (1981).
Am. J. Vet. Res. 42, 918-922.

Fitzgerald, P.R. and Mansfield, M.E. (1972).
Am. J. Vet. Res. 33, 1391-1397.

Fitzgeorge, R.B., Baskerville, A. and Lander, K.P. (1981).
J. Hyg. Camb. 86, 343-351.

Fleming, M.D. (1980).
Vet. Rec. 107, 202.

Flewett, T.H. (1978).
J.A.V.M.A. 173, 538-543.

Flewett, T.H., Bryden, A.S., Davies, H., Woode, G.N., Bridger, J.C. and Derrick, J.M. (1974).
Lancet, 2, 61-63.

Florent, A. (1953).
Compte rendu Societe Belge de biologie, 147, 2066-2069.

Florent, A. (1959).
Proc. 16th Int. Vet. Congr. Madrid, 2, 953-957.

Foggie, A. (1977).
Vet. Rec. 100, 315-317.

Fumarola, D., Miragliotta, G., Jirillo, E. and Monro, R. (1981).
Abstract - Proc. Inter. Workshop on campylobacter infection - 24-26th March - University of Reading.

Gardiner, M.R. and Craig, J. (1961).
Vet. Rec. 73, 372-373.

Garvie, E.I. (1967).
J. appl. Bacteriol. 30, 255-260.

Gay, C.C. (1965).
BACT. REV. 29, 75-101.

Gay, C.C., McKay, K.A. and Barnum, D.A. (1964).
Can. vet. J. 5, 297-308.

George, R.H., Symonds, J., Dimock, F., Brown, J.D. Arabi, Y., Shinagawa, N., Keighley, M.R.B., Alexander-Williams, J. and Burdon, D.W. (1978).
Br. Med. J. 1, 695.

Gerring, J.C. (1947).
Aust. Vet. J. 23, 122-124.

Gibson, E.A. (1961).
Vet. Rec. 73, 1284-1296.

Gills, N., Borland, E.D. Counter, D.E. and Gibson, E.A. (1977).
Vet. Rec. 100, 336-337.

Gilmour, N.J.L. (1965).
Vet. Rec. 77, 1322-1326.

Gilmour, N.J.L. (1976).
Vet. Rec. 99, 433-434.

Gilmour, N.J.L. and Angus, K.W. (1976a).
Res. vet. Sci. 20, 6-9.

Gilmour, N.J.L. and Angus, K.W. (1976b).
Res. vet. Sci. 20, 10-12.

Gilmour, N.J.L., Nisbet, D.I. and Brotherston, J.G. (1965).
J. COMP. PATH. 75, 281-286.

Griner, L.A. and Bracken, F.K. (1953).
J.A.V.M.A. 122, 99-102.

Griner, L.A., Aichelman, W.W. and Brown, G.D. (1956).
J.A.V.M.A. 129, 375-376.

Gubina, M., Zeleznik, Z., Mehle, J., Zajc-Satler, J. and Dragas, A.Z. (1981).
Abstract - Proc. Inter. Workshop on campylobacter infection - 24-26th May, University of Reading.

Hallgren, Av. W., Karlsson, N. and Wramby, G. (1954).
Nord. vet. med. 6, 469-480.

Hauschild, A.H.W., Niilo, L. and Dorward, W.J. (1967).
J. INFEC. DIS. 117, 379-386.

Hauschild, A.H.W., Niilo, L. and Dorward, W.J. (1968).
APPL. MICROBIOL. 16, 1235-1239.

Hauschild, A.H.W., Niilo, L. and Dorward, W.J. (1970a).
CAN. J. MICROBIOL. 16, 331-338.

Hauschild, A.H.W., Niilo, L. and Dorward, W.J. (1970b).
CAN. J. MICROBIOL. 16, 339-343.

Hauschild, A.H.W., Niilo, L. and Dorward, W.J. (1971).
CAN. J. MICROBIOL. 17, 987-991.

Hedstrom, H. and Isaksson, A. (1951).
Cornell Vet. 41, 251-253.

Henning, M.W. (1953).
Onderstepoort J. Vet. Res. 26, 25-44.

Hepple, J.R. (1952).
Vet. Rec. 64, 633-634.

Hoadley, A.W. and McCoy, E. (1968).
Cornell Vet. 58, 354-463.

Holdeman, L.V. and Moore, W.E.C. (1974).
Bergey's Manual of Determinative Bacteriology. 8th ed.
Eds. R.E. Buchanan and N.E. Gibbons, Baltimore, Williams
and Wilkins. pp. 641-657.

Hole, N.H. and Maclay, M.H. (1959).
Vet. Rec. 71, 1145-1149.

Hormaeche, E. and Edwards, P.R. (1960).
INTER. BULL. BACTERIOL. NOMENCLATURE and TAXONOMY. 10, 71-74.

Horner, G.W., Hunter, R. and Kirkbridge, C.A. (1975).
N.Z. vet. J. 23, 98.

Huck, R.A. (1957).
J. COMP. PATH. 67, 267-276.

Hughes, L.E., Gibson, E.A. Roberts, H.E., Davies, E.T., Davies, G.
and Sojka, W.J. (1971).
Br. vet. J. 127, 225-237.

Inoue, M. and Kurose, M. (1975).
Jap. J. vet. Sci. 37, 91-93.

Jayne-Williams, D.J. (1979).
J. Appl. Bacteriol. 47, 271-284.

Jensen, R., Flint, J.C. and Griner, L.A. (1954).
J.A.V.M.A. 54, 5-14.

Johnston, K.G. (1959).
Aust. Vet. J. 35, 101-110.

Jones, F.S. (1933).
Cornell Vet. 23, 117-122.

Jones, F.S. and Little, R.B. (1931a).
J. Exp. Med. 53, 835-843.

Jones, F.S. and Little, R.B. (1931b).
J. Exp- Med. 53, 845-851.

Jones, F.S., Little, R.B. and Orcutt, M. (1932).
J.A.V.M.A. 81, 610-619.

Jones, F.S., Orcutt, M. and Little, R.B. (1931).
J. Exp. Med. 53, 853-863.

Jubb, K.V.F. and Kennedy, P.C. (1970).
PATHOLOGY OF DOMESTIC ANIMALS. Vol. 2, 2nd ed.
Academic Press, New York and London.

Julian, R.J. (1975).
Can. vet. J. 16, 33-43.

Julian, R.J., Harrison, K.B. and Richardson, J.A. (1976).
MOD. VET. PRACT. 57, 711-718.

Kanoe, M., Imagawa, H. and Toda, M. (1975).
BULL. OF THE FAC. OF AGRIC. YAMAGUTI UNIV. 26, 161-172.

Karnovosky, M.J. (1965).
J. CELL BIOLOGY. 27, 137A-138A.

Keast, J.C. and McBarron, E.J. (1954).
Aust. Vet. J. 30, 305-306.

Kerry, J.B. (1964).
Vet. Rec. 76, 396-397.

King, E.O. (1957).
J. INFEC. DIS. 101, 119-128.

Kluge, J.P., Merkal, R.S., Monlux, W.S., Larsen, A.B., Kopecky, K.E., Ramsey, F.K. and Lehmann, R.P. (1968).
Am. J. Vet. Res. 29, 953-962.

Lambert, G., Fernelius, A.L. and Cheville, N.F. (1969).
J.A.V.M.A. 154, 181-189.

Lambert, M.E., Schofield, P.F., Ironside, A.G. and Mandal, B.K. (1979).
Br. Med. J. 1, 857-859.

Lander, K.P. and Gill, K.P.W. (1979).
Vet. Rec. 105, 333.

Lander, K.P. and Gill, K.P.W. (1980).
J. Hyg. Camb. 84, 421-428.

Lauwers, S., Vlaes, L. and Butzler, J.P. (1981).
Lancet. 1, 158-159.

Lawson, G.H.K. and Rowland, A.C. (1974).
Res. vet. Sci. 17, 331-336.

Lawson, G.H.K., Rowland, A.C. and Wooding, D. (1975).
Res. vet. Sci. 18, 121-126.

Lawson, G.H.K., Rowland, A.C. and Roberts, L. (1976).
J. MED. MICROBIOL. 9, 163-171.

Lederle, G. (1963).
Inaug. Diss. Munich pp. 44. Abstract 3151 in VET.BULL. (1964). 34, pp. 505.

Loesche, W.J. Socransky, S.S. and Gibbons, R.J. (1964).
J. Bacteriol. 88, 1329-1337.

Logan, E.F. (1974).
Br. vet. J. 130, 405-412.

Logan, E.F. and Penhale, W.J. (1971).
Vet. Rec. 88, 222-228.

Logan, E.F. and Penhale, W.J. (1972).
Vet. Rec. 91, 419-423.

Lozano, E.A., Catlin, J.E. and Hawkins, W.W. (1970).
Cornell Vet. 60, 347-359.

Love, R.J., Love, D.N. and Edwards, M.J. (1977).
Vet. Rec. 100, 65-68.

Macpherson, L.W. (1957).
Can. J. Comp. Med. 21, 184-192.

Macrae, D.R., Murray, E.G. and Grant, J.G. (1943).
Vet. Rec. 55, 203-204.

Mattson, D.E. (1973a).
Am. J. Vet. Res. 34, 623-629.

Mattson, D.E. (1973b).
J.A.V.M.A. 163, 894-896.

Matthews, P.R.J. and Fitzsimmons, W.M. (1964).
Res. vet. Sci. 5, 171-174.

Mebus, C.A. (1976a).
Proc. Minisymp., Neonatal Diarrhoea in Calves and Pigs,
Univ. of Saskatchewan, May 3-4. VET. INFEC. DIS. ORGANISATION.
pp. 13-21.

Mebus, C.A. (1976b).
J. Dairy Sci. 59, 1175-1178.

Mebus, C.A., Newman, L.E. and Stair, E.L. (1975).
Am. J. Vet. Res. 36, 1719-1725).

Mebus, C.A., Stair, E.L. Rhodes, M.B. and Twiehaus, M.J. (1973).
Vet. Pathol. 10, 45-64.

Mebus, C.A., Stair, E.L., Underdahl, N.R. and Twiehaus, M.J. (1971).
Vet. Pathol. 8, 490-505.

Merchant, I.A. and Barner, R.D. (1971).
Infectious Diseases of Domestic Animals. 3rd Ed.
IOWA STATE UNIV. PRESS, AMES, IOWA, U.S.A.
Chapter 60 p. 337-341.

Merkal, R.S., Larsen, A.B., Kopecky, K.E. and Ness, R.D. (1968).
Am. J. Vet. Res. 29, 1533-1538.

Merkal, R.S., Kopecky, K.E., Aarsen, A.B. and Ness, R.D. (1970).
Am. J. Vet. Res. 31, 475-485.

Merriman, G.M. (1-53).
VET. MEDICINE 48, 51-52.

Meuten, D.J., Van Kruiningen, H.J. and Lein, D.H. (1974).
J.A.V.M.A. 165, 914-917.

Meyer, R.C., Symon, J. and Byerly, C.S. (1975).
Vet. Pathol. 12, 46-54.

Miles, A.A. and Misra, S.S. (1938).
J. Hyg. Camb. 38, 732-749.

Mills, J.H.L. and Hirth, R.S. (1967).
J.A.V.M.A. 150, 862-870.

Mittal, K.R., Barnum, D.A. and Tizard, I.R. (1980).
Am. J. Vet. Res. 41, 1607-1610.

Moon, H.W. (1974).
Adv. Vet. Sci. and comp. med. 18, 179-211.

Moon, H.W., Whipp, S.C. and Skartvedt, S.M. (1976).
Am. J. Vet. Res. 37, 1025-1029.

Moore, W.E.C., Cato, E.P. and Holdeman, L.V. (1969).
J. INFEC. DIS. 119, 641-649.

Morin, M., Lariviere, S. and Lallier, R. (1976).
Can. J. comp. med. 40, 228-240.

Morin, M., Lariviere, S., Lallier, R., Begin, M., Roy, R. and Ethier, R. (1978).
Proc. 2nd Inter. Symp. on Neonatal Diarrhoea. University of Saskatchewan, Oct. 3-5. by VET. INFEC. DIS. ORGANISATION. pp. 347-369.

Morris, J.A. and Park, R.W.A. (1971).
The Society for Applied Bacteriology Technical Series No. 5. Isolation of anaerobes, edited by Shapton, D.A. and Board, R.G. Academic Press. London and New York. pp. 207-217.

Moscovici, C. and La Placa, M. (1962).
Am. J. Vet. Res. 23, 645-647.

Mullen, P.A. (1970).
Vet. Rec. 86, 587-588.

Mumford, D.H. (1961).
Aust. Vet. J. 37, 122-126.

Murphy, T. (1955).
IRISH VET. J. 9, 60-67.

Murray, M., Rushton, A. and Selman, I. (1972).
Vet. Rec. 90, 210-216.

Naess, V. and Hofstad, T. (1981).
Abstract - Proc. Inter. Workshop on campylobacter infection 24-26th May. University of Reading.

Newell, D.G. and Pearson, A.D. (1981).
Abstract - Proc. Inter. Workshop on campylobacter infections 24-26th March. University of Reading.

Niilo, L. (1971).
INFECT. IMMUN. 3, 100-106.

Niilo, L. (1972).
Res. vet. Sci. 13, 503-505.

Niilo, L. (1973).
J. COMP. PATH. 83, 265-269.

Niilo, L. and Avery, R.J. (1963).
Can. vet. J. 4, 31-36.

Niilo, L. and Dorward, W.J. (1971).
Res. vet. Sci. 12, 376-378.

Niilo, L. Harries, W.N. and Jones, G.A. (1974).
Can. vet. J. 15, 224-226.

Olander, H.J., Hughes, J.P. and Biberstein, E.L. (1966).
Path. vet. 3, 421-450.

Osbaldiston, G.W. and Walker, R.D. (1972).
Cornell Vet. 62, 364-371.

Osborne, J.C. (1967).
Cornell Vet. 57, 227-238.

Osborne, J.C. and Watson, D.F. (1967).
Cornell Vet. 57, 217-227.

Ørskov, I., Ørskov, F., Smirk, H.W. and Sojka, W.J. (1975).
Acta path. microbiol. SCAND. SECT. B, 83, 31-36.

Panciera, R.J., Thomassen, R.W. and Garner, F.M. (1971).
Vet. Path. 8, 479-484.

Patterson, D.S.P. and Berrett, S. (1969).
J. MED. MICROBIOL. 2, 327-334.

Patterson, D.S.P., Allen, W.M. and Lloyd, M.K. (1967).
Vet. Rec. 81, 717-718.

Payne, J.M. and Rankin, J.D. (1961).
Res. vet. Sci. 2, 175-179.

Pearson, G.R. and Logan, E.F. (1978).
Vet. Rec. 103, 212-213.

Pearson, G.R. and Logan, E.F. (1979).
Vet. Rec. 105, 159-164.

Pearson, G.R., Logan, E.F. and McNulty, M.S. (1978).
Proc. 2nd Inter. Symp. on Neonatal Diarrhoea.
University of Saskatchewan Oct. 3-5 sponsored by VET. INFEC. DIS. ORGANISATION. pp. 331-346.

Pearson, A., Suckling, W.G., Ricciardi, I.D., Knill, M. and Ware, E. (1977).
Br. Med. J. 2, 955-956.

Pedersen, M.M., Marso, E. and Pickett, M.J. (1970).
Am. J. Clin. Path. 54, 178-192.

Phillips, J.E. (1961).
J. Path. and Bact. 82, 205-208.

Pierson, R.E., Storz, J. McChesney, A.E. and Thake, D. (1974).
Am. J. Vet. Res. 35, 523-525.

Plowright, W. (1968).
J.A.V.M.A. 152, 795-803.

Pohlenz, J., Moon, H.W., Cheville, N.F. and Bemrick, W.J. (1978).
J.A.V.M.A. 172, 452-457.

Powell, H.S., Holscher, M.A., Heath, J.E. and Beasley, F.F. (1976).
VET. MED./SMALL ANIM. CLIN. 71, 205-207.

Prescott, J.F. and Barker, I.K. (1980).
Vet. Rec. 107, 314-315.

Quin, A.H. (1954).
VET. MEDICINE. 49, 271-274 and 297.

Radeleff, R.D. (1970).
Veterinary Toxicology. Second edition, Lea and Febiger, Philadelphia.

Rankin, J.D. and Taylor, R.J. (1966).
Vet. Rec. 78, 706-707.

Roberts, S.J. (1957).
Cornell Vet. 47, 372-388.

Robinson, R.A. (1966).
N.Z. vet. J. 14, 33-39.

Rogosa, M. (1974).
Bergey's Manual of Determinative Bacteriology 8th ed.
Eds. R.E. Buchanan and N.E. Gibbons, Baltimore,
Williams and Wilkins, pp. 522-525.

Rollinson, D.H.L. (1948).
Vet. Rec. 60, 191-192.

Rose, J.H. (1972).
Res. vet. Sci. 13, 362-366.

Rose, A.L. and Edgar, G. (1936).
Aust. Vet. J. 12, 212-220.

Rovozzo, G.C. Luginbuhl, R.E. and Helmboldt, C.F. (1965).
Cornell Vet. 55, 121-130.

Rowland, A.C. and Lawson, G.H.K. (1974).
Res. vet Sci. 17, 323-330.

Rowland, A.C. and Lawson, G.H.K. (1975a).
Vet. Rec. 97, 178-180.

Rowland, A.C. and Lawson, G.H.K. (1975b).
Res. vet. Sci. 18, 263-268.

Ryan, A.J. (1970).
Vet. Rec. 86, 650-651.

Sanyal, S.C., Singh, S.J. and Sen, P.C. (1975).
J. MED. MICROBIOL. 8, 195-198.

Schofield, F.W. (1955).
J.A.V.M.A. 126, 192-194.

Schipper, I.A. and Eveleth, D.F. (1957).
VET. MEDICINE. 52, 73-74.

Schmitz, J.A. and Smith, D.H. (1975).
J.A.V.M.A. 167, 731-732.

Schulze, F. and Günther, H. (1978).
Arch. exper. Vet. Med. Leipzig 32, 251-272.

Scott, F.W. Kahrs, R.F., Campbell, S.G. and Hillman, R.B. (1973).
The Bovine pract. 8, 40-43.

Sebald, M. and Veron, M. (1963).
ANN. DE L'INSTITUT PASTEUR. 105, 897-910.

Sellwood, R., Gibbons, R.A., Jones, G.W. and Rutter, J.M. (1975).
J. MED. MICROBIOL. 8, 405-411.

Sharpee, R.L., Mebus, C.A. and Bass, E.P. (1976).
Am. J. vet. Res. 37, 1031-1041.

Shirley, G.N. (1958).
Vet. Rec. 70, 478-480.

Simmons, G.C. and Tammemagi, L. (1964).
Aust. Vet. J. 40, 123-127.

Skirrow, M.B. (1977).
Br. Med. J. 2, 9-11.

Skirrow, M.B. (1979a)
Oxoid Newsletter. March 1979.

Skirrow, M.B. (1979b).
Personal communication.

Skirrow, M.B. (1981).
Vet. Res. commun. 5, 13-19.

Smibert, R.M. (1974).
Bergey's Manual of Determinative Bacteriology. 8th ed.
Eds. R.E. Buchanan and N.E. Gibbons,. Williams and Wilkins,
Baltimore p. 207-212.

Smibert, R.M. (1978).
Ann. Rev. Microbiol. 32, 673-709.

Smith, H.C. (1965).
MOD. VET. PRACT. 46, 51-57.

Smith, H.W. and Halls, S. (1967).
J. PATH. BACT. 93, 499-529.

Smith, H.W. and Halls, S. (1968).
J. MED. MICROBIOL. 1, 61-78.

Smith, H.W. and Jones, J.E.T. (1967).
J. PATH. BACT. 93, 141-156.

Smith, L.D.S. and Hobbs, G. (1974).
Bergey's Manual of Determinative Bacteriology. 8th ed.
Eds. R.E. Buchanan and N.E. Gibbons. Baltimore, Williams
and Wilkins. pp. 551-557.

Smith, L.D.S. and Holdeman, L.V. (1968).
THE PATHOGENIC ANAEROBIC BACTERIA. CHARLES C. THOMAS
PUBLISHER, Springfield, Illinois, U.S.A. pp. 85-91.

Smith, L.D.S., Safford, J.W. and Hawkins, W.W. (1962).
Cornell Vet. 52, 62-68.

Smith, P.C., Cutlip, R.C. and Page, L.A. (1973).
Am. J. Vet. Res. 34, 615-618.

Snodgrass, D.R., Herring, J.A., Linklater, K.A. and Dyson, D.A. (1977).
Vet. Rec. 100, 341.

Sojka, W.J. and Field, H.I. (1970).
VET. BULL. 40, 515-531.

Sojka, W.J., Wray, C., Hudson, E.B. and Benson, J.A. (1975).
Vet. Rec. 96, 280-284.

Soltys, M.A. and Jennings, A.R. (1950).
Vet. Rec. 62, 5-6.

Soulsby, E.J.L. (1968).
HELMINTHS, ARTHROPODS & PROTOZOA OF DOMESTICATED ANIMALS.
(6th ed. of Mönnig's Vet. Helminthology and Entomology).
London, Bailliere Tindall and Cassell. pp. 627-636.

Soulsby, E.J.L., Venn, J.A.J. and Green, K.N. (1955).
Vet. Rec. 67, 1124-1125.

Spradbrow, P.B. (1963).
Aust. Vet. J. 39, 398-399.

Stair, E.L., Rhodes, M.B., White, R.G. and Mebus, C.A. (1972).
Am. J. Vet. Res. 33, 1147-1155.

Stockdale, P.H.G. (1976).
Vet. Parasitol. 1, 367-376.

Stockdale, P.H.G. and Niilo, L. (1976).
Can. vet. J. 17, 35-36.

Storz, J. and Bates, R.C. (1973).
J.A.V.M.A. 163, 884-886.

Storz, J., Leary, J.J., Carlson, J.H. and Bates, R.C. (1978).
J.A.V.M.A. 173, 624-627.

Storz, J., Smart, R.A., Marriott, M.E. and Davis, R.V. (1966).
Am. J. Vet. Res. 27, 633-641.

Sugar, A.M. and McCloskey, R.V. (1977).
J.A.M.A. 238, 1180-1187.

Summers, B.A. (1981).
Vet. Rec. 108, 166-167.

Svedhem, A. and Norkrans, G. (1980).
Lancet. 1, 713-714.

Taylor, D.J. (1980).
Proc. Inter. Pig Vet. Soc. June 30-July 3, Copenhagen, Denmark.
p. 235.

Taylor, R.H., Boriello, S.P. and Taylor, A.J. (1971).
Br. Med. J. 283, 412.

Turner, A.J., Caple, I.W. and Craven, J.A. (1973).
Aust. Vet. J. 49, 544.

Tutt, J.B. (1972).
Vet. Rec. 90, 91-93.

Tutt, J.B. and Hoare, D.I.B. (1974).
Vet. Rec. 95, 334-337.

Udall, D.H. (1954).
The practice of veterinary medicine. 6th ed.
Published by the author Ithaca, New York. pp. 152-155.

Vance, H.N. (1967).
Can. J. Comp. Med. Vet. Sci. 31, 260-264.

Van Der Maaten, M.J. and Packer, R.A. (1967).
Am. J. Vet. Res. 28, 677-684.

Veron, M. and Chatelain, R. (1973).
INTER. J. SYST. BACTERIOL. 23, 122-134.

Waldhalm, D.G., Meinershagen, W.A. and Frank, F.W. (1969).
Am. J. Vet. Res. 30, 1573-1575.

Weiser, M.F., Preston, T.R., MacDiarmid, A. and Rowland, A.C. (1966).
Anim. Prod. 8, 411-423.

Williams, B.M. (1964).
Vet. Rec. 76, 591-596.

Williams, B.M. (1975).
Vet. Rec. 96, 318-321.

Williams, R.E.O., Hirch, A. and Cowan, S.T. (1953).
J. gen. Microbiol. 8, 475-480.

Wilson, G.S. and Miles, A.A. (1975).
Topley and Wilson's principle of Bacteriology and Immunity .
6th ed. Publ. Edward Arnold, Ltd. London. Vol.1, 1129-1130.

Withers, F.W. (1953).
Br. vet. J. 109, 122-131.

Withers, F.W. (1959).
Vet. Rec. 71, 1150-1153.

Woode, G.N. (1976).
The Vet. Annual. 16, 30-34.

Woode, G.N. (1978).
Vet. Rec. 103, 44-46.

Woode, G.N. and Bridger, J.C. (1975).
Vet. Rec. 96, 85-88.

Woode, G.N. and Bridger, J.C. (1978).
J. MED. MICROBIOL. 11, 441-452.

Woode, G.N. and Crouch, C.F. (1978).
J.A.V.M.A. 173, 522-526.

Woode, G.N., Bridger, J.C., Hall, G. and Dennis, M.J. (1974).
Res. vet. Sci. 16, 102-105.

Woode, G.N., Jones, J. and Bridger, J. (1975).
Vet. Rec. 97, 148-149.

Wooley, R.E., Shotts, E.B. and McConnell, J.W. (1980).
Am. J. Vet. Res. 41, 1667-1668.

Wray, C. and Sojka, W.J. (1977).
J. Dairy Res. 44, 383-425.

Wray, C. and Thomlinson, J.R. (1974).
Br. vet. J. 130, 189-198.

York, C.J. and Baker, J.A. (1951).
J. Exp. Med. 93, 587-604.

Young, B.J. (1969).
J. Med. Lab. Technol. 26, 248-252.

Zymet, C.L. (1969).
VET. MED./SMALL ANIM. CLIN. 64, 883-887.

APPENDIX 1.

Appendix 1. Detailed results of the survey

Animal Number	Age of Animal	Appearance of faeces	State of Animal	Clinical Diagnosis	Isolation and Identification of Bacteria	Abomasum			Small Intestine			Large Intestine			Others
						Isolation	Lesions seen Macro	Micro	Isolation	Lesions seen Macro	Micro	Isolation	Lesions seen Macro	Micro	
74589*	5y	MD	K	Mucosal disease	Bacillus licheniformis	+	UL/CO	+	+	CO	+	+	UL/CO		
					Escherichia coli	-			+	CO		+			
					Branhamella catarrhalis	-			+	CO		-			
					Campylobacter fetus ss.jejuni	-			-			+		+	
11	6w	FM	K	Part of parasitology experiment	B.licheniformis	+	Oed/Er	+	+			+			
					C.f.ss.jejuni	-			+	CO	+	+	CO	+	
					E.coli	-			+			+			
50	6w	FM	K	Pneumonia	B.licheniformis	+	CO	+	-			-			
					E.coli	+			+	SCO	+	+			
					Streptococcus bovis	+			+			+			
					C.f.ss.jejuni	-			-			+	N	+	

Key to symbols on final page.

Appendix 1 (Continued)

291	6w	F	K	Pneumonia	B.licheniformis	+	CO	+	-			-		
					E.coli	+			+			+		
					Peptostrepto-coccus productus	+			-			-		
					Staphylococcus epidermidis	+			-			-		
					Aeroccoccus viridans	-			-			+		
					C.f.ss. jejuni	-			+	N	+	+	N	+
75257*	3w	SM	K	Pneumonia ? salmon-ellosis	Streptococcus zooepidemicus	+	CO		+	CO		-		
					B.catarrhalis	+			-			-		
					E.coli	+			+			+		
					Bacteroides vulgatus	-			+			+		
					Bacteroides fragilis	-			+			+	N	+
					C.f.ss.jejuni	-			+		+	-		
					Corynebacterium pyogenes	-			-			+		
75258*	2w	SM	K	Pneumonia	Aeromonas hydrophilia	+	CO	+	-			-		
					Corynebacterium bovis	+			-			-		

Appendix 1 (Continued)

75258*(Cont'd)					S.epidermidis	-			+			+				
					C.f.ss. jejuni	-			+	SCO	+	-				
					C.f.ss. intestinalis	-			-			+	N	+		
					B.vulgatus	-			+			+				
					B.fragilis	-			+			+				
45	1-2w	VSD	K	Rotavirus diarrhoea	Cl.perfringens Type A.	+	CO	+	+	SCO	+	+	SCO	+	No rotavirus seen by E.M.	
					E. coli	+			+			+				
					Veillonella spp.	-			+			+				
					C.f.ss. jejuni	-			+		+	+		+		
					B.fragilis	-			-			+				
22P	6m	SBM	K	Exp. infection with O.ostertagi	E.coli	+	SCO									
					Cl.perfringens Type A.	+	-	+	Not Done			Not Done				
					C.f.ss.jejuni	+		+								
31P	6m	SBM	K	Exp. infection with O.ostertagi	E. coli	+	SCO									
					Cl.perfringens Type A.	+	-	+	Not Done			Not Done				
					C.f.ss.jejuni	+		+								
					Unidentified campylobacter	+										

Appendix 1 (Continued)

34P	6m	F	K	Exp. infection with O.ostertagi	E.coli	+							
					Cl.perfringens Type A.	+	CO	+	Not Done		Not Done		
					C.f.ss.jejuni	+		+					
37P	6m	SMB	K	Exp. infection with O.ostertagi	E.coli	+							
					C.f.ss.jejuni	+	SCO	+	Not Done		Not Done		
					Unidentified campylobacters	+		+					
38P	6m	F	K	Exp. infection with O.ostertagi	C.f.ss. jejuni	+	CO	+	Not Done		Not Done		
					E.coli	+							
44P	6m	SMB	K	Exp. infection with O.ostertagi	E.coli	+		-					
					Cl.perfringens Type A.	+	CO	+	Not Done		Not Done		
					C.f.ss. jejuni	+		+					
46P	6m	SMB	K	Exp. infection with O.ostertagi	E.coli	+							
					Cl.perfringens Type A.	+	CO	+	Not Done		Not Done		
					C.f.ss.jejuni	+		+					
					Unidentified campylobacters	+		+					
													Mesenteric L.N.
82773*	1y	WSD	K	Salmonellosis	B.licheniformis	+	CO	+	+	CO	+	+	CO	+	
					C.f.ss.jejuni	+		+	+		+	+		+	+
					E.coli	-			+			+			

Appendix 1 (Continued)

83444*	6m	SM	(D)	Vaccinated against (IBR)	Cl.perfringens Type A.				+	SCO/NE	ND			
					C.f.ss.jejuni				+					
					C.fecalis				+					
					S.bovis		Not Done		+				Not Done	
					E.coli				+					
83613*	3-5y	SMD	K	Salmonel-losis	Clostridium butyricum	+	CO/Oed		+	CO		-		
					E.coli	+			+			+		
					S.bovis	+			+			+		
					C.fecalis	+		+	-			+	N	+
					C.f.ss.jejuni	-			+		+	+		+
49*	4m	SMD	K	Diarrhoea	B.licheniformis	+	N	ND	+	CO	ND	+	CO	ND
					C.f.ss.jejuni	-			+			+		
					E.coli	+			+			+		
					B.vulgatus	-			-			+		
25	1-2w	VSD	K	Rotavirus diarrhoea	Cl.perfringens Type A				+	SCO	+	+	SCO	+
					C.f.ss.intestinalis		Not Done		+		+	+		+
					E.coli				+			+		
33	1-2w	VSD	K	Rotavirus diarrhoea	E.coli	+	CO/Oed		+	CO		+	CO	
					Cl.perfringens Type A.	-			+		+	+		+
					C.f.ss. *intestinalis*	-			+		+	+		+

Not Done

Not Done

Appendix 1 (Continued)

36	1-2w	VSD	(D)	Rotavirus diarrhoea	Cl.perfringens Type A	+	CO	+	+	SCO	+	+	SCO	+	Rotavrius seen by E.M.
					E.coli	+			+			+			
					C.f.ss. intestinalis	-			+		+	+		+	
75715*	2w	YD	K	Pneumonia ? salmonel-losis	A.hydrophila	+	CO	+	+	SCO	+	-			
					C.pyogenes	+			+			+			
					E.coli	-			+			+			
					C.f.ss. intestinalis	-			+		+	+	SCO	+	
83555*	1-2y	SM	K	Salmonel-losis	Fusobacterium necrophorum				+	NE	+				
					Cl.perfringens Type A.	Not Done			+		+	Not Done			
					E.coli				+						
					C.f.ss.intestinalis				+		+				
75205*	1.5y	BWD	K	Salmonel-losis	Enterobacter aerogenes	+	Ab	+	+	CO/Er	+	+	CO/Oed	+	
					Pseudomonas aeruginosa	+			+			-			
					C.bovis	+			+			-			
					Fusobacterium spp.	+		+	-			-			
					C.fecalis	+		+	-			-			
					A.viridans	-			-			+			

Appendix 1 (Continued)

																		Lung	Liver
78530*	9y	SD	(D)	Necro-bacillosis	E.coli	+		Ab		+		Ul		+				-	-
					B.licheniformis	+			+	+		Ul	+	+		CO	+	-	-
					B.vulgatus	+				+		Ul		+				-	-
					Bacteroides oralis	+				+		Ul		+				+	+
					Bacteroides melaninogenicus	+				-				-				+	+
					B.fragilis	-				+		Ul		+				+	+
					C.fecalis	+			+	-				-				-	-
					S.bovis	+				-				-				-	-
					Ps.aeruginosa	-				-				-				+	+
79894*	1.2y	WD	K	Necrotic enteritis	E.coli	+		Ab		+		CO		+					
					B.catarrhalis	+				+				-					
					S.bovis	+				+				+					
					C.fecalis	+			+	+			+	-					
					Cl.perfringens Type A.	+			+	+			+	+		CO	+		
80958*	2m	SF	(D)	Salmonel-losis	S.epidermidis	+				-				-					Excess thick mucus seen in small and large intestine
					Cl.perfringens Type A.	+		CO	+	+		CO	+	+		CO	+		
					C.fecalis	+			+	+			+	+			+		
					E.coli	-				+				+					
					B.vulgatus	-				-				+					
					Clostridium sordelli	-				+			+	+			+		

Appendix 1 (Continued)

75067*	14w	F	K	Pneumonia ?Salmonellosis	C.bovis	+	CO		+	CO		+	CO		
					B.catarrhalis	+			-			-			
					Unidentified campylobacters	-			+		+	-			
					B.fragilis	-			+			+			
					Veillonella spp.	-			+			+			
					Pasteurella multocida	-			-			+			
					S.epidermidis	+			-			-			
74331*	16y	WMD	K	Severe diarrhoea	Cl.perfringens Type A	+	UL	+	+	Th	+	+	Ul/CO	+	
					S.bovis	+			+			+			
					B.licheniformis	+			+			+			
					E.coli	+			+			+			
75478*	15m	SF	K	Oral ulceration	Cl.perfringens Type A	+	CO	+	+	CO/Th	+	+	CO/Th	+	
					Bacillus coagulans	+			-			-			
					S.bovis	+			-			-			
					Ps. aeruginosa	-			+			+			

Appendix 1 (Continued)

75911*	15m	PD	K	Salmonel-losis	E.coli	+	Ab	+	+			+			
					Ps.aeruginosa	+			−			−			
					S.bovis	+			+			−			
					Eubacterium aerofaciens	−			+			+			
					Cl.perfringens Type A	−			+	SCO	+	+	CO	+	
76217*	9y	SF	K	Haemor-rhagic/ syndrome (IBR)	E.coli	+			+			+			
					Actinobacillus lignieresii	+	Ul	+	−			−			
					Cl.perfringens Type A	+			+	CO	+	+	N	+	
76457*	2-3y	WMD	K	Winter dysentery	E.coli	+			+			+			Mesenteric L.N. +
					Cl.perfringens Type A	+	CO	+	+	CO	+	+	HE	+	+
					B.fragilis	+			+			+			−
24	1-2w	VSD	(D)	Rotavirus diarrhoea	E.coli	+			+			+			No rotavirus seen by E.M.
					S.bovis	+			−			−			
					Cl.perfringens Type A	+	CO/Oed	+	+	CO	+	+	CO	+	
29	1-2w	VSD	(D)	Rotavirus diarrhoea	E.coli		Not Done		+			+			No rotavirus seen by E.M.
					Cl.perfringens Type A				+	SCO	+	+	CO	+	

Appendix 1 (Continued)

30	1-2w	VSD	(D)	Rotavirus diarrhoea	Cl.perfringens Type A		Not Done		+	SCO	+	+	SCO	+	Rotavirus was seen by E.M.
					E. coli				+			+			
79383*	3w	SMD	(D)	Salmonellosis	E. coli	+			+			+			
					Cl. perfringens Type A	+	Ab	+	+	SCO	+	+	SCO	+	
					Veillonella spp.	-			+			+			
B *	2y	BD	(D)	Haemorragic diarrhoea	Cl. perfringens Type A		Not Done		+	HE	+		Not Done		
					Cl. sordellii				+						
					E. coli				+						
10	6w	F	K	Exp. parasitic infection	B.licheniformis	+	Oed/Er	+	+	N	+	-			
					Cl. perfringens Type A	+			+	N		+	N	-	
					E. coli	-			+	N		+	N	-	
22	6w	F	K	Exp. parasitic infection	B.licheniformis	+	Oed/Er	+	+	N	+	+	CO	+	
					Cl. perfringens Type A	-			-			+		+	
					E.coli	-			-			+			
31	6w	F	K	Exp. parasitic infection	B.licheniformis	+	Oed/Er	+	+	CO	+	+	N	+	
					Cl. perfringens Type A	-			+			-			
					E.coli	-			-			+			

Appendix 1 (Continued)

75186*	2y	F	K	Peritonitis	E.coli	+			+			-		
					Ps. aeruginosa	+			+			-		
					Cl.perfringens Type A	+	CO/Oed	+	+	CO/Er/Oed	+	+	CO/Er	
					P.productus	+			-			-		
					A.viridance	-			+			-		
42P	6m	F	K	Exp. infection with O.ostertagi	E.coli	+								
					Cl. perfringens Type A	+	CO	+		Not Done			Not Done	
43P	6m	F	K	Exp. infection with O.ostertagi	E.coli	+			-					
					Cl. perfringens Type A	+	CO	+		Not Done			Not Done	
76585*	6y	F	K	Spinal abscess	E.coli	+			+			+		
					S.epidermidis	+			+			+		
					Cl.perfringens Type A	+	Ul	+	+	HE	+	+	HE	+	
79249*	1.5y	MD	(D)	Diarrhoea	E.coli	+			+			+			Excess mucus seen on mucosa of small and large intestine
					Clostridium bifermentans	+	Ab	+	+	CO/Er	+	+	CO	+	
					Unidentified clostridium	-			+			+			

74989*	6w	MD	K	Diarrhoea	B.licheniformis	+	Oed	+	+	CO	+	-				No virus identified by E.M.
					S.epidermidis	-			+			+	N		+	
					E.coli	-			+			+				
75799*	3-4y	PD	K	Johne's disease	B.licheniformis	+	CO	+	-			-				Acid fast bacteria seen
					E.coli	+			+	V.Th.		+	CO		+	
					C.bovis	-			+			+				
76245*	20y	WSD	K	Diarrhoea	B.licheniformis				+	CO	+					
					E.coli		Not Done		+				Not Done			
					Moraxella non-liquefaciens				+							
3	6w	F	K	Exp. parasitic infection	B.licheniformis	+	Ul/Oed	+	+	N	+	+	N		-	
					A.viridans	+			-			-				
					E.coli	-			+			+				
54	8w	F	K	Pneumonia	B.licheniformis	+	CO	+	+	N	+	-				
					E.coli	-			-			+	N		+	
293	6w	F	K	Pneumonia	B.licheniformis	+	CO	+	-			-				
					S.epidermidis	+			+	CO		+	N		-	
					A.viridans	-			-			+				
294	6w	F	K	Pneumonia	B.licheniformis	+	N	+	-			-				
					B.catarrhalis	-			+	N		+	N		-	
					E.coli	-			-			+				

Appendix 1 (Continued)

296	8w	F	K	Pneumonia	B.licheniformis	+	N	+	-			-		
					S.epidermidis	+	N		+	N		-		
					E.coli	-			-			+	N	+
75516*	3y	F	K	Peritonitis	B.licheniformis	+	Er/Oed	+	-			-		
					E.coli	+			+	CO		+	CO	+
					A.viridans	+			+			+		
					B.fragilis	-			+			+		
AL*	1.5y	D	K	Johne's disease	E.coli				+	V.Th.	+	+	CO/Er	+
					B.fragilis		Not Done		+			+		
					Actinomyces bovis				-			+		
52R	6m	SF	K	Exp. infection with lungworm	β-haemolytic E.coli		Not Done		+	SCO	+	+	SCO	+
					E.coli				+			+		
53R	6m	SF	K	Exp. infection with lungworm	β-haemolytic E.coli		Not Done		+	CO	+	+	CO	+
					S.bovis				+			+		
75065*	3m	F	K	Pneumonia	S.epidermidis				+			+		
					A.hydrophila		Not Done		+	CO	+	-		
					E.coli				-			+	N	+

	Acid-fast bacteria seen
	Mucoid fluid contents seen in small and large intestine
	Excess mucus seen in the small and large intestine

Appendix 1 (Continued)

74381*	1-2y	F	K	Mucosal disease	A.viridans	+	Ul	+	+	N	-	+	N	+	
					E.coli	-			+			+			
					Alcaligenes odorans	-			+			+			
73948*	8m	F	K	C.C.F./ Diarrhoea	Acinetobacter lwoffii		Not Done		+	CO/Th	ND		Not Done		Excess mucus seen
					A.odorans				+						
75003*	3y	SD	K	Johne's disease	C.bovis	+			-			-			Acid-fast bacteria seen.
					B.catarrhalis	+	CO	+	+	Oed/Th.	+	-			
					A.bovis	-			-			+	N		
34	1-2w	VSD	(D)	Rotavirus diarrhoea	E.coli	+	N		+	CO			Not Done		
					S.epidermidis	+	N		+	CO					
A *	1-2y	D	K	Haemon-chosis	E.coli	+	Oed/Er	+							
					A.viridans	+						Not Done		Not Done	

* Clincal case
F = Firm faeces
M = Presence of mucus
S = Soft faeces
D = Diarrhoea
P = Pasty faeces

B = Presence of blood
Y = Yellowish faeces
W = Watery faeces
VS = Very soft faeces
CO = Congested mucosa
SCO = Severe congested mucosa

Ul = Ulcerated mucosa
Oed = Oedematous mucosa
Er = Eroted mucosa
Ab = Abomasitis
N = Normal mucosa
ND = Not Done

NE = Necrotic enteritis
HE = Haemorrhagic enteritis
Th = Thickened mucosa
V.Th= Very thickened mucosa
K = Killed
(D) = Died