A STUDY OF THERMOPHILIC CAMPYLOBACTER IN CATTLE, SHEEP AND LABORATORY ANIMALS

A Thesis presented in partial (70%) fulfilment
of the requirements for the degree of
Master of Philosophy in Veterinary Microbiology
at Massey University

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1986

ABSTRACT

A total of 1107 samples (rectal, intestinal and bile) from rats, mice, guinea pigs, rabbits and cats were examined for the presence of intestinal thermophilic campylobacter.

The investigation showed that 20.6% of the total of 503 laboratory animals examined were positive for *Campylobacter jejuni*. The prevalence rate was found to be highest amongst the cats (51.7%) with rats being the next in order (23.2%), whereas 4/52 (7.7%) of guinea pigs and a single rabbit (1%) were positive for *C. jejuni*. One of the four isolates recovered from guinea pigs was from bile. Campylobacter-like organisms were cultured from 5/50 (10%) of the mice, but these failed to grow on subsequent subculturing. By using bacterial restriction endonuclease DNA analysis (BRENDA) for their identification, a single type of *C. jejuni* was identified (pattern 25) from all isolates recovered from the rats, guinea pigs and a rabbit, whereas there were five different BRENDA patterns from cat isolates.

Sawdust was suggested as a likely vehicle through which the organism may have spread within the Unit and infected the rats. The spread to other species was thought to have been from the guinea pigs which were initially infected from contaminated hay or green feed.

The isolation of *C. jejuni* organisms having a multiplicity of BRENDA patterns from the cats, suggests that the cat organisms had been present in the cat colony from the time the colony was started or from the occasional bird which accidentally flew into the cat cages and was eaten.

A single isolate of *C. laridis* from a house fly was also isolated but there was no evidence of this organism in any of the laboratory animals.

Seasonal variations in the isolation of thermophilic *Campylobacter* species in dairy cows was also studied and showed that

there were 17/72 (23.6%), 33/106 (31.13%) and 11/95 (11.5%) positive for campylobacter during summer, autumn and winter respectively. The prevalence rate of Campylobacter species is highest in warmer months of the year.

The isolates recovered from dairy cows were also identified by the BRENDA technique and compared with those from sheep. Seventeen different BRENDA patterns were identified from 48 isolates from dairy cows and six from 27 isolates from sheep. Of the total of 21 different BRENDA patterns, only two were common to both animals suggesting cross infectivity between these two animals. Organisms having the same BRENDA pattern could also be isolated from dairy cows and sheep on more than one occasion establishing the stability of BRENDA patterns.

No similarities were seen between the BRENDA patterns recovered from laboratory animal isolates and those of dairy cows and sheep.

ACKNOWLEDGEMENTS

I am very thankful to Professor B.W. Manktelow who kindly made available the facilities of the Department of Veterinary Pathology and Public Health, Massey University, for this study.

I would particularly like to thank Dr R.B. Marshall, my supervisor for his continuous help, encouragement and readiness to discuss problems whenever required. The assistance of Mr P.J. Winter, who taught me the BRENDA technique is greatly appreciated.

I am indebted to Mr J.E. Ormsby and the other members of staff of the Small Animal Production Unit (SAPU) and Dr M.F. Tarttelin, Department of Physiology and Anatomy for allowing me to collect the samples from his cat colony. I am also thankful to Mr D. Hislop of Massey University No. 1 Dairy Farm and Mr P.H. Whitehead of Sheep and Beef Cattle Research Unit, Massey University, for arranging for the collection of samples from the sheep and cattle.

I would also like to thank Miss L. Cullinane and Mr R. Holdaway for their assistance in the laboratory; Mrs Jan Schrama for preparation of glassware; Mr P. Wildbore for technical administration and Mr T. Law for photographic work.

I am very grateful to Mrs Olive Harris for her care and attention to detail whilst typing this manuscript.

Finally, I wish to thank my family for their patience, encouragement and support.

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CHAPTER I

INTRODUCTION

Campylobacter species are widespread in the animal kingdom, both as pathogens and as commensals (Smibert, 1978). Their natural habitat is as yet unknown, but the organisms can be isolated from a wide range of wild and domestic animals and birds (Luechtefeld et al, 1980; Bauwens et al, 1981).

The taxonomy and classification have been studied and much has been learnt concerning the nutritional requirements and metabolism of these organisms; however, the epidemiology and pathophysiology of the thermophilic Campylobacter spp is largely obscure (Karmali et al, 1979).

Campylobacter infection in man has been associated with the consumption of contaminated water (Tieham et al, 1978; Mentzing, 1981), unpasteurised milk (Blaser, 1979; Robinson et al, 1981) and treated milk following temporary failure of pasteurization equipments (Porter et al, 1980). Other animal products also serve as potential sources of infection. Suggestions have been made that C. jejuni may be a cause of mastitis in cows (Lander et al, 1979). The organisms have been recovered from retail cuts of chicken (Smith and Muldoon, 1974; Simmons and Gibbs, 1979) and from lamb carcases (Stern, 1981b). Campylobacter have also been recovered from eviscerated pork, lamb and beef carcases (Stern, 1981a). The evidence that C. jejuni causes enteritis in cattle is strong (Skirrow, 1977), however, campylobacter are also commensals of the intestinal tracts of animals. They have been isolated from faeces voided by healthy cattle (Florent, 1959), sheep (Smibert, 1965), pigs (Pejtschev 1969), pups (Blaser et al, 1978; Bruce and Zochowski, 1980; Fox et al, 1983), cats (Svedhem and Norkrans, 1980; Skirrow et al, 1980a), monkeys (Tribe and Frank, 1980), primates (Tribe et al, 1979) and zoo animals (Luechtefeld et al, 1981).

The occurrence of Campylobacter species in rodents has not been assessed, possibly because campylobacter require for their

isolation, gaseous conditions that are not normally employed during routine bacteriological procedures. However, campylobacter have been isolated from rats killed on premises where there was an outbreak of dysentery in pigs (Pejtschev, 1969) and from laboratory rats (Fernie and Park 1977; Kakoyiannis, 1984). Now with the development of simple techniques for the isolation of campylobacter from faeces by Skirrow (1977) the work should become easier. From the work carried out so far it appears that campylobacter are widespread in the animal kingdom.

During this study two aspects of the occurrence of campylobacter infection in animals were carried out. Since cattle and sheep are two common animals man comes in contact with in New Zealand, there are many chances for cross transmission of the organisms to man. Transmission can take place in three different ways. Firstly, during the everyday farming of these species; secondly, by means of contact with sick animals and finally by consumption of infected meat or milk.

The first aspect covered in this thesis involves an investigation to determine the prevalence of campylobacter in dairy cows and sheep on Massey Dairy Farm 1 and Massey Sheep Farm. The campylobacter isolated were classified to sub-species level by the use of DNA restriction enzyme analysis and analysed as to whether the same subspecies occurred in these animals throughout the year.

The second aspect forms the major part of the work outlined in this thesis and deals with laboratory animals. The recognition that campylobacter can cause disease in domestic animals and man has led to the use of laboratory rodents for pathogenicity studies with these organisms. For example, guinea pigs and hamsters have been used as models for abortion caused by *Campylobacter fetus* (Ristic *et al*, 1954; Hraback *et al*, 1976), rats and cats have been used for crossinfectivity studies of *Campylobacter jejuni* (Kakoyiannis, 1984; Prescott and Karmali, 1978). It is therefore necessary to know if *Campylobacter* spp are normally present in the intestinal flora of such animals. The epidemiology of disease involving campylobacter has not been adequately determined and the role of rodents as a source of infection has not been assessed.

An extensive survey was carried out at the Small Animal Production Unit at Massey University to determine the prevalence of campylobacter in rats, mice, rabbits, guinea pigs and cats. Samples were also taken from the people working in the unit and the area in and around the unit. An attempt was made to ascertain whether or not crossinfectivity by campylobacter naturally occurs between these different species and between these animals and man.

CHAPTER II

REVIEW OF THE LITERATURE

HISTORY

Spirillum fetus was the original name suggested for the organism which later became known as Vibrio fetus. This name was originally suggested because of the typical spiral-like character; short, bent rods occurring singly or in short chains, and which are usually motile by means of single or, occasionally, two or three polar flagella (Genus Vibrio, Merchant and Packer). In 1913 McFadyean and Stockman found a spiral micro-organism, which caused abortion in ewes and cows, and Smith (1918) in the U.S.A. confirmed their finding. Smith and Taylor (1919), characterised these organisms and named them Vibrio fetus. Subsequently Vibrio jejuni was isolated in the U.S.A. by Jones and Little (1931a) from a cow and calves with enteritis (1931b). Doyle (1944) isolated a microaerophilic vibrio in apparently pure culture from the mucosa of the colon of hogs with dysentery which was later named Vibrio coli by Doyle (1948). These three organisms were microaerophilic and catalase positive. In subsequent years catalase negative Vibrios such as Vibrio sputorum and Vibrio bubulus were discovered. King (1957) classified the thermophilic microaerophilic vibrio which do not grow at 25°C. as 'Related Vibrios'. Campylobacter was classed as a genus distinct from Vibrios by Sebald and Veron (1963) following an extensive study of the genus. The thermophilic campylobacter were later divided into two, Campylobacter coli and Campylobacter jejuni (Veron and Chatelain, 1973). Smibert (1978) however, reunited these two groups as Campylobacter fetus subsp. jejuni and subsp. coli. Skirrow and Benjamin (1980b) classified the enteropathogenic thermophilic campylobacter into four biotypes, namely nalidixic acid resistant thermophilic campylobacter (NARTC), Campylobacter jejuni biotype 1 and 2, and Campylobacter coli. Since the last work reported by Skirrow and Benjamin (1980b), there have been no further attempts made by anyone to reclassify the enteropathogenic thermophilic campylobacter.

CAMPYLOBACTER IN SMALL ANIMALS

Rats

Parr (1923) found that the intestinal tract of several mammals including man, contained many spiral shaped organisms. Pijtschev (1969) was the first person to confirm this finding and isolated campylobacter from rats killed on premises where there was an outbreak of dysentery in pigs. His isolate resembled C. coli and this organism was thought to be associated with swine dysentery. Gordon and Dubos (1970) observed four types (A to D) of spiral shaped organisms in normal mice of which type A had similar characteristics to that of campylobacter, but they did not confirm this finding. Gustafsson and Maunsbach (1971) reported an organism similar to type A of Gordon and Dubos (1970) in the crypt of Lieberkuhn. Davis et al (1972) were able to isolate three types of spiral shaped organisms of which type 1 and 3 were morphologically similar to the type found by Gordon and Dubos (1970). They suggested that type 3 may be a spirillum or a Vibrio, but they did not confirm their finding.

Fernie et al (1977) reported the isolation of campylobacter from three of 29 rats that had been bred and reared for use in the laboratory. This confirmed the finding by Pijtshev (1969), however electrophorelograms of APS protein revealed distinct differences between rat isolates and the C. coli of Pijtshev. Kakoyiannis (1984) was able to grow 18 isolates from wild rats (Rattus norvegicus), which he identified as C. jejuni. Based on bacterial restriction endonuclease DNA analysis (BRENDA), 15 of his 18 isolates recovered from rats had identical DNA patterns and that two of the four BRENDA types seen in rats were identical with isolates from humans, chickens and a horse. He suggested that rats may be a source of infection for other animals and humans, but did not directly relate human isolates with those of rat origin.

Mice

Savage et al (1968) studied the presence of bacteria in the murine large bowel and came to a conclusion that the 'Spiral'

organisms required further study as they are numerous and exist in 'intimate' association with intestinal mucosa and mucus layer. In the same year, Lee et al (1968) reported cultivating spiral shaped organisms from normal mice, but did not differentiate these into types. Two types of spiral shaped organisms from normal mice were described by Davis (1969). These were morphologically similar but exhibited different colony morphologies when cultured. James et al (1970) in his study of the anaerobic bacterial flora of the mouse caecum observed four morphological types of spiral organisms. One (type A) had, as a rule, two or three bends along its length and were rapidly motile. Most type A's were about half the length and were thinner than the other campylobacter. The second type (type B) was twice as long and about one-third the width of type A, thereby resembling true Treponema. The third kind (type C) was very short with only one bend while a fourth (type D) was twice as long and about twice as wide as type A. Type A produced a small (0.5 mm), grey, round, slightly elevated colony. Usually it was a finely granular, spreading growth with a greening effect on blood plates. The spiral shape of the organism began to disintegrate or assume a coccoid shape upon exposure to air. The spiral shaped bacteria could be observed in the caecum and colon of mice as early as seven days (Davis et al, 1973) and 12 days after birth and inhabited the mucus layer of the epithelium of the large bowel and were present there throughout the remainder of the animal's life. Lee and Philip (1978) were able to isolate and subculture spiral shaped organisms in vitro several times. Roach and Tannock (1979) were able to isolate 14 different gram negative spiral shaped cells with a flagellum at each pole of the cell. The isolates did not produce acid from fumerate and they came to the conclusion that the isolates were campylobacters. Their isolates resembled C. faecalis in their growth characteristics and biochemical attributes (Smibert, 1974), but the bacteria were about 0.2 µm wide, which is smaller than the measurement given by Smibert (1974) for C. faecalis.

Field et al (1981) were able to detect colonization in the caecum and large bowel for a period of 2 to 3 weeks following experimental infection with C. jejuni. Blaser et al (1983) experimentally infected adult mice with C. jejuni, but no clinical disease was seen although there was rapid development of bacteraemia

and the mice were not able to naturally clear intestinal colonization. The presence of a biliary reservoir explains chronic carriage and it was concluded that the host defences of these mice were adequate to contain acute C. jejuni infection without serious consequences (Blaser et al, 1983b). Recently Newell et al (1983) reported that a campylobacter with a greater than usual width measurement and known as a wide type strain C. jejuni (8116) colonizes the gastrointestinal tract of day-old Balb/c mice more successfully than an a flagellate variant. Morooka et al (1985) were also able to infect mice experimentally with a wide-type strain and several non-motile mutant strains. Mice are experimentally susceptible to C. jejuni and it is expected that particular mouse strains of C. jejuni will become recognised (Leanne et al, 1981).

Monkeys

There have been no publications to date reporting the presence of campylobacter infection in non-human primates in their native habitat (Morton et al, 1983). It appears that healthy well nourished animals, after being captured and kept under good conditions free from stress and other infectious enteric organisms, are highly susceptible to C. jejuni (Bryant et al, 1983). Morton et al (1979), Tribe et al (1979) and Tribe and Frank (1980) were first to report the presence of campylobacter in the stools of simians. C. jejuni has been recovered from clinically normal monkeys and from those with diarrhoea (Tribe et al, 1979; Tribe and Frank, 1980; Ackerman et al, 1982), but the prevalence of infection is significantly higher in animals with diarrhoea (Luechtefeld et al, 1981; Tribe and Fleming, 1983). Bryant et al, (1983) reported C. jejuni isolates from 46% of 24 Patas monkeys with chronic diarrhoea. Tribe et al (1983) were able to detect campylobacter isolates in 53% and C. jejuni/coli alone from 42.9% of Macaca fascicularis. Infection mainly appears to be via the faecal oral route (Bryant, 1983).

Thermophilic campylobacter organisms have so far been isolated from blood, liver (Fitzgeorge et al, 1981), duodenum, ileum, caecum, colon, gall bladder, urinary tract, vaginal swabs, placenta and stomach of fetus (Tribe and Frank, 1981). Infected animals seem to

exhibit either persistent or intermittent enteritis (Tribe and Frank, 1981). Clinically the disease in monkeys is similar to that seen in humans (Skirrow, 1977; Butzler and Skirrow, 1979; Blaser and Reller, 1981) in that it is characterised by a haemorrhagic, watery diarrhoea that affects the jejunum, ileum and colon (Butzler and Skirrow, 1979; Blaser and Reller, 1981). The chronic and recurrent carrier states in monkeys is similar to that reported in humans in undeveloped countries (Bokkenheuser et al, 1979; Blaser et al, 1980b). Campylobacter enteritis has also been found in Macaca nemenstrina neonates and infants (Morton et al, 1983). It has been reported that Saguinus oedipus is more susceptible to C. jejuni than M. fascicularis. Large numbers of the latter species survive the infection and frequently become asymptomatic excretors (Tribe and Fleming, 1983). In monkeys, the carrier state is common (Bryant et al, 1983) and the length of time that C. jejuni is shed appears to be long (Tribe and Frank, 1980; Fitzgeorge et al, 1981). It is difficult to reproduce disease experimentally in monkeys (Tribe and Frank, 1980; Fitzgeorge et al, 1981).

Hamsters

Fernie et al (1977) carried out a widespread survey looking for campylobacter in rodents, but failed to isolate any in hamsters. Frisk and Wagner (1977) observed a campylobacter-like organism in the ilea of hamsters with enteritis. They also suggested that the campylobacter-like organisms did not produce a potent toxin. They were unable to classify the organisms since they failed to isolate them. James et al (1981) was able to isolate campylobacter from hamsters for the first time. He was able to isolate C. jejuni from three groups of golden Syrian hamsters, but not from a closed colony of 11 White Syrian hamsters. He suspected that a large percentage of asymptomatic hamsters may harbour this pathogen of man.

Rabbits

Moon et al (1974) reported the presence of curved and spiral rods resembling Vibrio species in Levediti stain sections from 10 of the 12 caeca from young rabbits thought to be suffering from Typhlitis. Bryner et al (1971) experimentally infected rabbits with

Campylobacter fetus and came to a conclusion that rabbits were resistant to the lethal effects of C. fetus. No reports of C. jejuni isolations from rabbits could be found.

Guinea Pigs

No isolation of Campylobacter jejuni/coli has been reported from Guinea pigs to date. Bryner et al (1971) experimentally infected Guinea pigs with Vibrio fetus to find out the infectivity of three Vibrio fetus biotypes (type 1, Subtype 1, and type 2) for gall bladder and intestine of guinea pigs. They suggested that guinea pigs were the most susceptible to the lethal effect of V. fetus amongst the laboratory animals that they tested.

Cats

Campylobacter jejuni has been recovered from diarrhoeic (Ferreira et al, 1979; Karmali and Skirrow, 1984) and non-diarrhoeic cats (Garcia et al, 1983; Donna et al, 1985). Donna et al (1985) observed no difference in prevalence between adult and immature domestic cats or between confined, institutionalised animals and pets in homes. Although Campylobacter jejuni has been isolated from the faeces of up to 45% of non-diarrhoeic cats, depending on their sources (Blaser et al, 1980c; Bruce et al, 1980; Patton et al, 1981), in general the rate of infection seems to be low in non-diarrhoeic cats (Bruce et al, 1980). Other groups of authors (Hasting, 1978; Hosie et al, 1979; Blaser et al, 1979b) reported a 4 to 10 percent isolation rate. Cats seem to be more likely to be infected when in an unhygienic environment (Blaser et al, 1980b; Gruffyld et al, 1980). Naturally occurring Campylobacter jejuni diarrhoea in kittens is uncommon (Skirrow, 1981). Cats obtained from kennels and animal control centres may be shedders of Campylobacter jejuni in the absence of diarrhoea (Donna et al, 1985).

In contrast to the situation in dogs, there have been few authenticated reports of transmission of Campylobacter jejuni from diarrhoeic kittens to their owners (Skirrow, 1981), although cats have been suspected as a source of infection for humans leading to diarrhoea (Blaser et al, 1978; Bruce et al, 1980; Hay and Ganguli,

1980; Skirrow and Benjamin, 1980; Svedhem and Norkrans, 1980; Blaser et al, 1980b). One report has suggested that feline and canine pets are responsible for 5% of cases of C. jejuni infection in humans (Prescott and Munroe, 1982). But, since the factor responsible for virulence is unknown, it is quite possible that isolates from dogs and cats may not be pathogenic for man, even though they are serologically identical to human isolates (Bjorn et al, 1985).

Attempts to bring about campylobacter diarrhoea in kittens using strains of organisms recovered from children with diarrhoea have been tried, but no detectable clinical disease could be produced and the animals shed the organisms for only two to three days which suggests that cats are not very susceptible to human strains (Prescott and Karmali, 1978).

Dogs

The presence of so-called "Spirochetal organisms" from the faeces of both healthy and diarrhoeic dogs was reported more than 30 years ago and cited by Blaser et al (1984). The bacteria concerned were later identified as C. jejuni and other Campylobacter spp. (Blaser et al, 1984). Since then several articles identifying C. jejuni as one of the possible causes of diarrhoea in dogs have been published (Prescott and Karmali, 1978; Blaser et al, 1978; Slee, 1979; Fox et al, 1983a), but it has not been well substantiated in view of the failure to differentiate their clinical condition from toxic enteritis and other intestinal infections. It has also been suggested that campylobacter may act synergestically with canine parvovirus (Skirrow, 1981; Schifferli et al, 1982; Simpson and Burnie, 1983). The absence of a well defined clinical disease syndrome and the frequent presence of other known causes present in campylobacter infected dogs suggests that Campylobacter spp may be pathogens of an opportunist nature (Fleming, 1980). Prevalence rates ranging from 3% in faeces from clinically unaffected pets to 90% in diarrhoeic strays have been reported (Doyle, 1981). Campylobacter rates of 13 to 75% for puppies, 5 to 50% for dogs have also been reported (Hasting, 1978; Blaser et al, 1979b; Ferreira et al, 1979; Hosie et al, 1979; Bruce and Zochowski, 1980).

In two studies performed in England, C. jejuni was recovered with about equal frequency from diarrhoeic dogs and nondiarrhoeic dogs, 10.4% and 8.0% vs 11.1% and 6% respectively (Hosie et al, 1979; Holt, 1980). In pet animals, the carrier rate is generally lower than that reported in animals found in pounds or shelters (Prescott and Mosch, 1981). A series of studies (Hosie et al, 1979; Bruce and Zochowski, 1979; Skirrow and Benjamin, 1980a; Fox et al, 1983a; Fleming, 1983; Garcia et al, 1983) has demonstrated comparatively higher rates of recovery in immature dogs, kennelled or confined subjects, stray dogs and in animals with diarrhoea. Thus there is a difference in the level of infection between populations of dogs, depending on their age and their environment (Prescott and Munroe, 1982). Prevalence values of up to 50% have been obtained in immature, kennelled and diarrhoeic populations compared with a low rate of recovery in mature clinically unaffected pets (Ferreira et al, 1979).

The first attempts to infect puppies by feeding them with campylobacter strains isolated from human cases of enteritis were unsuccessful (Prescott and Karmali, 1978; Hasting, 1978). However, campylobacter enteritis has been reproduced using pure cultures of human and canine isolates of C. jejuni in conventional as well as gnotobiotic pups. Clinically the disease induced in gnotobiotic pups was mild, being associated with transient diarrhoea and tenesmus (Hasting, 1978), and in conventional pups (Macartney et al, 1981) produced soft to watery diarrhoea with mucus. Although there are no detailed descriptions of the natural disease in dogs, short reports describe vomiting as a characteristic feature, with loose faeces occurring for about ten days, in some cases the faeces contain blood (Macartney et al, 1981). The site of infection in dogs was described by Macartney et al (1981) as being jejunal and ileal mucosa, with histologic changes being similar to the milder lesions described in man. In gnotobiotic dogs, histological changes resembled those described in man, but were confined to the large intestine (Prescott and Barker, 1980). Campylobacter jejuni has also been reported to cause abortion in the dog. The organisms have been reported from the liver, lungs and placenta (Bulgin et al, 1984).

Wheeler and Borcher (1961) were the first to report an association between human and canine campylobacter. After the first finding of thermophilic campylobacter (King, 1957), a number of studies (Skirrow, 1977; Blaser *et al*, 1978; Lindquist, 1978; Peel and McIntosh, 1978; Slee, 1979; Ferreira, 1979; Bruce and Zochowski, 1980; Blaser *et al*, 1980b; Skirrow *et al*, 1980; Svedhem and Norkrans, 1980; Hay and Ganguli, 1980) have associated infection in dogs with that in humans. Most of these cases involved children who had been in close contact with a pet animal suffering from diarrhoea (Skirrow, 1981). These and other findings indicate that domestic dogs may act as a reservoir for human campylobacter infection (Skirrow, 1977; Bruce and Zochowski, 1980). However, according to Skirrow (1981) and Prescott and Munroe (1982), possibly no more than 5% of the human cases in Britain have been associated with dogs.

CAMPYLOBACTER JEJUNI/COLI IN CATTLE AND SHEEP

Cattle

The disease potential of the microaerophilic Vibrios (*Campylobacter fetus* subspp) was first recognised by McFadyean and Stockman (1913), who reported that such organisms were associated with abortion in cattle and sheep. Their observations were later confirmed by Smith (1918), who isolated similar organisms from aborted bovine fetuses. Smith and Taylor (1919) subsequently characterised these organisms and named them *Vibrio fetus*.

Since then microaerophilic Vibrios have been associated with a variety of diseases. Jones and Little (1931a, 1931b) associated these organisms with winter dysentery in cattle and calves. To definitely prove that such organisms caused dysentery in cattle, Jones *et al* (1932) reproduced the disease in healthy cattle after feeding them a pure culture of a *Vibrio* species which had been isolated from diseased animals. These investigators judged that the jejunum was the first site in the intestinal tract to be infected, hence they proposed that these Vibrios be designated *Vibrio jejuni*. Their observation was confirmed by Stalnikov (1939). However

Rollingson (1948) did not agree that Vibrio jejuni was the cause of winter dysentery as he was not able to isolate the organism from adult cattle with a similar clinical disease. Also McPherson (1957) in Canada, Komarov (1959) in Israel, Charton (1963) in France and Scott (1973) in the U.S.A. have discounted the bacterial cause and have concluded that winter dysentery has a viral aetiology. Recently Al-Mashat et al (1980) was able to provide some evidence for the association of Campylobacter spp. with inflammatory lesions in the bovine intestinal tract. Firehammer et al (1981) was able to produce diarrhoea experimentally in three calves out of 12 calves. He suggested C. jejuni may represent the organism that researchers associated with winter dysentery years ago (Jones and Little, 1931a, 1931b; Jones et al, 1932; Stalnikov, 1939). McPherson (1957) was able to transmit winter dysentery with a filtrate of faeces and suggested therefore that it was caused by a virus. Dekeyser (1972) was able to isolate the organisms for the first time from stools by filtering the faeces through a 0.65μm filter. It would appear therefore, that the aetiological agent of winter dysentery has not been determined (Campbell and Cookingham, 1978), and that there may be more than one causal agent for this condition. It is difficult to attribute epizootics of diarrhoeal disease to specific agents on the basis of clinical signs. If infection with C. jejuni is relatively widespread in certain populations of both cattle and sheep, it is likely that colostral antibody against it is commonly present. It is also likely that C. jejuni could be more pathogenic in colostrum deprived animals. Jones and his co-workers gave a clear account of what was apparently C. jejuni enteritis in calves whether or not they were colostrum deprived was not reported. The clinical description of this enteritis agrees well with the recent description of experimental C. jejuni infection in cattle (Al-Mashat and Taylor, 1980; Al-Mashat and Taylor, 1981). The following description of C. jejuni enteritis in cattle is based on the description of several investigations (Al-Mashat and Taylor, 1980; Firehammer and Myers, 1981; Al-Mashat and Taylor, 1981).

The clinical appearance in calves is often characterised by an irregular and moderate increase in temperature and unthriftiness. Diarrhoea may last up to 14 days. The faecal consistency is often limited to a softness or pastiness which occurs during the course of

the disease, however faeces can become dark, fluid, and contain large quantities of thick, tenacious mucus. It is not uncommon to see flecks of bright blood in the faeces. At times, the faeces may be well formed even though mucus and occasionally blood may be in the faeces for up to 14 days or even longer. Experimentally, the disease may be so mild as to be almost inapparent, without fever, and is manifested only by mild depression and soft faeces with occasional strands of mucus.

A rise in agglutinating antibody titre against homologous infecting organisms has been consistently found in experimentally infected calves (Al-Mashat and Taylor, 1980, Al-Mashat and Taylor, 1981).

Although the part played by campylobacter as a cause of dysentery in cattle is not clear, a widespread survey for the presence of campylobacter still goes on. Firehammer et al (1981) were able to isolate campylobacter from 51 (40%) of diarrhoeic calves and from three clinically healthy calves (Firehammer and Myers, 1981). Svedhem and Kaijser (1981) from Norway, Luechtefeld and Wang (1982) in U.S.A. and Munroe et al (1983) in Canada reported isolation rates of: 19% of 90, 43% of 130 and 25% of 421 in clinically normal cattle, respectively. Thus, besides being present in clinically diseased animals, Campylobacter jejuni is often part of the normal intestinal flora of cattle (Bryner, 1964; Smibert, 1978).

Milk

Campylobacter infection in man has been associated with the consumption of unpasteurised milk (Blaser et al, 1979a; Robinson and Jones, 1981). Robinson and Jones (1981) described 13 episodes of illness involving schools and whole villages following the consumption of unpasteurised milk and there have been descriptions of episodes of campylobacter enteritis involving an entire town following temporary failure of pasteurization equipment (Porter and Reid, 1980).

Experimentally, Robinson (1981) was able to produce infection in a human volunteer just by inoculating 500 organisms in 180 ml of milk. Milk may act as a protective agent during the passage through the stomach (Blaser et al, 1980a) and thus help establish the infection.

A herd survey by Banford (1982) in Australia showed three percent prevalence rate in 36 milking cows. In England, Robinson (1982) in a long term survey of two milking herds, found 10% of the cows to be infected. It is possible that campylobacter may get into milk during milking as a result of faecal contamination if proper hygienic methods are not followed. Such incidences may more frequently occur in countries such as Fiji where milking machines are not common and the old traditional methods of milking are still being used. The milk is also distributed unpasteurised by individual farmers. The incidences of campylobacter enteritis of bovine milk origin is greatly reduced when the milk is collected hygienically and pasteurised (Doyle and Roman, 1981; Christopher et al, 1982).

Campylobacter does not grow in milk, but the organisms are likely to survive in raw milk long enough to reach consumers (Blaser et al, 1980a; Barrett, 1981; Doyle and Roman, 1981; Christopher et al, 1982; Doyle and Roman, 1982). Blaser et al (1980a) reported that C. jejuni is able to survive for up to three weeks at 4 C, but for no longer than three days at 25 C and hence he suggested that milk is a suitable biological milieu for the survival of campylobacter.

Suggestions have also been made that C. jejuni may be a cause of mastitis in cows. Inoculation of just 2-6 colony forming units into the udder of a cow resulted in moderately severe and acute mastitis and the organism was reisolated in milk from one infected quarter, but they failed to do so from other quarters, faecal samples and blood. This was the first recorded case of mastitis caused by C. jejuni/coli (Lander and Gill, 1980). Waterman and Parker (1982) failed to reveal the presence of C. jejuni from 600 milk samples of cows with mastitis. The organism has been isolated only once from incriminated milk (McNaughton et al, 1982).

Sheep

Smibert (1965) isolated Campylobacter from the faeces of clinically normal sheep. Firehammer et al (1981) found that the tissue section from lambs intestinal loops showed increased intralaminal leukocytes, defoliated epithelial cells, and mucus production which may indicate some degree of pathologic potential for Campylobacter jejuni.

Campylobacter jejuni has been recognised for many years as an important cause of abortion in sheep (Bryner et al, 1964; Al-Mashat and Taylor, 1981). In one outbreak 25 of 79 ewes lost their lambs through abortion, still birth, or neonatal death (Ryff, 1940). The infection is believed to have followed ingestion of the organisms, with the subsequent development of a bacteraemia (Miller, 1959), followed by foetal septicaemia and hepatitis, death of foetus and its expulsion.

Bacteria resembling C. jejuni were isolated from the gall bladder in 20 of 186 sheep at slaughter (Bryner et al, 1972). Gall bladder isolates caused abortion when given as an oral inoculum to pregnant ewes.

Diarrhoea did not occur in ewes given C. jejuni isolates as an oral inoculum, but Firehammer and Myers (1981) observed mucus production and intermittent flecks of blood in faeces of lambs. Smibert (1965) isolated C. jejuni from the faeces of clinically normal sheep thus the suggestion that besides being present in infected animals C. jejuni is often part of the normal intestinal flora of sheep.

In New Zealand, thermophilic campylobacter were isolated from the intestinal content of 20 and 35% of two of four groups of 70 sheep (Gill and Harris, 1982). Luechtefeld and Wang (1982) recovered C. jejuni at an isolation rate of 23% from the faeces of 35 sheep from several farms.

Pregnant ewes that ingest C. jejuni by mouth often develop a bacteraemia, the placenta becomes infected, and the lamb is aborted.

These events indicate the invasive ability of C. jejuni, an observation that is also supported by the fact that some people with enteric Campylobacter infections develop bacteraemia (Firehammer and Myers, 1981).

Birds

Wild birds are claimed to form the main natural reservoir of campylobacter infection (Kniel et al, 1982; Skirrow, 1982). Intestinal infections with C. jejuni in healthy birds of all types is generally common (Prescott and Munroe, 1982). Birds, particularly domestic poultry, are increasingly being perceived as a major source of infection for people in developed countries (Prescott and Munroe, 1982; Munroe et al, 1983). Grant et al (1980) isolated C. jejuni from the faeces of 38 of 46 chickens before slaughter. Prescott and Mosch (1981) reported C. jejuni from 50 of 210 chickens and from 83 of 94 ducks. Luechtefeld and Wang (1981) isolated C. jejuni from the caeca of 100% of 600 turkeys going through a processing plant.

Campylobacter jejuni/coli has also been isolated from other birds such as pigeons, blackbirds, starlings and sparrows (Smibert, 1978) and canaries (Pearson et al, 1977), Peruvian penguins and Blue-crowned motmots (Bauwens and Meurichy, 1981; Luechtefeld et al, 1981). From 20 to 70% of seagulls have been found to harbour Campylobacter, many of which are strains of C. jejuni indistinguishable from those found in sheep, cattle, poultry and man (Skirrow and Benjamin, 1980b; Skirrow and Benjamin, 1980a; Fenlon, 1981; Fenlon, 1982). C. jejuni has also been found in 35% of migratory water fowl in the U.S.A. (Leuchtefeld et al, 1980), and in 45% of rooks and 50% of urban pigeons in Britain (Fenlon, 1981). Luechtefeld et al (1981) reported the isolation of C. jejuni from Black-necked swans, spur-winged geese, Ruddy shelduck, Blue-crowned motmot and Upland geese. Kapperud and Rosef (1983) recovered campylobacter from 11 of 40 species of wild birds they captured; crows, gulls, puffins and pigeons and from seven sparrows. Luechtefeld et al (1980) isolated campylobacter from approximately 35% of caecal specimens from 445 wild ducks.

Other Animals

Most isolates of campylobacter from the intestine of swine are C. coli (Skirrow and Benjamin, 1980a, 1980b). Skirrow and Benjamin (1980a, 1982) reported 94% of their pig isolates to be C. coli. Munroe et al (1983) found that 97.5% of their isolates from pigs were C. coli and only 2.5% was C. jejuni. Many healthy pigs have C. coli in their faeces, isolation rates in such pigs generally have been high; 60.7% (Oosterom, 1980), 72.1% (Jorgensen, 1979) and 72% (Kniel et al, 1982). Rosef (1981) recovered C. coli from 58% of pigs' gall bladders at slaughter. While conducting a survey on a pig farm, Rosef and Kapperud (1983) were able to isolate C. jejuni from house flies (Musca domestica) and suggested that house flies could play an important part in the dissemination of the infection.

Bruce and Ferguson (1980) reported the isolation of C. jejuni from a number of zoo animals, including Capybaras, Oatimundis polar bears, Wallaroos, Wolves and several species of primates. In an extensive study carried out by Luechtefeld et al (1981) in a zoo they were also able to isolate C. jejuni from Lawland gorilla, Black lemur, Celebus monkeys, Chimpanzee, Pale-faced saki, Patas monkeys, Orangutan, Celebes macque, Cheetah, Bobcat, Damagazelle, Rocky mountain bighorn sheep, Llama, Reindeer, Roan antelope, Brazillian tapir and Red panda. Atherton and Ricketts (1980) isolated C. jejuni from the faeces of foals in a limited outbreak of diarrhoea characterised by fever and colic with occasional fresh blood in the faeces. Rosef et al (1983) recovered C. jejuni from one of 32 hares but failed to isolate the organisms from horses, goats, cervids and rodents.

Humans

Curtis, possibly reported the first case of human campylobacter infection occurred in 1913 (Curtis, 1913). He observed large numbers of curved, motile, gram-negative rods in vaginal discharges from two patients. Levy (1946) reported the first association of microaerophilic vibrios with diarrhoeal disease in humans, when he described a large outbreak of gastroenteritis in Illinois, organisms were isolated from the blood of some of the patients. Vinzent et al

reported Vibriosis in man in 1947. King (1957) isolated the organisms from blood and associated the organisms with a diarrhoeal disease and named the organisms "Related Vibrios". Cooper and Slee (1971), in Australia were the first to isolate the organisms from stools. The full extent of the association between C. jejuni and diarrhoeal illness could not be appreciated until simplified and improved methods for isolating these organisms from stools of patients with gastroenteritis was first reported by Dekeyser et al (1972) and further improved by Skirrow (1977).

Campylobacter enteritis has been reported from Australia (Cooper and Slee, 1971; Slee, 1972; Steele and McDermott, 1978), Belgium (Butzler et al, 1973; Lauwers et al, 1978), Canada (Pai, 1979), Holland (Severin, 1978), New Zealand (Brieseman, 1984 and 1985), Rwanda (De Mol and Bosman, 1978), Sweden (Lindquist et al, 1978), South Africa (Hallet et al, 1977; Schewitz and Roux, 1978; Bokkenheuser et al, 1979), the United Kingdom (Skirrow, 1977), the United States of America (King, 1957; Middlekamp and Wolf, 1961; Wheeler and Borchers, 1961; King, 1962; Mandel and Ellison, 1963; Park et al, 1973; Smith et al, 1977; Blaser et al, 1978; Guerrant et al, 1978) and Zaire (Butzler, 1973) which indicates that this disease is widely distributed in tropical as well as temperate areas of the world. Studies in Africa, Australia, North America and Europe have shown this agent to be involved in 3% - 11% of patients with diarrhoea (Butzler et al, 1973; Steele and McDermott, 1978; Pai et al, 1979; De Mol et al, 1979). In New Zealand and other overseas countries, it has been reported to be 4% to 15% (Vinzent et al, 1947; Walder, 1982; Wundt and Kasper, 1982; Shmilovitz et al, 1982; Kalman et al, 1983; Faoagali, 1984. In some countries, 74 to 84% of cases occurred in children and infants (Shmilovitz et al, 1982; Kalman et al, 1983), whereas in New Zealand the number of cases is highest in young adults, and children are the next most frequently affected group (Brieseman, 1985). However Campylobacter enteritis affects all age groups (Butzler et al, 1973; Skirrow, 1977; Dale, 1977) but the incidence is highest in young children (Butzler et al, 1973; Skirrow, 1977). Bokkenheuser et al (1979) recovered Campylobacter jejuni from 35% of children with diarrhoea and from 16% of asymptomatic children. The significantly higher prevalence of C. jejuni isolations in 0 to

8-month-old children with diarrhoea than in the asymptomatic children strongly suggests that the organisms are a causative agent of diarrhoea in these very young children (Bokkenheuser et al, 1979; Karmali and Fleming, 1979; Mentzing, 1981). The prevalence of C. jejuni increases dramatically after 9 months of age (Bokkenheuser et al, 1979). Jones et al (1984) reported the rate for children under one year of age as five times higher than that of adults, whilst for children aged 1-4 years the rate was three times higher than the adult rate (Jones et al, 1984). It has also been suggested that males are more commonly affected than are females in a male to female ratio of 3:2 (Karmali and Fleming, 1979).

In developed countries, there is seasonal variation in the incidence of C. jejuni enteritis, with highest rates in the summer, and the lowest in the winter (Butzler and Skirrow, 1979; Walder and Forsgren, 1982; Kist, 1982; Blaser et al, 1982b).

Campylobacter jejuni enteritis is a zoonosis with a world-wide distribution, and there are a number of ways by which man can become infected. Wild birds are probably the main source of campylobacter for natural waters (Kniel et al, 1982). Animals of special importance as sources of human infection are probably poultry, cattle, sheep, pigs and dogs, but not necessarily in that order of importance. In developed countries, the isolation rates of thermophilic campylobacters from water samples has been shown to be 43% for cattle, 91% for poultry, 88% for pigs, 49% for dogs, 53% for cats but in only about 1.6% for humans (Proc. Int. Workshop in Campylobacter Infection, 1982).

In the United Kingdom, most infections are sporadic, case to case transmission is uncommon and convalescent carriage of the organism seems to be unimportant as a source of new infections (Jones et al, 1984). Cross transmission from mother to children has been reported (Mentzing, 1981) but failure to isolate C. jejuni from 272 women by Blaser et al (1980c) suggested that it is not part of the normal vaginal flora and that transmission from maternal vaginal flora to fetus is probably not a common mode of human infection.

Infection can be acquired through direct contact with infected animals, but this accounts for only a small minority of infections. Contact may be occupational as with farmers, veterinarians and those engaged in meat processing (Jones and Robinson, 1981) or domestic in which case the animals concerned are almost always a newly acquired family puppy, or very occasionally a kitten, which is itself suffering from campylobacter enteritis (Skirrow, 1981). But most day to day infections are in patients without any direct contact with animals, so indirect pathways of infections presumably operate in these cases. It has therefore been suggested (Skirrow, 1982) that transmission is mainly through the food chain from raw animal products - milk, beef and sheep carcase meat, poultry meat, and offal. The ability of the organism to survive at normal refrigeration temperature, virtually guarantees that everybody handling raw chicken meat is exposed to the infection, unless it has been thoroughly decontaminated. The organism may well be found on many cutting boards and butchers knives (Grant et al, 1980). In Christchurch, New Zealand, Brieseman (1985) carried out analyses of the campylobacter infections among a number of occupations and found the following groups to be infected:

Housewives	23	Management, accountants, etc.	7
Meat handlers	19	Sales	6
Tradesmen	12	Labourers	6
Unemployed	12	Engineering	5
Office and clerical	10	Retail shop assistant	4
Farm workers	11	Government employees	4
Health service	9	Teachers	4
		Drivers	3

It appears that those associated with handling raw meat (butchers, freezing workers and housewives) are at greatest risk.

Campylobacter infections in humans are usually associated with C. fetus subsp. intestinalis, C. jejuni and C. coli (Bokkenheuser et al, 1979; Rettig, 1979). The two main clinical forms are:

1. Campylobacter enteritis
2. Localised form.

Campylobacter coli is a much less common cause of enteritis than C. jejuni (Jones et al, 1984).

Campylobacter enteritis

Although Campylobacter jejuni is very rarely pathogenic for animals, it is a common human enteric pathogen. Evidence for its pathogenic role in humans is based on the following observations.

Campylobacter jejuni has been simultaneously recovered from faeces and the blood stream in several diarrhoeal patients (Levy, 1946; King, 1957; Wheeler, 1961; King, 1962; White, 1967; Darrel et al, 1967; Evans and Dadswell, 1967; Bokkenheuser, 1970; Dekeyser et al, 1972; Butzler, 1973; Smith, 1977; Communicable Disease Surveillance Centre, 1978) specific missing serum antibody titres have been demonstrated in infected diarrhoeal patients (Skirrow, 1977; Steele and McDermott, 1978; Karmali and Fleming, 1979; Blaser et al, 1979b) and a human volunteer who ingested C. jejuni, developed a typical clinical illness and the infecting organism was recovered from his stools (Steele and McDermott, 1978). The frequent observation that blood and polymorphonuclear leukocytes occur in the stools of affected individuals (Blaser et al, 1979b) and the occasional demonstration of bacteraemia, suggests that the organism may be cytotoxic and/or tissue invasive.

Skirrow (1977) estimated the incubation period of campylobacter enteritis as being from 2 to 11 days. Other investigators (Prescott and Karmali, 1978; Karmali and Fleming, 1979; Blaser et al, 1980a) suggested that a typical incubation period is 2 to 5 days.

The major clinical manifestation are abdominal pain, diarrhoea and fever (Rettig, 1979). Diarrhoea usually occurs at the onset of illness or may develop within a few days after the onset of abdominal pain and fever. Typically, the diarrhoea is mild to moderate but may be profuse, watery and frequently one to three days after the onset of diarrhoea, blood may also appear in the stools. Other symptoms that may be expressed include malaise, headache, musculoskeletal pain, rigors and dilirium (Skirrow, 1977; Karmali and Fleming, 1979). Although vomiting may occur, it is not common

and is normally observed in less than 30% of the cases. The severity of the illness is quite variable but in most cases it is brief and self-limiting (King and Bronsky, 1961; Blaser et al, 1980c). Clearance occurs within two months in 90% of cases (Brieseman, 1985).

The principal site of disease in man seems to be the jejunum and ileum (Butzler and Skirrow, 1979). The disease is not, however, limited to the small intestine, but commonly also involves the colon, even extending to the rectum (Blaser and Reller, 1981).

The mechanisms by which C. jejuni causes disease are not yet known. The finding of dysenteric stools suggests that mucosal damage due to an invasive process analogous to that seen in shigellosis is important in the pathogenesis. The frequent occurrence of profuse water stools in many cases suggests that an enterotoxin may also be involved in the pathogenesis. Preliminary studies have shown that C. jejuni also produces substances that are cytotoxic for tissue culture cells (Anon, 1984).

In contrast to the frequent incidence of campylobacter enteritis, systemic campylobacteriosis appears to be an infrequent human disease.

Systemic Campylobacteriosis

In 1947, the first proven human case of Systemic Campylobacteriosis was reported. By early 1979 less than 150 cases of nonenteric human campylobacter infection have been documented (Bokkenheuser et al, 1979; Rettig, 1979) and C. fetus subsp. intestinalis is primarily responsible for this syndrome, having been isolated from most patients afflicted with this illness.

The disease is primarily associated with adults with most cases having occurred in individuals between 35 and 70 years of age. Most of these individuals had one or more underlying major medical conditions such as alcoholism or cirrhosis, diabetes mellitus, rheumatic heart disease, leukaemia or tuberculosis before infections. Increasingly, there appears to be a predilection

towards males as most cases (69% of 102) have occurred in males (Rettig, 1979).

The most common manifestation of systemic campylobacteriosis is bacteraemia without localized infections (Kahler and Sheldon, 1960; King, 1962; Bokkenheuser, 1970; Guerrant et al, 1978). However, several cases have been reported in which the organism localized and produced infection in specific tissues of the host. Examples of such localized infections include endocarditis (King, 1957; Loeb et al, 1966; Chung and Lee, 1970; Lee et al, 1970), meningitis (King and Bronsky, 1961; Eden, 1962; Collins et al, 1964; Gubina et al, 1976), spetic arthritis (King and Bronsky, 1961; Kutner and Arnold, 1970), thrombophlebitis, (Kahler and Sheldon, 1960; Vesily, 1975; Steele and McDermitt, 1978), salpingitis (Brown and Sautter, 1977), and abscess of the lung (Lawrence et al, 1971).

Symptoms commonly expressed by individuals affected with systemic campylobacteriosis includes fever, malaise, headache, confusion, lethargy and abdominal pain (Rettig, 1979). Diarrhoea is not common in these systemic infections.

Food

The increasing awareness of the presence of C. jejuni in patients with gastroenteritis has led to attempts to recover the pathogens from incriminated food. The organism has been mainly associated with food of animal origin such as pork, ground beef, chicken and milk.

Unpasteurised milk is the most frequently implicated vehicle of campylobacter infection. Campylobacter enteritis was first suspected as being attributed to the consumption of milk in 1946 (Levy, 1946). A number of other reports have documented the association of C. jejuni enteritis with unpasteurised milk (Blaser et al, 1979a; Taylor et al, 1979; Porter and Reid, 1980; Robinson and Jones, 1981; Tosh et al, 1981). Butzler and Skirrow (1979) mentioned that the consumption of milk was implicated in five major campylobacter outbreaks in Britain during a 6 months' period.

King (1962) suggested chicken as the primary source of campylobacter infection in man. Smith and Muldoon (1974) were the first to report the incidence of C. jejuni from commercially processed poultry. Simmon and Gibbs (1977) reported recovery rates of 48% from processed chickens and 92% of turkeys. Studies from Canada (Park et al, 1981), Sweden (Norberg, 1981) and United States of America (Grant et al, 1980; Luechtefeld et al, 1981; Norberg, 1981) have reported incidences of 22 to 92% of C. jejuni from retail market poultry meat. Soaking turkey carcases overnight in 340 ppm chlorine wash water did not decrease the number of infected carcases (Luechtefeld, 1981). C. jejuni survived refrigeration and freezing on processed turkeys and chickens (Simmon and Gibbs, 1979).

Stern (1981b) reported organisms from lamb carcases, beef and swine carcases, 24%, 2% and 22% respectively. Stern (1982) in his second survey recovered C. jejuni from unwashed carcases of pig, lamb and beef at rates of 38, 24 and 2% respectively. Hudson and Roberts (1981) did not find C. jejuni on beef or lamb carcases but did find it on 59% of the pig carcases they examined. Turnbull and Rose (1981) reported that 1.6% of meat samples from both abattoirs and retail outlets were positive for campylobacter. Only a limited amount of data has been published on the contamination of red meat carcases by C. jejuni (Stern, 1981a, 1981b). Chicken meat can be contaminated with strains of Campylobacter species (Grant, 1980) and they are common in healthy pigs (Oosterom, 1980). Both poultry and pork have been suggested as vectors.

Consumption of cake, particularly the icing, was associated with an outbreak of campylobacter enteritis (Blaser et al, 1979b). Raw hamburger has also been implicated as a source of campylobacter enteritis in a military camp (Oosterom, 1980).

Although food has been implicated in the transmission of campylobacter disease (Doyle, 1981; Stern, 1982), the presence of C. jejuni in foods has rarely been confirmed (Doyle and Roman, 1981; Gill and Harris, 1982).

Kniel et al (1978) assayed 84 samples of water for the presence of C. jejuni and found the organisms to be present in 7 of 34

samples of sea water and 37 of 50 samples of fresh water. Blaser et al (1980a) reported that C. jejuni could survive in stream water at 4°C for 1-4.5 days. Water has been implicated as a medium for conveying campylobacter infection.

Thus in summary, we can say that thermophilic campylobacter are widely spread in the animal kingdom and there are many ways humans can become infected. The animals dealt with in the thesis could therefore also take part in this zoonoses.

CHAPTER III

GENERAL MATERIALS AND METHODS

INTRODUCTION

Samples from rectal faeces, intestinal content, stomach content and bile were used to determine the presence of thermophilic campylobacter in the animals under investigation.

The use of rectal, intestinal and stomach swabs was judged to be the best method for collecting samples from individual animals and carcases for subsequent bacteriological examination in order to determine their Campylobacter status. It was necessary, however, to ensure that the time between taking the swabs and making primary cultures was not great enough to affect the survival of the micro-organisms under investigation (Kakoyiannis, 1984). The viability of the Campylobacter under study maybe sensitive to environmental conditions as they do not grow at less than 30.5°C, (Skirrow and Benjamin, 1980a), and exposure to air has been observed to cause rapid coccal transformation and degeneration (Karmali et al, 1981).

MATERIALS

ANIMAL SAMPLES

Cattle, Sheep, Cats

In cattle, sheep and cats, only rectal swabs were used. Sterilised cotton wool swabs (Hospiswabs)* were used to collect rectal contents from cattle on the Massey Dairy Farm No. 1 while the cows were being milked. In the case of sheep, the rectal content was collected from sheep from one of the Massey flocks. These

* Medical Wire and Equipment Co. Ltd., Corsham, England.

samples were taken after the animals had been driven into an enclosure and then allowed to pass through a race one at a time. Rectal swabs from cats were collected when hand-held by an assistant. On all occasions cotton swabs were moistened in 1% alkaline peptone water (pH 8.4) as recommended by Tanner and Bullin (1977). Each swab was rotated within the rectum for approximately 30 seconds, ensuring its contact with the mucosa. The swabs were then placed in a universal bottle containing 5 ml of sterilized 1% alkaline peptone water sufficient to immerse the swabs. One percent alkaline peptone water (pH 8.4) is a satisfactory enrichment medium and allows campylobacter to multiply in the presence of much larger numbers of Escherichia coli and Streptococcus faecalis (Tanner and Bullin, 1977). Rectal swabs were transferred to the laboratory and cultured within two hours. All the samples after being cultured were kept in a refrigerator at 4°C for 48 hours so that if necessary, they could be further investigated.

Rats, Mice, Guinea Pigs and Rabbits

In laboratory animals intestinal, stomach, caecal and rectal swabs were taken. Bile from guinea pigs was also examined for the presence of these organisms.

Rectal Swabs

Swabs similar to those used for cats were used to collect material from the rectum of rats, guinea pigs and rabbits. The swabs were transferred to the laboratory within one hour and cultured.

Intestinal Swabs

Rats, guinea pigs and rabbits were euthenased by injecting 1 ml of Euthesate* intraperitoneally. Mice were euthenased in an

* Euthesate (Pentabarbiton Sodium); Willows Francis, Westhoughton, Bolton, BLS 3SL, U.K.

airtight chamber filled with pure carbon dioxide*.

Intestinal swabs were taken from six sites after the animals had been eviscerated on a dissection board. Sites selected for the collection of material were: stomach, approximately half way along the duodenum, jejunum, ileum, caecum and colon. Using an aseptic technique, a 2 cm incision was made through the wall of the intestine and a swab was inserted into the lumen. Each swab was rotated within the intestine ensuring its contact with the mucosa and the intestinal content. The swabs were then immediately cultured.

Collection of Bile

The bile duct was removed aseptically from the guinea pigs and placed in a petridish. Bile was transferred to a bottle of thiol broth** by means of a syringe and then incubated at 42°C. for 48 hours before being subcultured onto agar.

Capturing House flies

House flies were captured from the Small Animal Production Unit by knocking them down with a fly swat.

COLLECTION OF SAMPLES FROM IN AND AROUND THE SMALL ANIMAL PRODUCTION UNIT

Water Samples

Water samples of 50 ml were collected in a universal bottle from the drinking water used by the rats, mice, guinea pigs, rabbits, cats and from drains within the Unit. Water was transferred to laboratory for culture within one hour of collection.

* NZIG

** Fort Richardson Laboratories Ltd., Auckland, New Zealand.

Feed Samples

Feed samples of about 50 gms were collected in plastic bags from the rat, mice, guinea pig, rabbit and cat feeders and were transferred to the laboratory for culturing within one hour. Samples were also collected from bulk samples of feed bought in for these animals.

Sawdust Samples

Sawdust samples of about 50 gms were collected in plastic bags from the rat, mice, and rabbit cages and guinea pig pens and were transferred to the laboratory for culturing within one hour. Samples were also collected from bulk samples.

Formite Samples

Washings from the sinks, benches, table tops, bins, shovels, brooms, floors, soles of gumboots were collected in sterile universal bottle and cultured within an hour.

Samples from the Workers

There are four regular workers at the Unit and samples of hand washings were collected from each of these during their work period in sterile universal bottles and transferred to the laboratory and cultured within 2 hours.

Every worker was also given a sterile wool swab for the collection of rectal swabs and these were cultured within one hour after collection.

Samples of rectal swabs from some sheep kept within the unit for experimental purposes were also collected. Samples of waste (excreta, used sawdust, etc.) being stored in a large disposal bin within the unit was also collected and cultured within one hour.

METHODS

CULTURE METHODS

Selective Media

Three antibiotic selective media were used for primary cultures (see Chapter IV).

Culture of Intestinal, Stomach and Faecal Swabs

Each swab sample was well mixed with 5 ml of 1% alkaline peptone. The same swab was then used to carry a sufficient quantity of the mixed material to the surface of a selective agar plate. The agar was inoculated until the complete surface was well spread with the material.

Culture of Bile

After incubating for 48 hours at 42°C in a thiol broth, 1 ml was transferred to the selective medium by means of a syringe and well spread over the surface of the agar selective media by means of a sterile cotton wool swab.

Culture of Flies

Each housefly (10 from inside the unit and 10 from the area around the garbage bin) was macerated with 5 ml of 1% alkaline peptone. The macerated material was inoculated onto a selective agar medium by means of a cotton swab. The other 10 flies (5 flies from inside the unit and 5 from the area around the garbage bin) were allowed to walk about on individual selective agar plates which were covered with a beaker.

Culture of Feed, Sawdust and Waste

Each sample of feed, sawdust (used and unused) and waste were macerated with 50 ml of water by means of a mortar and pestle and allowed to settle. The supernatent liquid was collected and

cultured by the same method as used for the water samples (see below).

Culture of Water Samples

Water samples were examined by the method of Kniel et al (1978). Fifty millilitres of water was passed through a 0.45 µm millipore* filter. The membrane was placed upside down on the surface of the selective agar medium plates. The plates were incubated at 42°C. for 48 hours in a microaerophilic atmosphere. The membrane was then removed and the plates reincubated for a further 24 hours.

Culture Conditions

Gaspack jars with no catalyst were used, each jar was capable of holding 12 plates. The inoculated culture plates were placed upside down in the jars and the air pressure reduced on two occasions to 600 mm of Hg by using a water vacuum pump. After each evacuation the jars were filled with a mixture of 5% O_2, 10% CO_2 and 85% N_2. They were then incubated at 42°C for 48 hours.

IDENTIFICATION OF CAMPYLOBACTER

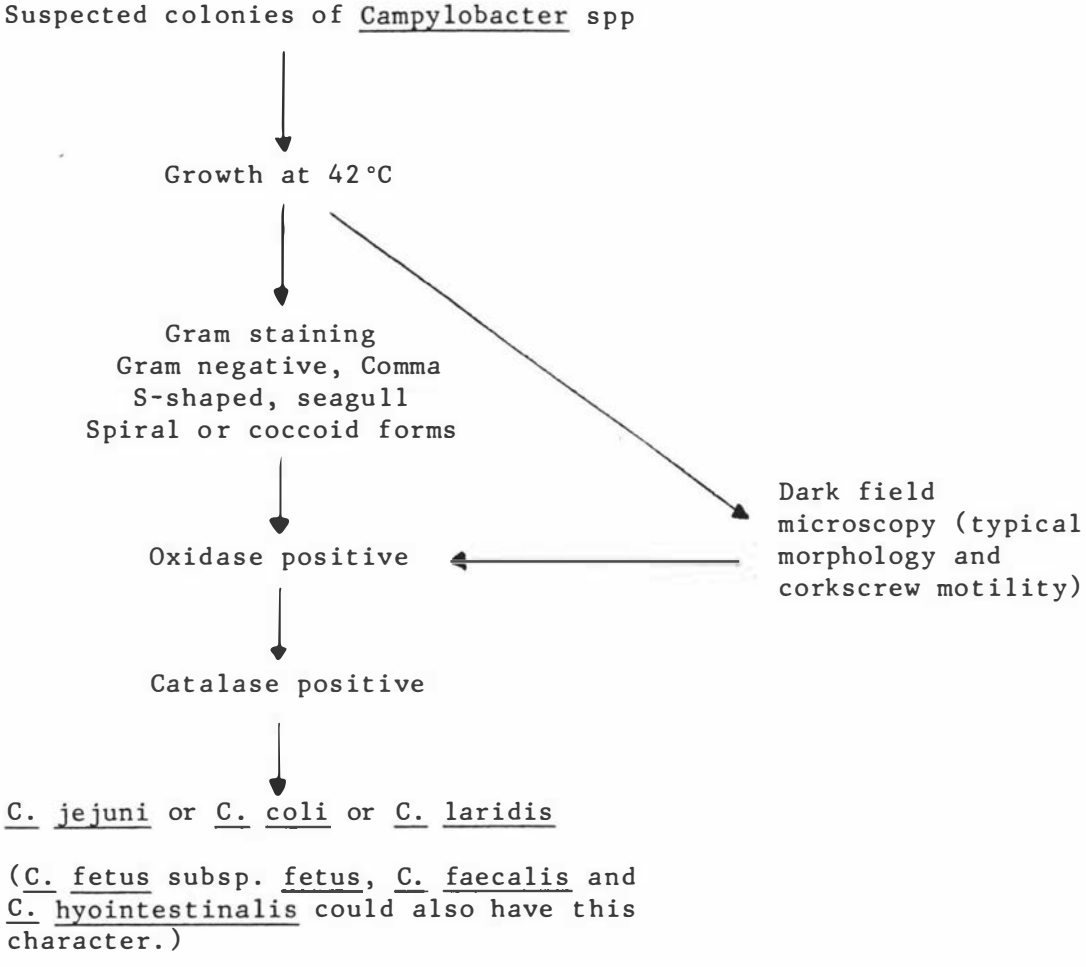
The following criteria were used for the identification of the Campylobacter species (Figure 3.1). Two colonial types are recognised and can be described as follows:

(a) Flat, pale grey, transparent, irregular edges, 2-4 mm in diameter. Swarming and spread along the line of inoculation.

(b) Circular, raised convex, shiny grey colonies 1-2 mm in diameter.

* Millipore Corporation, Bedford, Massachussets, 01730, U.S.A.

Figure 3.1 : **IDENTIFICATION OF CAMPYLOBACTER**



Colonies which were considered likely to be C. jejuni/coli were recultured for 24 hours and were examined by gram staining and by dark field microscopy using a wet preparation of the suspected micro-organism in saline. Microscopic examination was at a magnification of 600x.

Preparation of a Pure Culture

Individual colonies from the selective media were subcultured onto blood agar (BA) plates (Difco Columbia blood agar base with 7% sheep blood and 1 ml of 0.05% solution of ferrous sulphate, sodium metabisulphite and sodium pyruvate (FBP supplement) per 500 ml of agar), which were incubated at 42°C for 48 hours. The primary culture was also reincubated at 42°C for 48 hours to make sure of the growth of the suspected bacteria. These isolates were given a further three subcultures to make sure that they were pure. They were then subjected to a series of tests in order to determine their species identification.

Oxidase Test (Kovac Method 1956)

Filter papers were saturated with freshly prepared 0.5% aqueous solution of NNN N-tetramethyl-P-phenylenediamine dihydrochloride* and dried. A visible amount of growth from a suspected campylobacter colony was removed with a loop and smeared on to the filter paper. In a positive reaction, a deep purple colour appeared within a few seconds indicating the presence of cytochrome oxidase. When no colour change occurred it was classified as a negative reaction (Veron and Chatelain, 1973).

Catalase Test

One drop of a 3.5% aqueous solution of H_2O_2 was added to a microscope slide, placed on a black background and a loop of micro-organisms was transferred to the drop. The reaction was positive when effervescence or bubbles appeared within a few seconds (Skirrow and Benjamin, 1980a).

* BDH Chemical Ltd., Poole, England.

Table I : **DIFFERENTIATION OF CATALASE POSITIVE INTESTINAL THERMOPHILIC CAMPYLOBACTER (ISOLATED AT 42°C)**

(Based on data from: Firehammer, 1965; Smibert, 1978; Skirrow and Benjamin, 1980a, 1980b; Karmali *et al*, 1981; Gebhart *et al*, 1983)

	OBLIGATORY TEST				
	Growth at 42°C	Growth at 25°C	30 µg Nalidixic Acid Disc	30 µg Cephalothin Disc	Hippurate Hydrolysis
C. jejuni	+	-	S	R	+
C. coli	+	-	S	R	-
C. laridis (NARTC)	+ / - (+)	- / +	R	R	-
C. fetus subsp. *fetus*	- (+)	+	R	S	-
C. faecalis	+	-	NK	NK	NK
C. hyointestinalis	- (+)	+ (-)	R	S	-

\+ = positive test or growth
\- = negative test or growth
- (+) = few isolates growth at 42°C
+ (-) = few isolates fail to grow at 25°C

S = sensitive
R = resistant
NK = not known

Differentiation of Catalase Positive Thermophilic Campylobacter

The catalase positive thermophilic Campylobacter isolated at 42°C were differentiated on the bases of the parameters given in Table I.

Growth Temperature Test at 25°C and 42°C

A colony from a 24 hours culture was subcultured onto four BA plates supplemented with 0.05% FBP supplement. Two plates were incubated at 25°C and two plates at 42°C for 48 hours and 24 hours respectively. Visible growth along the sites of inoculation was recorded as positive.

Sensitivity to 30 µg Nalidixic Acid and Cephalothin Discs

The sensitivity to 30 µg nalidixic acid* and cephalothin discs* was determined by a disc diffusion test. Growth from the 37°C temperature test plates was removed on a 4 mm loop and closely streaked horizontally across the BA plates supplemented with 0.05% FBP. A 30 µg nalidixic acid and a cephalothin disc was placed opposite one another at a distance of about 3 cm on the surface of the inoculated plates. The plates were incubated at 37°C for 48 hours. The absence of a clear zone of inhibition around the disc was considered to be indicative of resistance (Karmali et al, 1980).

Hippurate Hydrolysis Test (Skirrow and Benjamin method 1980b)

A 2 mm loop full of organisms from a 48 hours culture at 37°C was suspended in 2 ml of sterile distilled water in a bijou bottle. To this suspension was added 0.5 ml of 5% of an aqueous solution of sodium hippurate** which was then incubated in a water bath at 37°C for 2 hours. After removal from the bath, 1 ml of a ninhydrin

* Alpha Biological Ltd., Auckland, New Zealand.

** Pfizer Diagnostic Div., Pfizer and Co. Inc., New York, N.Y. 10036, U.S.A.

solution (3.5 g ninhydrin in 100 ml 1:1 mixture of acetone and butanol) was slowly overlaid and left on the bench for a further 2 hours. A deep purple colour was considered as positive. This method was found to be the most sensitive for identifying C. jejuni isolates, which are not strongly hippurate negative.

Growth in the Presence of TMAO (Lior, 1984)

This test was applied to the hippurate hydrolysis negative, nalidixic acid and Cephalothin resistant organisms only.

A 100 mg amount of trimethylamine N-oxide (TMAO) dihydrate (Sigma Chemical Co.) was added to 100 ml of semisolid yeast extract nutrient broth agar medium (Appendix V). The medium was distributed in 4 ml amounts into screw capped tubes.

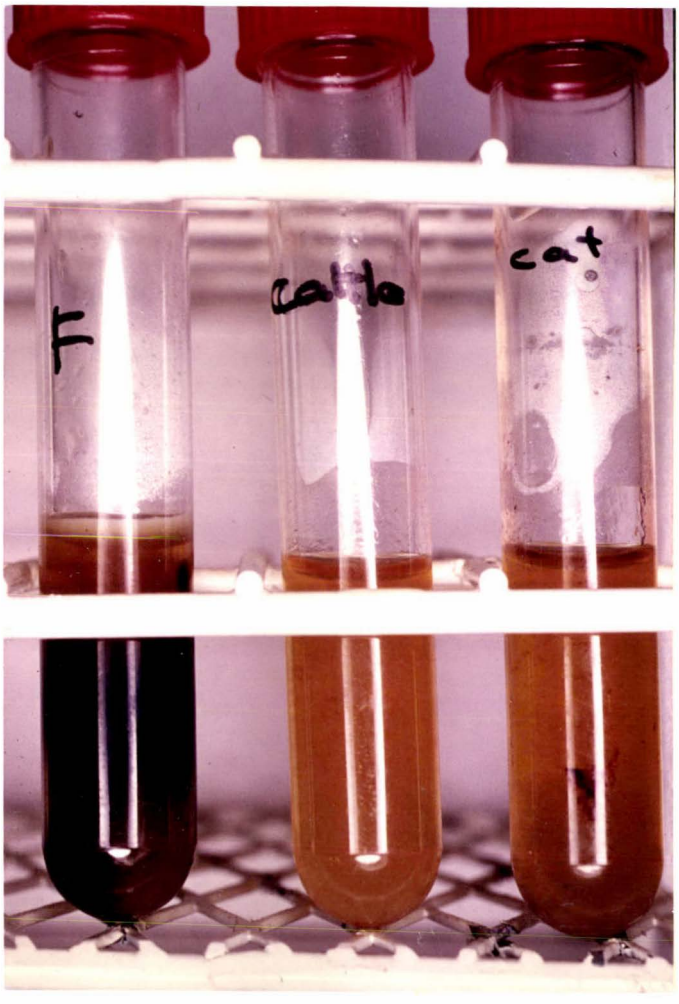
Cultures, 24 h old, were inoculated by stabbing (two to three times) into the semisolid agar to about 1 cm below the surface of the medium. The tubes were incubated anaerobically and examined periodically for up to seven days looking for growth throughout the medium in addition to growth just below the surface. The presence of growth identified the organisms as C. laridis. Figure 3.2, Tube 1, shows growth of C. laridis in presence of TMAO.

Long-term Storage and Preservation of C. Jejuni and C. Coli

Previous work has indicated that Campylobacter are able to survive for a long time at temperatures below 0°C. Skirrow and Benjamin (1980a) preserved their cultures in FBP broth (Appendix IV) which included 15% glycerol either at -179°C in liquid nitrogen or at -20°C. Wang (1981) stated that cultures of Campylobacter in glycerol Brucella broth remain viable for several years when they were preserved at -70°C. His findings were confirmed by Kakoyiannis (1984) when he succeeded in preserving the organisms in FBP broth at -70°C for more than a year.

It was decided to preserve the organisms in FBP broth at -70°C. FBP broth was selected as the storage medium since it contains nutrient broth and agar. The agar was added to the broth at

Figure 3.2 : TEST FOR GROWTH OF C. LARIDIS IN THE PRESENCE OF TMAO



1 2 3

Tube 1 shows growth of C. laridis from a house fly. No growth of isolates of C. coli (Tube 2) and C. jejuni (Tube 3) is seen in the other two tubes.

concentrations of between 0.12% and 0.16% which created microaerophilic conditions particularly suitable for the growth and survival of campylobacter (Butzler 1979, Luechtefeld et al, 1981). The addition of 0.05% of FBP supplement increases the aerotolerance of these organisms (George et al, 1978), while glycerol has cryoprotective properties, which have been found to increase the viability of C. jejuni when added to frozen ground beef (Stern and Kotula, 1982). The other factors which are likely to contribute to a better survival at -70°C could be the lower temperatures, and the more rapid freezing of the organisms at -70°C which induces smaller intracellular ice crystals. When the organisms are thawed at room temperature, rupture of the organisms is less likely to occur when such ice crystals are small.

Preservation Method

Thermophilic Campylobacter were subcultured three times under microaerophilic conditions at 42°C to get a pure growth. The organisms were harvested in 3 ml of FBP broth including 15% glycerol. The suspension was divided into three aliquots and placed in 1 ml microcentrifuge plastic tubes with tightly fitting lids. These had been washed with 70% alcohol and dried before use. The tubes were stored in special aluminium racks in a -70°C deep freeze (Forma, U.S.A.).

BACTERIAL RESTRICTION ENDONUCLEASE DNA ANALYSIS (BRENDA) TECHNIQUE

All the isolates recovered throughout the study were differentiated to a subspecies level by means of restriction endonuclease DNA analysis (BRENDA).

The steps involved in the technique are:

1. Preparation of DNA.

2. Extraction of DNA.

3. Digestion of DNA.

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measured by a spectrophotometer* in a quartz-glass cell with a 1 cm light path. From a standard curve using known concentrations of calf thymus DNA**, it was found that a reading of one optical density unit was equivalent to double stranded DNA at 50 µg/ml. In order to calculate the contribution of RNA to the absorbance at 260 nm, the DNA content of each preparation was also measured by fluorimetry# (Le Pecq and Paoletti, 1966). It was concluded that the difference between the DNA concentration as measured by the two methods represented the contribution of RNA.

Digestion of DNA

Restriction endonuclease Hsu II, prepared by the method of Greene et al (1978) from Haemophilus suis, was used to digest 2 µg of bacterial DNA to completion at 37°C for 1 hour in 100 µl enzyme buffer, pH 7.5 (Appendix VI). Bacteriophage (C1857 S7) obtained from a lysogenic strain of E. coli (Miller 1972) and was used as a reference marker for each series of digest.

Gel Electrophoresis and Photography

Gel electrophoresis was performed by the method of McDonnel et al (1977). Each sample was placed in one of nine wells in a horizontal slab of 0.7% agarose## and electrophoresis was maintained at 4v/cm until the bromophenol blue, which was used as a tracking dye, travelled a distance of 12.5 cm. The buffer pH 7.8 used for electrophoresis (Appendix VI)and which contained Ethidium Bromide, was also used for dissolving the agarose. To prepare the agarose, it was boiled in a flask fitted with a reflux condenser. On the completion of electrophoresis, the gel was illuminated with ultraviolet light and photographed by a method similar to that of

* Unican Spectrophotometer, SP500

** Sigma Chemicals Co., St. Louis, MO.63178, U.S.A.

Turner

Bio-Rad, Richmond, Ca 94804, U.S.A.

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4. Electrophoresis of the digested DNA.

5. Photography of the resultant pattern of 'fingerprint' of the DNA.

Preparation of DNA

A 24 hour growth under microaerophilic condition at 42°C of a pure culture of an isolate of thermophilic campylobacter on a FBP plate, was harvested from two plates in 10 ml of PBS, pH 7.2. The harvest was centrifuged at 1200 for 30 min and the pellet resuspended in PBS to remove any trace of soluble agar constituents which might be present in the harvest. The final pellet was mixed with 1 ml of Tris-Ethylenediaminetetraacetic acid sodium salt buffer (TEB) (Appendix VI), 100 µl of an aqueous solution of grade 1 lysozyme* (3 mg/ml) added, and the suspension incubated at 37°C for 15 min. Subsequently 100 µl of a 10% aqueous solution of sodium lauryl sulphate** and 100 µl of an aqueous solution of protease# (10 mg/ml) were added and the mixture incubated overnight at 50°C. The next morning sodium perchlorate# was added to produce a final concentration of 1 ml, the mixture incubated for another hour and then made up to a volume of 5 ml with Saline-Tris-EDTA (STE) (Appendix VI).

Extraction of DNA

DNA was extracted from the above preparation three times with a mixture of phenol, chloroform, and isoamyl alcohol (25:24:1) previously saturated with STE which largely follows the method of Younghusband and Bellett (1971).

After extraction, each sample was dialysed exhaustively against Tris-EDTA (TE) (Appendix VI) at 4°C and absorbance at 260 nm was

* Sigma Chemicals Co., St. Louis, MO.63178, U.S.A.

** BDH Chemicals, Poole, England

\# Calbiochem, San Diego, Ca 92112, U.S.A.

Sharp et al (1973). Gels were placed on a transilluminator plate* measuring 15 x 15 cm, and this was illuminated from below by four 15 w germicidal ultraviolet lamps**, and photographed on Kodak Tris-X film by means of a 120 formate plate camera through a Wratten 23A gelatin filter.

* Ultraviolet Products, San Gabriel, Ca 91778, U.S.A.

** Philips, Eindhoven, Holland

CHAPTER IV

A COMPARATIVE STUDY OF THREE SELECTIVE MEDIA

INTRODUCTION

Until 1972, Campylobacter species had not been established as an important cause of enteritis in man partly due to the inadequency of bacteriological techniques routinely used in medical diagnostic laboratories. The first method for the isolation of Campylobacter from human faeces was differential filtration of a saline extract through a 0.65 µm filter, which allows Campylobacter and other small bacteria to pass through (Dekeyser et al, 1972; Butzler et al, 1973). Thereafter the introduction of selective agars (Skirrow, 1977; Blaser et al 1978; Bolton and Robertson, 1982) proved successful for their isolation from human and animal faeces and so established Campylobacter species as an important cause of enteritis in man. On occasions however, all of these selective media can be overgrown by other organisms present in the faeces (Bolton and Robertson, 1982). A recent study indicates that Preston's medium is more successful than Skirrow's medium for the isolation of campylobacter from human faeces because of better selectivity (Bolton and Robertson, 1982). Preston's medium is also superior for the isolation of campylobacter from cattle, pigs and seagulls, but it is similar to Skirrow's medium for their isolation from sheep and chickens (Bolton and Robertson, 1982). To date, the selectivity of Campy BAP medium of Blaser et al (1978) has not been compared with the other two media. Thus it was not certain which one would be best suited for the isolation of Campylobacter jejuni and C. coli from faecal and rectal samples from cattle and sheep.

This experiment was carried out to find out the relative selectivity of the modifications of Skirrow's, Campy BAP and Preston's for the isolation of Campylobacter jejuni/coli from faecal and rectal samples from both cattle and sheep.

MATERIALS AND METHODS

Media Modification

Modified Skirrow's, modified Campy BAP and modified Preston's media were prepared according to the methods described in the Appendix (I, II, III). The modification of the selective medium of Skirrow's (1977) involves the use of Difco Columbia blood agar base No. 2 and sheep blood instead of Oxoid blood agar base No.2 and defibrinated horse blood (George et al, 1978). Campy BAP medium was modified by the addition of cephalothin and amphotericin B, as recommended by Blaser et al (1978). The third medium is similar to the modified Skirrow's medium, but includes rifampicin and actidione rather than the vancomycin recommended by Bolton and Robertson (1982). Table I shows the quantities of the different antibiotics added to each medium.

Specimens

Rectal and faecal samples from ten cattle and ten sheep from which Campylobacter species had previously been isolated were re-examined by direct culture. The specimens were kept at 4°C for 48 hours between these two bacteriological examinations.

Each sample was inoculated onto three plates of each selective medium and incubated at 42°C for 48 hours under microaerophilic conditions.

RESULTS

Table II shows the comparative rate of isolation of Campylobacter with these three media and the rate of growth of other organisms. Modified Preston's medium was more successful than modified Skirrow's and modified Campy-BAP medium for isolating Campylobacter jejuni/coli from both the cattle and sheep rectal and faecal samples and showed the least growth of other organisms.

Table I : **COMPARISON OF VARIOUS QUANTITIES OF DIFFERENT ANTIBIOTICS ADDED IN EACH MEDIUM**

		Modified Skirrow's Medium*	Modified Campy-BAP Medium**	Modified Preston's Medium#
1.	Vancomycin	10 mg/l	1 mg/dl	
2.	Polymyocin B	2.5 IU/ml	2.5 IU/ml	10 IU/ml
3.	Trimethoprin	5 mg/l	0.5 mg/dl	10 µg/ml
4.	Amphotericin B		2 µg/ml	
5.	Rifampicin			10 mg/ml
6.	Actidione			100 µg/ml
7.	Cephalothin		7.5 mg/ml	

* Skirrow, 1977
** Blaser et al, 1978
\# Bolton and Robertson, 1982

Table II : **COMPARISON OF THREE MEDIA FOR THE ISOLATION OF CAMPYLOBACTER FROM 20 KNOWN POSITIVE RECTAL AND FAECAL SAMPLES FROM CATTLE AND SHEEP**

Direct Plating	Trial No.	Growth of Campylobacter						Growth of other organisms					
		xxx	xx	x	0	% of Isolation for individual trial	Average % of isolation	xxx	xx	x	0	% of isolation for individual trial	Average % of isolation
Modified Skirrow's Medium	1	4	2	3	11	45		4	10	3	3	85	
	2	2	5	6	7	65	55	5	8	3	4	80	85
	3	3	4	4	9	55		9	4	5	2	90	
Modified Campy-BAP Medium	1	2	4	8	6	70		3	5	6	6	70	
	2	3	5	7	5	75	71.7	1	4	9	6	70	63
	3	4	6	4	6	70		4	7	3	10	50	
Modified Preston's Medium	1	6	3	9	2	90		2	3	7	8	60	
	2	5	6	5	4	80	85	0	6	4	10	50	53
	3	4	3	10	3	85		1	3	5	10	45	

xxx = Growth over the whole of the inoculated area
xx = Scattered growth all over the plate
x = Countable number of colonies present
0 = No growth

Figure 4.1 is a bar graph showing the percentage isolation rate of Campylobacter jejuni/coli for the individual trials carried out. the modified Preston's medium isolated C. jejuni/coli from 17/20 (85%), modified Campy BAP medium isolated 14/20 (71.7%) while modified Skirrow's medium isolated 11/20 (55%) of samples. Modified Preston's medium also produced heavier growth of the organisms and they produced more distinctive colony characteristics.

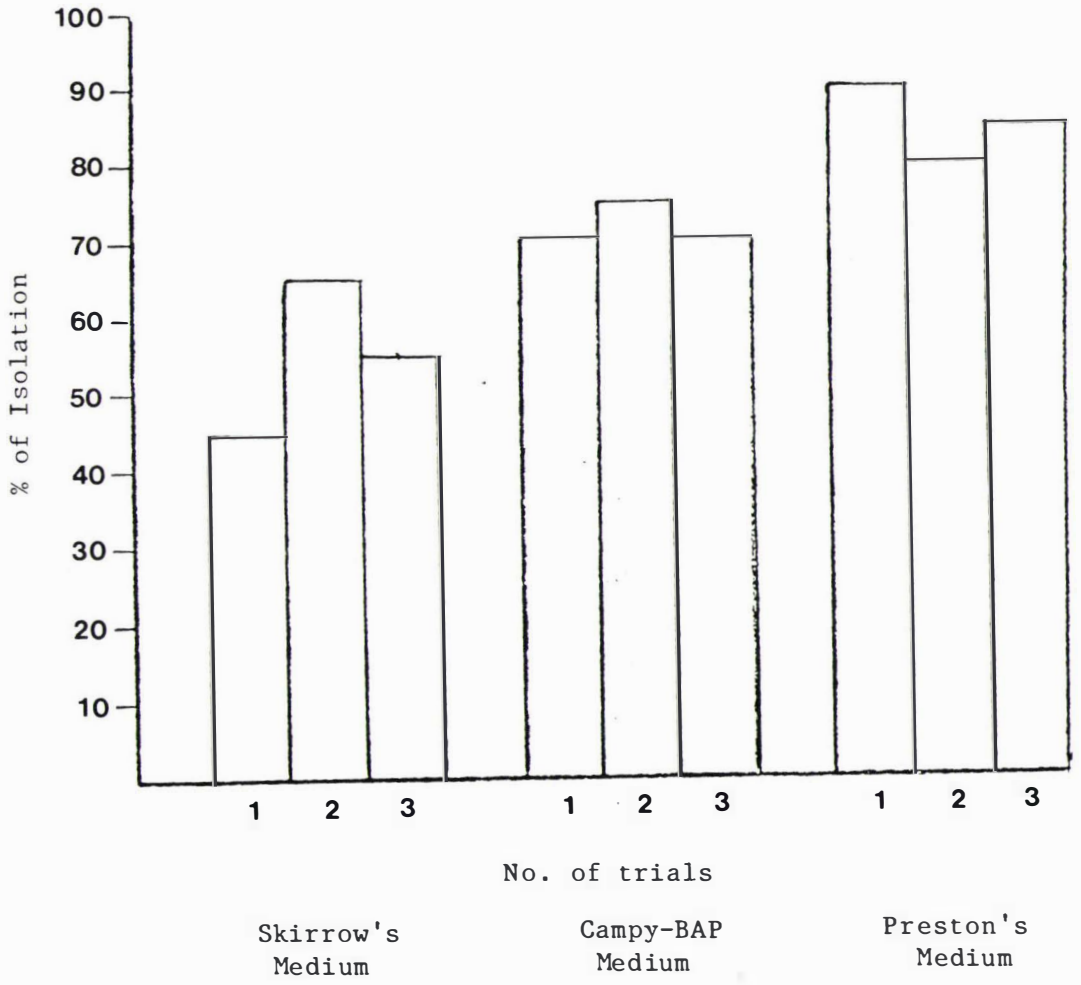
Organisms other than campylobacter were found from 85% of cultures on modified Skirrow's medium in comparison to 63% and 53% of cultures on modified Campy BAP and modified Preston's medium respectively. The non campylobacter organisms most frequently encountered on modified Skirrow's medium were Pseudomonas spp coliforms, fungi and spreading Bacillus spp and Streptococci were the most common on the modified Preston's medium.

DISCUSSION AND CONCLUSION

Skirrow's medium and Campy BAP medium have proven successful for the isolation of campylobacter from human faeces (Skirrow, 1977; Blaser et al, 1978) but in these experiments with animals, a number of contaminants were encountered, thus indicating the need for a more selective medium. Bacillus spp frequently isolated from cattle and sheep faecal and rectal samples produced a spreading growth over the surface of the modified Skirrow's medium thereby obscuring the campylobacter colonies. The most immediate obvious difference between the campylobacter colonies and those of the spreading Bacillus spp was their odour. Bacillus spp and yeasts were a greater problem in specimens taken during the winter when the animals were fed on hay and silage. The addition of amphotericin B and cephalothin to the modified Campy BAP medium to some extent prevented the growth of the yeasts and Pseudomonas spp.

From this study it is evident that the modified Preston's medium is more selective than the modified Skirrow's and modified Campy BAP media and is more successful for the isolation of C. jejuni and C. coli from faecal and rectal samples from cattle and sheep. Modified Preston's medium eliminated many of the

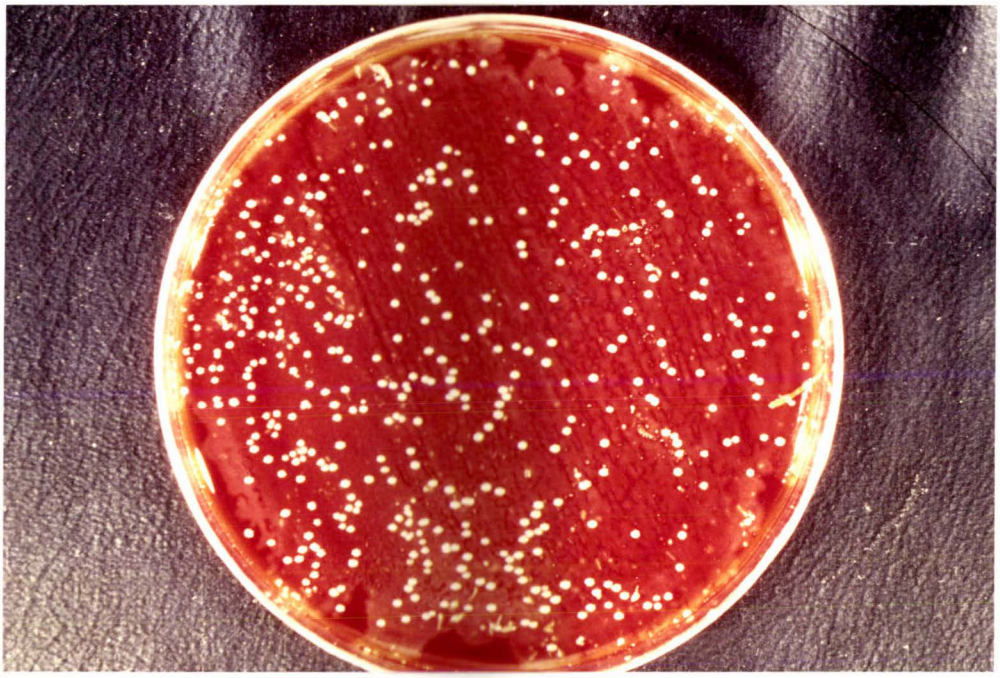
Figure 4.1 : PERCENTAGE ISOLATION RATE OF CAMPYLOBACTER FROM INDIVIDUAL TRIALS CARRIED OUT COMPARING THE SELECTIVITY OF THREE MEDIA



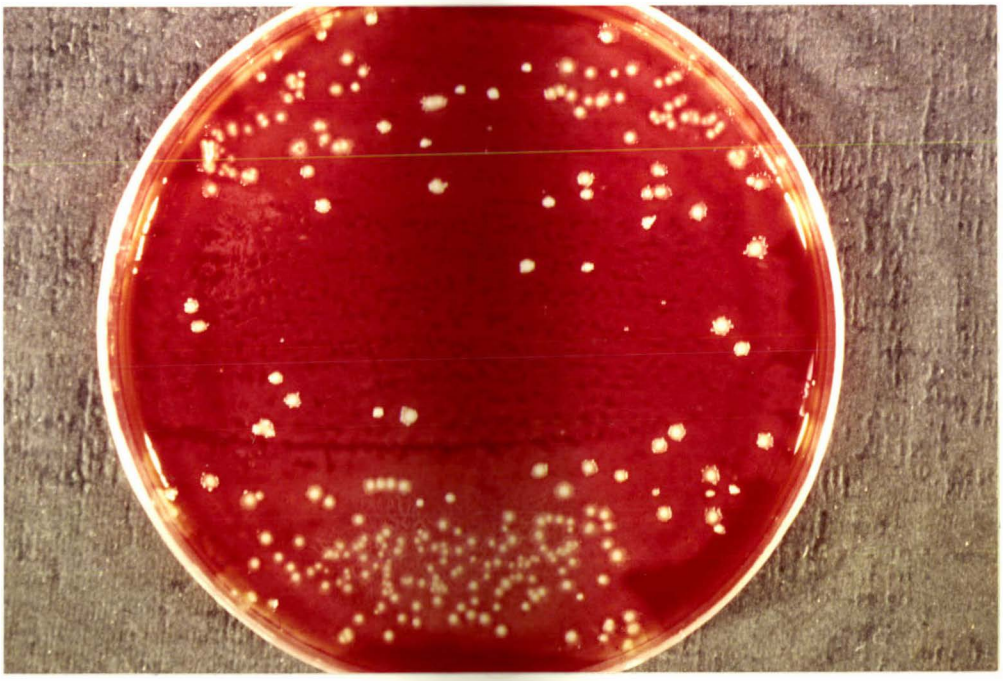
contaminating organisms which can mask the presence of *C. jejuni/coli* but it is not totally selective because some *Pseudomonas* spp, coliforms and faecal streptococci were still found to be able to grow. Figures 4.2 and 4.3 show a comparison of the selectivity of the three media for the isolation of *Campylobacter* species from cattle and sheep respectively.

It was therefore decided to use modified Preston's medium for the epidemiological studies.

Figure 4.2 : A COMPARISON OF THE SELECTIVITY OF THREE MEDIA FOR THE ISOLATION OF CAMPYLOBACTER SPECIES FROM CATTLE RECTAL SWABS

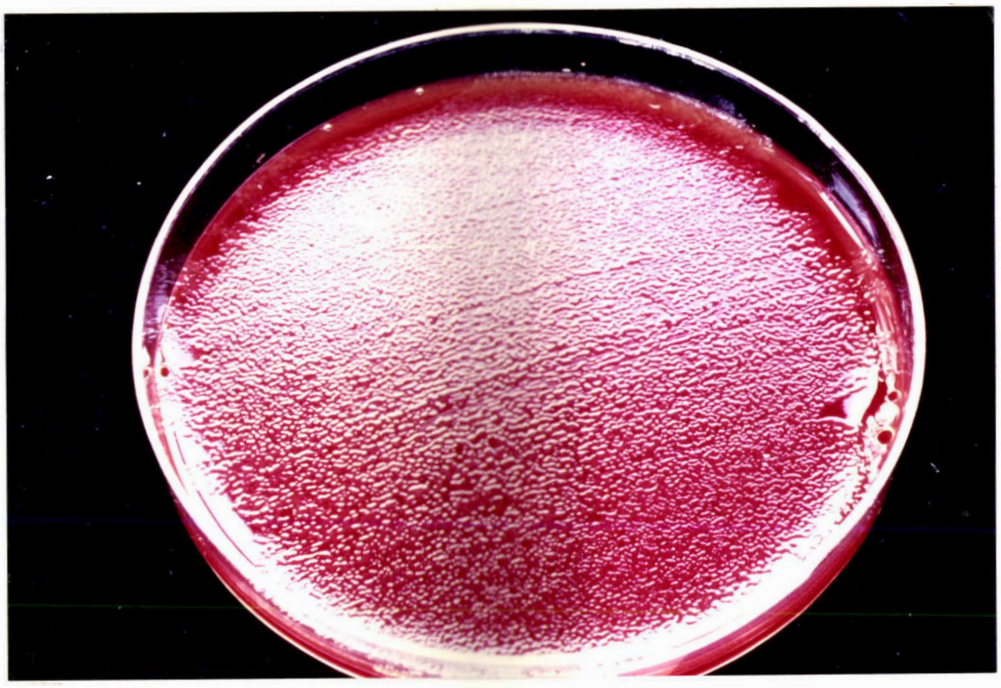


MODIFIED SKIRROW'S MEDIUM



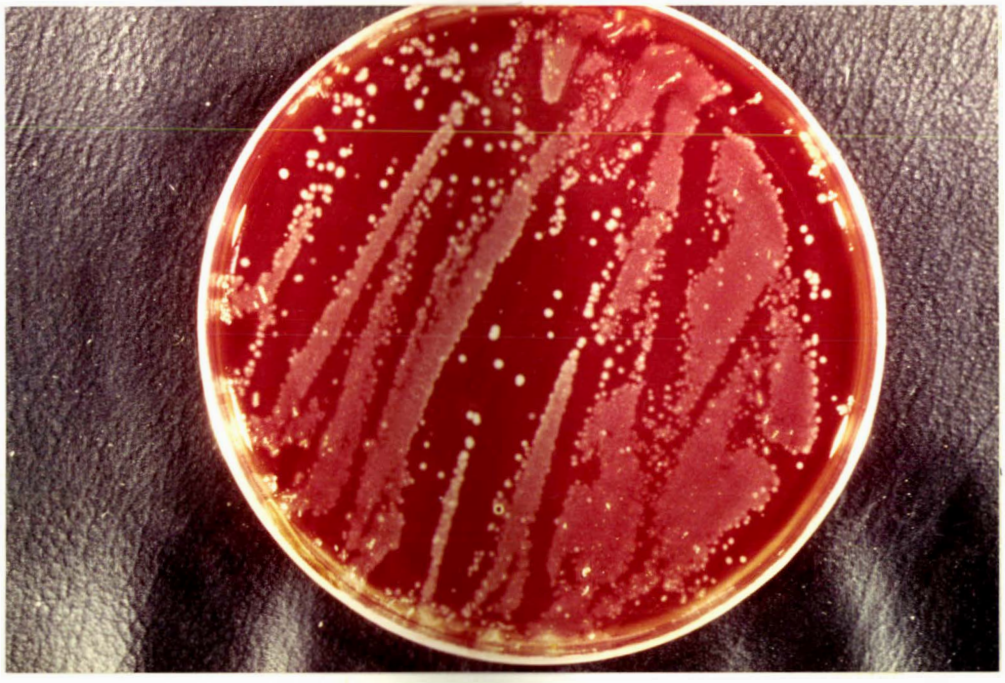
MODIFIED CAMPY BAP

Figure 4.2 (Continued)



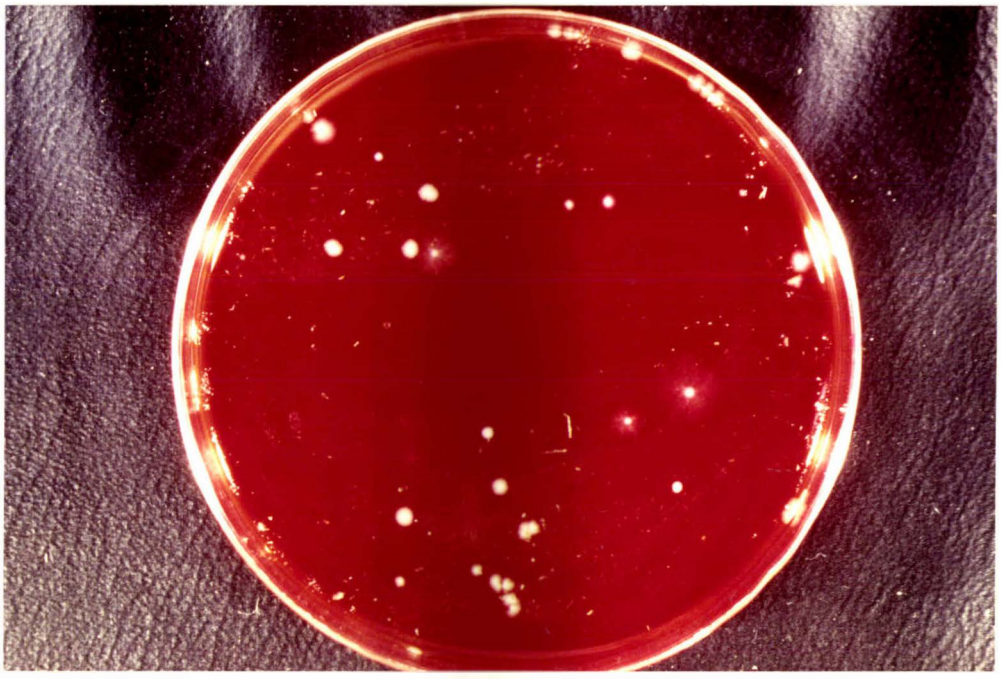
MODIFIED PRESTON'S MEDIUM

Figure 4.3 : A COMPARISON OF THE SELECTIVITY OF THREE MEDIA FOR THE ISOLATION OF CAMPYLOBACTER SPECIES FROM SHEEP RECTAL SWABS

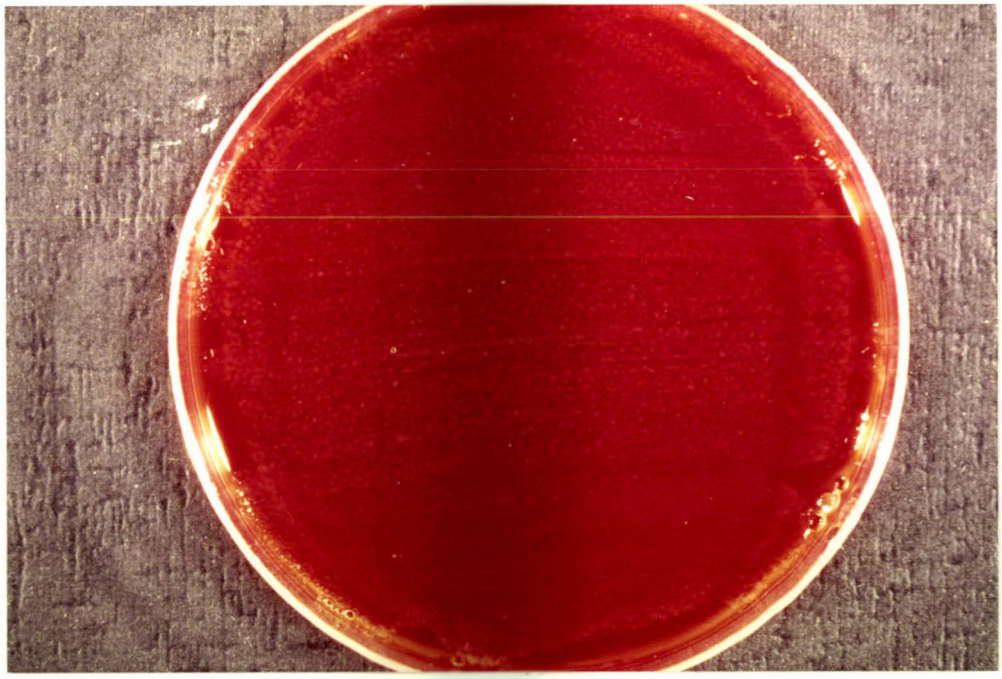


MODIFIED SKIRROW'S MEDIUM

Figure 4.3 (Continued)



MODIFIED CAMPY BAP



MODIFIED PRESTON'S MEDIUM

CHAPTER V

SEASONAL VARIATION IN THE ISOLATION OF CAMPYLOBACTER JEJUNI/COLI FROM DAIRY COWS AND A COMPARISON BETWEEN BRENDA PATTERNS OF ISOLATES FROM COWS AND SHEEP

INTRODUCTION

Epidemiological data have provided strong evidence that animals and food products of animal origin are the main reservoirs for human campylobacter infections (Skirrow and Benjamin, 1982; Newell, 1982; Kist, 1982). Campylobacter enteritis is therefore considered to constitute a zoonosis of major public health concern and indeed, has been shown to be a greater problem than Salmonellosis in several countries (Svedhem and Kaijser, 1980; Skirrow and Benjamin, 1982; Newell, 1982; Blaser et al, 1983a). Among domestic livestock, high faecal carriage rates of Campylobacter jejuni and Campylobacter coli have been reported in poultry, pigs, cattle and sheep (Butzler and Skirrow, 1979; Lior et al, 1982; Luechtefeld and Wang, 1982; Prescott and Munroe, 1982). Eviscerated carcases at slaughter are frequently contaminated (Lior et al, 1982). However, Stern (1981) in U.S.A., Gill and Harris (1982) in New Zealand and Oosterom and Becker (1982) in the Netherlands were unable to isolate any thermophilic campylobacter from 31, 65 and 200 cattle at abattoirs, respectively. To date little work has been done to determine the occurrence of any seasonal variation in the prevalence of thermophilic campylobacter in dairy cows.

Raw and improperly pasteurized milk from cows has been incriminated as the infection bearing vehicle in several outbreaks of Campylobacteriosis (Robinson and Jones, 1981; Skirrow and Benjamin, 1982). Since cows may be intestinal carriers of these bacteria, the faecal contamination of milk represents a potential route leading to human infection. Carriage rates ranging from 0 to 100% have been reported in dairy cows (Doyle and Roman, 1982; Prescott and Munroe, 1982). In Britain, Robinson (1982) found a marked seasonal variation in faecal carriage among lactating cows. Campylobacter jejuni could be isolated from 10% of each herd in the

summer, declining to 0% during the winter and re-emerging in the spring. A similar pattern of infection seems to occur in humans (Brieseman, 1985).

The biotyping scheme of Skirrow and Benjamin (1980b) provides a method for differentiating between C. jejuni, C. coli and C. laridis. It also allows for the subdivision of C. jejuni into biotypes I and II. Weaver et al (1982) divided the thermophilic campylobacter into eight types. Biotypes 1 to 4 represent the C. jejuni biotypes I and II of Skirrow and Benjamin (1980b) and biotypes 5 to 8 are subdivisions of C. coli. Lior et al (1982b) developed a slide agglutination test for heat labile antigens which can differentiate 53 serogroups and serotypes, but it is still incapable of typing approximately 7.9% of isolates examined from humans, animal, and environmental sources (Patton et al, 1985). The serotyping system of Penner and Hennesy (1980) uses a passive haemagglutination technique based on the extraction of soluble heat stable antigens, and at present recognises 59 different serotypes (42 C. jejuni and 17 C. coli) (Penner et al, 1983), but it is still incapable of typing approximately 3.9% of isolates examined from human, animal and environmental sources (Patton et al, 1985). Both Lior's and Penner and Hennesy's systems are dependent on the hippurate hydrolysis test (Skirrow and Benjamin, 1980b) to predetermine isolates as being either C. jejuni or C. coli. Lauwers et al (1981) scheme was based on O serotyping by passive haemagglutination. Although these systems provide a basis for classification, they are unable to differentiate between all strain differences of a genotypic nature.

Bacterial restriction endonuclease DNA analysis (BRENDA) has been shown to be a most useful technique for the subspecies typing of a broad spectrum of different organisms, including Leptospira interrogans (Marshall et al, 1981; Robinson et al, 1982), Vibrio cholerae (Kaper et al, 1982), Rickettsia prowazeki (Rignery et al, 1983), Neisseria meningitidis (Bjorvtn et al, 1984) and Morexella bovis (Marshall et al, 1984). One of the advantages of the BRENDA technique is that a DNA fingeprint of any isolate can be obtained and therefore it is reasonable to expect that the total number of types will be more than the number created by a serotyping system.

Also it is unlikely that any routinely used system of subspecies identification will be as sensitive as BRENDA typing (Kakoyiannis, 1984). Thus it was decided to differentiate all the isolates for the work carried out in this thesis by this technique. Each different BRENDA pattern obtained was assigned a number which was used as an identification number for each different strain.

Information was lacking on the seasonal prevalence of intestinal campylobacter in dairy cows in New Zealand and the cross infectivity between different subspecies carried by sheep and cows and the year round variation in the subspecies of campylobacter carried by animals kept on the same paddock. There is also little information on sheep and dairy cows as sources of campylobacter infection for humans. The objectives for the work outlined in this Chapter are as follows:

1. To determine the seasonal prevalence of Campylobacter jejuni/coli in dairy cows.

2. BRENDA typing of intestinal thermophilic Campylobacter jejuni/coli received from sheep and dairy cows to determine any possible relationmship between isolates from sheep and dairy cattle.

3. To determine the variation in the DNA fingerprints of C. jejuni/coli isolates from sheep and dairy cows from the same animal, recovered during three different occasions in the year.

MATERIALS AND METHODS

The animals observed in this study were the dairy cows of the Massey No. 1 Dairy Farm and sheep on the Terrace Farm (the Sheep and Beef Cattle Research Unit, No. 1 Sheep Unit).

The Farm Property

Massey No. 1 Dairy Farm

The farm is located on river flats in a bend of the Manawatu River occupying 117 hectares at a height of 35 m above sea level.

The field along the length of the river boundary has a mixed vegetation of rank weeds, fern and willow trees. There are also several pine plantations on the farm. These areas of plantations and wasteland support small populations of possums (Trichsurus vulpecula), black and brown rats (Rattus rattus and Rattus norvegicus), mice (Mus musculus), hedgehogs (Erinaceus europaeus) and rabbits (Oryctolagus caniculus), mustelids hares (Lepus timidus) and stoat (Mustela erminea) are probably also present from time to time. In addition, there is a small population of feral cats (Felis catis) living in the barns and out buildings.

A large piggery is located in the centre of the dairy farm and though it runs as a separate unit, there is contact between pigs and cattle through a single fence, and some drainage from the pig farm runs onto dairy pastures. There are also two large paddocks at the western end of the farm on which sheep and occasionally steers are grazed. Some dogs are occasionally housed in large concrete kennels in one area. There is no contact with any other livestock and the farm is entirely bounded by the river, highways or cropland. All the waterways flowing through or beside the farm have no stock pastured near them for at least two kilometres upstream of the farm. Effluent from both the piggery and the dairy shed is discharged directly into the Palmerston North Municipal sewer.

The Herd

The herd consists of approximately 250 Friesian cows of which 180 are lactating at any one time. The herd is run as a commercial unit for town supply. All animals are run at pasture on the main farm throughout the year and calves are born out of doors. Some groups of younger animals are sent to other properties when the farm

manager considers that the pastures cannot cope with feeding all age groups.

The Sheep and Cattle Research Unit

The unit is located adjacent to the Massey University No. 1 Dairy Farm. The vegetation is similar to that described as occurring on the dairy farm and supports a similar type of small animal population. Although the sheep and cattle Research Unit runs as a separate unit, there is contact between dairy cows from No. 1 Dairy Farm through a single fence, and at times the dairy cows are allowed to graze on the same pasture. The deer farm is also situated on the same unit. There is no contact with any other livestock and the unit is entirely bounded by fences.

The Sheep Flock

The flock consists of 2,460 ewes and is run as a research unit. All animals are run at pasture throughout the year.

Sampling Procedure

On each occasion, rectal swabs were collected at random from an average of 90 dairy cows and 100 sheep. Rectal swabs from dairy cows were collected on four occasions at an interval of 2-3 days during three seasons of the year - summer, autumn and winter. The samples for summer were collected during the last weeks of December 1985 and the first week of January 1986. The autumn samples were collected during the last week of March and the first week of April and the winter samples were collected during the last week of July and the first week of August 1985.

The rectal swabs from sheep were collected over two to four consecutive days (50 at a time) on three occasions during the year - April, July and December.

RESULTS

The Seasonal Prevalence of Campylobacter jejuni/coli in Dairy Cows

The seasonal prevalence of Campylobacter jejuni/coli in dairy cows in this survey is indicated in Table I. A total of 273 rectal swabs were cultured for Campylobacter jejuni/coli. The survey showed that 17/72 (23.6%), 33/106 (31.13%) and 11/95 (11.57%) were campylobacter positive during summer, autumn and winter respectively. From the total of 17 Campylobacter isolates recovered during the summer survey, 6 (35%) were C. jejuni and 10 (59%) were C. coli. One isolate which had been identified as a Campylobacter was lost before the species had been established. From the total of 33 Campylobacter isolated during autumn 19 (58%) were C. jejuni and 14 (42%) were C. coli. The prevalence rate was lowest during winter. A total of 11 campylobacter were isolated, 7 (64%) were C. jejuni and 4 (36%) were C. coli. No other species of campylobacter were identified.

Variations in the BRENDA patterns of C. jejuni/coli isolates from dairy cows and sheep from the same paddocks recovered on different occasions

Forty nine isolates of thermophilic campylobacter from dairy cows (32 C. jejuni and 17 C. coli) and 27 isolates from sheep (2 C. jejuni and 25 C. coli) were examined by bacterial restriction endonuclease DNA analysis (BRENDA) with HSU II. Two isolates from dairy cows and sheep kept in the same enviroinment had identical patterns. Different colonies isolated from the same animal on each occasion gave the same pattern. Altogether, 21 patterns of Campylobacter jejuni and C. coli were identified. Of these, there were 10 different patterns of C. jejuni and 11 of C. coli. These findings are shown in Table II and illustrated in Figures 5.1 and 5.2.

Dairy cow isolates

Seventeen different BRENDA patterns (9 C. jejuni and 8 C. coli were obtained from the 32 isolates of C. jejuni and 17 of C. coli examined.

Table I : **SEASONAL PREVALENCE OF CAMPYLOBACTER JEJUNI/COLI IN DAIRY COWS ON MASSEY NO. 1 DAIRY FARM (1985-86)**

Seasons	No. Examined	No. Positive	Prevalence C. Jejuni	Prevalence C. coli	Overall Prevalence Rate
Summer	72	17	6/72 (8.33%) 6/17 (35%)	10/72 (13.88%) 10/17 (59%)	17/72 (23.6%)
Autumn	106	33	19/106 (17.92%) 19/33 (58%)	14/106 (13.2%) 14/33 (42%)	33/106 (31.13%)
Winter	95	11	7/95 (7.36%) 7/11 (64%)	4/95 (4.2%) 4/11 (36%)	11/95 (11.57%)

Table II : **DIFFERENT BRENDA PATTERNS PRESENT ON THREE DIFFERENT OCCASIONS OF THE YEAR IN DAIRY COWS AND SHEEP**

		Different BRENDA Patterns	
		Dairy Cows	Sheep
April	C. jejuni	1, 2, 4, 6, 12, 15	14
	C. coli	3, 7, 8, 9, 11	3, 10, 13
August	C. jejuni	4	4
	C. coli	9	10, 13
December	C. jejuni	1, 24, 28, 30	
	C. coli	3, 29, 31, 32	27

The nine BRENDA patterns of C. jejuni differentiated were identified as numbers 1, 2, 4, 6, 12, 15, 24, 28 and 30 and those of C. coli as 3, 7, 8, 9, 11, 29, 31 and 32. Patterns 1, 2, 4, 6, 12 and 15 of C. jejuni and patterns 3, 7, 8, 9 and 11 of C. coli were found present during the autumn and patterns 4 of C. jejuni and No. 9 of C. coli were found present during the winter sampling. During summer sampling patterns 1, 24, 28 and 30 of C. jejuni and patterns 3, 29, 31 and 32 of C. coli were present.

Pattern 4 of C. jejuni was found present at both the autumn and winter sampling, whereas pattern 1 was found present at the autumn and summer sampling.

Sheep isolates

Of six different BRENDA patterns produced by sheep isolates, there were two different BRENDA patterns of C. jejuni and four of C. coli. These were obtained from the 27 isolates of C. jejuni/ C. coli examined. The two BRENDA patterns of C. jejuni which were differentiated, were 4 and 14 and those of C. coli were 3, 10, 13 and 27. At the April sampling, pattern 14 of C. jejuni was present and during the August sampling pattern 4 of C. jejuni was present. No common patterns of C. jejuni were found to be present at the three different samplings. Patterns of 3, 10, and 13 of C. coli were present during April sampling and patterns 10 and 13 were present during the August sampling. Patterns 10 and 13 were therefore the two common patterns of C. coli found during both samplings. From the samples taken during December only isolates of C. coli were obtained and all had a common BRENDA pattern (27). No common patterns of C. coli were seen present during the summer samplings. An isolate giving pattern 27 had not previously been recovered from either the sheep or the cattle. Table II shows the different BRENDA patterns present in sheep and dairy cows at the three samplings during the year.

DISCUSSION

The prevalence rate of campylobacter infection in dairy cattle has been reported from England to be high in the summer and low in the winter (Robinson, 1982). In the present study, the prevalence rate was 23.6%, 31.3% and 11.57% in summer, autumn and winter respectively. These figures do not agree with the English report. This may be because the New Zealand autumn is longer and milder than an English autumn and more like an English summer. The organisms may be able to thrive better in this moderate weather.

Studies in man in England, Belgium, the U.S. and South Africa have shown a summertime peak of Campylobacter infection (Blaser et al, 1983a; Butzler and Skirrow, 1979; Mauff and Chapman, 1981). In Zaire where mean temperatures are constant throughout the year, isolation of C. jejuni from patients with diarrhoea was much more frequent in the wet than in the dry season (Bokkenheuser, 1970). In New Zealand, Brieseman (1985) reported the prevalence rate of campylobacter infection to be highest in summer and lowest in winter. The autumn prevalence rate reported was only a little lower than that of summer rate. The present finding in dairy cows does not exactly correspond to that of humans (Brieseman, 1985) but seems to have a close relation since there is little variation between the prevalence rate in human and dairy cows in autumn. The prevalence rate for both humans (Brieseman, 1985) and dairy cows is lowest in winter.

Many studies reviewed elsewhere (Prescott and Munroe, 1982) have recorded the common occurrence of C. jejuni or C. coli in the intestinal tracts of the domesticated animals and in wild animals and birds (Luechtefeld et al, 1980, 1981). Besides being present in clinically diseased animals, Campylobacter jejuni is often part of the normal intestinal flora of cattle (Bryner, 1964; Smibert, 1978) and sheep (Smibert, 1965). In this study, 49 isolates of Campylobacter from clinically normal dairy cows (32 C. jejuni and 17 C. coli and 27 isolates from sheep (2 C. jejuni and 25 C. coli produced 21 BRENDA patterns (10 BRENDA patterns of C. jejuni and 11 of C. coli). Persistence of C. jejuni and C. coli with the same BRENDA pattern in dairy cows and sheep sampled on two different

occasions of the year (Table IV and Figures 5.1 and 5.2) indicates the ability of the same strain of organisms to thrive for some months irrespective of the environmental conditions. The stability of the BRENDA patterns in this field study for a period of months and probably for much longer is in agreement with Kakoyiannis (1984) who, after repeated subculturing of campylobacter in the laboratory, found the BRENDA patterns to remain stable. Stability is further substantiated by the finding in sheep that C. jejuni and C. coli isolated from different parts of the intestinal tract of the same animal yielded identical BRENDA patterns. The finding that isolates with the same BRENDA patterns were recovered 2-3 months apart is in agreement with the report of Robinson (1982). No evidence was found to substantiate Robinson's (1982) suggestion that colonisation may occur for life.

Two of the BRENDA patterns from sheep resembled the BRENDA patterns from the 49 isolates from dairy cows (Table III and Figure 5.1). Both the animals in question were grazed at one time or another on the same paddock and this might explain the colonisation of both cattle and sheep with the same strains. It also demonstrates the ability of the cross infectivity for at least some strains between these two species of animals.

CONCLUSION

1. The prevalence rate of campylobacter infection in cows is highest in the warmer months of the year.

2. The prevalence rate in dairy cows appears to be in proportion to that in humans and thus the seasonality of campylobacter infection in humans may relate to the frequency of contamination of the animal products used for human food.

3. The same strain of C. jejuni and C. coli can be found in the same animal on three different occasions.

4. There is strong evidence that there is cross infectivity of C. jejuni and C. coli strains between dairy cows and sheep.

5. The BRENDA patterns produced by isolates from dairy cows and sheep appeared to remain stable throughout the study period.

Table III : **COMMON AND DIFFERENT BRENDA PATTERNS IN COWS AND SHEEP**

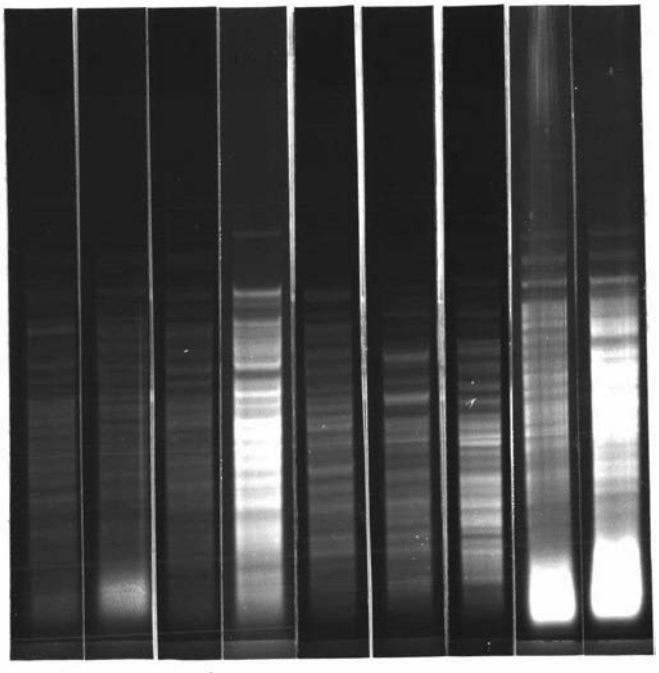
	C. jejuni
Cows	1, 2, 4, 6, 12, 15, 24, 28, 30
Sheep	4, 14
	C. coli
Cows	3, 7, 8, 9, 11, 29, 31, 32
Sheep	3, 10, 13, 27

Table IV : **BRENDA PATTERNS FOUND ON MORE THAN ONE OCCASION**

	Dairy Cows		Sheep	
	C. jejuni	C. coli	C. jejuni	C. coli
April	1, 4	3, 9		10, 13
August	4	9		10, 13
December	1	3		

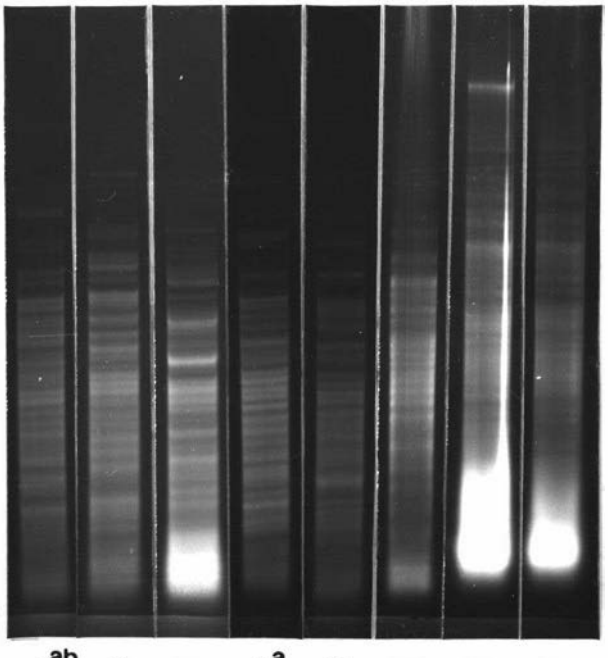
Figure 5.1 : DNA FINGERPRINTS PRODUCED BY THE ISOLATES OF C. JEJUNI/COLI RECOVERED FROM DAIRY COWS

C. jejuni



1[a] 2 4[ab] 6 12 15 24 28 30

C. coli

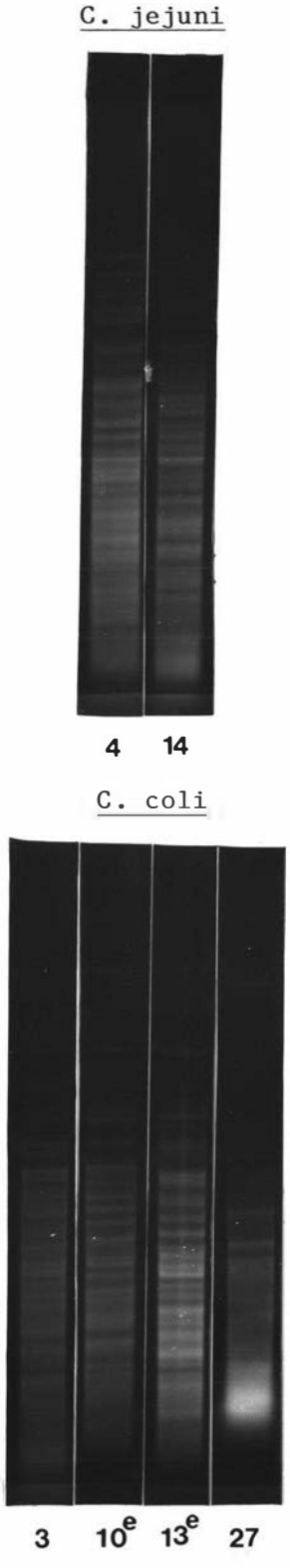


3[ab] 7 8 9[a] 11 29 31 32

b DNA fingerprints common to both dairy cows and sheep.

a DNA fingerprints found on more than one occasion in dairy cows.

Figure 5.2 : DNA FINGERPRINTS PRODUCED BY THE ISOLATES OF C. JEJUNI/COLI RECOVERED FROM SHEEP



e DNA fingerprints found on more than one occasion in sheep.

CHAPTER VI

EPIDEMIOLOGY OF CAMPYLOBACTER IN LABORATORY ANIMALS

INTRODUCTION

In recent years there has been increasing recognition of the role of C. jejuni (Veron and Chatelain, 1973) as a cause of enteritis in man (see Chapter II). An understanding of pathogenesis of C. jejuni infection and its natural history is extremely important and could help lead to the control of this disease. Because there is no suitable animal model, little is known about the pathogenesis. Only a few animals have been found susceptible to experimental infection, but even here success has been reported by only a few workers, others have failed to confirm their findings (Butzler and Skirrow, 1979). Monkeys (Boneyk et al, 1972), puppies (Prescott and Karmali, 1978), kittens (Prescott and Karmali, 1978), gnotobiotic puppies (Madge, 1980), mice (Field et al, 1981), and chickens (Butzler and Skirrow, 1979) have been tested without uniform result, but the most reliable experiment have been those using newborn animals (Prescott and Barker, 1980; Madge, 1980; Field et al, 1981; Ruiz-Palacios et al, 1981; Prescott et al, 1982; Soerjadi et al, 1982) or adult rabbits (Caldwell et al, 1983). But before attempting to determine the ability of a species of animal to act as a model, it is necessary to discover if thermophilic Campylobacter spp are normally present in the intestinal flora of these animals. There appear to be a number of animal reservoirs of these organisms since it has been isolated from faeces and/or carcases of chickens, cattle, and from faeces of cats, dogs, wild ducks and other animals (see Chapter II). Puppies and kittens with diarrhoea have been linked to human diseases (Blaser et al, 1978; Hay and Ganguli, 1980; McKinley et al, 1980; Skirrow et al, 1980). Also the epidemiology of diseases involving campylobacter has not been adequately determined and the role of rodents as a source of infection has not been assessed.

The purpose of the present investigation was to:

1. Determine the occurrence of thermophilic campylobacters among rats, mice, guinea pigs, rabbits and cats from the Small Animal Production Unit, Massey University.

2. Differentiate isolates to a species level by the biochemical reactions and to a subspecies level by bacterial restriction endonuclease DNA analysis (BRENDA) technique (see Chapter V) in order to recognise epidemiologically related strains.

MATERIALS AND METHODS

Small Animal Production Unit

The animals observed in this study were part of the Small Animal Production Unit of Massey University. Mainly four types of laboratory animals are bred and maintained in the unit:

Rats (Rattus norvegicus) strain type; Sprague Dawley
Mice (Mus musculus) strain type; Black - C57
Brown - C3H, CHI, CBA
White - Massey strain
Guinea pig (Cavia parcellus) strain type; Duncan Hartley
Rabbits (Oryctolagus cuniculus) strain type; N.Z. White.

A closed colony of about 60 cats (Felis catus) has also been maintained within the unit for the last 7 years.

The rat colony was started over 20 years ago with 20 pairs of Sprague Dawley rats which were obtained for breeding purposes from the U.S.A. From time to time rats are received from the Dairy Research Institute (DRI) but they are kept separated so as to maintain the original colony as a closed colony. The mice were originally obtained from Sydney and Britain and except for C3H strain, the mice colony has been closed since the setting up of the Unit. The brown mice strain C3H were obtained from the Veterinary Pathology Department two years ago. Hybrid strains of mice are

being produced in the unit for research purposes. The original rabbits were obtained from a private breeder, Palmerston North, three years ago and they came from Britain and U.S.A. The guinea pigs were obtained from the Animal Research Centre, Wallaceville, 10 years ago.

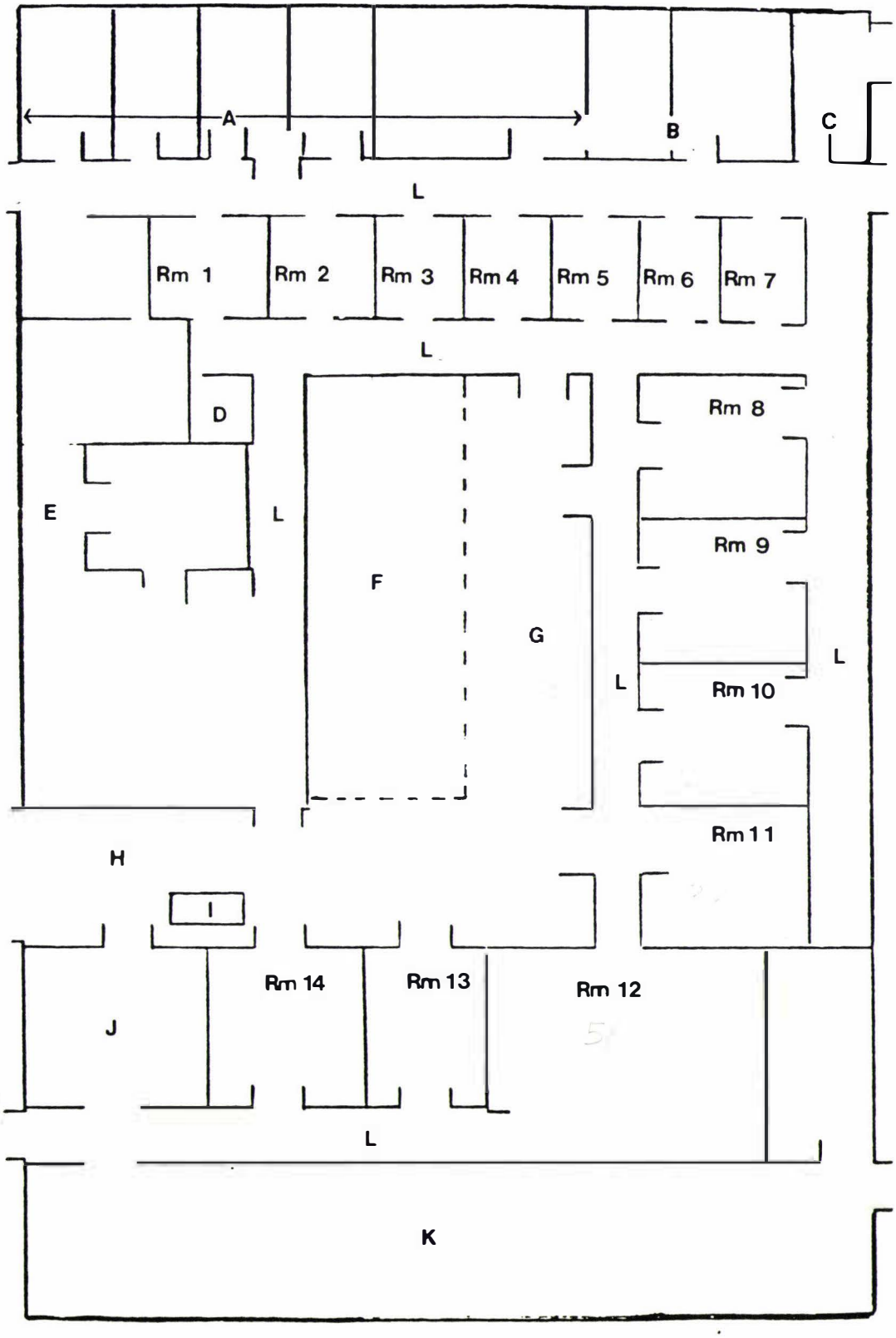
A closed colony of all the animals is maintained from the time they are received, not allowing mixing with any other strains. The total number of each species of animal within the unit varies depending on the time of the year and the demand. No fixed number of animals are maintained at any one time of the year. The description given in this chapter applies to the Unit at the time of the study only.

The laboratory animals are mainly used for teaching, research and antiserum production purposes by various University departments, research institutes such as Ministry of Agriculture and Fisheries (MAF) and Dairy Research Institute (DRI), colleges, schools and other universities around New Zealand. Surplus rats are killed and frozen and sold to schools for dissection purposes. Most of the rabbits used for antiserum production are housed in the Unit.

The unit is located as a separate building on the Massey University campus on University Avenue. Originally the building was planned as a veterinary clinic and later turned into the Small Animal Production Unit. The building is approximately square-shaped and occupies an area of 574.96 m^2.

Within the building are located offices, staff common room, a sawdust storage room, feed store, cage store rooms, a washdown area, two furnace rooms, three rat rooms, four mice rooms, five rabbit rooms, one guinea pig room and one sheep room for physiological experiments and a laboratory (Figure 6.0). The cats are maintained in an enclosed partly covered area in the courtyard. The drainage system is of an open type, discharging effluent directly into the Palmerston North Municipal sewer. Ventilation is of a semiclosed type circulating fresh air. The building is surrounded by well maintained gardens frequently manured with fowl manure.

Figure 6.0 : FLOOR PLAN OF THE SMALL ANIMAL PRODUCTION UNIT



Mice Rooms

There are four mice rooms; No. 1 (12.1 m^2), No. 2 (10.52 m^2), No. 3 (8.83 m^2) and No. 7 (8.46 m^2). Adjacent to the mice room No. 7 is a rabbit room and across the passage from mice room No. 7 is a rat room.

There are two types of polycarbonate cages* used, the small cage having a floor area of 824 cm and is 15 cm in height, the large cage has a floor area of 1815 cm x 20 cm. Five to ten young and adult mice are accommodated in each cage. The cages are provided with a stainless steel lid and are placed on wooden racks fixed to the wall (Figure 6.1). Sawdust is used as a bedding and feed is placed in a feeder in the lid. The mice are watered by means of an inclined polycarbonate and glass bottles with stainless steel sippers resting on the lid. There are about 20-30 cages per room. There is no special breeding room for mice. Each room has two doorways which are located on opposite walls and kept closed all the time.

Rat Rooms

There are three rat rooms; No. 5 (8.64 m^2), No. 8 (22.98 m^2) and No. 14 (23.62 m^2). Rat room No. 5 is situated between room No. 4 (mice) and room No. 6 (rabbits) but has no direct access to either of them. This room accommodates rats received from outside sources to ensure that none are ever mixed with rats bred in the unit. Room 8 is a large room situated next to room No. 9 which contains rabbits. Room No. 8 mainly accommodates rats just after weaning (21 days) until they are ready to be used. Room No. 14 is situated between room No. 13 housing guinea pigs and the sheep room, but there is no direct access between these three rooms. All the rooms are provided with two doorways with two wooden doors which are not able to be shut properly thus causing problems in maintaining the ideal temperature during winter and allowing access to flies in summer. Room 14 is used for the breeding and maintenance of parent stock.

* Maknolon, Germany

Figure 6.1 : INTERIOR OF MICE ROOM SHOWING THE TYPE OF MICE CAGES

Figure 6.2 : INTERIOR OF RAT ROOM SHOWING THE TYPE OF RAT CAGES

Five to eight and ten to twelve rats are accommodated respectively in small and large polycarbonate cages. The number of rats per cage are reduced as they grow. In room 14 where the breeding stock is maintained, only two rats (male and female) are accommodated per small cage. Two females and one male rat may be housed together for mating purposes. When the young ones are born, they are kept with the parents for 21 days before they are weaned. The rats in room 5 are accommodated in steel cages. There are about 20-25 rat cages per room (Figure 6.2).

Sawdust is used as bedding in the case of polycarbonate cages, but no bedding is used in steel cages as these are provided with stainless steel mesh floors through which the faecal pellets can drop onto a plate below. Feed and water are provided in much the same way as has been described for the mice.

Guinea Pig Room

Room No. 13 (18.13 m^2) accommodates the guinea pigs. This room is situated between a rat breeding room (14), and rabbit room (12). One of its doors opens onto the courtyard where a large metal bin is used to hold the unit's refuse, the other doorway opens onto a passage.

There are two stainless steel floor pens each accommodating 15 to 20 guinea pigs. The pens are provided with sawdust bedding and green hay (Figure 6.3). Feed for the guinea pigs is held in stainless steel containers placed above floor level and water is provided in a 2 l glass bottle inverted, dripping onto steel containers. The same type of pen is used for both breeding and maintenance.

Rabbit Room

Room No. 9 (23.30 m^2), No. 10 (23.09 m^2), No. 11 (23.14 m^2) and No. 12 (55.49 m^2) accommodate rabbits. Room 9 is situated adjacent to a rat room 8, but there is no direct access between these two rooms. Room 12 is situated adjacent to the guinea pig room. This room has no direct access to the outside.

Figure 6.3 : INTERIOR OF GUINEA PIG ROOM SHOWING THE TYPE OF PEN

Figure 6.4 : INTERIOR OF RABBIT ROOM SHOWING THE TYPE OF RABBIT CAGES

The rabbits are maintained individually in stainless steel cages. Rabbits in room 9 and 11 are not provided with sawdust but are kept in cages on wire gauze floors. Feed is provided in a container and they are watered by means of an inverted glass bottle (Figure 6.4).

The room next to the rat breeding room is used by the Physiology Department for experiments and at times sheep are maintained in this room. The people who work in this room also have access.

The rest of the rooms are used for management purposes and as store rooms as designated in Figure 6.0.

Cats

Cats are maintained in the courtyard in the centre of the building and are enclosed by wire fences, about one-third of this enclosure is covered by a roof (Figure 6.5). The area is divided into four sections accommodating about 15-30 cats within each section. Although cats are provided with processed cat food they can at times eat a wild bird which may inadvertently fly into a cat cage. The cats are watered by means of a trough.

MANAGEMENT OF THE UNIT

Hygiene Practice

Generally, routine cleaning procedures are carried out by two regular workers. The first worker does the cleaning of the cat enclosure and then she goes to the rat rooms and finally to the mice rooms. The second worker cleans the rabbit rooms first in the morning and then she goes to the guinea pig room. In the case of a staff member being absent, this routine may be upset.

Floors, corridors, drains and the cat enclosure are cleaned every day by means of a jet of water and are then well doused with

Figure 6.5 : TYPE OF CAT CAGES

an iodoform disinfectant and at the same time the animals are fed* and watered. Rats and mice get a complete change of both bedding and cage each week. In the case of the rabbits, the tray collecting droppings is cleaned twice a week and the cages are changed once a week. The guinea pig pens are cleaned once a week and include a complete change of bedding.

All the cages are sent once a week to the washdown area, where the cages are washed by means of a jet of water and cleaned with detergent. After washing, the cages are dipped in a strong solution of iodoform and finally stacked for drying. The steel cages used for the rabbits are cleaned in an HCL acid bath.

Health Status

Overall health status of the animals at the unit is good. No chronic problems are being experienced by any of the animal species, although at times rats are found having tumours or to be suffering from respiratory disease. The respiratory problem occurs occasionally following overcrowding and because of the poor ventilation. Some years ago there was an outbreak of snuffles among the rabbits, but it has now been controlled. No other major disease problems have been seen in the unit. A few wild rats and mice have from time to time gained entry into the unit, they have the potential to act as a source of disease.

An open garbage bin (3 m x 1.5 m) kept in the courtyard close to the cat enclosure is used for the collection of the unit's refuse and an empty bin is replaced every week. Due to rotting refuse, the bin attracts flies which can gain access to the various animal rooms and constitute a possible means of spread of infectious disease agents.

* Rats, mice, rabbit and guinea pigs - Pellet diet -317, 319 dry. Harvey Farms, Palmerston North.

SAMPLING PROCEDURES

Rats

Two hundred and sixty two (262) rats from three different rooms (5, 8 and 14) were examined by taking rectal swabs. The rats were one to 60 days of age. Of these rats, 17 were from room 5. These were part of a consignment bred at the Department of Scientific and Industrial Research (DSIR). They were surplus to their requirements and were kept isolated so as not to mix them with the other rats in the unit.

A further thirty one (31) rats from room 8 were euthenased and a cultural examination was carried out on rectal swabs taken immediately after death and before evisceration. Intestinal swabs were taken from various sites of the gastrointestinal tract immediately after evisceration of these animals. In all cases swabs were taken from the stomach, middle of the duodenum, jejunum, ileum, caecum and rectum. Cultural examination was carried out on 186 samples of intestinal swabs (31 rats x 6 sites).

Mice

Three hundred intestinal swabs were collected from 50 mice (50 mice x 6 sites) by the same procedure described for rats. The mice for sampling were selected from each mouse room.

Guinea Pigs

Twenty seven rectal swabs were collected at random from 27 guinea pigs taken from the two pens. Intestinal swabs were collected from a further 25 guinea pigs from seven sites (stomach, middle of duodenum, jejunum, ileum, caecum, rectum and gall bladder) making a total of 175 samples.

Rabbits

Ninety seven (97) rectal swabs were collected at random from rabbits held in three different rooms. An average of 30-35 rabbits per room were sampled.

Cats

Sixty rectal swabs were collected from cats selected at random from four different cat cages.

The rectal swabs, intestinal swabs and other samples collected were transported and cultured as already described in Chapter II.

Flies

Thirty house flies were captured from the small animal production unit. Of these 30 flies, 20 were captured from the corridors and inside different rooms, ten were live and ten were dead. The other ten flies were alive and captured from the courtyard and from the area around the garbage bin.

Humans

See Chapter III.

RESULTS

Rats

Thermophilic campylobacter were isolated from 59 (22.5%) of the 262 rats examined. Of 181 rats kept in room 8 in cages on the left wall, 57 (31.5%) were positive for campylobacter. Only one rat was found positive from 40 of the rats examined from those kept on the right wall of the room. No rats were found positive for campylobacter from the 17 examined out of the 60 rats in room 5. These rats were received from DSIR. Only one adult rat was found positive from the 24 breeding rats examined from the breeding room. Table I shows the isolation of campylobacter from rats according to their rooms.

Campylobacter were isolated from different parts of the intestines from nine rats of a further 31 rats examined. The site of isolation was different for individual rats, but the rectum was

Table I : **THE ISOLATION OF CAMPYLOBACTER FROM RATS ACCORDING TO THEIR ROOMS**

No. of Rats Examined	Room No.	No. +ve	*C. jejuni*	*C. coli*
221	8	58	58	-
17	5	0	-	-
24	14 (Breeding)	1	1	-

the most common site from which campylobacter were isolated. Ileum, jejunum and caecum were next most common sites of isolation. The duodenum was the least likely intestinal region to be colonised by the organisms. There were no isolates from the stomach. Table II shows the different sites of isolation of the thermophilic campylobacter from rats. All the isolates recovered from the different parts of the intestine were C. jejuni.

The overall prevalence rate of campylobacter in the rats examined from SAPU was 68 from 293 (23.2%).

Mice

Campylobacter-like organisms were isolated from 5 of 50 (10%) mice being examined. The most common site of isolation were the caecum and rectum. There was a single isolate from the ileum. No other sites of the intestinal tract showed the presence of the organisms. Morphologically, the isolates were like campylobacter, but their species could not be ascertained because they would not grow after primary isolation. Table III shows the sites of isolation of these campylobacter-like organisms from mice.

Guinea Pigs

Thermophilic campylobacter were isolated from four (7.7%) of the 27 rectal swabs and 175 intestinal swabs (25 guinea pigs x 7 sites) examined. There were no isolates from animals examined by means of rectal swabs only. Isolates were recovered from four different sites of the intestine, duodenum, jejunum, ileum and rectum. One isolate was also recovered from the gall bladder. All the isolates recovered from the guinea pigs were C. jejuni. Table IV shows the different sites of isolation of the campylobacter.

Rabbits

Campylobacter jejuni was isolated from 1 of 97 rectal swabs examined, the rabbit positive for campylobacter was from room 10.

Table II : **THE DIFFERENT SITES OF ISOLATION OF CAMPYLOBACTER JEJUNI FROM THE INTESTINAL TRACTS OF RATS**

Rat No.	Stomach	Duodenum	Jejunum	Ileum	Caecum	Rectum
RJ 207	-	-	-	+	-	+
RJ 210	-	-	-	-	+	+
RJ 213	-	+	+	+	+	+
RJ 214	-	-	-	-	+	-
RJ 217	-	-	-	-	-	+
RJ 219	-	-	-	-	-	+
RJ 220	-	+	+	+	+	+
RJ 222	-	-	+	+	-	-
RJ 226	-	-	+	-	-	-

Table III : **THE DIFFERENT SITES OF ISOLATION OF CAMPYLOBACTER-LIKE ORGANISMS FROM THE INTESTINAL TRACTS OF MICE**

Mice No.	Stomach	Duodenum	Jejunum	Ileum	Caecum	Rectum
JM 11	-	-	-	+	+	+
JM 12	-	-	-	-	+	+
JM 13	-	-	-	-	+	+
JM 14	-	-	-	-	+	+
JM 16	-	-	-	-	+	+

Table IV : **THE DIFFERENT SITES OF ISOLATION OF CAMPYLOBACTER JEJUNI FROM THE INTESTINAL TRACTS OF GUINEA PIGS**

Guinea Pig No.	Stomach	Duodenum	Jejunum	Ileum	Caecum	Rectum	Gall Bladder
JGP 11	-	-	-	+	-	-	+
JGP 14	-	-	+	-	-	-	-
JGP 15	-	-	-	-	-	+	-
JGP 18	-	+	-	-	-	-	-

Cats

Of the 60 cats examined, 31 (51.7%) were positive for C. jejuni and they were equally distributed within the three cat cages.

During identification procedures, a subculture of one of the 31 isolates exhibited resistance to nalidixic acid. After a further subculture it remained fully resistant to nalidixic acid but remained hippurate positive. Of the 31 campylobacter originally isolated, three were lost during subculture.

The overall prevalence of thermophilic campylobacter in the various species of laboratory animals is summarised in Table V.

House Flies

Of 30 house flies captured inside the production unit, a single fly was found positive for the organisms. The organism was identified as C. laridis.

Humans

No isolates were recovered.

Samples from in and around the Small Animal Production Unit

No isolates were recovered.

BRENDA Analysis

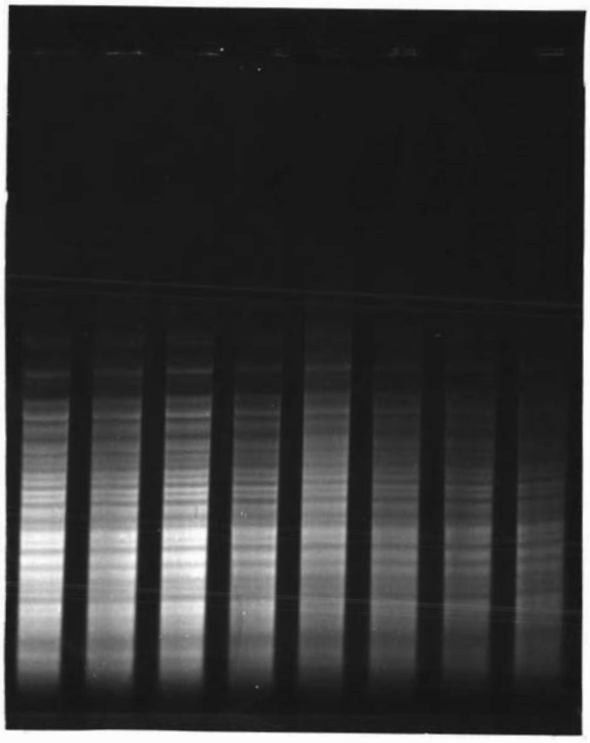
Only one (1) BRENDA pattern (pattern 25) of C. jejuni was recovered from the 84 isolates examined from rats, rabbits and guinea pigs. The isolates from different parts of the intestine in the case of rats and guinea pigs also had the same BRENDA pattern. Five different BRENDA patterns were recovered from 28 cat isolates. Figure 6.6 shows the BRENDA patterns of campylobacter isolates recovered from rats, guinea pigs, and the rabbit.

Table V : **OVERALL PREVALENCE OF CAMPYLOBACTER IN LABORATORY ANIMALS**

Species	No. Examined	No. Positive	Prevalence *C. jejuni*	Prevalence *C. coli*	CLO*	Overall Prevalence
Rats	293	68	68	-	-	23.2%
Mice	50	5	-	-	5	10.0%
Guinea Pigs	52	4	4	-	-	7.7%
Rabbits	97	1	1	-	-	1.0%
Cats	60	31	31	-	-	51.7%

* Campylobacter-like organisms

Figure 6.6 : DNA FINGERPRINTS PRODUCED BY CAMPYLOBACTER JEJUNI ISOLATES RECOVERED FROM RATS, GUINEA PIGS AND A RABBIT



25

DISCUSSION

Of a total of 39 BRENDA patterns of C. jejuni recovered from cattle, sheep, rats, guinea pigs, rabbits and cats, a single BRENDA pattern of Campylobacter jejuni was recovered from rats, guinea pigs and a rabbit within the Small Animal Production Unit (SAPU). This strongly suggests that a single strain of organism has at some time been introduced into the production unit providing a common source of infection.

Five different BRENDA patterns produced by the Campylobacter jejuni isolates recovered from cats were all different from the single pattern produced by the isolates recovered from rats, guinea pigs and the rabbit. This finding indicates that the strains of campylobacter associated with the cat colony are different and appear to be confined to the cat colony. The chances of transmission of cat organisms to other laboratory animals are considered to be small since the cats are maintained in an enclosure in the courtyard and there is no contact with the other laboratory animals. The only possible routes of transmission would be via the handlers and house flies. But since the BRENDA pattern of the isolates from rats, guinea pigs and the rabbit was dissimilar to those from the cat isolates, it is unlikely that the cats are responsible for the infection found in the other laboratory animals.

The single isolate recovered from the fly was that of C. laridis. No C. laridis isolates were recovered from any of the other animals.

The three animal species involved in this outbreak are rats, guinea pigs and rabbits. The infection could have entered the SAPU through any of these animals.

Since the prevalence rate in the rats was found to be the highest of the three species, it could perhaps be assumed that the rats were infected first and that infection in the other species was the result of cross infection from the rats.

There are reports which indicate the natural presence of campylobacter in rats. Pijtschev (1969) reported the presence of C. coli in rats and suggested that rats could be a reservoir for C. coli infection for pigs. The present finding of C. jejuni from rats agrees with the finding of Kakoyiannis (1984) who also isolated C. jejuni from a number of wild rats, thus it is equally likely that laboratory rats could be a reservoir.

The isolation rate of C. jejuni from rats in this study was highest for the rats kept on the left wall racks in room 8 (31.5%). Forty rats examined from those kept on the right wall had a much lower isolation rate (2.5%). There was a single isolation from the breeding rats, making the overall isolation rate 23.2% which is much higher than the 10.3% reported by Fernie and Park (1977).

If the rats were indeed the first to be infected, then what could be the origin of this infection? Because it was not possible to identify the parents of the infected rats, an overall survey of the breeding rats in room 14 was carried out. A single breeding female rat was found positive for C. jejuni. The BRENDA pattern of this isolate was the same as those of the rats in room 8. It therefore can be argued that the first possible source of infection was this breeding female. Her offspring could have become infected either in the uterus, or by direct contact with the dam via faecal pellets, sawdust or water contamination.

To date no intrauterine infections of C. jejuni have been reported either naturally or experimentally. Experimental intestinal colonization of neonatal rats has, however, been reported (Field et al, 1981). Cross transmission from human mothers to their children has been reported (Codranel et al, 1973) but failure to isolate C. jejuni from the vagina of 272 women by Blaser et al (1980c) suggested that it is not part of normal vaginal flora and that transmission from maternal vaginal flora to fetus is probably not a common mode of human infection. It is not known if this is also true for rats. In this study, it was considered unlikely that intrauterine transmission had occurred.

The young rats are weaned after 21 days and if the mother was carrying the organisms at the time of birth, it is possible and likely, that she could have transmitted the infection by direct contact to her young ones or indirectly through the feed, water and sawdust following contamination with faecal pellets. Because rats were pooled together at weaning, it was not possible to relate individual animals to their parents in room 14. All the rats bred during that time were mixed up by the time the investigation was carried out, thus it is difficult to say whether or not the single breeding female which was positive for C. jejuni was in fact the origin of all the infections. All or some may have acquired the infection from an external source.

Just because the rats have the highest infection, it must not automatically be assumed that they were the first species to become infected.

The organism may have entered via guinea pigs, on hay or other green food and then spread to other animals. The infection could have started in one of the guinea pigs housed in either of the two pens and then spread from animal to animal. Because the guinea pigs are kept free in the pens, transmission is possible via direct contact or through feed, water, bedding and faecal pellets. Cross transmission between infected and non infected chickens kept in the same cage has been demonstrated by Kolb and Willinger (1967), a similar mode of infection in guinea pigs is possible. Since only 7.7% of the guinea pigs were found to carry C. jejuni, either the sampling was carried out at the beginning or end of an epidemic or it is more likely that guinea pigs do not readily become infected.

Transference to the rabbit colony could have taken place via the animal handlers because the same worker that cleans the rabbit cages also looks after the guinea pigs. Because the animal handler attends to the rabbits before the guinea pigs, it seems unlikely that the infection in the rabbit would have directly come from the guinea pigs.

Humans have been found to be relatively unimportant secondary reservoirs for direct or indirect transmission (Skirrow, 1982). In

the present study, the humans were all found to be negative for the organism and hence it is unlikely that they had played any part in the transmission.

Initially, the possibility of transmission of infection from guinea pigs or the rabbit to rats, seemed to be remote since there is no contact at all between rats and the other laboratory animals. However, it may be significant that the rat room from which the highest rate of infection was detected is only one room away from the rabbit room (10) in which the single infected rabbit was housed, but the guinea pig room (8) is five rooms away (see Figure 6.0). The workers appointed to the task of cleaning the rat room (8) are different from those who clean the guinea pig and rabbit rooms. A possible transfer of infection from rabbits to rats could take place at the washdown area where all the cages are collected together for washing. There are no guinea pig cages so the possibility of transfer of infection from guinea pigs to rats is difficult to explain by means of the cages. However, it is very likely that the organisms could have been transmitted to the clean sawdust by the shovel used for cleaning soiled sawdust from the guinea pig pen, which is also occasionally used to handle clean sawdust. New, but contaminated, sawdust could have been used for bedding in rat cages, thus making the transmission to rats possible. The same could be true for the single rabbit found in room 10 since the rabbits in this room are provided with sawdust bedding.

The sawdust or feed used in the SAPU could have become contaminated at the site of production, during transit, or while handling in the unit. Campylobacter have been found to thrive for at least 7 days on completely desiccated swabs (Ullman and Kischkel, 1981), thus they could conceivably survive in feed or sawdust for about the same length of time. However, subsequent examination of these items proved them to be negative. Water could also be a vehicle introducing the organisms into the unit. Water has been found to be the cause of outbreaks of Campylobacter in humans in Vermont (Brouwer *et al*, 1979) and Sweden (Mentzing, 1981). Kniel *et al* (1978) assayed 84 samples of water for the presence of *C. jejuni* and found the organisms to be present in 7 of 34 samples of sea water and 37 of 50 samples of fresh water. Blaser *et al*

(1980a) reported survival of Campylobacter in water at 4 C for 1-4.5 days. Keeping this in mind, water samples from taps, drains and water bottles were examined for the presence of C. jejuni but all were found to be negative.

House flies have also been found as carriers of C. jejuni and C. coli (Rosef and Kapperud, 1983). From the 30 houseflies captured from the SAPU none were found to be carrying C. jejuni.

The four regular staff members were found to be negative for the organisms on both occasions at which they were tested and none complained of having suffered from diarrhoea in the immediate past. Humans have been found to acquire infection through direct contact with animals (Jones and Robinson, 1981), but transmission is mainly through the food chain from raw animal products - milk, beef and sheep carcases, poultry meat, and offal (Skirrow, 1982). Individuals with campylobacter enteritis who are not treated excrete the organism in their faeces for several weeks (Karmali and Fleming, 1979). Clearance seems to occur spontaneously in most cases within two months (Brieseman, 1985). Therefore it seems highly unlikely that the staff of the SAPU have brought the infection into the unit.

The animal handlers do not seem to play any role in the spread of infection because the workers who clean the guinea pig room do not clean the rat and rabbit rooms, but still there has been infection in the rat and a rabbit. Looking at the pattern of the infection in the three species of laboratory animals, it appears that there must be a common source of infection. Feed for these animals is different thus it cannot form the common reservoir of infection. House flies are mainly found in the courtyard and do not have free access to the animal rooms. The materials which are common to all the animals are sawdust and water. There are reports indicating survival of campylobacter in sea and fresh water (Kniel et al, 1978). Chlorinated water is obtained from a single source and is used for all the purposes in the unit. It is difficult to imagine that the Campylobacter organism could survive in this chlorinated water. If by chance the organisms had survived it would have spread the same type of infection to all the animals including cats and mice, but these were found to be negative for the strain of

C. jejuni found in the rats, guinea pigs and the rabbit. The water therefore, does not seem to be the vehicle for the introduction of this infection.

The sawdust used in the Unit has not been autoclaved. It is therefore possible that this could have been contaminated at the site of production or it could have also become contaminated during its tranport to the Unit. However, because the sawdust is kept covered at the mill and arrives at Massey in a covered truck, this seems unlikely. Sawdust is unloaded at the Unit manually which takes approximately half an hour. During this period the sawdust could have been contaminated with bird droppings. Birds are claimed to form the main natural reservoir of campylobacter (Kniel et al, 1982; Skirrow, 1982). It is also possible that this could have been contaminated at some other stage. The guinea pigs are fed with green feed as well. It is likely that these greens carried the organisms from soil or animal manure thereby infecting the guinea pigs. Campylobacter have been found to survive in soil and dung for up to 30 days (Anon, 1984). Sawdust is unloaded at the Unit manually by means of a shovel. It is known that the shovel used for handling soiled sawdust from the guinea pig pens was sometimes used in the clean sawdust room. Though the washings from shovels were found negative for the organisms, it is likely that at some stage the organisms were carried to the clean sawdust by the shovel contaminating the sawdust. Sawdust seems therefore to be the most likely vehicle by which the infection moved around the Unit and that the greens fed to the guinea pigs initially brought the infection into the Unit.

Infection was most common in rats (23.2%) suggesting that the type of organism was able to infect rats better than guinea pigs (7.7%), rabbits (1%) or mice (0%).

In the present study an attempt was made to isolate campylobacter from the intestinal tract of mice using the campylobacter selective media of Bolton and Robertson (1982) under micro-aerophilic conditions. The organisms isolated from five mice of 50 (10%) were campylobacter-like spiral shaped microbes. They were all from the rectum and caecum with only one isolate coming

from the ileum. Spiral shaped microbes have been detected repeatedly in the large bowel of mice (Lee et al, 1968, 1971; Savage et al, 1968, 1971; Gordon and Dubos, 1970). Such organisms have been shown to colonize the mucus layer in the large bowel (Savage, 1971). The spiral shaped bacteria could be observed in the bowels of mice as early as 12 days after birth (Lee et al, 1971).

These spiral shaped organisms which were isolated could not be recultured. It is possible that these organisms could only grow in the presence of intestinal content and thus these could not be sustained at a second subculture. Gordon and Dubos (1970) were unable to grow an aerobic bacterial flora of the mouse caecum at first, but by using a medium enriched with rumen fluid they were successful. Thus, it could be said that organisms isolated in this present study could also have the same growth characteristics as those of Gordon and Dubos or Roach and Tannock (1979). These workers were able to grow spiral shaped bacteria from the caecum of mice in liquid E medium (with or without added blood) and also in thioglycolate broth under anaerobic condition. Experimental mice are susceptible to C. jejuni infection (Field et al, 1981; Newell et al, 1983 and Morooka et al, 1985) and it is expected that particular mouse strains will become recognised.

The recovery of C. jejuni from 51.7% of a sample of 60 healthy cats maintained at the SAPU is in close agreement with that of Blaser et al (1980b), Bruce et al (1980), Patton et al (1981), who were able to isolate campylobacter from up to 45% of non-diarrhoeic cats. Other groups of authors (Hasting, 1978; Hosie et al, 1979; Blaser et al, 1979c) reported a 4 to 10 percent isolation rate. Cats obtained from catteries and animal control centres may shed C. jejuni in the absence of diarrhoea (Donna et al, 1985). The same could be said about the cats in this present study since they were all clinically healthy. The five BRENDA patterns obtained from the C. jejuni isolates from cats indicates that there are at least five subspecies of C. jejuni within the colony. No cat organisms were transmitted to the people handling the cats, but there are reports indicating that feline pets are responsible for up to 5% of the C. jejuni infections in humans (Prescott and Munroe, 1982). The isolation of C. jejuni organisms having a multiplicity of BRENDA

patterns from the cats, suggests that the cat organisms had been present in the cat colony from the time the colony was started, or from the occasional bird which accidentally flew into the cat cages and was eaten.

House flies may play a linkage role in the epidemiology of campylobacter infection in humans by transmitting these organisms from animals and animal excreta to human food (Rosef and Kapperud, 1983). Flies could also spread infection between animals maintained under very unhygienic conditions. In the present study an isolate of C. laridis was detected in flies from the SAPU. This is not the first time C. laridis has been isolated from flies. A similar finding was reported by Rosef and Kapperud (1983). C. laridis is mainly found in the intestinal contents of gulls, but there are reports indicating its isolation from dogs, monkeys, cows, goats, ducks and humans (Benjamin et al, 1983).

The close proximity of the SAPU to a duck pond and the behaviour of the ducks which at night move even closer to the unit makes the ducks the most likely source of C. laridis. It is therefore highly likely that the flies have picked up these organisms from duck droppings.

CONCLUSIONS

1. The greens fed to the guinea pigs initially brought the infection into the Unit and the sawdust seemed to be the most likely vehicle by which the infection moved around the Unit.

2. There was only one strain of organism found in the laboratory animals within the Unit. The cats housed in the courtyard had five unique strains which were all different from that of the other laboratory animals.

3. Infection was most common in rats suggesting that the organism was able to infect rats better than guinea pigs, rabbits or mice and the cross infection amongst different species of animal was possible.

4. Cat organisms had been present in the cat colony from the time the colony started or they were introduced by the birds.

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APPENDIX I

PREPARATION OF THE MODIFIED ANTIBIOTIC SELECTIVE MEDIUM OF SKIRROW (1977)

The components of this medium are:

(a) Columbia blood agar base No. 2.
(b) Sheep blood.
(c) Skirrow's antibiotic supplement.
(d) Campylobacter growth supplement (FBP supplement)

(a) Columbia blood agar base No. 2

This is a standard Difco product.

(b) Sheep blood

Blood was only collected from healthy sheep which had not been subjected to any form of prior medication. The blood was collected from a jugular vein by a needle connected by a rubber tube to a blood bag[5].

The blood was dispensed into 100 ml bottles which were kept at 2-3°C.

(c) Skirrow's antibiotic supplement

A vial of freeze dried antibiotic supplement (SR 69, Oxoid) contains 5 mg vancomycin 2.5 mg trimethoprim lactate and 1250 I.U. of polymyxin B and is dissolved in 2 ml of sterile distilled water.

(d) Campylobacter growth supplement (FBP supplement)

Four grams of each of ferrous sulphate[1], sodium pyruvate[2], sodium metabisulphate[3] were dissolved in 16 ml of distilled water (250 mg/ml). The solution was passed through 0.45 µm milipore filter[4] and concentrated in a 20 ml sterile universal bottle and the supplement was kept at 2-3°C until used. One ml (250 mg) of the FBP supplement was added to 500 ml of the medium.

To rehydrate the Columbia blood agar base No. 2, 22 g was suspended in 500 ml of distilled water and heated to boiling until completely dissolved. Autoclaved for 15 minutes at 121°C. The base was allowed to cool at 43-45°C and sterile sheep blood (component b), one vial of the Skirrow's antibiotic supplement (component c) dissolved in 2 ml of sterile distilled water and 1 ml of the FBP growth supplement (component d) was added. After thorough mixing, 12-15 ml of the medium was poured into sterile plastic petri-dishes. The plates were allowed to cool for a few hours and stored at 2-3°C for up to two weeks.

1 BDH Chemicals Ltd., Poole, England.

2 Sigma Chemicals Co., Product No. P-2526, St Louis, Mo 63178, U.S.A.

3 May & Baker Ltd., Product No. 61689, Dagenham, England.

4 Millipore Corporation, Bedford, Massachussets, 01730, U.S.A.

5 'Terumoç Corporation, Tokyo, Japan.

APPENDIX II

PREPARATION OF THE MODIFIED CAMPY-BAP ANTIBIOTIC SELECTIVE MEDIUM (Blaser et al, 1978)

The modified CAMPY-BAP selective medium was prepared in a similar manner to the modified selective medium of Skirrow, but with the addition of cephalothin and amphotericin B.

Seventy five (75) mg of cephalothin[a] was dissolved in 10 ml distilled water (7.5 mg/ml). The solution was passed through 0.45 µm millipore filter and kept in a universal bottle at 2-3 °C, and 1 ml was added to 500 ml of medium.

Fifteen (15) mg of amphotericin B[b] was first dissolved in 1-2 ml ethylalcohol and distilled water was then added to make up to 15 ml (1 mg/ml). Subsequent steps are similar to the preparation of cephalothin solution. One ml of the solution was added to 500 ml of medium. Both antibiotics were added to the medium after it had been cooled to 43-45 °C (see Appendix I).

a and b = Sigma Chemicals Co., Product Nos C-4520 and A-4888 respectively, St. Louis, MO 63178, U.S.A.

APPENDIX III

PREPARATION OF THE MODIFIED PRESTON'S MEDIUM (Bolton and Robertson, 1982)

The modified Preston's medium was prepared in a similar manner to the modified selective medium of Skirrow, but with the addition of antibiotics recommended by Bolton and Robertson (1982) rather than Skirrow's antibiotic supplement.

A solution was prepared containing 3.125 mg of polymyxin B* dissolved in 10 ml of distilled water (.3125 mg/ml). The solution was kept in a universal bottle at 2-3°C, and 1 ml was added to 500 mls of medium (51 µg/ml.

Fifty (50) mg of rifampicin* was first dissolved in 1 ml methanol and distilled water was then added to make up to 10 ml (5 mg/ml). The solution was kept in a universal bottle at 2-3°C, and 1 ml was added to 500 ml of medium (10 µg/ml).

Fifty (50) mg of trimethoprim* was dissolved in 10 ml of distilled water (5 mg/ml). The solution was kept in a universal bottle at 2-3°C and 1 ml was added to 500 ml of medium (10 µg/ml).

Five hundred (500) mg of actidione* was dissolved in 10 ml of distilled water (50 mg/ml). The solution was kept in a universal bottle at 2-3°C, and 1 ml is added to 500 ml of medium (100 µg/ml).

* Sigma Chemicals Co., Product Nos P1004, No. R-3501, T-7883 and C-6255 respectively, St. Louis, Mo 63178, U.S.A.

APPENDIX IV

PREPARATION OF THE FBP BROTH

(a) Nutrient broth
(b) Agar
(c) Glycerol
(d) FBP supplement

Twelve point five grams (12.5 g) of nutrient broth (CM 67, oxoid) and 0.6 g Bacto-agar (Difco) was rehydrated in 500 ml of distilled water. It was heated to boiling until dissolved. Fifteen percent (15%) (v/v) glycerol was added and autoclaved for 15 minutes at 121°C. The ingredients were cooled down to 45-50°C and 1 ml (250 mg) of the FBP supplement was added (see Appendix I). Then it was dispensed into sterile universal bottles and was stored at 2-3°C.

APPENDIX V

PREPARATION OF SEMISOLID YEAST EXTRACT NUTRIENT BROTH AGAR MEDIUM (Benjamin et al, 1983)

(a) Nutrient broth
(b) Agar
(c) Yeast extract
(d) FBP supplement

Twelve point five grams (12.5 g) of nutrient broth (CM67, Oxoid), 5 g of yeast* and 1 g of Bacta agar (Difco) were rehydrated in 500 ml of distilled water. It was heated to boiling until dissolved. Autoclaved for 15 minutes at 121°C. The ingredients were cooled down to 45-50°C and 1 ml (250 mg) of the FBP supplement was added (see Appendix I) and stored at 4°C.

* DYC Foods, Auckland.

APPENDIX VI

REAGENTS USED FOR BRENDA TECHNIQUE

TEB = Tris-Ethylene Diaminetetraacetic Acid Disodium Salt Buffer

100 mM Tris (hydroxymethyl) aminomethane (Tris[a], pH lowered to 7.5 by the addition of HCl

100 mM Ethylenediaminotetraacetic acid disodium salt (EDTA[b])

STE = Saline-Tris-EDTA

100 mM NaCl

50 mM Tris, pH lowered to 7.5 by the addition of HCl

1 mM EDTA

TE = Tris-EDTA

10 mM Tris, pH lowered to 7.5 by the addition of HCl

1 mM EDTA

Enzyme Buffer

60 mM NaCl

10 mM MgCl

10 mM Tris, pH lowered to 7.5 by the addition of HCl

100 µg/ml bovine serum albumin[c].

Electrophoresis Buffer

40 mM Tris, pH lowered to 7.8 by acetic acid

5 mM sodium acetate

1 mM EDTA

0.5 µg/ml ethidum bromide[d].

Protease[e] Solution

After preparation incubate at 37°C for 2 hours before use or store at -20°C.

Sodium Perchlorate

May add as crystals or as 5M solution.

Phenol

Before use redistill and store under N_2 at -20°C.

Alcohol

Ethanol 95%.

a, c, d = Sigma Chemicals Co., St. Louis, MO 63178, U.S.A.

b = Ethylenediaminetetraacetic acid, BDH Chemicals Ltd., No. 28021, Poole, England.

e = From Streptomyces griseus. Callico Chem. Behring Co., San Diego, California 92112, U.S.A.