THERMOPHILIC CAMPYLOBACTER IN ANIMALS AND MAN

A thesis presented in partial fulfilment
of the requirements for the degree
of Doctor of Philosophy
in Veterinary Pathology and Public Health
at Massey University.

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1984

ABSTRACT

During the course of the work reported in this thesis, more than 2,000 samples and isolates from animals and from humans, were examined for the presence of intestinal thermophilic Campylobacter. This figure does not include samples and isolates examined during the course of experimental studies.

Culture of either rectal swabs from pigs or cloacal swabs from poultry usually provides an accurate assessment of the prevalence of distal gastro-intestinal (G.I.) tract infection by C. coli and C. jejuni, provided that the swabs are kept at $4^{o}C$ and cultured within 6 hours. Cultures of isolates of C. jejuni can be preserved for at least 21 months if stored in FBP broth or PBS at $-70^{o}C$.

The major site of colonisation by C. coli in pigs, is the distal part of the G.I. tract and the proximal G.I. tract in the case of C. jejuni. In poultry, the major sites of colonisation by both C. jejuni and C. coli are the distal ileum and the large intestine, especially the caeca.

Surveys of pigs showed that 60 - 100% of post weaning pigs are infected with C. coli. In neonates the rates of infection increase with age, and infected sows are the major source of infection for piglets. C. jejuni is rarely isolated from pigs.

Twenty eight percent (28%) of all poultry flocks examined were infected with intestinal thermophilic Campylobacter. In infected broiler flocks the rate of infection is almost 100%, while in older birds lower rates were found. Of all the isolates examined, 86% were C. jejuni and 14% C. coli.

All poultry carcases and edible viscera, derived from infected broiler flocks, are contaminated with Campylobacter. The levels of contamination of these products by both C. jejuni and C. coli are approximately 10^{6} and 10^{4} cfu per whole carcase and packet of edible viscera respectively. Poultry chilled products for sale in supermarkets were also heavily contaminated by these two species of Campylobacter which remained viable for the 'shelf life' of the products (ten days).

The prevalence of infection by intestinal thermophilic Campylobacter in gulls was 59%, in ducks 29%, and in rats 60%. Infected sparrows were not detected. With the exception of an isolate from one pig, C. laridis is a species apparently host specific for sea birds, and isolates from gulls of what, on conventional taxonomic criteria, would be classified as C. coli are shown on more detailed examination to be C. laridis.

The level of excretion in faeces of Campylobacter in most infected pigs is between 10^2 - 10^5 cfu/g, in poultry approximately 10^8 cfu/g, in rats 10^5 cfu/g, and in gulls 10^3 cfu/g.

The infectivity of C. jejuni isolated from poultry for experimental chickens is in the region of 5×10^2 cfu and that of C. coli approximately 5×10^5. Both species of Campylobacter were a 100 to 1000-fold less infective for rats. Infection in both chickens and rats was self-limiting and attempts to reinfect chickens with the same species or organism, were usually unsuccessful. C. laridis was not infective for either chickens or rats at dose rates of up to 10^9 organisms.

Campylobacter infections are the most common notified human enteric infection in New Zealand with an annual incidence rate of 31/100,000. Approximately 94% of the human cases of campylobacteriosis are due to C. jejuni and 6% to C. coli. People between 1 to 4, and 15 to 35 years of age are the most commonly affected, and patients who no longer have clinical signs of infection may shed up to 10^8 cfu of C. jejuni/g of faeces. By use of a bacterial restriction endonuclease DNA technique (BRENDA), up to 80 different types of C. jejuni and C. coli were identified from 338 isolates from humans. One hundred and ninety four (61%) of the 316 isolates of C. jejuni from humans had similar BRENDA patterns to isolates of C. jejuni from animals. Poultry appear to be the major source of C. jejuni, and possibly C. coli, for humans, while pigs apparently are an insignificant source of C. coli for humans. Rats can be infected with strains of C. jejuni which can infect poultry, humans and other animals. BRENDA types recovered from gulls and ducks were not similar to any of the isolates from humans or other animals examined.

Some strains of *C. jejuni* and *C. coli* developed an *in-vitro* resistance to nalidixic acid. This is an important finding in relation to conventional taxonomic criteria for differentiating *C. laridis* from other intestinal thermophilic *Campylobacter*. Isolates of '*C. coli*' from gulls are phenotypic variants of *C. laridis* which may be either resistant, or non resistant, to nalidixic acid. Only by determining whether or not, anaerobic growth in the presence of TMAO occurs, can *C. coli* be differentiated from *C. laridis*.

ACKNOWLEDGEMENTS

The author wishes to thank the N.Z. Universities Grants Committee for the award of a Commonwealth Scholarship, and Dr K. Polydorou, Director of Department of Veterinary Services, Cyprus, for leave of absence. Without this assistance the work would not have been possible. Some financial assistance from General Foods Poultry Ltd is acknowledged.

Special thanks are due to my supervisors, Professor D.K. Blackmore and Dr R.B. Marshall, for unbridled enthusiasm and encouragement, and for their help and readiness to discuss problems whenever required. The assistance of Mr P.J. Winter, who introduced and taught me the BRENDA technique and also carried out many of the BRENDA analyses, is greatly appreciated.

I am indebted to the many individual poultry and pig farmers, who allowed me to visit their farms to collect samples during the field investigations. In particular, I would like to thank Mr S. Burgess of the Golden Coast Poultry Industries, and Mr D. Gill, manager of the poultry slaughterhouse in Otaki. Particular thanks are also due to Mr C. Tempest and Mr M. Austin, veterinarians, of General Foods Poultry Ltd and Golden Coast Poultry Industries for their co-operation, and the manager of the Kiwi Bacon factory at Longburn. Many thanks are also due to Miss D.M. Norris of the National Health Institute and Mr M. Robertson of the Hamilton Medical Laboratory, who provided the isolates of Campylobacter from humans.

I am particularly grateful to Prof. D.K. Blackmore for the many hours he spent with me in 'sharp-shooting' rats, collecting seagull faeces and for assistance with the handling and inoculation of animals. I wish to thank Professor J.L. Penner for serotyping certain isolates and Dr R.B. Marshall for help in the preparation of antigens and making appropriate arrangements. I am also grateful to Dr G.V. Petersen for his advise on biometrics and statistical analysis.

I would like also to thank Mrs Jan Schrama for the preparation of glassware, Mr P. Wildbore for administrative assistance, Mr T. Law for photographic work and Miss L. Cullinane and Mr R. Holdaway for many pleasant hours in the laboratory.

The typing of this thesis was carried out by Mrs Olive Harris. For her cheerful and efficient co-operation in the face of a Greek Cypriot, whose written English was far from perfect, many thanks are due.

Finally, I wish to thank my family for their patience and support and particularly my wife, Anastasia, who on numerous occasions acted as an unpaid technician, and for her love and care of my experimental animals. To her and to my three children Vikky, Photios and Leda, a debt of time and neglect will hopefully be repaid.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

XhoI

CHAPTER I

INTRODUCTION

Campylobacter were originally classified within the genus Vibrio and the taxonomy of this group of organisms has been subjected to considerable change. This has tended to cause some confusion for those not directly involved with this group of organisms.

At present, the genus Vibrio of the family Vibrionaceae, includes two species of particular public health importance. One is Vibrio cholerae, the aetiological agent of cholera, discovered by Robert Koch in 1883. Cholera, which probably originated in India in the 18th century, has been the cause of many pandemics. The other is the halophilic organism, Vibrio parahaemolyticus, which can be isolated frequently from coastal marine waters and seafoods. V. parahaemolyticus is a major cause of gastro-enteritis in Japan, and to a lesser extent, other countries.

In the U.S.A. in 1919, Smith and Taylor isolated a spiral organism from aborted bovine foetal fluids which they named Vibrio fetus. In 1931, a similar organism, Vibrio jejuni was isolated from cattle with enteritis and in 1944, in the U.S.A. a third 'vibrio' was recovered from the intestine of pigs with swine dysentery, which was later named Vibrio coli. All three organisms, were subsequently shown to be microaerophilic and catalase positive.

Other microaerophilic, but catalase negative, 'vibrio like' organisms were isolated from the oral cavity of humans and from the genital tract of cattle and sheep. These were named respectively, Vibrio sputorum and Vibrio bubulus.

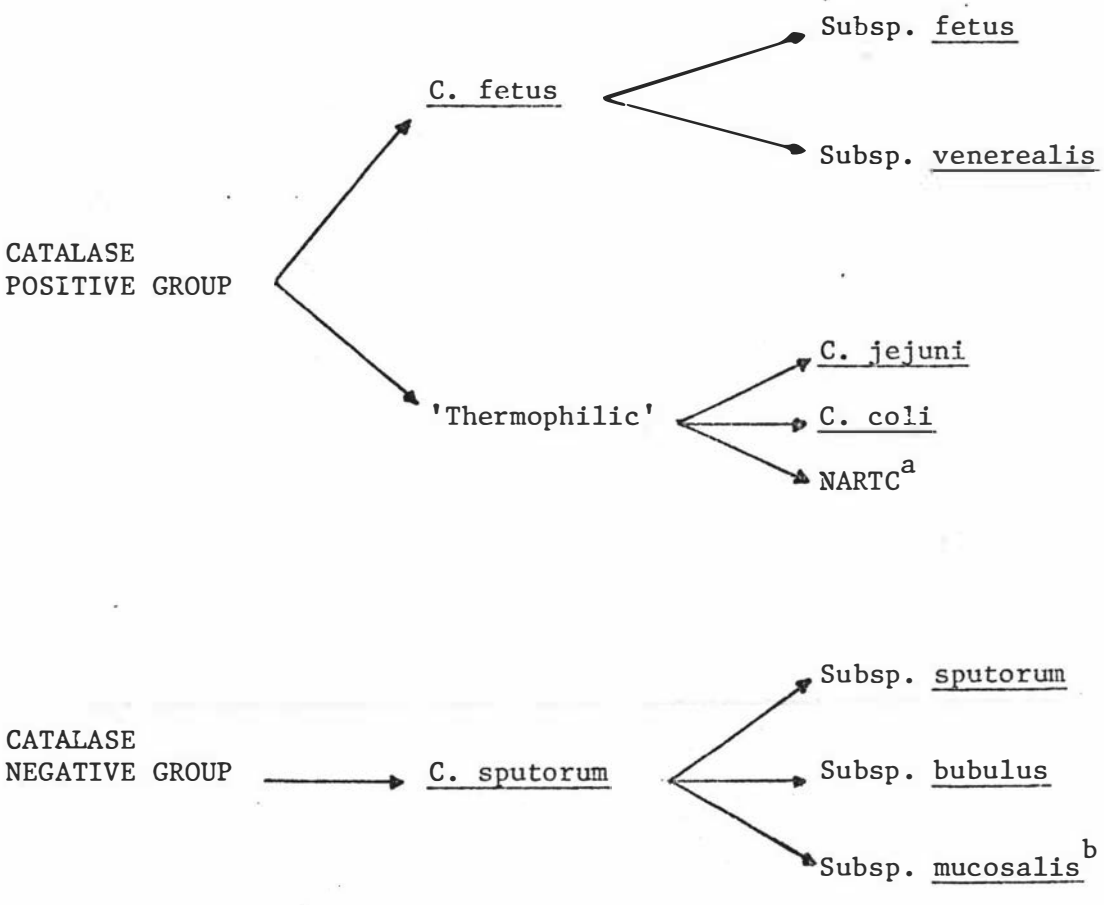
Up to 20 years ago, all these organisms, of similar morphology, irrespective of whether or not they were facultative anaerobes or microaerophilic, were classed together within the genus Vibrio.

In 1963 Sebald and Veron, in an extensive study of the genus

Vibrio and other similar organisms, proposed that two genera, Vibrio and Campylobacter, be established. The former including the facultative anaerobic fermentative organisms such as V. cholerae, and the latter the microaerophilic non fermentative organisms such as V. fetus.

The word Campylobacter is derived from the Greek word Kampylos, meaning curved. Since Sebald and Veron's original proposal, the Campylobacter have been reclassified several times as will be discussed in Chapter II.

Based on the current Approved Lists of Bacterial names (ALBN) (Skerman et al 1980), the genus Campylobacter includes the following groups, species, and subspecies.



a Nalidixic acid-resistant thermophilic Campylobacter (Skirrow and Benjamin 1980b), not in the ALBN.

b Lawson et al (1975a), not included in the ALBN.

For about 70 years, bacteria within the genus Campylobacter have been associated with diseases of animals in many parts of the world. Until comparatively recently Campylobacter were not, to any great extent, associated with disease in humans, and early reports of human infections were rare.

The development of a simple technique for the isolation of Campylobacter from faeces by Skirrow (1977), gave impetus to an interest in human infection. This led to an appreciation that Campylobacter infections were one of the most common infectious causes of human gastro-enteritis. The species now known to most commonly infect man are C. jejuni and, to a lesser extent, C. coli.

C. jejuni and C. coli are common intestinal infections of a wide variety of animals including domestic stock, and are frequent contaminants of food products from such animals. It is thus widely believed that Campylobacter infections of humans are zoonotic, and infection can be acquired from direct animal contact, from contamination of animal products or indirectly from a contaminated environment.

In New Zealand, Campylobacter infections of humans were made notifiable in May 1980, and by 1981 they were the sixth most common notifiable human infectious disease in the country with 442 cases being reported. However, until recently there was little information on the prevalence of the enteric Campylobacter infections of animals in New Zealand and no comprehensive studies had been carried out to determine the source of human infections.

The major part of the work outlined in this thesis was designed to investigate three main areas of the epidemiology of Campylobacter in animals and man. First, to investigate the prevalence of the different species of intestinal thermophilic Campylobacter in poultry, pigs, wild rats (Rattus norvegicus) and certain species of wildbird. Secondly, to study the infectivity of various species and strains of thermophilic Campylobacter, and the course of infection, in experimental chickens and rats. Finally, by the use of DNA restriction enzyme analysis, of isolates of Campylobacter from animals and man, an attempt was made to ascertain whether or not,

cross infection by Campylobacter naturally occurs between different species of animal, and between animals and man. It was hoped that this latter work would be of public health importance.

Before these major studies were carried out, it was necessary to investigate and develop certain techniques, and during the course of the work it became necessary to reappraise certain of the conventional taxonomic criteria on which the classification of the thermophilic Campylobacter had been previously based.

CHAPTER II

REVIEW OF THE LITERATURE

HISTORY OF CAMPYLOBACTER

The disease potential of microaerophilic vibrio-shaped organisms was recognised for the first time at the beginning of this century. MacFadyean and Stockman (1913), as part of a committee appointed to inquire into epizootic abortion in England, cultured vibrios from cases of abortion in sheep. These authors were also the first to observe vibrio-shaped organisms from aborted bovine foetuses. Five years later, their observations on bovine vibriosis were confirmed by Smith (1918) in the U.S.A. Subsequently Smith and Taylor (1919) in a study of infectious abortion in cattle, characterised these organisms and named them Vibrio fetus.

Subsequent to these early reports, microaerophilic vibrios had been associated with a variety of diseases. In the U.S.A., Jones and Little investigated the aetiology of infectious diarrhoea (winter scours) in cows (1931a) and enteritis in calves (1931b), and associated these intestinal disorders with vibrios. Jones and Little (1931a, 1931b) and Jones et al (1932) reproduced the disease in healthy cattle by feeding them cultures of vibrio which had been isolated from diseased animals. These investigators showed that the jejunum was the predilection site of this infection in the intestinal tract, and proposed that these 'vibrios' be designated Vibrio jejuni (Jones et al 1931). The same authors (1931) found that Vibrio jejuni was serologically distinct from the V. fetus of Smith and Taylor (1919). Their observations were confirmed by Stalnikov (1939) who induced diarrhoea in young calves by feeding them intestinal contents and suspensions of faeces from field cases of calf diarrhoea. However Rollinson (1948) was unable to isolate the organism from adult cattle with a similar clinical disease.

The concept that vibrios were the cause of winter dysentery in cows was largely discounted when MacPherson (1957) in Canada, Komarov (1959) in Israel, Charton (1963) in France and Scott (1973)

in the U.S.A., concluded that winter dysentery had a viral aetiology. More recently Campbell and Cookingham (1978), in a review of winter dysentery of cattle, were of a similar opinion.

Doyle (1944) isolated a microaerophilic vibrio from the mucosa of the colon of pigs affected with dysentery, and succeeded in inducing diarrhoea in six out of eight pigs which were fed cultures of vibrios isolated from affected pigs. Although the diarrhoea and dysentery was less severe than in pigs fed the colon from affected pigs, he proposed that swine dysentery was caused by these microaerophilic vibrios. In more extensive investigations involving 60 pigs Doyle (1948), found that in five out of six experiments, the feeding of cultures of vibrios mixed with gastric mucin, reproduced the clinical and pathological signs of naturally occurring swine dysentery. He suggested that these vibrios be named Vibrio coli.

The observations of Doyle were confirmed by some investigators (Jones and Doyle 1947, Roberts 1956) but not by others (Deas (1960, David 1961, Söderlind 1965). The theory that swine dysentery is caused by vibrios was largely dismissed after the work of Andress and Barnum (1968a), and Andress et al (1968b) failed to reproduce swine dysentery in either conventional or gnotobiotic pigs even though vibrios became established in the intestinal tract.

Swine dysentery is now considered to be caused by Treponema hyodysenteriae (Harris et al 1972, Taylor 1970, 1976, 1978), in association with other organisms of the normal colonic flora (Meyer et al 1974, Braudensburg et al 1977, Harris et al 1978).

More recently Taylor and Olubunmi (1981) and Taylor (1982) claimed that Campylobacter coli is the cause of enteritis in non immune unweaned piglets, in which primary lesions occur to the small intestine. Forty years after Doyle's work, the role of V. coli as a pathogen for pigs is still unclear and more work concerning its role as an intestinal pathogen of pigs is required.

An anaerobic vibrio with a long terminal flagellum was isolated from the sputum of a patient with acute bronchitis

(Tunnicliff 1914). Later Prevot (1940) assigned the name V. sputorum to this organism. MacDonald (1953) demonstrated the frequent occurrence of V. sputorum in the oral cavity of humans and described it as a nonfermentative, strictly anaerobic, catalase negative and H_2S positive organism.

Catalase positive, H_2S negative microphilic vibrios were considered to be V. fetus (Bryner and Frank 1955, Kuzdas and Morse 1956) and it was proposed by Thouvenot and Florent (1954), that the catalase negative and hydrogen sulphide positive microaerophilic vibrios, which had been isolated from the normal reproductive organs of cattle in Belgium by Florent (1953), be named Vibrio bubulus. Other investigators also reported the isolation of similar organisms from the genital tract of cattle (Lindqvist 1955, Bryner and Frank 1955, Akkermans et al 1956 and Kuzdas and Morse 1956) and from the genital tract of sheep (Firehammer et al 1956), and Firehammer and Lovelace (1961) proposed that they be named V. bubulus.

In 1956, Kiggins and Plastridge studied the effect of the gaseous environment on the growth of V. fetus of bovine origin. They found that the optimum environment was microaerophilic, consisting of 5% oxygen and 10% carbon dioxide.

As pointed out earlier, from the beginning of the century until the late 1940's, the microaerophilic vibrios were incriminated as the aetiological agents of several diseases of domestic animals. Hofstad (1956) was the first investigator to isolate vibrios from the livers of diseased adult chickens and to associate them with hepatitis. Peckham (1958) confirmed these findings and coined the term, avian vibrionic hepatitis. Kŭlbl (1964), Winkerwerder and Maciak (1964), Truscott and Stockdale (1966) and Kŭlbl and Willinger (1967) demonstrated the presence of vibrios in the caeca, intestine and faeces of infected birds. Winkerwerder and Maciak (1964) demonstrated that 36.4% of chickens, from clinically normal flocks, carried vibrios in their intestinal tract. Truscott and Stockdale (1966) suggested that the presence of vibrios in the intestine of chickens indicated that infection was transmitted by the oral - faecal route. Subsequently, Kŭlbl and Willinger (1967), showed that infection was spread by contact with

infected faeces, and that caecal infection occurred, in initially uninfected chickens, within 24 hours of contact with infected birds.

Florent (1959) divided V. fetus of bovine origin into two subspecies; V. fetus venerealis which was venereally transmitted and V. fetus intestinalis which was apparently not transmitted by this route.

On the basis of the guanine and cytosine (G + C) content of their DNA and phenotypic characteristics (Sebald and Veron 1963) suggested that V. fetus and V. bubulus should be classified in a genus to be termed Campylobacter.

Loesche et al (1965) studied the catalase negative Vibrio sputorum and V. bubulus and demonstrated that the former was not a strict anaerobe as previously described by MacDonald (1953), but was microaerophilic. The authors recommended combining the two in the one species; Vibrio sputorum, with two subspecies; V. sputorum subsp. sputorum and V. sputorum subsp. bubulus. These recommendations were accepted by Veron and Chatelain (1973) and Smibert (1974) who also recommended that they be transferred to the genus Campylobacter.

Another catalase negative Campylobacter was isolated in large numbers from the lesions of porcine intestinal adenomatosis (PIA) (Lawson and Rowland 1974) and subsequently named C. sputorum subsp. mucosalis by Lawson et al (1975a). The same organism was isolated from the intestinal lesions of necrotic enteritis and regional ileitis, and was suspected as being associated with the lesions of proliferative haemorrhagic enteropathy (PHE) of pigs (Rowland and Lawson 1975). In Australia, Love et al (1977) also associated the isolation of subsp. mucosalis with PHE.

Campylobacter sputorum subsp. mucosalis is neither present in the alimentary tract of clinically normal pigs, even if these pigs are obtained from herds where PIA was previously endemic (Rawland and Lawson 1975), nor from pigs affected by non-adenomatous enteritis (Lawson and Rowland 1974). Occasionally the organisms can be

isolated from the oral cavity of pigs from herds where clinical intestinal adenomatosis is endemic (Lawson *et al* 1975b). Conversely subsp. *sputorum* and subsp. *bubulus* are considered part of the normal flora of the mouth of humans, (Loesche *et al* 1965) and the genital tract of cattle and sheep, respectively (Smibert 1978).

In 1965 Firehammer reported the isolation of a *Campylobacter* from the faeces of sheep which was similar to *C. sputorum* subsp. *bubulus*, except that it was catalase positive. He suggested the name *V. faecalis* which was amended to *Campylobacter faecalis*, by Smibert (1974).

Disease in humans were associated with vibrios before the beginning of this century, and the microaerophilic vibrios were recognised as a cause of diarrhoea in humans in the mid 1940's. In 1946, in two penal institutions in Illinois U.S.A., approximately 6% of the 6019 inmates were affected with acute gastroenteritis, and although Levy (1946) could not culture vibrios from the faeces of affected persons, he observed 'vibrio-like' organisms in 31 of 73 faecal specimens examined. The isolation of morphologically similar microaerophilic organisms from the blood of 13 of the 39 affected persons indicated that vibrios were the cause of their infection. Although the vibrios, which were isolated, were lost before being fully characterised, contaminated milk was incriminated as the probable source of infection. Levy (1946) suggested at this time, that the microaerophilic vibrios were probably the same as the *V. jejuni* associated with winter dysentery in cattle by Jones *et al* (1931).

The first well documented case of human infection by microaerophilic vibrios was reported by Vinzent *et al* (1947). They isolated *V. fetus* from the blood and the uterus of a woman suffering with an influenza-like condition, and showed for the first time an association between this organism and human abortion. Further sporadic cases of *V. fetus* in humans were described until 1957. King (1957, 1962) studied microaerophilic vibrios isolated from humans and other sources and described two distinct groups; one group identical to *V. fetus* and the second group which he termed the 'related vibrios'. The optimum temperature for the culture of the 'related vibrios' was 42^{o}C, (thermophilic) whereas for *V. fetus* it

was 37°C. Both groups grew at 37°C but only V. fetus grew at 25°C. Although the 'related vibrios' were isolated only from blood cultures of patients with gastroenteritis, King speculated that they were a more common cause of gastroenteritis than was recognised at the time.

The isolation of Campylobacter from faeces, is more difficult than from blood, because the Campylobacter have to be differentiated from other intestinal organisms. For this reason the hypothesis put forward by King (1957, 1962) was not properly investigated for a further ten years. Cooper and Slee (1971) in Australia were the first to isolate Campylobacter from patients with diarrhoea. By inoculating faeces on blood agar plates supplemented with cephalothin discs, they were able to grow Campylobacter within the zone of inhibited bacterial growth produced by the disc.

The first major breakthrough in isolating Campylobacter was made by Dekeyser et al (1972) in Belgium, by filtering supernatants of faeces, through 0.65 mm filters. Butzler et al (1973) showed that 'related Campylobacter' were isolated in significantly higher numbers from 900 patients with diarrhoea (5.1%), compared with only 1.3% from 1,000 people without diarrhoea. Butzler et al (1973), found serological similiarities between isolates from both humans and poultry.

Veron and Chatelain (1973) transferred V. jejuni of Jones et al (1931) and V. coli of Doyle (1948) to the genus Campylobacter and named them C. jejuni and C. coli respectively, which included the 'related vibrios' of King (1957). However, Smibert in Bergeys Manual (1974), considered both C. jejuni, C. coli and the 'related vibrios' to be only one species; C. fetus subsp. jejuni.

Smith and Muldoon (1974) were the first investigators to isolate C. fetus subsp. jejuni from poultry carcases (1.8%) from retail shops. The results of Butzler (1973) were confirmed by Skirrow (1977) in England. Rather than using the laborious filter technique of Dekeyser et al (1972), Skirrow developed a culture medium containing different antibiotics, which was capable of selectively permitting the growth of C. jejuni and C. coli from faecal cultures. Isolates of C. jejuni or C. coli were obtained from approximately 7% of 803

patients with diarrhoea examined. No isolates were obtained from 194 controls without diarrhoea. Skirrow assumed that the source of infection for nine patients was chickens, or their carcases, or contact with dogs, and suggested undercooked food as a source of infection.

The work of Skirrow (1977) on this 'new disease' stimulated an interest among the medical profession, and the rate of recorded isolations of Campylobacter jejuni and C. coli rapidly increased to a level equal to, or greater than, that of the other recognised microbial enteric pathogens.

In 1979 Neil et al designated to the genus Campylobacter the aerotolerant Campylobacter-like organisms which had been collected from bovine and porcine abortions by Ellis et al (1977, 1978) in Ireland.

Skirrow and Benjamin (1980a) recognised among the 'thermophilic Campylobacter', (C. jejuni and C. coli of Veron and Chatelain or C. fetus subsp. jejuni of Smibert), a distinct group; the nalidixic acid resistant thermophilic Campylobacter (NARTC), which they frequently isolated from wild seagulls. Subsequently Skirrow and Benjamin (1980b) used the hippurate hydrolysis test of Harvey (1980) to separate C. jejuni from C. coli. In 1983, Benjamin et al proposed the NARTC group to be designated as a new species, which they named Campylobacter laridis; from the Greek name laros meaning seabird. Also in 1983, a nitrogen fixing bacterium, associated with the roots of a plant, was proposed as another species; Campylobacter nitrofigilis (McClung et al 1983). In the same year, a new species of Campylobacter was isolated from pigs affected with lesions of proliferative ileitis which was thought to be associated with the pathogenesis of this disease. This new species was named C. hyointestinalis (Gebhart et al 1983). In 1981, Tanner et al, isolated another species of Campylobacter from the gingival crevices of humans, and they proposed that it be named C. concisus.

TAXONOMY OF CAMPYLOBACTER

Many different criteria for the classification of Campylobacter have been proposed. It now appears that the taxonomy

of Veron and Chatelain (1973), is most widely accepted, although that of Smibert (1974) is also recognised by some workers. The former has been adapted for the Approved Lists of Bacterial Names (A.L.B.N.), edited by Skerman et al (1980) on behalf of the International Committee on Systematic Bacteriology. Table 2.1 compares the official nomenclature adopted for the A.L.B.N. with the other previous nomenclatures.

All members of the genus Campylobacter are Gram-negative non spore forming microaerophilic curved-to-spiral shaped rods 1.5 - 3.5 µm long by 0.2 - 0.4 µm wide. Single cells are typically vibroid being curved or twisted, but also may be 'S'-shaped or gull-shaped (Smibert 1978). In old cultures cells may become coccoid (Ogg 1962, Tritz and Ogg 1967). Rapid coccal transformation is particularly prominent with the thermophilic Campylobacter, but not with C. fetus (Karmali et al 1981). This coccal transformation appears to be degenerative, as cultures composed mainly of coccoid forms are nonviable (Smibert 1978). The viable organisms have a unique corkscrew-like motibility by means of a single polar flagellum two to three times the length of a cell. Some cells may have more than one flagellum at each end of the cell (Smibert 1978).

The optimum microaerophilic environment for growth is 5% oxygen and 10% carbon dioxide (Kiggins and Plastridge 1956), and the presence of more than 21% oxygen is bacteriostatic (Smibert 1978).

Campylobacter are not able to ferment or oxidise carbohydrates (Kiggins and Plastridge 1958, Loesche et al 1965, Smibert 1978). They obtain their energy for growth from the tricarboxylic acid cycle (Smibert 1978). They are oxidase positive (Holdeman 1977) and nitrates are reduced (Loesche et al 1965, Smibert 1978).

There are several criteria used for the differentiation of Campylobacter into groups, species, subspecies and biotypes. One of the most important characteristics is the production of catalase, and on the basis of this activity, Campylobacter are separated in two major groups; the catalase positive and the catalase negative Campylobacter (Florent 1953, Veron and Chatelain 1973, Smibert 1974, Skerman et al 1980). Growth at 25°C versus growth at 42 -

TABLE 2.1 : COMPARISON OF PRESENT NOMENCLATURES OF CAMPYLOBACTER (ALBN) WITH PREVIOUS CLASSIFICATIONS

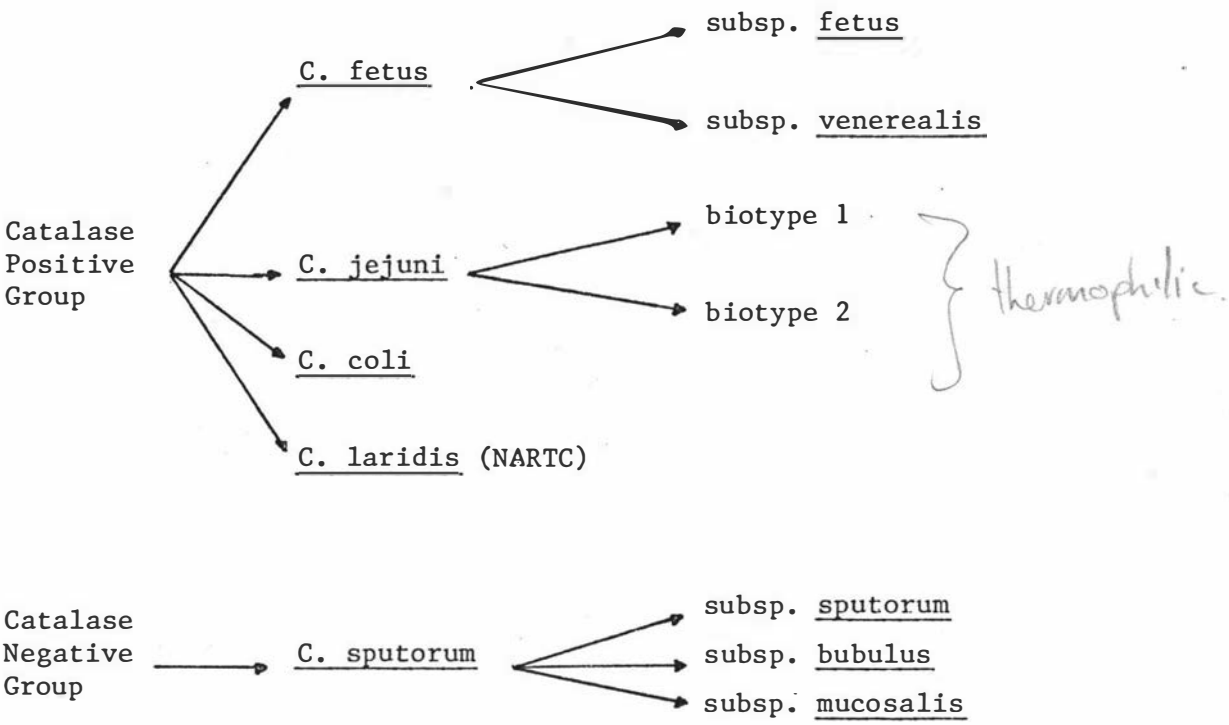
A.L.B.N. (Skerman et al 1980	Smibert (1974)	Lawson et al (1975a)	Veron and Chatelain (1973)	Firehammer (1965)	Loesche et al (1965)	Sebald and Veron (1963)	King (1957)	Florent (1953, 1959)	Doyle (1948)	Prevot (1940)	Jones et al (1931)	Smith and Taylor (1919)
C. fetus subsp. fetus	C. fetus subsp. intestinalis		C. fetus subsp. fetus		V. fetus	Campylobacter fetus	V. fetus	V. fetus subsp. intestinalis (1959)				V. fetus
C. fetus subsp. venerealis	C. fetus subsp. fetus		C. fetus subsp. venerealis		V. fetus	Campylobacter fetus	V. fetus	V. fetus subsp. venerealis (1959)				
C. jejuni	C. jejuni subsp. jejuni		C. jejuni				"related vibrios"				V. jejuni	
C. coli			C. coli						V. coli			
C. sputorum subsp. sputorum	C. sputorum subsp. sputorum		C. sputorum subsp. sputorum		V. sputorum subsp. sputorum					V. sputorum		
C. sputorum subsp. bubulus	C. sputorum subsp. bubulus		C. sputorum subsp. bubulus		V. sputorum subsp. bubulus	Campylobacter bubulus	V. bubulus	V. bubulus (1953)				
	C. sputorum subsp. mucosalis	C. sputorum subsp. mucosalis										
	C. faecalis			V. faecalis								

43°C is another important characteristic, which can usually be used to differentiate C. fetus from the thermophilic Campylobacter group (C. jejuni, C. coli and C. laridis), (King 1962, Veron and Chatelain 1973, Smibert 1974). Absolute differentiation of these species is achieved by a combination of the temperatures of growth, the sensitivities to nalidixic acid and cephalothin 30 µg, and hippurate hydrolysis (Harvey 1980, Skirrow and Benjamin 1980b). The hippurate hydrolysis test is the only simple test capable of differentiating between C. jejuni and C. coli (Skirrow and Benjamin 1980b, 1982). On the basis of H_2S production in an iron-metabisulfite pyruvate medium (FBP), C. jejuni is divided into two biotypes, and C. coli is differentiated from C. laridis (Skirrow and Benjamin 1980b). Other characteristics of taxonomic importance are tolerance to 1% glycine, NaCl and 1% bile. All three tests are useful in the differentiation of the subspecies of the catalase negative group, while tolerance to glycine and detection of sodium selenite reduction are important for the differentiation of C. fetus sub species. The detection of nitrate reduction and the H_2S production in Triple sugar iron (TSI) agar slants either in the medium or with lead acetate impregnated strips or the use of a 'sensitive medium' containing 0.02% cysteine hydrochloride and the lead acetate strips (Veron and Chatelain 1973) and the tolerance to 0.1% triphenyltetrazolium chloride (TTC) are additional tests which help to differentiate Campylobacter. Morphological differences based on the amplitude and wavelengths of spiral forms have been used to classify the catalase positive group in 'large', 'medium', and 'small' size Campylobacter (Karmali et al 1981a). Table 2.2 shows a broad presentation of the groups, species, subspecies and biotypes of the genus Campylobacter of the family Spirillaceae.

Catalase Positive Campylobacter

These organisms, which do not produce H_2S in TSI agar or reduce nitrates beyond nitrites (Smibert 1978), are divided into two groups. One group, which is represented by C. fetus is divided into two subspecies, which both grow at 25°C and 37°C, but not at 42°C (King 1962, Veron and Chatelain 1973, Smibert 1974). The other group, the thermophilic Campylobacter have an optimum temperature of 42°C. They grow at 37°C, but not at 25°C (King 1962, Veron and Chatelain 1973, Smibert 1974).

TABLE 2.2 : A BROAD PRESENTATION OF THE GROUPS, SPECIES, SUBSPECIES AND BIOTYPES OF THE GENUS CAMPYLOBACTER[a] OF THE FAMILY SPIRILLACEAE



C. faecalis[b]	(Smibert 1978)
C. hyointestinalis[b]	(Gebhart et al 1983)
C. concisus[b]	(Tanner et al 1981)
C. nitrofigilis[b]	(McLung et al 1983)

The aerotolerant Campylobacter-like organisms (Neil et al 1979)

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a The classification of catalase positive/catalase negative Campylobacter, with the exception of C. laridis (Benjamin et al 1983), the biotypes 1 and 2 of C. jejuni (Skirrow and Benjamin 1980b) and C. sputorum subsp. mucosalis (Lawson et al 1975a), is that of the official nomenclature in the A.L.B.N. (Skerman et al 1980).

b Their taxonomy is either not absolutely clear or they were described after the preparation of the A.L.B.N.

Table 2.3 shows the important characteristics of the two C. fetus subsp. The upper (A) part of the table presents those characteristics which are shared by both subspecies, while the lower part, those which are not shared. These latter characteristics, although not infallible, permit the differentiation of the two subspecies.

C. fetus subsp. fetus: This organism is tolerant to 1% glycine (Florent 1959, Veron and Chatelain 1973, Karmali et al 1981). Chang and Ogg (1971) have transferred glycine tolerance by transduction from C. fetus subsp. fetus to subsp. venerealis, which indicates that this test may not be a reliable differential test, however Smibert (1978) suggested that since the mutations to glycine tolerance are step wise (Chang and Ogg 1971) this test may still be useful. The organism is positive for the production of H_2S which is detected by using lead acetate strips suspended above both the 'sensitive medium' (Veron and Chatelain 1973, Butzler 1979) and TSI agar slants (cited in Kaplan 1980) and growth occurs in 0.1% sodium selenite which is also reduced (Veron and Chatelain 1973). Morphologically C. fetus subsp. fetus represents the 'medium' sized organism described by Karmali et al (1981a). A few isolates of this subspecies will grow at 42°C (Smibert 1978, Skirrow and Benjamin 1980, 1980b, 1980c, Smibert and Graevenitz 1980).

C. fetus subsp. venerealis: This organism is unable to grow in glycine and sodium selenite (Veron and Chatelain 1973). Although Veron and Chatelain (1973) found that this subspecies is generally negative for H_2S in the 'sensitive medium', they also observed a few positive isolates. Three to four percent of C. fetus subsp. venerealis isolates are weakly H_2S positive when detected with lead acetate strips (cited in Kaplan 1980). C. fetus subsp. venerealis isolates are weakly H_2S positive when detected with lead acetate strips (cited in Kaplan 1980). C. fetus subsp. venerealis represents the 'large' sized organism described by Karmali et al (1981). Neither of the two subspecies grew on plates supplemented with 0.1% T.T.C. (Veron and Chatelain 1973), although a few exceptions have been reported (Leaper and Owen 1981). Both subspecies are resistant to discs containing 30 µg nalidixic acid, but on the other hand, are sensitive to 30 µg cephalothin discs (Veron and Chatelain 1973, Skirrow and Benjamin 1980b, 1982; Karmali et al 1980, 1982).

TABLE 2.3 : CHARACTERISTICS OF C. FETUS SUBSP.

A : Characteristics shared by subsp. of C. fetus

	Catalase	Reduction of Nitrate	Reduction of Nitrite	H_2S (TSI)	NaCl 3.5%	Growth at 25°C	Growth at 42°C	Nalidixic 30 µg disc	Cephalothin 30 µg disc	Hippurate Hydrolysis	TTC 0.1%
C. fetus subsp. fetus	+	+	-	-	-	+	-(+)	R	S	-	S(R)
C. fetus subsp. venerealis	+	+	-	-	-	+	-	R	S	-	S

B : Characteristics permitting differentiation of subsp. of C. fetus

	Reduction of 0.1% Sodium Selenite	H_2S with Lead Acetate Strips: 0.02% Cysteine Hydrochloride	H_2S with Lead Acetate Strips: TSI	Glycine 1%	Amplitude/Wavelength of Spiral Forms
C. fetus subsp. fetus	+	+	+	+	Medium, 0.55 mm/1.80 mm
C. fetus subsp. venerealis	-	-(+)	-(+)	-(+)	Large, 0.73 mm/2.43 mm

\+ = Positive test or growth
\- + Negative test or growth
S = Sensitive
R = Resistant
S(R) = Sensitive few strains resistance
-(+) = Negative except for few strains positive

Thermophilic Campylobacter

Table 2.4 shows the most important characteristics of thermophilic Campylobacter which allow them to be distinguished from each other. C. fetus subsp. fetus is also included in the table, first because few strains grow at 42°C, and secondly as it is a cause of disease in humans, although not usually with gastroenteritis as are C. jejuni and C. coli.

The fact that resistance to nalidixic acid has been recently reported in an occasional strain of C. jejuni (cited in Benjamin et al 1983) suggests that this characteristic is not an absolutely reliable differential test. We were able to induce such C. jejuni strains in the laboratory. However, these strains still remain positive for hippurate hydrolysis and this could assist in the differentiation.

C. laridis have been reported to grow anaerobically in the presence of 0.1% trimethylamine N-oxide hydrochloride (TMAO) while all the other thermophilic Campylobacter and the C. fetus subsp. do not grow anaerobically in the presence of this substance (Benjamin et al 1983).

Differences in the susceptibility of 'thermophilic Campylobacter' to TTC have been observed. C. laridis (NARTC) has generally been reported to be sensitive to 0.1% TTC and resistant to 1.5% NaCl (sensitive to 3.5%), while the reverse, whilst not absolute, has been reported for isolates of C. jejuni and C. coli (Skirrow and Benjamin 1980a, Benjamin et al 1983).

Catalase Negative Campylobacter

Unlike the catalase positive Campylobacter, they are capable of reducing nitrates beyond nitrites and they produce H_2S in TSI agar (Loesche et al 1965). Table 2.5 shows the characteristics of Campylobacter sputorum subspecies.

C. sputorum subsp. bubulus grows well at 2.5% and 3.5% NaCl (Loesche 1965, Veron and Chatelain 1973, Smibert 1978), while C. sputorum subsp. sputorum does not (Smibert 1978). This latter subspecies only grows poorly in 2% NaCl (Kudzas and Morse 1956, Loesche et al 1965), but it grows in 1% bile, whereas the former does not (Veron and

TABLE 2.4 : DIFFERENTIATION OF "THERMOPHILIC CAMPYLOBACTER", AND C. FETUS SUBSP. FETUS

(KING 1962, SKIRROW AND BENJAMIN 1980b, 1982; KARMALI ET AL 1980, 1982; BENJAMIN ET AL 1983)

	Growth at 25°C	Growth at 42°C	Nalidixic Acid 30 µg	Cephalothin 30 µg	Hippurate Hydrolysis	H_2S in Iron/ Metabisulite Pyruvate (FBP) Medium
C. jejuni Biot. 1	-	+	S	R	+	-
C. jejuni Biot. 2	-	+	S	R	+	+
C. coli	-	+	S	R	-	-
C. laridis (NARTC)	-	+	R	R	-	+
C. fetus subsp. *fetus*	+	-(+)	R	S	-	-

\+ = positive
(+) = few isolates grow at 42°C.
S = sensitive
\- = negative
R = resistant

TABLE 2.5 : CHARACTERISTICS OF SUBSPECIES OF CAMPYLOBACTER SPUTORUM

(RECEIVED FROM SMIBERT 1978, MODIFIED)

Subspecies	Catalase	Nitrate Reduction	Nitrite Reduction	H_2S (TSI)	Growth at 25^o	Growth at 45^o	Yellow Colonies	Glysine 1%	NaCl 3.5%	Bile 1%
Sputorum	-	+	+	+	+	-	-	+	-	+
Bubulus	-	+	+	+	+	-	-	+	+	-
Mucosalis	-	+	+	+	Nk	-	+	-	-	-

+ = positive test or growth Nk = Not known - = negative test for growth

Chatelain 1973). Both subspecies grow in the presence of 1% glycine, but subsp. sputorum is less tolerant of this substance (Loesche et al 1965, Veron and Chatelain 1973). Subsp. sputorum and bubulus grow at 25°C and 37°C, but not at 45°C (Loesche et al 1965).

The third subspecies is C. sputorum subsp. mucosalis (Lawson et al 1975a), which unlike the other two subspecies, does not grow in the presence of 1% glycine (Lawson et al 1975a). Another important characteristic of this third subspecies, is that its colonies are a dirty yellow colour, whereas the colonies of the other two subspecies have a white to tan colour (Smibert 1978). Conversely, Lawson et al (1975a), indicated that the dirty yellow colour is a characteristic of all the subspecies of C. sputorum. It grows in 1.5% but not in 2.5% NaCl (Lawson et al 1976). Subsp. mucosalis grows at 37°C, but not at 45°C (Lawson et al 1975a), however there is now evidence that is also grows at 25°C (Smibert 1978).

C. faecalis, according to Firehammer (1965) and Smibert (1978), is catalase positive, does not reduce nitrites, is strongly H_2S positive and grows at 42°C, but not at 25°C. It is tolerant to 1% glycine and 2% NaCl. As H_2S production is a common characteristic of the catalase negative Campylobacter (Loesche et al 1965, Lawson et al 1975a), the positive catalase activity and the lack of nitrite reduction separates C. faecalis easily from them. The H_2S (TSI) production and the growth temperature separate this species from C. fetus while only the first test from C. jejuni, C. coli and C. laridis.

C. hyointestinalis is catalase positive and nitrite reduction is negative. These features distinguish it from the catalase negative C. sputorum. It is differentiated from C. fetus on the basis of being H_2S positive. The H_2S production, the non-susceptibility to nalidixic acid and the sensitivity to a cephalothin 30 µg disc permits its differentiation from C. jejuni and C. coli, whereas only the first and the last of these tests, permit differentiation from C. laridis. It is tolerant to 1% glycine and susceptible to 1.75% NaCl (0/15 isolates), while the majority of the isolates (11/14) grow well at 25°C and only a few isolates (3/14)

grow at 43°C and these, rather poorly (Gebhart et al 1983). On the basis of what is known about the biochemical characteristics of C. faecalis, only the susceptibility to NaCl and probably differences in growth temperature can assist in their differentiation.

C. concisus has been isolated from gingival crevices of humans. It is catalase negative, it reduces nitrates and nitrites and it is H_2S positive (Tanner et al 1981). The site of isolation of C. concisus is similar to the site of C. sputorum subsp. sputorum (Loesche et al 1965) and according to its biochemical characteristics as presented by Tanner et al (1981), it would probably be difficult to distinguish these two. But no significant DNA homology has been found between C. concisus isolates and reference strains of C. sputorum subsp. sputorum (Tanner et al 1981).

C. nitrofigilis is a nitrogen-fixing Campylobacter which has been described by McClung et al (1983). Unlike C. fetus it grows in the presence of 3.5% NaCl, and it is susceptible to nalidixic 30 µg disc and resistant to vancomycin. It can be easily differentiated from the 'thermophilic Campylobacter' by its ability to grow at 25°C and not at 42°C and its susceptibility to cephalothin 30 µg disc. Unlike C. sputorum and C. concisus, nitrogen-fixing Campylobacter are catalase positive. Compared with the latter one, C. nitrofigilis is susceptible to nalidixic acid and it grows on the presence of 3.5% NaCl.

The aerotolerant Campylobacter-like organisms were isolated from porcine and bovine abortions by Ellis et al (1977, 1978) and have been designated to the genus Campylobacter by Neil et al (1979). They are catalase positive, H_2S negative and did not grow at 42°C. However they are sensitive to nalidixic acid and grow aerobically after their primary isolation, features which distinguish them from C. fetus (Neil et al 1978). They can be easily differentiated from the 'thermophilic Campylobacter' because they don't grow at 42°C and unlike C. faecalis they are H_2S negative and they don't grow at 42°C. C. hyointestimals is H_2S positive and nalidixic acid resistant. C. sputorum and C. concisus are catalase negative and H_2S positive.

CAMPYLOBACTER INFECTIONS IN HUMANS

Human infections with Campylobacter are usually associated with C. fetus subsp. fetus (intestinalis), C. jejuni and C. coli (C. fetus subsp. jejuni), (Bokkenheuser 1979, Butzler 1979, and Retting 1979), and have two main clinical forms; systemic infection and a localised enteritis (Retting 1979).

Systemic Campylobacteriosis

Systemic campylobacteriosis is uncommon and only 134 cases have been documented up to early 1979 (Retting 1979, Bokkenheuser et al 1979). In older publications it is not always clear what species of Campylobacter was isolated, but more recent publications indicate that C. fetus subsp. fetus is the most likely cause of the condition (Bryner 1975).

Systemic campylobacteriosis occurs most frequently in the old or the very young and is rare in young adults (Bokkenheuser and Sutter 1978). King (1957, 1962), Bokkenheuser (1970) and Guerrant et al (1978) suggested that infection with C. fetus subsp. fetus (V. fetus) is more likely to occur in debilitated patients. Butzler (1979) also considered that this subspecies is an opportunist pathogen and that systemic infections were confined to debilitated patients with some pre-existing disease and impaired defence against infection.

The most common clinical manifestation of systemic campylobacteriosis is a bacteraemia without localised infection, and in such cases C. fetus subsp. fetus is easily isolated from the blood (Bokkenheuser 1970, File et al 1979, Hallet et al 1977, Kahler and Sheldon 1960, and King 1957). However, localised infections have been reported, (Butzler 1979) which include endocarditis (Loeb et al 1966, Chung and Lee 1970, King 1957), meningitis and meningoencephalitis (Eden 1962, Cubina et al 1976, Burgert and Hagstrom 1964, Collins et al 1964), septic arthritis (King and Bronsky 1961, Kilo et al 1965), pulmonary abscess (Targan et al 1977, Laurence et al 1971), and salpingitis (Brown and Sautter 1977, Retting 1979).

Pregnant women appear to be particularly susceptible to infection with C. fetus subsp. fetus and infection often results in premature

birth, abortion or stillbirth (Eden 1966), however males are twice as susceptible to infection as are females (Retting 1979). Death in adults is rare as the septicaemia usually responds to antibiotics treatment. If death occurs, it is usually associated with the deterioration of an underlying pre-existing disease (Butzler 1979). In infants and young children, systemic campylobacteriosis due to C. fetus subsp. fetus may be fatal (Eden 1966).

Consumption of foods including, raw beef (Chung and Lee 1970), raw liver (Soonattrakul et al 1971) and raw milk (Willis and Austin 1966), has been associated with three cases of systematic campylobacteriosis. More recently, the consumption of raw liver was associated with Campylobacter septicaemia in ten debilitated patients receiving 'nutritional therapy'. C. fetus subsp. fetus was isolated from blood cultures of nine patients and from the peritoneal fluid of one (Anon 1981).

Campylobacter Enteritis

Unlike systemic campylobacteriosis, over the last ten years Campylobacter enteritis has become recognised as a common disease of humans. Although Campylobacter spp have been associated with diarrhoea in humans for more than three decades (Levy 1946), it was not until 1973 in Belgium that they were shown to be an important cause of diarrhoea (Butzler et al 1973) and it was not for another four to five years, largely through the work of Skirrow (1977), that it became generally recognised that Campylobacter were a common cause of acute diarrhoea in humans. C. jejuni and C. coli, are the species most frequently associated with enteritis in humans, and although C. fetus subsp. fetus has been implicated with this type of disease, such occurrences are rare. Of 22,000 faecal specimens examined in Brussels in a five year period, C. fetus subsp. fetus was isolated from only three cases (Lauwers et al 1978), and was not associated with diarrhoea (Butzler and Skirrow 1979).

In England, C. coli accounts for only 5% of the total isolates from humans (Skirrow and Benjamin 1982) and 3% and 14% from Canada and Japan respectively, (Karmali et al 1983, Itoh et al 1982).

There is strong evidence that C. jejuni is a human enteric

pathogen (Blaser et al 1978, Butzler and Skirrow 1979, Steele and McDermott 1978, Karmali and Fleming 1979). In many developed countries, C. jejuni has been isolated from the faeces of 2.1% to 14% of patients with diarrhoea, compared with up to only 1.6% (Lauwers et al 1978) from healthy case controls. However, the situation is different in developing countries where, especially in children, C. jejuni has been isolated from up to 40% of both those with and without diarrhoea (Bokkenheuser et al 1979). Tables 2.6 and 2.7 list some of the major surveys on the prevalence of enteric Campylobacter in healthy persons and those with signs of enteric disease in both developed and developing countries. It also indicates the association of such infection with other enteric pathogens where such information was available.

The reason for the higher prevalence of infection in developing countries is not known (WHO/CDD/BEI/82.4). Prescott and Munroe (1982) discussed whether or not, there was an analogy between the differences in the ratio of infection to morbidity, in humans in developing and developed countries, and domestic stock kept in intensive conditions and animals kept as companion animals under higher standards of hygiene. They suggested that in domestic stock, sub-clinical infections due to C. jejuni, would be common, but in dogs, cats and horses, infection would be more likely to result in signs of enteritis.

Owing to the difficulties in knowing the time when infection occurred, it is difficult to precisely define the incubation period of human campylobacteriosis. Based on circumstantial evidence, Skirrow (1977), estimated the incubation period to be 2 to 11 days. Mouton et al (1982), presenting clinical data from an outbreak involving 13 patients, suggested that the mean incubation period was 3.1 days, with a range of 2 to 5 days. Other investigators suggested a similar range of 2 - 5 days (Butzler and Skirrow 1979, Blaser et al 1978, Oosterom and Becker 1982). Thus it appears that the incubation period is longer than that of most other common enteric pathogens. Some possible explanations for this comparatively long incubation period will be discussed later in this thesis (Chapter VIII).

TABLE 2.6 : ISOLATION OF INTESTINAL THERMOPHILIC CAMPYLOBACTER FROM FAECAL CULTURES OF PEOPLE WITH AND WITHOUT DIARRHOEA IN DEVELOPED COUNTRIES

Reference	Country	Population	WITH DIARRHOEA				WITHOUT DIARRHOEA	
			No. examined	% with Campylobacter	% with Salmonella	% with Shigellae	No. examined	% with Campylobacter
Butzler et al (1973)	Belgium	Children	800	5.1	-	-	1,000	1.3
		Adults	100	4.0	-	-	-	-
Skirrow (1977)	England	All ages	803	7.1(6.7)	-	-	194	0
Telfer-Brunton and Heggie (1977)	Scotland	All ages	196	8.7(7.1)	2.5	6.7	50	0
Bruce et al (1977)	England	All ages	280	14.0(12.2)	4.3	3.9	156	0.6
Watson et al (1979)	New Zealand	All ages	102	5.9(5.9)	1.0	1.0	-	-
Blaser et al (1980a)	United States	All ages	956	4.1	-	-	300	0
Mavromichalis et al (1980)	Greece	Children	134	4.5	-	-	110	0
Savopoulou (1980)	Greece	Children	779	7.2(7.0)	1.5	4.9[a]	72	0
Cavanagh et al (1980)	Australia	All ages	871[b]	12.0	20.0	4.0	-	-
Itoh et al (1982)	Japan[c]	Children	846	9.0	7.7	-	-	-
		Adults	840	4.6	2.7	0.1	-	-
Walter and Forsgren (1982)	Sweden	All ages	5571	6.9	4.1	1.7	2,000	0.25
McGechie et al (1982)	Hong Kong	All ages	587	4.6	7.2	-	-	-
Smilovitz and Kretzer (1982)	Israel	All ages	6258[d]	3.9	2.8	10.6	222	0
Kist (1982)	West Germany	All ages	3388	4.9[e](4.8)	-	-	-	-

For Legend - see next page.

Legends for Table 2.6

a Approximately 65% of the cases were from a common outbreak.
b Number calculated from a bar chart.
c Vibrio parachaemolydicus was recovered from 0.6% and 10.8% of the children and adults respectively.
d Patients with multiple gastrointestinal infections were excluded (Smilovitz and Kretzer 1982).
e Four of the patients were also infected with either Salmonella or Shigella (two from each species).
( ) Infected only with intestinal thermophilic Campylobacter.

TABLE 2.7 : ISOLATION OF INTESTINAL THERMOPHILIC CAMPYLOBACTER FROM FAECAL CULTURES OF PEOPLE WITH AND WITHOUT DIARRHOEA IN DEVELOPING COUNTRIES

Reference	Country	Population	WITH DIARRHOEA				WITHOUT DIARRHOEA	
			No. Examined	% with Campylobacter	% with Salmonella	% with Shigella	No. Examined	% with Campylobacter
Bokkenheuser et al (1979)	South Africa	Children (8-24 mths)	-	39	-	-	-	44
		> 2 yrs	-	44	-	-	-	16
De Mol (1982)	Zaire	Children						
		< 1 yr	140	19.9	-	-	55	5.4
		2-14 yr	171	5.9	-	-	145	1.5
Olusanya et al (1983)	Nigeria	All ages	412	12.4	5.6	10.2	83	2.4
Blaser et al (1980b)	Bangladesh	Children						
		1 year	-	-	-	-	18	38.9
		2 years	-	-	-	-	37	16.2
		3 years	-	-	-	-	46	19.6
		4 years	-	-	-	-	40	7.5

The signs and symptoms of Campylobacter enteritis in humans has been extensively described (Skirrow 1977, Butzler and Skirrow 1979), Karmali and Fleming 1979, Blaser et al 1978, Cavanagh et al 1980, Savopoulou 1980, Kist 1982, Pai et al 1979). In developed countries the disease is usually a moderate to mild, self-limiting condition rarely lasting more than one week, although recurrences of abdominal pain may occur. Diarrhoea is the commonest sign and usually lasts less than one week and develops 1 - 2 days after the prodromal signs. The prodromal signs occur in approximately two-thirds of patients and consist of a fever of up to 40^{o}C, central or lower abdominal pain, general malaise, headache, backache, myalgia and arthralgia. These latter signs occur more frequently in adults than in children (Kist 1982). The faeces rapidly become fluid, foul smelling, and often contain mucous and blood. Vomiting occurs in approximately one-third of cases. The abdominal pain may be similar to that associated with appendicitis. Table 2.8 summarises the major signs of disease associated with enteric Campylobacter infections.

The principle sites of infection in man appear to be the jejunum and ileum (Karmali and Fleming 1979). An autopsy of a chicken farmer, who died from Campylobacter enteritis, revealed a severe enteritis of the whole of the jejunum which was haemorrhagic and congested (King 1962). The colon also may be frequently affected (Price et al 1979, Blaser et al 1980c). Recently Karmali et al (1984) described an enterocolitis in four newborn infants in a nursery.

Untreated patients gradually eliminate C. jejuni from the intestinal tract. In a study of 230 recently infected patients, all were still infected at the end of the first week, approximately 55% at the end of the second week, 30% at the end of the third, 20% at the end of the fourth until by the end of the ninth week, only 0.5% were still infected (Wright 1982a). Similar results have been recorded by other workers (Svedhem and Kaijser 1980, Richardson et al 1982, Walder and Forsgren 1982).

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 (Blaser et al 1982, Butzler and Skirrow 1979, Walder and Forsgren 1982, Kist 1982) and the summer rates are

TABLE 2.8 : MAJOR SIGNS AND SYMPTOMS ASSOCIATED WITH CAMPYLOBACTER ENTERITIS IN HUMANS (%)

Signs and Symptoms	GERMANY Kist (1982)	GREECE Savopoulou (1980)	GREECE Mavromichalis et al (1980)	AUSTRALIA Cavanagh et al (1980)	CANADA Karmali and Fleming (1979)	JAPAN Itoh et al (1982)		SWEDEN Walder and Forsgren (1982)	BELGIUM Mouton et al (1982)
Diarrhoea	92(b)	100(a)	100(a)	100(b)	95(a)	100(c)	100(a)	100(b)	73(d)
Blood in faeces	33	-	-	40	92	2.6	32	12	-
Fever	69	73	17	68	86	50	76	35	27
Abdominal pain	65	33	17	67	60	47	58	87	87
Vomiting	31	63	67	50	30	2.6	49	-	-
Headache	38	-	-	-	-	-	-	-	-
Myalgia	22	-	-	-	-	-	-	-	-
Arthralgia	16	-	-	-	-	-	-	-	-
Appendicitic signs	6	-	-	-	-	-	-	-	-
Dehydration	-	33	67	17	-	-	-	-	-
Anorexia	-	53	-	-	-	-	-	-	-
Convulsion	-	5	-	-	-	-	-	-	-
Recurrent bouts of diarrhoea	-	-	17	-	-	-	-	-	-

(a) Children
(b) All ages
(c) Adults
(d) Common outbreak

more than double those which occur in the winter (White and Gill 1982). One possible explanation for the high summer rate could be that more people are involved in outdoor activities with a greater chance of direct or indirect contact with infected animals and contaminated environments. Although C. jejuni survives less well in a warm than a cold environment (Blaser et al 1980d), the very low infective dose of approximately 500 organisms (Robinson 1981) would tend to make such differences in environmental survival, of little consequence.

ANIMAL RESERVOIRS OF THERMOPHILIC CAMPYLOBACTER

Intestinal infection by thermophilic Campylobacter, similar to those which affect humans, appear to be common in a wide variety of domestic stock, companion animals and wildlife throughout the world.

BIRDS

Poultry

Most investigations have shown that C. jejuni is the most common species of thermophilic Campylobacter isolated from poultry flocks (Shanker et al 1982), Svedhem and Kaijser 1981, Munroe et al 1983, Cruickshank et al 1982). C. coli has also been isolated and Skirrow and Benjamin (1982) and Rosef and Yndestad (1982) showed that approximately 20% of isolates from poultry were C. coli.

In infected flocks, the published rates of infection have been usually high, but often the investigators failed to report the age of infected birds, the number of flocks examined, and other factors which might influence the rate of infection within a flock.

From the reports available, it would appear that many flocks of young chickens are infected with Campylobacter. In Canada, Munroe et al (1983) found that 7 of 26 broiler flocks were infected. In England, Cruickshank et al (1982) reported 3 of 14 flocks to be infected, whereas in Yugoslavia Mehle et al (1982) found 19 (70%) of the 27 flocks harboured the organism. Lower rates of infection have been reported from Scandinavian countries. In Norway, in a survey of 100 flocks of both young and old birds, 10 were found to be positive

(Rosef and Kapperud 1982), while in Sweden only 1% of chickens examined were found to be infected (Person 1981).

Infection in adult poultry has also been reported. Eight per cent of such birds in Denmark were reported to be infected (Jorgensen 1980) and 6.6% in Sweden (Person 1981). In Germany, Winderwerder and Maciak (1964) examined 110 laying hens from 3 flocks and reported 17 (12%) isolations from the gastro-intestinal tract. In Greece, Savopoulou (1980) reported two isolates from 37 laying hens from different sources.

C. jejuni was isolated from the intestines of 38 (83%) of 46 broiler chickens from a poultry market in New York city, (Grant et al 1980). Other investigators reported rates of isolation from 14 to 91%, either from intestinal or caecal contents; (Simons and Gibbs 1977; Prescott and Pruin Mosch 1981; Goren and de Jong 1980; Shanker et al 1982; Svedhem and Kaijser 1981; Bruce et al 1977; Banford 1982; and Ribeiro 1978).

Large numbers of Campylobacter occur in the intestine of clinical normal poultry. Grant et al (1980) reported a range from $5.6 \times 10^4 - 1.2 \times 10^7$/g in the rectal content with a mean of 4.4×10^6, but suggested that the number was probably closer to 10^8 as the filter technique used in their isolation procedure, was likely to retain approximately 99% of the organisms.

This high level of intestinal infection which is often more than 10^6/g of caeca content, frequently results in carcase contamination during processing (Wempe et al 1983). Therefore, it is not surprising that Campylobacter have been isolated from 1.7% to 94% of poultry carcases and their offals (Smith and Muldoon 1974, Shanker et al 1982, Park et al 1981, Wempe et al 1983, Simmons and Gibbs 1979, Christopher et al 1982a, Smeltzler et al 1981).

In Australia, Shanker (1982), using a whole carcase rinse technique on 40 chicken carcases, showed that 45% were contaminated, and the level of whole carcase contamination was up to 4.8×10^6 per carcase with a mean of 8.6×10^4. Similar levels of contamination were found by Bamford (1982). C. jejuni on contaminated chicken

carcases has been found to remain viable at 3 - 4°C (refrigerator) for 5 to 7 days (Smith and Muldoon 1974, and Mehle et al 1982). The same authors recorded survival at -23°C to -25°C for at least three weeks.

When fresh chicken carcases were experimentally inoculated with thermophilic Campylobacter and stored at -20°C, the number of organisms decreased by 5 to 100 times over a three month period (Hänninen 1981).

Turkeys

It appears, from the limited literature available, that turkeys are also an important reservoir of Campylobacter. Rosef and Kapperud (1982) reported that 4 of 6 turkey flocks examined were positive for Campylobacter. Although they did not state the rate of infection within flocks, as only pooled samples had been examined, three of the four isolates were C. coli and the remaining one C. jejuni. In Denmark, Jorgensen (1980) recovered C. jejuni from 100% of turkeys examined. In U.S.A., Luechtefeld and Wang (1981), examined caecal cultures from 600 turkeys from a poultry processing plant over a 1-year period, and found 100% to be infected. Examination of 36 caecal contents showed 1.2×10^4 to 1.5×10^7 organisms per g with a mean of 2.7×10^6/g. Although different techniques were used, this level of infection was similar to that reported in chickens by Grant et al (1980).

In England, Simmons and Gibbs (1979) and in U.S.A., Luechtefeld and Wang (1981) reported contamination of turkey carcases of up to 100% and 94% respectively. Increasing the chlorine levels of washing water from 50 to 340 ppm, decreased the number of contaminated carcases, but did not eradicate the problem (Luechtefeld and Wang 1981).

Ducks

In Canada, Prescott and Pruin Mosch (1981) isolated what they described as C. jejuni (but could have been C. coli), from 83 (88%) of 94 ducks at a processing plant. Similar results have been observed in Denmark (Jorgensen 1980).

Gulls, Wild Ducks and Other Wild Birds

Smibert (1969) was the first to isolate thermophilic Campylobacter from 11 of 15 sparrows, 1 of 5 starlings, 1 of 5 pigeons and one blackbird, which were trapped around sheep and cattle houses. (The author only used the colloquial names of birds used in the U.S.A.)

Gulls have been found to harbour several species of thermophilic Campylobacter (Skirrow and Benjamin 1980a, and Fenlon et al 1982). The former investigators were the first to report the high proportion of NARTC from seagulls (Larus spp), (twenty six (44%) of 59 isolates examined). Puffins have been reported to be exclusively infected with NARTC (Kapperud and Rosef 1983).

In England, Fenlon (1981a) isolated thermophilic Campylobacter from 20 - 70% of gulls, 45% of crows and 50% of urban pigeons. In Norway, Kapperud and Rosef (1983) examined cloacal swabs from 540 wild birds, captured from urban and non-urban coastal areas. They isolated Campylobacter from 11 of 40 species of bird. Crows, gulls, puffins and pigeons were the main species infected, but no isolates were recovered from seven house sparrows.

Campylobacter were isolated from approximately 35% of caecal specimens from 445 wild ducks (Luechtefeld et al 1980). These investigators also found that the rate of isolation from caecal contents was significantly higher than from cloacal swabs. Considerable variation in the rates of isolation of Campylobacter has been observed in different species of wild ducks. The highest rate (66%), was found in those ducks whose diet included a significant proportion of animal food, while the lowest (15.2%) was in those which fed almost exclusively on vegetable matter.

Wild birds probably constitute a major reservoir of natural infection of thermophilic Campylobacter.

PIGS

Contrary to other animals and humans, where C. jejuni is the predominant species and C. coli is less frequently isolated, the predominant species in pigs is C. coli (Skirrow and Benjamin 1980a,

1982). These authors (1982) found that C. coli represented 94% of their isolates, while in Canada Munroe et al (1983) found that 97.5% of their isolates from pigs were C. coli and only 2.5% was C. jejuni.

With the exception of one report (Prescott and Pruin Mosch 1981), the prevalence of infection of C. coli in the faeces of pigs is generally higher than 50%. However, the investigation by Prescott and Pruin Mosch (1981) recorded only two positive samples from 208 pigs examined.

In an early report from Scotland, Deas (1960) reported 15 C. coli isolates from the colon of 28 bacon pigs. In Germany, Oosterom (1980), Teufel (1981) and Sticht-Groh (1982) reported isolation rates of 61% from the intestinal contents of 300 pigs, 63% from the faeces of 97 pigs and 70% from the intestinal content of 110 pigs from abattoir surveys. In U.S.A. Luechtefeld and Wang (1982) isolated Campylobacter from 41 (66%) of the faeces of 71 pigs. In Canada, Munroe et al (1983), isolated 100 (69%) positive samples of a total of 144 pigs examined. Stern (1981) reported from 38 pigs an isolation rate of 87%, while Svedhem and Kaijser (1981) in Norway, a rate of 95%. Tuefel (1981) showed that in the majority of infected pigs, the level of infection in faeces was between 10^3 and 10^5/cfu/g. A survey carried out in German abattoirs and butcher shops showed that Campylobacter could be isolated from 40 - 50% of pig intestines, stored in brine overnight (Sticht-Groh 1982).

Stern (1981) reported the contamination rate of 58 fresh pig carcases to be 38%. In a survey conducted by the Central Public Health Laboratory, London (Turnbull and Rose 1982), 32 of 49 pork carcases at an abattoir were contaminated. However, these investigators stated that the levels of contamination were generally low. Hudson and Roberts (1982) examined 100 carcases at an abattoir and found 59 carcases contaminated. Subsequently, the same investigators found that chilling of carcases for 24 h at $0^{o}C$ at a 95% relative humidity, decreased the number of contaminated carcases. Even when samples were taken from moist areas where adjacent carcases had been in contact, surface counts were half of those obtained before chilling and were less than $1/cm^2$.

Minced pork meat experimentally contaminated at a level of 10^3 - 10^4 cfu/g stored at 4°C, resulted in no reduction in numbers at 12 h, but by 72 h, this was reduced to 5 x 10^2 cfu/g and the total aerobic bacterial count of the mince rose to 10^9/g (Teufel 1981).

CATTLE AND SHEEP

Thermophilic Campylobacter (C. fetus subsp. jejuni) are relatively common intestinal isolates from cattle and sheep (Smibert 1978). Such infected animals provide two potential sources of human infection; meat contaminated during the slaughter process and the faecal contamination of milk.

C. jejuni is the predominant isolate from both cattle and sheep, but with the exception of pigs and possibly chickens, the proportion of isolates of C. coli is greater than in most other species. Skirrow and Benjamin (1982), who were pioneers in the study of thermophilic Campylobacter, found 6% and 18% of their total isolates from cattle and sheep respectively to be C. coli. In Canada Munroe et al (1983) observed a similar proportion of C. jejuni and C. coli from diarrhoeic cows, however C. coli were not observed in healthy cows. Several surveys of the prevalence of intestinal Campylobacter of cattle and sheep have been carried out throughout the world with somewhat different results.

Cattle

Gill and Harris (1982) in New Zealand, Stern (1981) in U.S.A. and Oosterom and Becker (1982) in the Netherlands were unable to isolate any thermophilic Campylobacter from 65, 31 and 200 cattle at abattoirs respectively. Approximately a three percent prevalence rate was recorded from 36 milking cows by Banford (1982) in Australia. However Svedhem and Kaijser (1981) from Norway, Munroe et al (1983) in Canada and Luechtefeld and Wang (1982) in U.S.A. reported isolation rates of; 19% of 90, 25% of 421 and 43% of 130 cattle respectively. In England, Robinson (1982), in a long-term survey of two milking herds, found 10% of the cows examined to be infected, the level of excretion to be low and intermittent, the duration of excretion to be for several months and probably for life, and

transmission to occur between calves and young heifers, but not between adult members of the herd.

Thermophilic Campylobacter were isolated from 51 faecal samples (50%) of 127 diarrhoeic calves and from three healthy calves from 14 (56%) of 25 herds with enteric disease (Firehammer et al 1981). Gill and Harris (1982) reported a 50% rate of intestinal infection in each of three groups of a total of 50 unweaned calves at an abattoir and the numbers of Campylobacter in faeces to be 10^3/g or less.

Sheep

In New Zealand, thermophilic Campylobacter were isolated from the intestinal content of 20 and 35% of two of four groups of 70 sheep examined at an abattoir (Gill and Harris 1982). In another investigation in 1983-84, C. jejuni was recovered from the faeces of 28% healthy sheep (Gumbrell 1984, personal communication). However, Gill and Harris (1982) found only one isolate in two groups of 42, three to four-month-old lambs at an abattoir. Conversely, Stern (1981) in U.S.A. reported 11 (73%) isolates from the faeces of 15 lambs, of unrecorded age, at an abattoir, but Firehammer et al (1981) were unable to isolate thermophilic Campylobacter from the faeces of 36 diarrhoeic and healthy lambs from eight ranches in the U.S.A. Luechtefeld and Wang (1982) recorded an isolation rate of 23% C. jejuni from the faeces of 35 sheep from several farms. In earlier work Smibert (1965a, 1965b), isolated thermophilic Campylobacter from sheep between three months and eight years old, but not from younger animals.

Carcases

No Campylobacter were isolated from the carcases of 100 cattle and 100 sheep examined by Hudson and Roberts (1982) in England. Stern (1981) in U.S.A., reported isolates from one of 58 and 14 (24%) of 59 eviscerated unwashed beef and lamb carcases respectively. The prevalence of contamination was estimated to be between one and ten organisms/cm^2 from the flank (abdominal wall), but rump areas were apparently free of contamination. Overnight chilling was shown to decrease the number of contaminated carcases by 50% (Gill and Harris 1982).

In an extensive survey in England by the Public Health Laboratory

Service (Turnbull and Rose 1982) of 6169 meat samples, 1236 of which were from abattoirs and 4933 from retail and other outlets, C. jejuni was isolated from 98 (1.6%). Four percent were from abattoir samples and only 1% from other samples. In comparison Salmonellae were found in 4.5% of abattoir and 2.1% from retail samples. The results from this survey, which included beef, sheep and pork raw meat indicated that;

1. the contamination rate of raw red meat by C. jejuni was low,
2. the numbers of organisms in contaminated samples was low, and
3. increased temperatures in summer did not result in any apparent increase in the prevalence of contamination.

Milk

As already discussed, C. jejuni is a relatively common intestinal organism of cattle and sheep (Smibert 1978). Although C. jejuni cannot multiply in milk under either laboratory or natural conditions, Blaser et al (1980d) showed that milk is a suitable biological milieu for the survival of Campylobacter. He reported that C. jejuni is able to survive for up to 3 weeks at $4^{o}C$, but for no longer than 3 days at $25^{o}C$. Pasteurisation inactivates C. jejuni (Gill et al 1981, Doyle and Roman 1981, Christopher et al 1982b and Waterman 1982).

The infective dose of C. jejuni in milk is apparently small. Robinson (1981) was able to produce classical signs of infection by ingesting 500 organisms of a known serotype in 180 ml of pre-pasteurised milk. It is possible that milk protects the micro-organism during his passage through the stomach (Blaser et al 1980d).

The prevalence of C. jejuni in the faeces of diarrhoeic and healthy goats has been recorded as 3% (Prescott and Pruin Mosch 1981). Consumption of raw goats' milk could therefore be a source of human infection in countries such as Cyprus and New Zealand where such milk is often not pasteurised.

Although C. jejuni has been implicated in many milkborne outbreaks of

enteritis in humans (Blaser et al 1979a, Taylor et al 1979, Porter and Reid (1980, Robinson and Jones (1981) and Jones et al (1981), the organisms have never been directly isolated from the milk suspected to be involved in such outbreaks. In two occasions C. jejuni was isolated from milking machine filters on the farm from which the suspect milk originated (Robinson et al 1979 and Porter and Reid 1980).

Doyle and Roman (1982a) used an enrichment media with recovery limits of 1.0 organism per ml of milk, for surveying nine farms of a university dairy herd. C. jejuni was isolated from 0.9% of bulk milk samples over a three-month period and from 64% of faecal swabs from the same herd. Lovett et al (1983) using a different technique, but as sensitive as the former, reported a prevalence rate of 1.5% C. jejuni in bulk milk samples in a survey of 195 farms.

Clinical mastitis has been produced experimentally by the intramammary inoculation of a strain of thermophilic Campylobacter isolated from a child who had consumed milk which was suspected to be the source of infection. Campylobacter were reisolated in large numbers from the milk from the infected quarters, but milk from the uninfected quarters and blood and faecal cultures did not contain organisms (Lander and Gill 1980). However, a survey in the U.K. (Waterman and Park 1982) of 600 milk samples of cows with mastitis, failed to reveal the presence of C. jejuni in spite of the use of enrichment media.

DOGS AND CATS

There are considerable variations in the reported rates of isolation of thermophilic Campylobacter from dogs. Fereira et al (1979) and Fleming (1980) reported isolation rates of 5.5% and 18.7% in diarrhoeic dogs, but failed to obtain isolates from healthy animals. Sandstedt and Wierup (1981) and Vandenberche et al (1982) reported isolation rates of 47% and 14%, and 54% and 8% from dogs with and without diarrhoea respectively. The latter workers suggested there was a synergist relationship between parvovirus infections and C. jejuni. Blaser et al (1980a) demonstrated higher prevalence rates of C. jejuni in young dogs, compared with old dogs, irrespective of whether they were kept as pets in the house or in kennels.

Hosie et al (1979), Holt (1980) and Bruce (1982), were unable to demonstrate differences between diarrhoeic and non-diarrhoeic dogs, and recorded rates of 10% and 11%, 8% and 6%, and 39% and 38% respectively (the last results were from puppies). Prescott and Pruin Mosch (1981) failed to isolate C. jejuni from 66 dogs with diarrhoea, but obtained one isolate from 190 healthy animals. Jorgensen (1982) and McOrist and Browing (1982) reported isolation rates of 17% from diarrhoeic dogs, compared with 11.5% without diarrhoea and 16.5% and 9.5% respectively.

Prescott and Karmali (1978) failed to produce disease in conventional puppies and kittens by feeding them with a strain of C. jejuni isolated from a human case of gastro-enteritis. Prescott and Parker (1980) observed a mild superficial colitis, and mesenteric lymphatenitis in six, two to three-week-old, gnotobiotic beagle puppies.

The isolation rate in clinically normal cats was found by Blaser et al (1979b) and McOrist and Browing (1982) to be 5% and 3% respectively. The later investigators reported significantly higher isolation rates from the diarrhoeic cats (12%), whereas Gruffydd-Jones et al (1980) obtained only two isolates from 92 cats with diarrhoea and from 10 normal cats. Contrary to the previous results, Bruce et al (1980) reported a 45% isolation rate from 56 normal cats. They speculated that this high prevalence was partly due to the fact that among the positive cats, 9 were from 14 strays captured close to a poultry processing plant where they may have been eating contaminated poultry products.

Campylobacter are more frequently isolated from the faeces of stray dogs, than from household pets (Simpson et al 1981), and rates of infection increase in both young and old dogs when they are impounded for 5 - 7 days (Burnie et al 1983). Dogs are the only species of domestic mammal to be infected with a relatively high rate of NARTC (Skirrow and Benjamin 1980a, Wright 1982b).

OTHER ANIMALS

Eleven percent of 144 healthy rabbits examined by Prescott and

Pruin Mosch (1981) were infected with Campylobacter. Atherton and Rickets (1980) reported the isolation of C. jejuni from five foals suffering with diarrhoea, from which other pathogens were not isolated. Tribe et al (1979) reported an overall isolation rate of Campylobacter spp. of 17.8% from the faeces of recently imported healthy non-human primates and a rate of 61.4% from those with diarrhoea. The induction of an experimental enteritis in Rhesus monkeys (Macaca mullata) by the oral administration of a human isolate of C. jejuni has been reported (Fitzgeorge et al 1981). The diarrhoea was of short duration, but Campylobacter could be recovered from the faeces for 45 days. Bacteraemia was present for 2 - 3 days and resulted in localisation in the liver and gall bladder for 30 and 46 days respectively. The animals, when rechallenged with the same strain or organisms, showed no clinical signs, no bacteraemia and excreted the organisms in their faeces for only three days.

Tribe and Frank (1980) reported abortion in three pregnant monkeys (Macaca fascicularis), shortly after their arrival at premises where other infected monkeys were kept. Thermophilic Campylobacter, presumably C. jejuni, were isolated from vaginal swabs from all three animals, from two of the placentae and from the stomach contents of one fetus, (C. jejuni is also a known cause of abortion in sheep (Smibert 1978)).

ENVIRONMENTAL RESERVOIRS

It is obvious from the findings so far reported, that thermophilic Campylobacter are frequently isolated from a wide variety of domestic and wild animals. When these species are infected, they usually excrete large numbers of organisms in their faeces. This must result in a considerable degree of environmental contamination including water, which may act as a vehicle for transmission.

Kniel et al (1978), employing a membrane filtration technique, examined 84 water samples and isolated thermophilic Campylobacter from 7 of 34 sea water samples and 37 of 50 fresh water samples. All positive samples also contained Escherichia coli type I, which suggested that the Campylobacter were of faecal origin and were

unlikely to exist as independent saprophytes. Marcola et al (1981) also isolated thermotolerant Campylobacters from sewage and river water.

Blaser et al (1980d) investigated the survival of C. jejuni (C. fetus subsp. jejuni) in autoclaved mountain stream water. They found that if a sample contained 10^7 bacteria per ml, organisms survived for up to 33 days at $4^{o}C$, but for only 4 days at $25^{o}C$. Contaminated faeces and urine may act as environmental reservoirs of Campylobacter (C. fetus subsp. jejuni) as the organism can survive for 3 to 5 weeks in faeces and urine respectively when kept at $4^{o}C$. but for lesser periods at $25^{o}C$. Contrary to the other biological mileus, C. jejuni survives at $37^{o}C$. in bile for up to two months (Blaser et al 1980d).

INFECTED ANIMALS, THEIR PRODUCTS AND CONTAMINATED ENVIRONMENT AS A SOURCE OF INFECTION FOR HUMANS

The large number of animal reservoirs, the high contamination rates of animal products and a contaminated environment could all constitute sources of Campylobacter infection for man. However, only in a small proportion of human cases, is a definite source of infection (vehicle of transmission) established (Blaser 1982). Two major reasons for this problem are that Campylobacter survives for only a few days in contaminated vehicles at ambient temperatures, and the incubation period of the disease in humans is approximately three days. Thus by the time an investigation is initiated, viable organisms may no longer be present in the initially contaminated source of infected.

Poultry are believed to be an important potential source of Campylobacter for humans (Blaser 1982, Skirrow 1982). Similar biotypes (Skirrow and Benjamin 1982) and serotypes (Lior et al 1982, Kosumen et al 1982, Munroe et al 1983) of C. jejuni have been isolated from both humans and poultry.

The death of a poultry farmer with haemorrhagic necrotic enteritis involving the small intestine, was associated with thermophilic Campylobacter (King 1962). Jones et al (1982)

provided serological evidence of occupationally acquired infection in workers in poultry and duck processing plants. Skirrow (1977) reported an association of six patients with Campylobacter enteritis and contact with live poultry or poultry processing. A 14-year-old boy developed Campylobacter diarrhoea four days after plucking chickens, which were infected with C. jejuni (Schaefer et al 1979), and an infant developed Campylobacter enteritis after contact with pet chickens harbouring thermophilic Campylobacter (Bruce and Ferguson 1980).

Several outbreaks of human Campylobacter infections have been associated with the consumption or handling of fresh chickens. In Japan left-over chicken meat was found to contain the same serotype of C. jejuni as that isolated from all four affected persons from the ten at risk (Itoh et al 1982). In Belgium, 123 soldiers developed enteritis three days after killing, and processing chicken for their own consumption which were also undercooked (Brouwer et al 1979). Mouton et al (1982) found a statistically significant relationship between the consumption of chicken livers and the occurrence of the disease. They reported an outbreak involving 19 guests who attended a party in a restaurant where 13 of the 14 people who ate chicken developed diarrhoea. The non-affected case was a person who had suffered from travellers' diarrhoea several times.

In Germany, Kist (1982) in an investigation of 114 patients who had been infected with Campylobacter and 90 case controls, found that significantly more patients (22) had eaten poultry, particularly broiler chickens, 48 hours before the onset of illness, compared with healthy controls (9). In Sweden, Norkrans and Svedhem (1982) demonstrated a statistical association between the preparation of fresh chickens and Campylobacter enteritis. Seven of nine patients who had prepared and consumed chicken became ill while family members, although also eating the chicken, did not become affected. In the Netherlands, Severin (1982) found that infection was more frequently associated with undercooked chicken compared with those cooked for a longer time.

C. coli from pigs is considered an important reservoir for

human C. coli infections (Skirrow and Benjamin 1980a, 1982). In certain European countries where salted, smoked and lightly cooked pork and edible offals are popular foods, such pig food products may account for many of C. coli human infections (Skirrow 1982). Isolates of C. coli account for up to 15% of the total Campylobacter infections of humans in Belgium (Skirrow and Benjamin 1980a).

Pork pickled in vinegar was suspected to be the most likely source of an outbreak in Japan, affecting about 800 school children, who developed diarrhoea, from a total of 2500 children who had consumed the product (Yanagisava et al 1980). Peel and McIntosh (1978) reported a case involving a person and a dog in the same house, both of whom ate luncheon and pork meat and the dog died.

Blaser et al (1980c) reported a case of a young man who developed diarrhoea due to Campylobacter, shortly after he began work in a cattle feedlot. Similarly, Butzler and Skirrow (1979) associated the high prevalence of Campylobacter in Morrocans living in Brussells with their cultural habits of keeping sheep in their houses at times of religious feasting. The infection of a shepherd with C. jejuni, who attempted mouth to mouth resuscitation on weak or stillborn lambs, has been reported by Duffell and Skirrow (1978).

Beef hamburgers eaten raw, were attributed as the vehicle of infection in an outbreak of Campylobacter enteritis involving 34 patients in The Netherlands (Oosterom and Beckers 1982).

Consumption of unpasteurised or incompletely pasteurised milk appears to be the most important vehicle of human Campylobacter gastro-enteritis. Robinson and Jones (1981) reviewed 13 outbreaks in England and Wales associated with the consumption of raw or incompletely pasteurised milk involving more than 3,000 people. Although Campylobacter were not directly isolated from the suspect milk, on two occasions the same serotype of C. jejuni was isolated from milking machine filters, as was isolated from the affected patients (Porter and Reid 1980, Robinson et al 1979). In an outbreak in Luton in Bedfordshire, 2,500 school children were affected, and this constitutes the largest outbreak of enteric Campylobacter infection so far documented (Jones et al 1981).

Campylobacter milk borne infections have been reported in U.S.A. (Blaser et al 1979a), Australia (Bamford 1982) and Canada (McNaughton et al 1982), and in the Canadian outbreak, C. jejuni was isolated from the suspected milk. Similar biotypes (Skirrow and Benjamin 1980a, 1982) and serotypes (Lauwers 1982, Lior et al 1981, 1982, Munroe et al 1983, Bradbury et al 1984) of C. jejuni have been isolated from both humans and cattle.

Skirrow (1981) estimates that in England less than 5% of Campylobacter enteritis cases in humans are acquired from dogs and cats. Dogs, (Wheeler and Brochers 1961), Skirrow 1977, Blaser et al 1978, Lindquist et al 1978, Blaser et al 1980a, Bruce et al 1980, Smilovitz and Kretzer 1982, Norkrans and Svedhem 1982) and less frequently cats, (Skirrow et al 1980, Svedhem and Norkrans 1980) have been reported as sources of human infection. In Belgium Vanderberche et al (1982) found that several of their isolates from both diarrhoeic and non diarrhoeic dogs shared common antigens with C. jejuni isolates from humans, indicating that dogs may act as possible sources of infection for man.

Occasionally a newly acquired puppy or kitten has developed an acute diarrhoea and subsequently, within a few days, the members of the household, particularly babies or children, have developed diarrhoea (Skirrow 1981, Blaser et al 1978, Bruce et al 1980).

Water has been incriminated in two major outbreaks of Campylobacter infections. The first occurred in Vermont U.S.A. It was estimated from a survey of households that about 2,000 people from a total of 10,000 were affected. Consumption of water from the town supply and diarrhoea were strongly associated ($P < 0.005\%$). Campylobacter were isolated from five of nine rectal swabs examined. None of the 20 healthy controls were infected and no other pathogens were isolated. The regular water supplies were chlorinated but not filtered, but when reserves were low, supplementary non-chlorinated water was diverted to the town supply. There was no residual chlorine in the incriminated water from several areas of the town, which was examined during this investigation (Anon 1978).

A second suspect outbreak of water-borne infection occurred in Sweden (Mentzing 1981) affecting approximately 2,000 people. C. jejuni was isolated from 221 of 263 patients from whom faeces were cultured. The outbreak occurred during a short period indicating a common source. The water was filtered through sand and distributed unchlorinated, and circumstantial evidence strongly indicated that surface water was introduced into the town water supply. Although Campylobacter were not isolated from the water, it was thought that a nearby poultry farm could have been the source of contamination. Unfortunately no attempts were made to isolate Campylobacter from the poultry or from the environment of the farm.

A third smaller outbreak (Anon 1982) involving 81 people, was recorded in a community in Illinois, U.S.A. The outbreak occurred after a water main broke and the water presumably became contaminated. C. jejuni was isolated from the faeces of several ill persons. Water samples were collected late in the outbreak and no Campylobacter were isolated. The only sample from which an isolate was obtained, was taken six weeks after the outbreak from a house which had been unoccupied during and after the incident. It is not clear from the report, if this isolation was related with the outbreak, but presumably it was assumed to be, as the report emphasised the importance of 'stored' water samples such as ice, or water from fire hydrants or unused spigots as a source of infection. However, it is difficult to believe in the light of the work of Blaser et al (1980d), that the isolation of Campylobacter six weeks after the outbreak was related to the incident. More recently two water-borne outbreaks have been reported from Israel (Rogol et al 1983) and England (Palmer et al 1983). From the English outbreak, a similar serotype was recovered from both the water and six of eleven isolates from infected humans (Penner et al 1983a). In another epidemiological study, untreated water was considered to be a significant risk factor in relation to the development of Campylobacter enteritis in humans (Hopkins et al 1984).

The work of many of the investigators presented in this chapter indicates that human Campylobacter enteritis is a zoonosis, and that animals and contaminated foods of animal origin are a direct or indirect source of human infection. However, person to person

transmission, particularly between children, or from an infected child or infant to its mother (Skirrow 1977, Blaser et al 1980a, Norkrans and Svedhem 1982) or from a nurse to an infant (Lauwers et al 1978) have been recorded. Karmali et al (1984) reported transmission from mothers to their infants during parturition.

Patients, suffering with Campylobacter enteritis, rarely remain infected for more than six weeks although diarrhoea persists for less than a week. However, Norkrans and Svedhem (1982), found no evidence of transmission of Campylobacter by indirect contamination of food, by asyptomatic excreters. Two infected female cooks returned to their work after recovering from diarrhoea due to infection with Campylobacter. Ten days later, faecal examination of all the 63 children and staff at the day centre, served by the two cooks were bacteriologically negative for Campylobacter. Based on such evidence, Skirrow (1982) recommends, because of the low transmissibility of C. jejuni and C. coli, and their inability to multiply in food at room temperatures, that food handlers should not necessarily be excluded from their normal work, as soon as they have recovered from diarrhoea.

CHAPTER III

GENERAL MATERIAL AND METHODS

COLLECTION OF INTESTINAL MATERIAL FOR CULTUR AL EXAMINATION

Introduction

To determine the prevalence of intestinal thermophilic Campylobacter in animals, three types of intestinal material were examined; rectal or cloacal faeces, intestinal content from large and small intestine, and samples of fresh faeces.

In pigs and poultry, investigations were based on the cultural examination of rectal and cloacal swabs respectively. In wild ducks, southern black back gulls (Laurus dominicanus), and house sparrows (Passer domesticus), investigations were based on the examination of fresh faeces, and in Norway rats (Rattus norvegicus), on the examination of caecal contents. However, to test the relative accuracy of different sampling procedures for the recovery and enumeration of Campylobacter from the intestine, the results obtained from the different types of intestinal material from each type of animal examined, were compared (see Chapter IV).

PIGS

Rectal Swabs

Cotton wool swabs (Hospiswabs)[a] were used to collect rectal contents from pigs either at the farm, or at abattoirs immediately after stunning and before slaughter. Each swab was rotated within the rectum for approximately 30 seconds, ensuring that it was in contact with the mucosa.

Rectal swabs were transferred to the laboratory on ice and cultured within six hours (Chapter IV).

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[a] Medical Wire and Equipment Co. Ltd., Corsham, Wilts., England.

Intestinal Swabs

Intestinal swabs were taken from five sites, after the pigs had been eviscerated, approximately 20 minutes after slaughter. These sites were the middle of the duodenum, the middle of the jejunum, the terminal ileum, the caecum and the colon. Using aseptic techniques, a 2 cm incision was made through the wall of the intestine and a swab was inserted into the intestinal lumen. Swabs were transported to the laboratory on ice and cultured within six hours.

Colonic Contents

Colonic contents were collected in 100 ml plastic containers from the same site and in a similar manner to that of the colon swab.

POULTRY

Cloacal Swabs

Swabs similar to those used for pigs were used to collect material from the cloaca of live chickens except that they were first moistened in FBP broth. (The need to use swabs moistened in FBP broth is discussed in Chapter IV.)

Caecal Contents

Caeca were obtained from the undamaged intestinal tracts of birds following evisceration at a poultry processing plant. Both caeca were removed and placed in a plastic container, and transported on ice to the laboratory where they were cultured within six hours. The distal end of each caeca was incised and the contents expressed into a universal container. Swabs for direct culture were taken from this material which was also used for enumeration of Campylobacter. (As described in Chapter VI, there are no significant differences between the total number of organisms in each caecum).

DUCKS, SEAGULLS, SPARROWS

Faeces

Fresh faeces, from places inhabited by ducks, gulls and sparrows, were collected individually, in sterile universal bottles

with the aid of cotton wool swabs. Transport to the laboratory was in ice and cultures were carried out within six hours.

RATS

Caecal Swabs

Norway rats (Rattus norvegicus) from a rubbish tip at an abattoir, were shot with a .22 calibre rifle equipped with telescopic sights and a hand-held spotlight (see Chapter VII). The dead rats were transferred to the laboratory the same night and placed in a refrigerator at 4°C. The following morning, the intestinal tracts were removed, the caeca incised and a cotton wool swab inserted from which primary cultures were prepared. Caecal content was collected in universal containers when enumeration studies were required.

COLLECTION OF SAMPLES FROM THE CARCASES AND VISCERA OF POULTRY

Poultry Carcases and Edible Viscera

Whole carcases, livers, gizzards and hearts were collected from poultry known to originate from infected flocks, after processing at a local works. These specimens were taken after they had been through the spin chiller, were placed in plastic bags and cultured within six hours.

Chicken Wings

Fresh, chilled chicken wings, in packages of six, were obtained over a period of 5 weeks from a local supermarket. Some packs were cultured immediately, while others were kept in the refrigerator at 4°C for up to ten days and cultured periodically during this time (see Chapter VI).

COLLECTION OF WATER FOR CAMPYLOBACTER EXAMINATION

Water samples of 250 ml were collected in 500 ml sterile bottles from the drinking water for poultry between the holding tanks and the drinking vessels (see Chapter VI), were transferred to the laboratory in ice and cultured within six hours. In the case of water which had been chlorinated, samples were collected in bottles containing a

crystal of sodium thiosulphate which was introduced into the bottle prior to sterilisation. This should have neutralised the bacteriostatic properties of any free chlorine.

COLLECTION OF CAMPYLOBACTER ISOLATED FROM HUMANS

Isolates of Campylobacter from humans on 5% blood agar plates, were received from several medical laboratories within 12 hours of despatch. On receipt, these cultures were subcultured on 7% blood agar plates (see preparation of pure cultures) and after this, were subjected to the same tests as the animal isolates (see identification of Campylobacter).

CULTURAL METHODS

Antibiotic Selective Media for Isolation of Campylobacter

Two antibiotic selective media were used for the primary cultural isolation of Campylobacter. The first was a slight modification of the selective medium of Skirrow (1977). The modification being the use of Difco Columbia blood agar base and defibrinated sheep blood instead of Oxoid blood agar base No. 2 and defibrinated horse blood. Also 0.05% solution of the following were added as a supplement (FBP supplement) to the medium; ferrous sulphate ($FeSO4.7H_2O$), sodium metabisulphite and sodium pyruvate (see Appendix I). The addition of the FBP supplement had been shown to increase the aerotolerance of Campylobacter (George et al 1978) by quenching superoxides anions and other radicals in the medium (Hoffman et al 1979a, 1979b). The second selective medium was the same as the modified Skirrow's medium described above, but with the addition of cephalothin and aphotericin B, as these antibiotics are included in the Campy-BAP selective medium of Blaser et al (1978). The formula of the two selective media is indicated in Appendices I and II.

The first selective medium was used for most of the work with pigs and for part of the work with poultry. The second medium was used for some of the later work in pigs, for the majority of the work with poultry and all other investigations.

Culture of Intestinal and Faecal Swabs

The material on each swab was mixed with 0.5 ml sterile saline in a bijou bottle by rotating the swab within the bottle for approximately 30 seconds. Using the same swab, from which excess fluid was expelled by pressing it against the inner wall of the bottle, one half of the surface of a selective medium was inoculated. The swab was discarded and the inoculum was spread on the other half of the medium using a loop and following standard microbiological procedures in order to achieve well separated Campylobacter colonies.

Enumeration of Campylobacter in Intestinal Content

One to two grams of intestinal content or faeces were placed in 20 ml universal containers, and a series of ten-fold dilutions in saline were made. At each dilution, 0.1 ml was spread over two plates of selective media as described by Cruickshank et al (1975). Final counts were based on plates which contained between 30 and 100 well separated colonies of Campylobacter.

Quantitation of Campylobacter in Processed Poultry Samples

Poultry carcases, edible viscera and chicken wings were removed from the bags in which they were either packaged or collected, placed in new plastic bags and examined on the basis of a whole washing rinse technique. Three hundred millilitres of phosphate buffered saline (PBS)[a], pH 7.2, were added; half into the body cavity of the carcase and half on the outside. The carcase was shaken to rinse the body cavity and rubbed by hand through the bag to rinse the external surface. Edible viscera were massaged in a similar manner in a bag containing 20 ml of PBS. Four wings from a package of six were massaged, two at a time, in 40 ml PPS while the other two were discarded. One corner of a bag was wiped with 70% alcohol, cut with sterile scissors and as much of the fluid rinse as possible was allowed to drain into either 500 ml, or 80 ml sterile bottles. From the rinse fluid in each bottle, two serial tenfold silutions were made by transferring 1 ml at the rinse to 9 ml sterile saline. Of the initial and the diluted rinse, 0.1 ml was spread on duplicated modified Campy-BAP selective medium plates. The remaining procedures were similar to those used for the enumerational Campylobacter in intestinal content.

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[a] Calcium and magnesium free

Any liquid present in each of the original commercial packages containing the chicken wings, was individually collected in McCartney bottles and enumeration studies carried out by the tenfold dilution technique already described.

Culture of Water Samples

Water samples were examined by the method of Knill et al (1978). Fifty millilitres of water were passed through a 0.45 Millipore filter.[a] The membrane was placed on the selective plate and the plate was incubated at 37°C for 15 - 18 hours, in the microaerophilic atmosphere (see cultural conditions). The membrane was then removed and the plate reincubated for a further 30 - 36 hours. Colonies were identified by the procedures to be described later in this chapter (Identification of Campylobacter).

Cultural Conditions

The inoculated culture plates were placed upside down in either McIntosh and Fildes' or BBL Gaspak jars with no catalyst. The former jars were capable of holding 12 plates, while the latter up to 39. Using a water vacuum pump, the air pressure in the jars was twice reduced to 600 mm Hg lower than atmospheric pressure, and each time refilled with a mixture of 5% O_2, 10% CO_2 and 85% N_2.

Both antibiotic selective medium plates were incubated at 42°C for 48 h. Plates with no growth of Campylobacter at the end of this period were incubated for a further 24 h. Figure 3.1 schematically represents these initial cultural procedures.

IDENTIFICATION OF CAMPYLOBACTER

Presumptive Identification of Intestinal Thermophilic Campylobacter

Figure 3.2 outlines the criteria used to reach a presumptive identification of intestinal thermophilic Campylobacter. Suspicious colonies on the selective medium plates were examined by Gram staining and by dark field microscopy using wet

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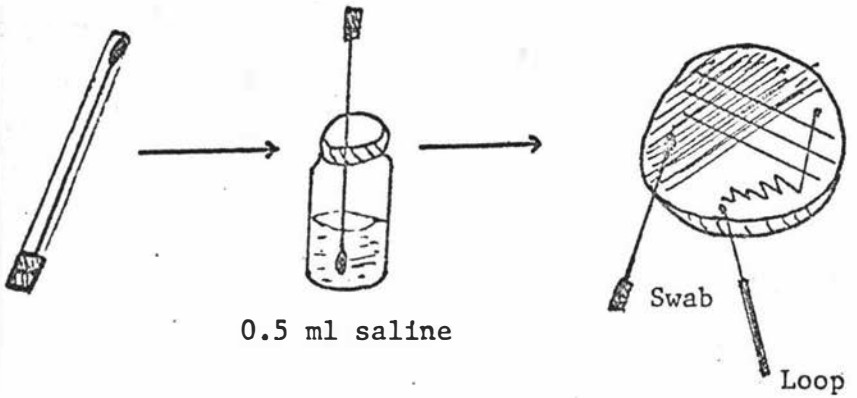
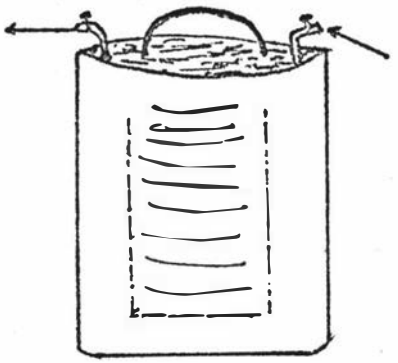
[a] Millibore Corporation, Bedford, Massachussets, 01730, U.S.A.

FIGURE 3.1 : A SCHEMATIC REPRESENTATION OF CULTURAL TECHNIQUES



ANTIBIOTIC SELECTIVE MEDIA

i. Skirrow's) Modified
ii. Campy BAP)



Filled with a MICROAEROPHILIC ATMOSPHERE (X2)

O_2 = 5%
CO_2 = 10%
N_2 = 85%

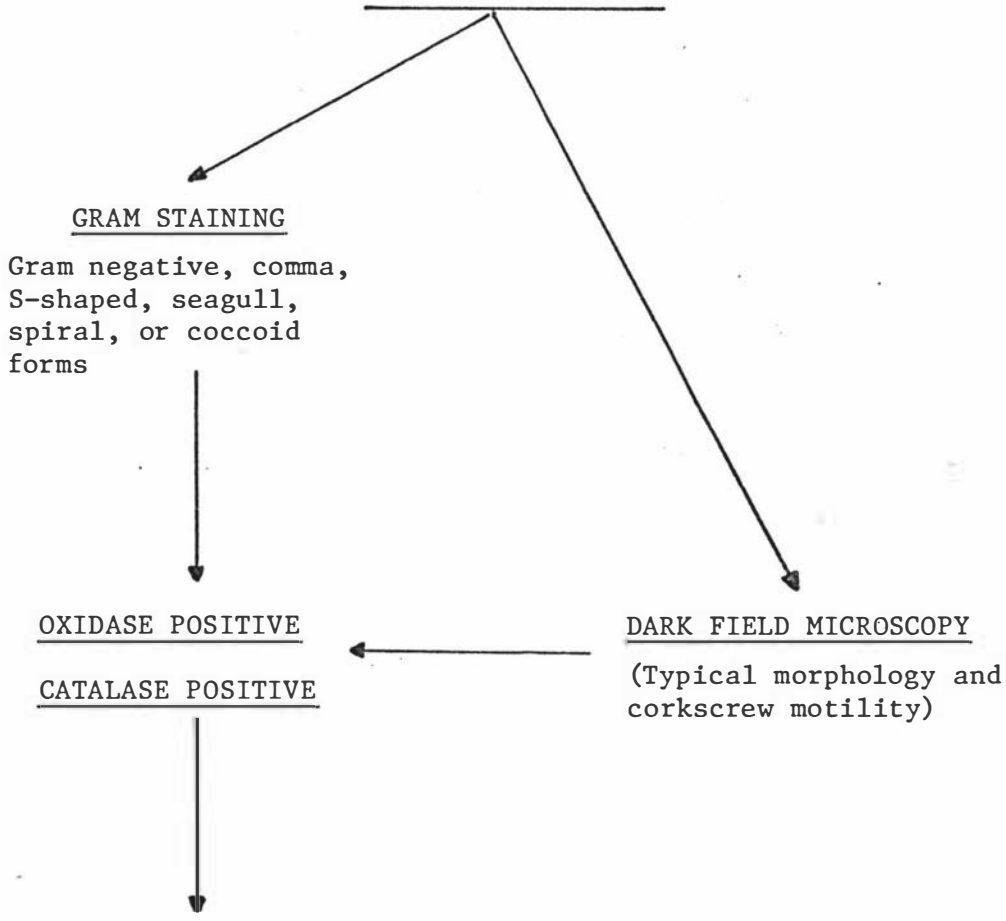
→ INCUBATION (at 42°C for 48 - 72 h)

FIGURE 3.2 : PRESUMPTIVE IDENTIFICATION OF INTESTINAL THERMOPHILIC CAMPYLOBACTER

Two colonial types:

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

(b) Circular, raised convex, shiny grey colonies 1 - 2 mm in diameter. No evidence of spreading.



preparations of the suspected micro-organism in saline and examined by a magnification of 600 times.

Oxidase reaction: The oxidase activity was tested by the method of Kovacs (1956). Filter papers were saturated with freshly prepared 0.5% aqueous solution of NNN' N'-Tetramethyl-p-phenylenediamine dihydrochloride[a] and dried. A visible amount of growth from a suspected Campylobacter colony, confirmed to be Gram negative and with characteristic morphology and motility was removed with a loop and smeared on the filter paper. A positive reaction (presence of cytochrome oxidase) was indicated by the appearance of a deep purple colour within a few seconds. No colour change was classified as a negative reaction (Veron and Chatelain 1973).

Catalase activity: The catalase activity was tested by a slide test. One drop of a 3.5% aqueous solution of H_2O_2 was added to a microscope slide, placed over a black background and a small amount of growth was transferred with a loop to the drop. The reaction was positive when effervescence and bubbles appeared within a few seconds (Skirrow and Benjamin 1980a).

Preparation of Pure Cultures

Preparation of pure cultures of presumptive intestinal thermophilic Campylobacter were made by subculturing individual colonies from the selective media on to blood agar plates (BA), (Difco Columbia blood agar base with 7% defribinated sheep blood), which were incubated at 37°C for 48 h. Alternatively, when the primary culture on the selective medium was heavily contaminated, an extra selective medium was used to aid the detection of individual colonies. These pure cultures provided the material for the subsequent tests.

Differentiation of Catalase Positive Intestinal Thermophilic Campylobacter

Table 3.1 lists the features forming the basis upon which the catalase positive intestinal thermophilic Campylobacter isolated at 42°C were differentiated. The growth temperature tests at 25°C and

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[a] BDH Chemicals Ltd., Poole, England.

TABLE 3.1 : DIFFERENTIATION OF CATALASE POSITIVE INTESTINAL THERMOPHILIC CAMPYLOBACTER

(isolated at 42°C)

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

	OBLIGATORY TESTS					NON OBLIGATORY TESTS		
	Growth at 42°C	Growth at 25°C	30 µg Nalidixic disc	30 µg Cephalothin disc	Hippurate hydrolysis	NaCl 3%	H_2S (TSI)	1% Glycine
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 grow at 42°C
+(-) = few isolates fail to grow at 25°C
S = sensitive
R = resistant
NK = Not known

42°C, the sentivities to nalidixic and cephalothin 30 µg discs and the hippurate hydrolysis tests were carried out routinely (obligatory tests). These tests are capable of differentiating between C. jejuni, C. coli and C. laridis (Skirrow and Benjamin 1980a, 1982 and Karmali et al 1980). However, if after the performance of these obligatory tests, an isolate could not be classified as one of the above species, the H_2S production in TSI agar and on lead acetate strips suspended over the TSI agar, the tolerance to NaCl and the growth in the presence of glycine were investigated (non obligatory tests). These later tests are capable of identifying C. fetus subsp. fetus, C. faecalis and C. hyointestinalis (see Table 3.1).

The growth temperature tests and the sensitivities to antibiotics were performed on BA plates alone and on BA plates supplemented with 0.05% FBP respectively (BA + FBP).

OBLIGATORY TESTS

Growth Temperature Test at 25°C and 42°C

A colony from a 48 h culture was subcultured on to three BA plates. Two of the plates were incubated at 25°C and 42°C for 96 and 48 h respectively. Visible growth along the sites of inoculation was recorded as positive. The third plate, which was heavily inoculated and incubated at 37°C for 48 h, was used for the hippurate hydrolysis and the antibiotics sensitivity tests.

Sensitivity to 30 µg Nalidixic Acid and Cephalothin Discs

The sensitivity to 30 µg nalidixic and cephalothin discs was determined by a disc diffusion test. Growth from the 37°C temperature test plate was removed on a 4 mm loop and closely streaked both horizontally and vertically across the surface of a BA + FBP plate. A 30 µg nalidixic acid and cephalothin discs[a] were placed on the surface of the inoculated plates. Incubation was at 37°C for 48 h. The absence of a clear zone of inhibition around the disc was considered to be indicative of resistance (Karmali et al 1980). The diameter of any zone of inhibition was also recorded to

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[a] Alpha Biologicals Ltd., Auckland, New Zealand

the nearest 0.5 mm with a graduated caliper. Plates 3.1 and 3.2 illustrate the 30 µg nalidixic acid and cephalothin disc diffusion test.

Hippurate Hydrolysis Test

The ability of Campylobacter to hydrolyse hippurate was tested by the method of Skirrow and Benjamin (1980b). With a 2 mm loop, growth from a 48 h 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[a] which was then incubated in a water bath at 37°C for 2 h. After a removal from the bath, 1 ml of a ninhydrin solution (3.5 g ninhydrin in 100 ml 1:1 mixture of acetone and butanol) was added, mixed and left on the bench for a further 2 h. A deep purple colour (crystal violet-like) was considered a positive result, whereas no colour change or a change to a light purple was considered as negative. As the work progressed, it was observed that the slow overlaying of the mixture with the ninhydrin solution was preferable because this resulted in the faster appearance of the deep purple colour which occurred initially in the overlay and later in the rest of the liquid. This method was found to be the most sensitive for identifying C. jejuni isolates, which are not strongly hippurate positive. Plate 3.3 shows the results obtained from both the hippurate hydrolysis tests.

NON OBLIGATORY TESTS

Hydrogen Sulphide Production

The butt and the slant of a triple sugar iron (TSI) agar in a tube were stab inoculated with a straight wire. A lead acetate indicator paper was suspended over the medium; the tube was capped and incubated at 37°C for 5 days under microaerophilic conditions. The appearance of a black colour either on the lead acetate indicator paper only, or in the medium and the indicator, was recorded as positive.

Growth in 1% Glycine

Five ml of Albimi brucella broth[a] with 0.15% agar as a base

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[a] Pfizer Diagnostics Div., Chas. Pfizer and Co. Inc., New York, N.Y. 10036, U.S.A.

PLATE 3.1 : ANTIBIOTIC DISC DIFFUSION TEST: ISOLATES OF C. JEJUNI SENSITIVE TO NALIDIXIC ACID AND RESISTANT TO CEPHALOTHIN

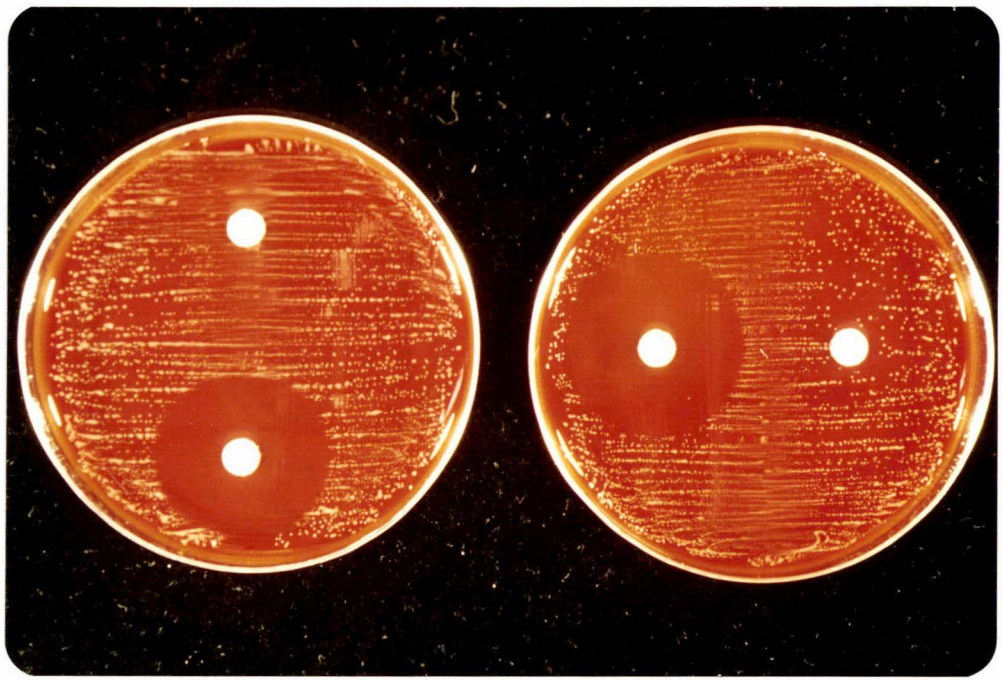


PLATE 3.2 : ANTIBIOTIC DISC DIFFUSION TEST: ISOLATE OF C. LARIDIS RESISTANT TO NALIDIXIC ACID AND CEPHALOTHIN

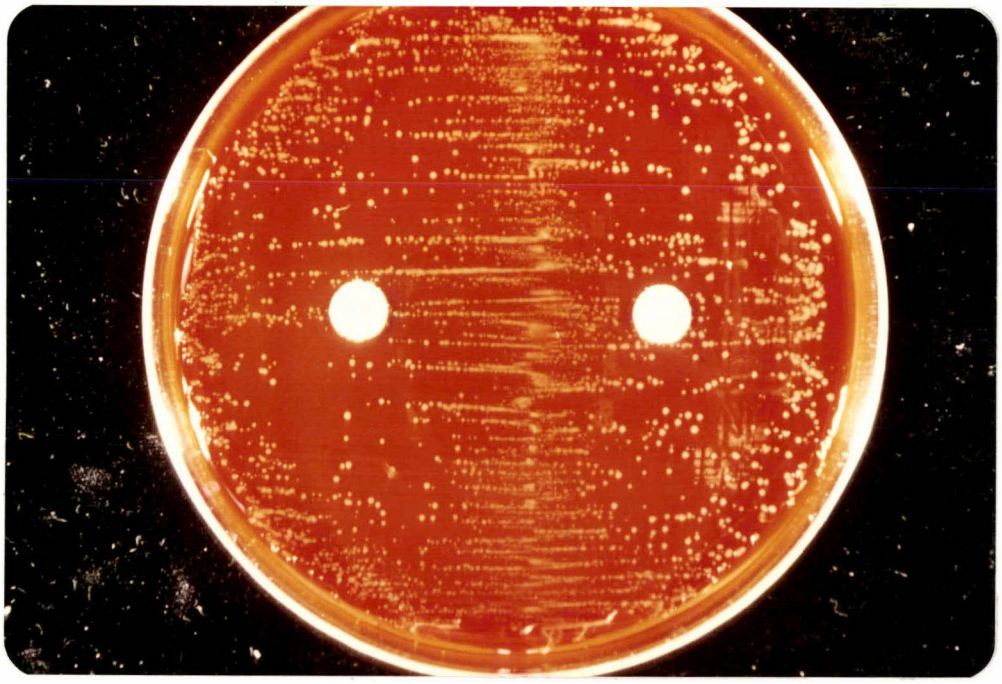
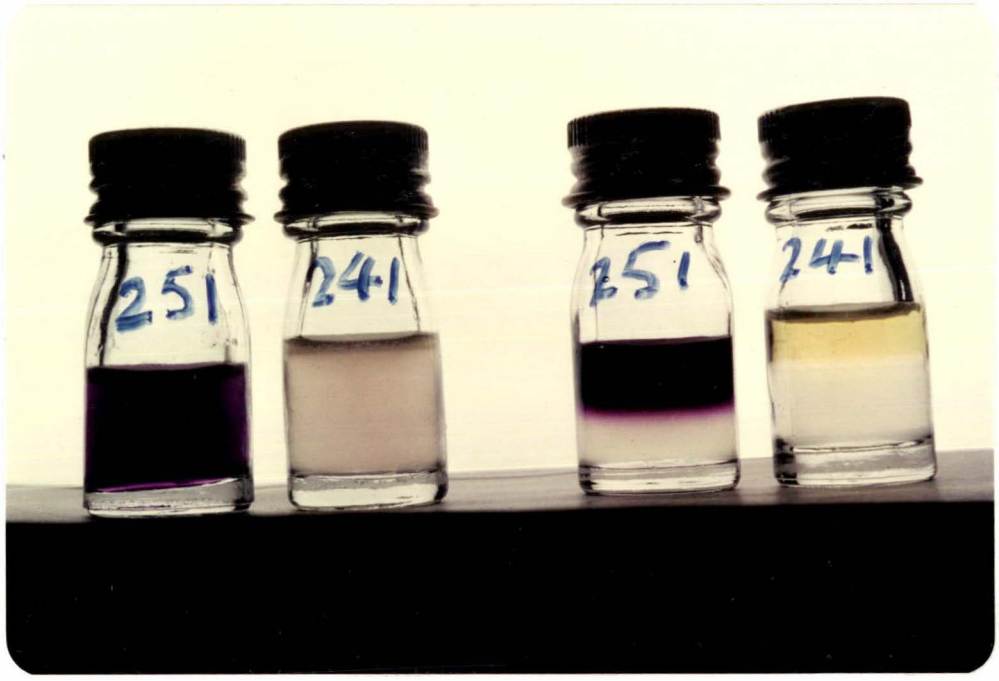


PLATE 3.3 : HIPPURATE HYDROLYSIS TEST



Deep purple denotes hydrolysis. Note the more sensitive overlay technique in culture 251 on the right (*C. jejuni* 251, *C. coli* 241).

and 1% glycine[a] in McCartney bottles were stab inoculated with Campylobacter growth using a straight wire. The test was read after six days' incubation at 37°C in aerobic condition and was considered positive when growth of the organism took place (Butzler 1979). Such growth was present in the medium a few millimeters under the surface, where microaerophilic conditions prevailed.

Tolerance to 3% NaCl

This test was performed in the same medium as that described in the preceding paragraph, but containing 3% NaCl instead of 1% glycine. Interpretation of results were similar as for those used in the glycine test.

H_2S Production in an Iron Metabisulfite Pyruvate Medium (FBP Medium)

The test was carried out by the method of Skirrow and Benjamin (1980b). A large loopful of an overnight growth of a pure culture of Campylobacter from a PA plate which had been incubated overnight at 37°C was inoculated as a single mass into the FBP medium (Appendix III). The tube of inoculated medium was left at room temperature for four hours and then the inoculated mass was examined for evidence of a change in colour to black.

Anaerobic Growth in the Presence of Trimethylamine N-Oxide Hydrochloride (TMAO)

The test was performed according to the method described by Benjamin et al (1983).

A small amount of a 24 hour growth on a PA plate incubated at 37°C in microaerophilic conditions, was removed with a 2 mm loop and stab inoculated with a straight wire, into a bottle containing yeast extract nutrient broth (YNB) supplemented with 0.1% trimethylamine N-oxide hydrochloride (TMAO) (Appendix IV). The bottles were incubated at 37°C and examined periodically for up to seven days. Growth throughout the medium in addition to growth just below the surface was considered as positive anaerobic growth. Growth restricted only a few millimeters below the surface of the medium was classified as microaerophilic growth (Razi et al 1981) and as a negative test result. Plate 3.4 illustrates this test.

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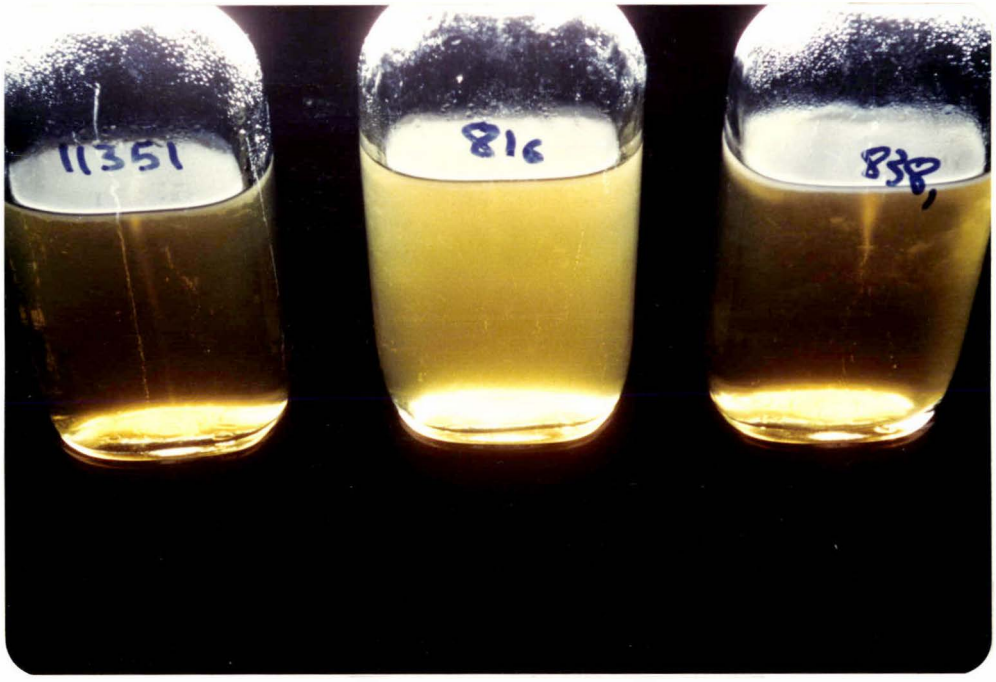
[a] Sigma Chemicals, St Louis, Mo 63178, U.S.A.

PLATE 3.4 : TEST FOR ANAEROBIC GROWTH IN THE PRESENCE OF TRIMETHYLAMINE N-OXIDE HYDROCHLORIDE (TMAO)



Bottle 816 shows anaerobic growth of '*C. coli*' from a gull. No growth of isolates of *C. jejuni* non-resistant and resistant to nalidixic acid is seen in the other two bottles. (See Chapter X for detailed explanations.)

Growth in the Presence of 1.5% Sodium Chloride (NaCl)

The test was performed according to the method of Benjamin et al (1983). Yeast extract nutrient agar (YNA) plates (Appendix V) were inoculated with Campylobacter. The plates were incubated at 37°C for 48 hours in the special atmosphere. Visible growth along the sites of inoculation was reported as positive (tolerance to NaCl).

These last three tests were used in the comparative taxonomic studies of C. coli, C. laridis and C. jejuni (see Chapter X).

PRESERVATION OF CULTURES

Pure growth of cultures on a BA plate, resulting from the incubation of a thermophilic Campylobacter at 42°C for 24 h or a non-thermophilic Campylobacter at 37°C for 48 h, were harvested in 3 ml FBP broth including 15% glycerol (see Appendix VI). Approximately 2 ml of recovered suspension were divided into three aliquots and placed in 1 ml microcentrifuge plastic tubes which had been washed with 70% alcohol and dried before use. These tubes were stored in small cardboard boxes at -70°C. The efficiency of the FBP broth in supporting the survival of Campylobacter at different temperattures is discussed in Chapter IV.

BACTERIAL RESTRICTION ENDONUCLEASE DNA ANALYSIS (BRENDA) TECHNIQUE

A bacterial restriction endonuclease DNA analysis (BRENDA) technique was used for subtyping different species of intestinal thermophilic Campylobacter. The initial steps are the preparation, extraction and digestion of the DNA organisms. The final stages of the technique involve electrophoresis of the digested DNA and photography of the resultant pattern of 'fingerprint' of the DNA.

The relative merits of the BRENDA technique compared with other typing systems are discussed in Chapter X.

Preparation of DNA

The growth of a pure culture of an isolate of intestinal thermophilic Campylobacter, on a PA and FBP plate incubated under

microaerophilic conditions at 42°C for 24 h, was harvested in 10 ml of PBS, pH 7.2. One heavily inoculated plate provides sufficient organisms (10^9 - 10^{10}) for the DNA analysis of an isolate. The harvest was centrifuged at 12100 g 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 TEB (Appendix VII), 100 µl of an aqueous solution of grade 1 lysozyme[a] (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[b] and 100 µl of an aqueous solution of protease[c] (10 mg/ml) were added and the mixture incubated overnight at 50°C. The next morning sodium perchlorate[b] was added to produce a final concentration of 1M, the mixture incubated for another hour and then made up to a volume of 5 ml with STE (Appendix VII). The preparation was extracted three times with a mixture of phenol, chloroform, and isoamyl alcohol (25:24:1) previously saturated with STE. After extraction, each sample was dialysised exhaustively against TE (Appendix VII) at 4°C and absorbance at 260 nm was measured by a spectrophotometer[d] in a quartz-glass cell with a 1 cm light path. From a standard curve using known concentration of calf thymus DNA (Sigma), 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 concentrations as measured by the two methods represented the contribution of RNA.

Restriction Endonuclease Digestion of DNA

Restriction endonuclease Hind III, 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 h in 100 µl enzyme buffer, pH 7.5 (Appendix VII). A bacteriophage C1857 S7 made from a lysogenic strain of E. coli (Miller 1972) with the DNA extracted by the method of Youghnsband and Bellett (1971) was used as a reference marker for each series of digest.

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a Sigma Chemicals Co., St Louis, Mo 63178, U.S.A.

b BDH Chemicals, Poole, England

c Calbiochem, San Diego, Ca 92112, U.S.A.

d UNICAM Spectophotometer, SP500

Gel Electrophoresis and Photography

Gel electrophoresis was performed by the method of McDonnell et al (1977). Each sample was placed in one of nine wells in a horizontal slab of 0.7% agarose[a] 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 for electrophoresis (Appendix VII) was also used to dissolve the agarose by boiling it 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 Sharp et al (1973). Gels were placed on a filter plate[b] measuring 15 x 15 cm, in a light box, illuminated from below by four 15W germicidal ultraviolet lamps[c], and photographed on Kodak Tri-X film by means of a 120 format plate camera through a Wratten 23A gelatin filter.

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

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

c Philips, Eindhoven, Holland.

CHAPTER IV

DEVELOPMENT OF TECHNIQUES

1. SURVIVAL OF CAMPYLOBACTER IN PIG FAECES ON SWABS

INTRODUCTION

The use of rectal and cloacal swabs is the most simple method of collecting samples of lower intestinal contents from individual animals for subsequent bacteriological examination, providing the time between taking the swabs and making primary cultures does not affect the survival of the micro-organisms under investigation. The viability of Campylobacter and in particular, thermophilic Campylobacter, is potentially sensitive to environmental conditions as they do not grow at less than 30.5°C, (Skirrow and Benjamin 1980a), and exposure to air causes rapid coccal transformation and degeneration (Karmali et al 1981a). This is in contrast to many other enteric pathogens where ambient temperatures and normal oxygen concentrations favour multiplication.

The objective of the following experiments was to study the survival of Campylobacter on swabs of rectal faeces, over periods of time which would be necessary for a survey of the prevalence of intestinal Campylobacter in pigs, in which swabs collected in the field would be transported to the laboratory and cultured several hours later.

MATERIALS AND METHODS

Source of Faeces

Approximately 250 g of faeces were collected in a plastic bag directly from the rectum of a pig from a nearby pig unit and transferred to the laboratory in less than 15 minutes. (The faeces of the 16-weeks-old pig were found to contain C. coli a few days earlier). On arrival at the laboratory, the presence of Campylobacter was confirmed by dark field microscopy. The faeces were thoroughly mixed and a small portion kept in the refrigerator at

4°C for later enumeration studies. The remainder was divided into two 100 ml plastic containers.

Types of Swab Used, Temperatures and Time Intervals

Fifty-six cotton swabs (Hospiswabs) which had been preweighed, were dipped and rotated in the faeces in one of the containers and weighed again. The same procedure was repeated with an equal number of swabs which had been kept in Amies semisolid transport medium (Transwabs)[a], using faeces from the other container. Half of the Hospiswabs and half of the Transwabs were kept at 25°C, while the other half were kept at 4°C. After intervals of one, two, four, six, 12, 24 and 48 h following their preparation, four of the Hospiswabs and four from the Transwabs kept at each temperature, were suspended individually in 2 ml of sterile saline and were streaked over the entire surface of plates containing modified Skirrow's selective medium. Enumeration of Campylobacter in the bulk faecal sample, was carried out on the faeces kept at 4°C, between one and two hours after collection.

RESULTS

Examination of the bulk faeces revealed 9.3×10^3 Campylobacter colony forming units (cfu) per g of faeces, and 15 randomly selected colonies were confirmed to be C. coli.

Table 4.1 shows the rates of survival of Campylobacter in rectal faeces on the two types of swabs, at the two temperatures. The maximum recoveries were obtained from swabs in the Amies semisolid transport medium kept at 4°C and the minimum from the cotton swabs with no transport medium kept at 25°C. The first failure to isolate from these latter swabs occurred at six hours and increased thereafter. The rates of survival on similar swabs kept at 4°C were better and the first definite negative result occurred at 48 h on hospiswabs. (At 12 hours one hospiswab was overgrown with a fungal contaminate which made detection of colonies of Campylobacter impossible.)

Table 4.2 shows the mean weights of rectal faeces taken on the swabs and the approximate numbers of Campylobacter colonies obtained from these swabs. Although it was not practical to undertake an

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[a] Medical Wire & Equipment Co. Ltd., Corsham, Wilts., England

TABLE 4.1 : DURATION OF DETECTION OF C. COLI IN FAECES ON SWABS WITH AND WITHOUT TRANSPORT MEDIA AT 4°C AND 25°C.

Time of Culture	25°C.		4°C.	
	No Transport Medium (Hospiswabs)	With Transport Medium (Transwabs)	No Transport Medium (Hospiswabs)	With Transport Medium (Transwabs)
1 h	$^4/_4$	$^4/_4$	$^4/_4$	$^4/_4$
2 h	$^4/_4$	$^4/_4$	$^4/_4$	$^4/_4$
4 h	$^4/_4$	$^4/_4$	$^4/_4$	$^4/_4$
6 h	$^3/_4$	$^4/_4$	$^4/_4$	$^4/_4$
12 h	$^2/_4$	$^4/_4$	$^3/_3$[a]	$^4/_4$
24 h	$^2/_4$	$^4/_4$	$^4/_4$	$^4/_4$
48 h	$^0/_4$	$^3/_4$	$^0/_4$	$^4/_4$

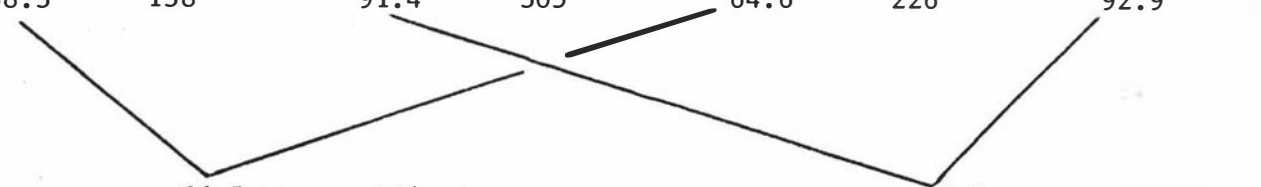
[a] One plate overgrown by fungi.

Note: Results expressed as fraction number of plates with growth of Campylobacter compared to total number of plates examined.

TABLE 4.2 : NUMBER OF COLONIES OF C. COLI DETECTED IN COMPARISON TO THE AMOUNT OF FAECES RETAINED ON DIFFERENT SWABS HELD AT 25°C AND 4°C FOR DIFFERENT TIMES

(Mean weights and numbers from groups of four swabs)

Time interval (hours)	Hospiswabs		Transwabs		Hospiswabs		Transwabs	
	Weight (mg)	No. of colonies	Weight (mg)	No. of colonies	Weight (mg)	No. of colonies	Weight (mg)	No. of colonies
1	62.5	62	97.5	76	65.0	67	92.5	78
2	65.0	38	80.0	68	75.0	69	87.5	58
4	65.5	16	95.0	42	70.0	29	85.0	48
6	87.5	11	92.5	36	62.5	26	100.0	47
12	65.0	7	92.5	31	62.5	16	92.5	40
24	65.0	4	95.0	39	62.5	19	92.5	33
48	62.5	0	87.5	13	55.0	0	100.0	27
Means or Totals	68.3	138	91.4	305	64.6	226	92.9	331



Overall Mean

66.5 mg + 2 ml saline — Dilution factor 30

92.1 mg + 2 ml saline — Dilution factor 22

absolute dilution technique, there was a good correlation between the relative reduction in the number of colonies and time. Figure 4.1 indicates the reduction in the numbers of Campylobacter obtained from the group of four swabs from each treatment group examined at each time interval.

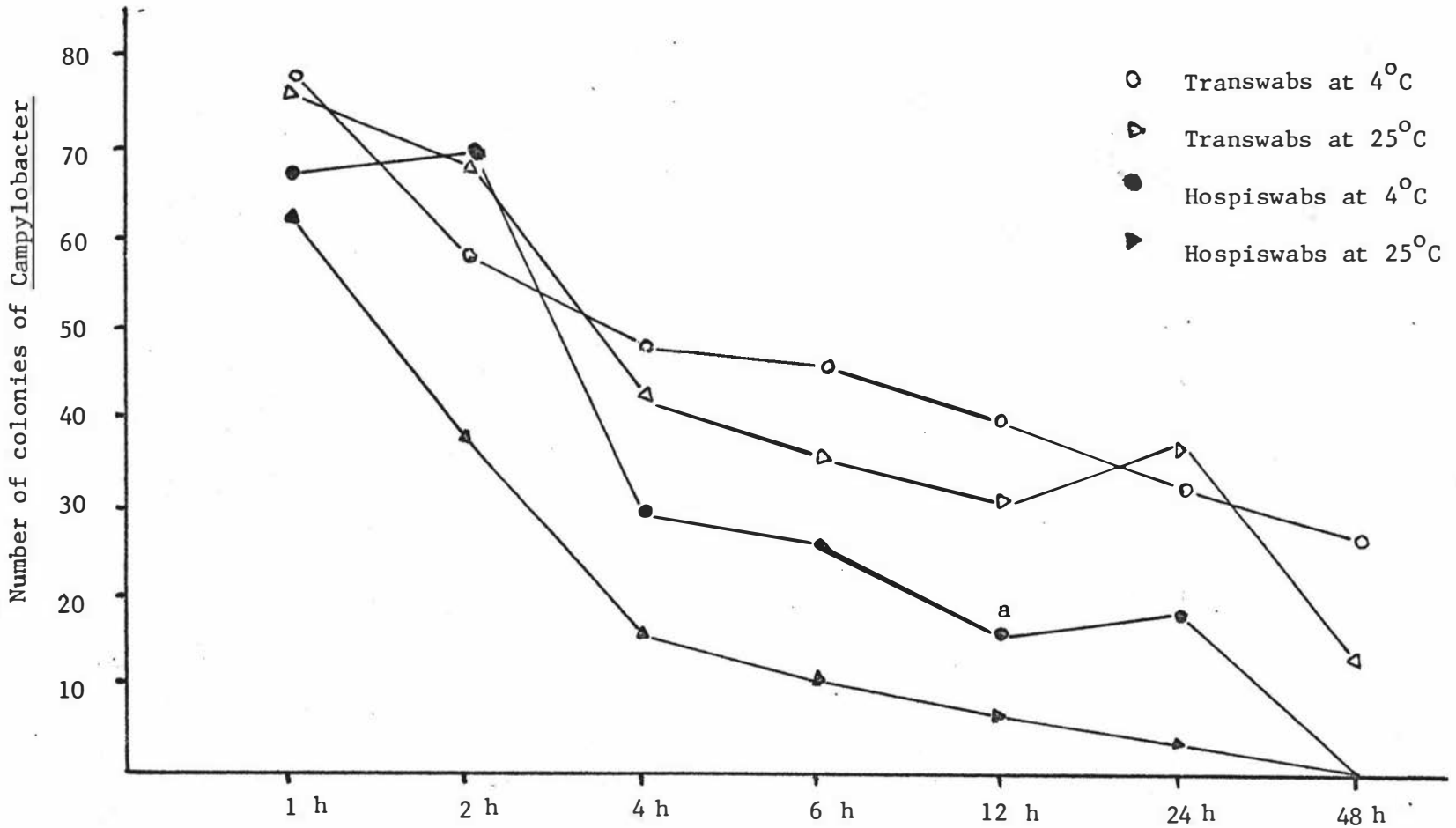
DISCUSSION

The faeces were divided into two separate containers to avoid the possible effects of the addition of small quantities of Amies transport media, on the survival of C. coli, in faeces on dry swabs. As the original sample of faeces was thoroughly mixed before being divided into two, it was assumed that both contained similar numbers of C. coli/g.

Although more faeces adhered to the transwabs, compared to the hospiswabs, resulting in a theoretical difference in the degree of dilution in the transport medium of 1 in 22 and 1 in 30 respectively, (see Table 4.2) this was not considered to have affected the results. This belief was based on the observation that approximately one third of the faeces from the transwabs remained in the transport medium while this loss did not occur with the hospiswabs. Thus differences in survival times were not apparently due to differences in dilution factors. Savopoulou (1980) stated that C. fetus subsp. jejuni remained viable in the faeces of a diarrhoeic child for at least 15 days when heavy suspension of these faeces was inoculated in the same transport medium as used in these experiments and kept at $4^{o}C$. Although the objective of this experiment was to investigate the survival of Campylobacter for up to 48 h only, it appears from the results, that survival for up to 15 days would not have been achieved. It is therefore probable that Savopoulou used a much larger inoculum and that the child's faeces contained more than 9.2×10^{3} Campylobacter per g of faeces. (Blaser et al (1980d) found that the numbers of C. fetus subsp. jejuni in the faeces of diarrhoeic patients were 6×10^{6} to 1×10^{9} per g of faeces.)

The increased survival of Campylobacter observed at $4^{o}C$ compared with $25^{o}C$ is in agreement with similar observations made by Blaser et al (1980d), Savopoulou (1980) and Luechtefeld et al (1981), working

FIGURE 4.1 : DECLINE IN NUMBERS OF COLONIES OF C. COLI CULTURED FROM DIFFERENT SWABS AND HELD AT 25°C AND 4°C FOR DIFFERENT TIMES



[a] Total of three swabs only, as the culture of the fourth swab was overgrown with a fungus

with human faeces and turkey caecal content respectively. Similar observations were made by Tanner and Bullin (1977).

One reason for the different survival rates observed on hospiswabs held at 25°C and 4°C could be associated with different rates of evaporation of water from the faeces on these swabs. It appeared that hospiswabs kept at 25°C started to dry out within a few hours of inoculation. By the 24 h and 48 h, they were almost completely dry. Conversely, hospiswabs held at 4°C were still moist at 24 h and had still not completely dried out at 48 h. In contrast to Campylobacter, Salmonella and Shigella survive well in dried faeces on filter paper strips, a method which can be used for the transportation of these pathogens (Bailey and Bynoe 1953, Ewing and Martin 1974). Luechtefeld et al (1981) found that C. fetus subsp. jejuni did not survive at room temperature in faeces dried on filter paper strips for more than two hours, and Doyle and Roman (1982a) also demonstrated that C. jejuni was sensitive to drying and storage at room temperature, but could survive for several weeks at refrigeration temperatures in a humid temperature.

The present results show that C. coli survives in rectal faeces on swabs without transport medium when kept at 4°C for at least 24 h and therefore the culture of such swabs within six hours is a reliable, economic and easy method to detect the presence of Campylobacter (C. coli) in faeces. If the time between collection of swabs and culture is longer than six hours, a transport medium should be used, and if faecal swabs from pigs are held in Amies semisolid transport medium at 4°C, satisfactory results can be obtained for up to 48 hours.

2. RECOVERY RATE OF CAMPYLOBACTER FROM DIFFERENT SITES OF THE G.I. TRACT OF PIGS

INTRODUCTION

After it was established that Campylobacter survived satisfactorily for at least 24 h in rectal faeces at 4°C on swabs with no transport medium, it was decided to investigate the sensitivity of culturing rectal swabs to determine the prevalence of intestinal infection of pigs, and the distribution of Campylobacter within the different parts

of the gastro-intestinal (G.I.) tract of pigs.

MATERIALS AND METHODS

Intestinal swabs (rectal, duodenal, ileal, caecal and colonic) were collected from a total of 13, four-months-old, bacon pigs on two consecutive days, (seven and six) at a local abattoir from animals originating from two different farms (A and B). The swabs were collected, transported and cultured as already described, except that the entire surface of the selective plate was inoculated from the original intestinal swab, after it had been immersed in 0.5 ml of sterile saline. Colonic contents were also collected from two pigs from each farm, for enumeration studies.

RESULTS

All pigs were infected with *C. coli* and four were concurrently infected with *C. jejuni*. Table 4.3 shows the rates of isolation of *Campylobacter* from different sites in the G.I. tract of pigs and Table 4.4 indicates the comparative degree of colonisation of the G.I. tract at the different sites investigated. Recovery rates from the small intestine were less than from the large intestine. Forty eight *Campylobacter* colonies were collected and subjected to more detailed investigation (one from each positive site of the G.I. tract). Five colonies, from four different pigs, were identified as *C. jejuni* and all were recovered from only the small intestine; four from the duodenum and one from the ileum. The remaining 43 colonies were confirmed to be *C. coli*.

In every pig examined, the highest rates of recovery were from the large intestines and the lowest from the small intestines with an increasing gradient of infection from duodenum to caecum and colon with the highest levels of recovery from the caecum.

The numbers of *Campylobacter* obtained from the colonic contents of the four pigs from the two farms, were 2.9×10^4, 8.8×10^4 and 2.1×10^2, 5×10^2 per g respectively.

TABLE 4.3 : ISOLATION RATE OF CAMPYLOBACTER FROM DIFFERENT SITES IN G.I. TRACT OF PIGS

Farm of Origin	No. of Pigs	Duodenum	Terminal Ileum	Caecum	Middle of Colon	Rectum (soon after Stunning)	No. of Campylobacter/ g of Colonic Content
A	7	3[a]	4[a]	7	7	7	$2.9 \times 10^4 - 8.8 \times 10^4$
B	6	1[a]	1	6	6	6	$2.1 \times 10^2 - 5 \times 10^2$
A + B	13	4	5	13	13	13	

[a] All the duodenal and one of the five ileal isolations (tested one colony from each site) were *C. jejuni*, the rest *C. coli*.

TABLE 4.4 : COMPARATIVE LEVELS OF CAMPYLOBACTER INFECTION WITHIN THE G.I. TRACT OF PIGS

Farm of Origin	No. of Pigs Examined	Duodenum	Terminal Ileum	Caecum	Middle of the Colon	Middle of the Rectum	Campylobacter per g of Colonic content
A	1	+ (a)	++ (a)	++++	++++	++++	
	2	+ (a)	+++	++++	++++	++++	8.8×10^4
	3	+ (a)	++++	++++	++++	+++	
	4	-	+	++++	++	++	
	5	-	-	+++	++	+++	
	6	-	-	++++	+++	+++	2.9×10^4
	7	-	-	++++	++++	++++	
B	8	-	-	++	++	++	
	9	-	-	++	++	++	
	10	+ (a)	++	+++	++	+++	
	11	-	-	+++	++	+++	
	12	-	-	+++	++	+++	2.1×10^2
	13	-	-	+++	++	++	5×10^2
A + B	13	4/13	5/13	13/13	13/13	13/13	

- No Campylobacter growth.
+ Light growth, less than 10 Campylobacter colonies/plate (in case of duodenum, one to three colonies).
++ Moderate growth, 10 - 50 Campylobacter colonies/plate
+++ Heavy growth, 51 - 200 Campylobacter colonies/plate
++++ Very heavy growth, more than 200 Campylobacter colonies/plate.
(a) C. jejuni isolations. All the others were C. coli (tested one colony from each site).

DISCUSSION

The observed levels of infection within the G.I. tract of pigs were similar to those recorded by Andress *et al* (1968a). These investigators were attempting to reproduce swine dysentery, by the oral administration of *V. coli*, (presumably *C. coli*) to three to eight-week-old gnotobiotic pigs. Although they failed to reproduce the disease, they examined the G.I. tract of 14 experimental pigs, a few days to three weeks post challenge. Light to moderate levels of infection were recorded from the duodenum and jejunum, and steadily increasing levels with progression distally down the intestinal tract, with highest levels in the caecum and colon. They also recorded, similarly to the results in this thesis, 100% recovery rates from the caeca and colon of the 14 pigs and 100% recovery rate from the 135 rectal swabs examined during the course of the three week experiment.

Although Andress *et al* (1968a) found lower rates of isolation from the small intestine compared with the large intestine, their recorded rates of infection from the small intestines, were approximately two and a half times higher than those recorded in the present work. This can be attributed probably, to the use of gnotobiotic pigs without any other gut flora which might inhibit the growth of *C. coli*. An alternative explanation is, that the four-month-old pigs examined in the present study may have been infected for longer than the experimental gnotobiotic pigs, (only a few days to three weeks) and some degree of natural elimination of *Campylobacter* from the proximal region of the G.I. tract may have started to occur.

Deas (1960) examined pigs which had died subsequent to attacks of severe diarrhoea and although he did not state the number of organisms present, he reported that although *Campylobacter* (vibrios) were recovered readily from the caecum, colon, rectum, they were less readily isolated from the ileum. Thus, there appears to be general agreement that *C. coli* in pigs is essentially a large intestinal inhabitant.

Conversely, *C. jejuni* was recovered from only the small intestine and their low numbers tend to indicate that the G.I. tract of the pig is not their natural niche. It is also interesting to speculate that

their presence might also be associated with an absence of C. coli. It is possible that C. jejuni was also present in the large intestine, but their growth and isolation was not detected due to the higher numbers of C. coli present in this part of the intestine. Alternatively, it is possible that the C. jejuni isolates had been ingested a few hours before culture and that their presence was not an indication of an active infection, but merely passive contamination.

A similar lack of recovery of C. jejuni was noted in a more extensive study of the G.I. tracts of 30 pigs reported in Chapter V. Although the objectives were different, the results relating to the prevalence and distribution of C. jejuni were similar to those recorded in this chapter.

3. LONG-TERM STORAGE AND PRESERVATION OF C. JEJUNI

INTRODUCTION

It was expected that during the course of this work, many isolates of Campylobacter would be recovered from animals and humans, which after preliminary identification, would need to be preserved for periods of more than a year for more detailed investigations and classification (see Chapter X).

Previous work indicated that Campylobacter are able to survive for long times at temperatures below $0^{o}C$. Wang (1981) stated that cultures of Campylobacter in glycerol-brucella broth remain viable for several years when they were preserved at $-70^{o}C$. Skirrow and Benjamin (1980a) preserved their cultures in FBP broth which included 15% glycerol, either in liquid nitrogen ($-179^{o}C$) or at $-20^{o}C$. However, neither of these reports gave details of the procedures used.

The overall aim of the work reported in this section was to determine an effective and economic method for the long-term storage of cultures of Campylobacter. Secondary objectives were to study the effect of different media on the survival of cultures of Campylobacter in the laboratory and to study the effect of temperature and time of storage on the survival of such cultures.

MATERIALS AND METHODS

Culture Studied

C. jejuni isolated from a poultry farm (flock No. 23a, see Chapter VI) which also appeared to be a strain often isolated from humans (see Chapter IX and X) was selected for use in these experiments.

Suspending Media

A 24 h pure growth of the isolate (No. 218) incubated at 42°C on a BA plate was used to heavily inoculate 80 BA plates which subsequently were incubated at 42°C for 24 h. The growth from 40 of these plates were harvested with PBS pH 7.2 (5 ml per plate) and the other 40 with FBP broth (5 ml per plate). The total harvest from each group of plates (approximately 150 ml) was collected separately into two 200 ml sterile bottles, mixed well and divided in aliquots of 0.5 ml in 300 sealed plastic microcentrifuge tubes.[a]

Storage Temperatures

Of the 300 tubes of each suspending medium, two groups of 50 were stored at 25°C and 4°C, and two groups of 100 at -20°C and -70°C.

Storage Times

Samples from five of the tubes kept at 25°C and 4°C were cultured at 24 h, and 2, 3, 7 and 10 days and at subsequent five day intervals for up to 60 days. A similar system was used for tubes stored at -20°C and -70°C, which were cultured at 24 h, 7 days, one month, three months and subsequently every three months until two consecutive sets of five tubes failed to reveal the presence of *C. jejuni* after culture.

Culture and Enumeration

The suspension in each of the five aliquots was well shaken in a mechanical mixer. The tubes were opened and a sterile cotton swab inserted, which was used to directly inoculate half the surface of a BA plate. (The other half of the plate was inoculated with a swab from another tube.) The remaining suspension from all five tubes in a group was collected in a sterile bijou bottle, mixed well and an enumeration of the viable *C. jejuni* cells was carried out according to methods previously described, except for the use of BA plates instead of a selective medium. Incubation was at 42°C for 48 h.

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[a] Eppendorf, Reaktionsgefasse 1.5 ml, Micro test tubes 3810. Postf. 650670.2000 Hamburg 65, W. Germany.

RESULTS

The duration of survival of C. jejuni in PBS are shown in Table 4.5 and FBP in Table 4.6.

Of the two suspending media examined, FBP broth appeared to support survival better than PBS. All suspensions in PBS held at 25°C, 4°C and -20°C were negative by 48 h, 10 days and 3 months respectively, whereas at these corresponding temperatures the FBP replicates were negative by 10 days, 45 days and 12 months respectively. At -70°C, both suspending media preserved viable organisms in all replicates for 21 months at which time the experiment was terminated. By this time it was noted that two of the PBS replicates yielded less than 30 colonies per plate. At this time (21 months), the inactivation factor (IF) for PBS was 503, while for FBP, it was only 3.4; a 148 times difference in favour of FBP broth. (The IF of the suspending medium for a particular time interval is calculated by dividing the initial count by the count at the end of the period of investigation.)

Except for the 24 h interval, at all other time intervals where enumeration of C. jejuni either in PBS or FBP broth was undertaken, the higher the temperature of preservation, the lower was the rate of survival. At the 24 h period, storage at 4°C resulted in a higher rate of survival than storage at -20°C, -70°C in PBS, and -20°C in FBP broth.

DISCUSSION

The superiority of FBP broth as a storage medium compared with PBS was to be expected. Although the latter is a good buffer, it has no nutritional value, while FBP broth contains nutrient broth and agar. Agar added to broth at concentrations of between 0.12% and 0.16% creates microaerophilic conditions which are particularly suitable for the growth and survival of Campylobacter (Butzler 1979, Luechtefeld et al 1981). As already discussed (Chapter III), the addition of 0.05% of FBP supplement increases the aerotolerance of these organisms (George et al 1978), while glycerol has cryo-protective properties, which have been found to increase the viability of C. jejuni when added to frozen ground beef (Stern and Kotula 1982).

TABLE 4.5 : SURVIVAL OF C. JEJUNI SUSPENDED IN PHOSPHATE BUFFERED SALINE (PBS) HELD AT DIFFERENT TEMPERATURES AND EXAMINED AT DIFFERENT TIMES (INITIAL COUNT 3.47×10^8 cfu PER ML OF PBS)

Time of Culture	25°C	4°C	-20°C	-70°C
24 h	5/5 (2.34×10^4)	5/5 (4.35×10^7)	5/5 (1.07×10^6)	5/5 (2.21×10^7)
48 h	0/5	5/5 (2.71×10^6)	ND	ND
96 h	0/5	5/5 (2.69×10^6)	ND	ND
7 days		2/5 (2.19×10^4)	5/5 (5.32×10^5)	5/5 (2.19×10^7)
10 days	ND	0/5 (10^2 neg)	ND	ND
15 days	ND	0/5 (10^2 neg)	ND	ND
1 month	ND	ND	3/5 (6×10^2)	5/5 (5.4×10^6)
3 months	ND	ND	0/5 (10^2 neg)	5/5 (5.0×10^5)
6 months	ND	ND	0/5 (10^2 neg)	5/5 (4.4×10^6)
9 months	ND	ND	ND	5/5 (3.2×10^6)
12 months	ND	ND	ND	5/5 (ND)
15 months	ND	ND	ND	5/5 (1.2×10^6)
18 months	ND	ND	ND	5/5 (ND)
21 months	ND	ND	ND	5/5 (6.9×10^5)

The nominator of the fraction represents the positive tubes, while the denominator represents the number of tubes examined.

In parenthesis is the cfu of C. jejuni per ml of suspension concentrated from aliquots of five tubes subsequent to their subculture.

TABLE 4.6 : SURVIVAL OF C. JEJUNI SUSPENDED IN FBP BROTH HELD AT DIFFERENT TEMPERATURES AND EXAMINED AT DIFFERENT TIMES (INITIAL COUNT 5.15×10^8 cfu PER ML OF FBP BROTH)

Time of Culture	25°C	4°C	-20°C	-70°C
24 h	5/5 (1.16×10^8)	5/5 (4.23×10^8)	5/5 (2.0×10^8)	5/5 (4.9×10^8)
48 h	5/5 (4.3×10^7)	5/5 ND	ND	ND
96 h	5/5 (1.31×10^4)	5/5 ND	ND	ND
7 days	4/5 (4.5×10^4)	5/5 (3.83×10^7)	ND	ND
10 days	0/5 (10^2 neg)	5/5 ND	ND	ND
15 days	0/5 (10^2 neg)	5/5 ND	ND	ND
20 days	ND	5/5 ND	ND	ND
25 days	ND	5/5 ND	ND	ND
30 days	ND	4/5 (3.1×10^4)	ND	5/5 (3.5×10^8)
35 days	ND	2/5 ND	ND	ND
40 days	ND	2/5 (10^2 neg)	ND	ND
45 days	ND	0/5 (10^2 neg)	ND	ND
50 days	ND	0/5 ND	ND	ND
3 months	ND	ND	5/5 (3.1×10^2)	5/5 (1.43×10^8)
6 months	ND	ND	4/5 (4×10^2)	5/5 (5.52×10^8)
9 months	ND	ND	2/5 (10^2 neg)	5/5 (ND)
12 months	ND	ND	0/5 (10^2 neg)	5/5 (4×10^8)
15 months	ND	ND	0/5 (10^2 neg)	5/5 (2.15×10^8)
18 months	ND	ND	ND	5/5 (ND)
21 months	ND	ND	ND	5/5 (1.5×10^8)

ND = Not Done

The nominator represents the positive tubes, while the denominator represents the number of tubes examined.

In parenthesis is the number of C. jejuni per ml of suspension which was concentrated from aliquots of five tubes subsequent to their subculture.

The greater survival of C. jejuni at 4°C than at 25°C, is in agreement with the previous finding related to the greater viability of C. coli in rectal faeces on swabs, kept at 4°C and 25°C (see Part 1 of this chapter). These findings are also in agreement with similar observations of others using a variety of different media or biological mileu (Tanner and Bullin 1977, Blaser et al 1980d, Savopoulou 1980, Luechtefeld et al 1981, WHO/CDD/BEI/82.4.

Two of the factors which are likely to contribute in a better survival at -70°C than -20°C could be first, the lower metabolic and enzymatic activities at lower temperature, and secondly, the more rapid freezing of the organisms at -70°C which induces smaller intracellular ice crystals compared with freezing at -20°C. When organisms are thawed at room temperature, rupture of the organisms is less likely to occur when such ice crystals are small.

The finding that the survival of C. jejuni was greater after 24 h at 4°C than at -20°C and -70°C was at first sight unexpected. However, the result could be explained if the IF of holding the organisms at 4°C for 24 hours was less than the combined effects of a lesser IF factor at lower temperature and the additional factors associated with a 'shock' due to freezing and thawing. At sub-zero temperatures of down to -20°C, the effects of a freeze-thaw shock are likely to be relatively severe, but the IF will decrease much more rapidly as temperatures of storage are lowered to below this level.

The finding that organisms survived in FBP broth, even at 4°C for more than three weeks, was exploited in further field investigations requiring the transport of faecal material to the laboratory for subsequent cultural examination. Swabs were dipped in FBP broth just prior to taking a sample, and then transported to the laboratory on ice. This technique is discussed further in the following section.

4. SURVIVAL OF C. JEJUNI IN POULTRY FAECES ON NON-MOISTENED SWABS AND SWABS MOISTENED WITH FBP BROTH

INTRODUCTION

It has been shown that the use of cotton swabs with no transport medium was a suitable method for the recovery of C. coli from rectal faeces of pigs providing these swabs were kept at 4°C and cultured within six hours (see Part 1 of this chapter). It was initially decided to use the same technique to isolate C. jejuni from the faeces of poultry. However, in light of the good performance of FBP broth in maintaining the viability of an isolate of C. jejuni from poultry (see previous section of this chapter), it was decided to investigate the use of cotton swabs moistened with FBP broth, as a better technique for collecting samples from poultry for subsequent laboratory examination.

MATERIAL AND METHODS

Source of Faeces

Three, 35-day-old, broiler chickens from a poultry farm (flock No. 5) which when sampled four days before, all birds were found to be infected with C. jejuni (see Chapter VI), were transferred to a room pre-fumigated with formalin and placed in individual sterile metal cages. Food and water were provided ad libitum and cloacal swabs were collected and cultured from the three chickens immediately after their arrival. Thermophilic Campylobacter were recovered from all swabs. The chickens were observed daily and on the fourth day after their arrival, freshly void faeces of normal appearance, and judged by eye to weigh more than 20 g, were collected from one of the birds in a small plastic container of 50 ml capacity. This sample was thoroughly mixed with a sterile glass rod and divided into two universal bottles and pressed to form a compact mass.

Type of Swab, Storage Temperatures, Time of Sub-Cultures

Eighty faecal swabs (cotton wool swabs with no transport medium), were prepared from one of the two faecal samples. The bottle with the remaining faeces was kept in the refrigerator at 4°C for enumeration of C. jejuni. Another hundred cotton swabs were

moistened by dipping and rotating them in FBP broth and were then used to sample the faeces in the other universal bottle. Half the number of each type of swab were kept at 25°C and the other half at 4°C. At 6 hours, 12 h, 24 h, 36 h, 48 h, 60 h, 96 h, 7 days and 10 days., five swabs of each type held at the two temperatures were removed, suspended individually in 0.5 ml sterile saline and cultured on the surface of a modified Campy-BAP plate as described in Chapter III.

Enumeration of Campylobacter

Enumeration of Campylobacter was carried out immediately after the preparation of the faecal swabs, by tenfold dilutions of two grams of faeces obtained from the universal bottle which had been kept at 4°C.

RESULTS

Enumeration of Campylobacter in the faeces revealed 1×10^5 colony forming units (c.f.u.) of Campylobacter per g of faeces and all five colonies examined were confirmed to be C. jejuni.

The results obtained from the cultures of the two types of swabs are summarised in Table 4.7. Although organisms remained viable on all swabs for longer periods at 4°C than at 25°C, swabs pre-moistened in FBS broth, increased by twofold the duration of viability compared with the dry swabs. On dry swabs loss of viability was recorded after 12 h at 25°C and after 48 h at 4°C, while on moistened swabs such detected loss of viability was extended to 60 h and 7 days respectively.

There was little difference in the degree of growth of organisms other than Campylobacter, on dry and moist swabs held at 4°C. However at 25°C, there was a marked increase in other enteric organisms on moist swabs compared to the dry. By 96 h, four of the five plates cultured from moistened swabs held at 25°C, were overgrown by extraneous organisms to such an extent that it would have been impossible to detect individual colonies of Campylobacter. This problem of contamination was first noted on cultures made at 48 h from moistened swabs held at 25°C.

TABLE 4.7 : SURVIVAL OF C. JEJUNI IN FAECES ON SWABS PREVIOUSLY IMMERSED IN FBP BROTH (MOISTENED) AND ON DRY SWABS (NON MOISTENED)

Time of Culture	25°C Cotton Swabs		4°C Cotton Swabs	
	Non Moistened	Moistened	Non Moistened	Moistened
6 h	5[a]	5	5	5
12 h	5	5	5	5
24 h	3	5	5	5
36 h	0	3	5	5
48 h	0 (ICP)	3 (2CP)	5	5
60 h		1 (2CP)	4	5
72 h		0 (4CP)	1	5
96 h		0 (1CP/ 4 CPC)	0	5
7 days			0	3
10 days				1

[a] Number of isolations per five swabs inoculated

CP = Contaminated plates, but colonies of Campylobacter still detectable

CPC = Contaminated plates completely overgrown

DISCUSSION

Grant et al (1980) found that the numbers of C. jejuni subsp. jejuni in faeces from five broiler chickens ranged from 5.6×10^4 to 1.2×10^7 per g (mean 4.4×10^6). The 1×10^5 cfu of C. jejuni per g of faeces recorded from the present investigation is compatible with these results, albeit in the lower range. Thus the chicken faeces used in the present study was appropriate and indicates that the use of moistened swabs is an accurate method of detecting birds infected at such a level. However the technique could be less accurate in detecting infection in chickens excreting smaller numbers of organisms.

Similar to the study on the survival of C. coli in pig faeces on swabs, this study has shown that the culture, six hours after collection, of swabs of chicken faeces kept at $4^{o}C$, should result in the detection of infected birds.

The longer periods, during which C. jejuni could be recovered in this experiment, compared with the previous experiment on pig faeces (see Part 1 of this chapter), may be associated with the approximately eleven times greater numbers of C. jejuni in the chicken faeces (1×10^5 cfu per g) compared with pig faeces (9.2×10^3 cfu per g). Another possible explanation is that the chicken faeces have usually a greater water content than pig faeces associated with the common excretion of faeces and kidney products from the cloaca of poultry (Sturkie 1965). Human urine has been found to be better biological mileu for the survival of C. fetus subsp. jejuni than human faeces (Blaser et al 1980d). C. jejuni may also be better able to survive outside the G.I. tract than C. coli, however, as reported in Chapter VI, such hypothetical differences could not be demonstrated.

The moistening of cotton swabs with FBP broth before sampling and the transportation at $4^{o}C$, was shown to be a very satisfactory transport medium for at least 96 h, and this supports the previous results obtained with pure cultures of C. jejuni (see Part 3 of this chapter).

Cotton wool swabs moistened with FBP would also be satisfactory for the transportation of faeces at ambient temperatures providing

they were cultured within 24 hours. This could facilitate the study of the prevalence of Campylobacter infection in poultry by others. However, the routine use of such a technique will encourage the growth of other intestinal organisms which could be a serious problem in certain circumstances. If the number of Campylobacter in the faeces is low and inoculated plates are less inhibitory than the modified Campy-BAP medium used in these studies, overgrowth with the competing organisms and fungi will become a serious problem. However, it is believed that such circumstances could be avoided by supplementing the FBP broth with antibiotics at levels similar to those used in the modified Skirrows and Campy-BAP selective media (see Appendix I and II).

5. COMPARISON OF THE CLOACAL AND CAECAL SAMPLING, AND CULTURAL TECHNIQUES FOR THE ISOLATION OF INTESTINAL THERMOPHILIC CAMPYLOBACTER FROM INFECTED POULTRY FLOCKS

INTRODUCTION

A comparison of two different cultural techniques to detect infection of 64 wild ducks with C. jejuni subsp. jejuni showed that significantly higher numbers ($P < 0.001$) were isolated from caecal contents than from cloacal swabs (Luechtefeld et al 1980). In a similar study of turkeys, Campylobacter were isolated from all the caecal and the cloacal swabs (Luechtefeld and Wang 1981).

The objective of this trial was to compare the rate of isolation of intestinal Campylobacter from cloacal swabs and caecal contents from birds from an infected broiler flock. Before this study could be undertaken, it was necessary to identify infected flocks and subsequently to carry out the trial at a poultry processing plant at the time the birds were being slaughtered and eviscerated.

MATERIALS AND METHODS

Source of Chickens

Three commercial broiler flocks, each of approximately 20,000 birds, were found to be infected. The prevalence of infection by C. jejuni in two of these flocks (A and B) was estimated as 100% and

in the third (C), which was infected with C. coli, as 85% (see Chapter VI). Birds were examined at the time of slaughter on three different occasions between four to eight days after the flocks had been identified as infected. The birds from flocks A and B were 41 days old when slaughtered and those from flock C, 60 days old. Although the flocks were owned by different people, all birds originated from the same hatchery and were fed the same food and subjected to similar systems of husbandry. The birds were delivered to the processing plant in crates, each of which contained approximately 20 birds.

Collection of Cloacal Swabs and Caecal Contents

Cloacal swabs were taken from 50 birds from each flock, while they were still in the crates, just prior to being slaughtered. Swabs were taken from five birds per crate and the swabs were premoistened in FBS broth as described in the preceding section of this chapter.

Approximately 20 minutes later, immediately after carcase evisceration, the G.I. tracts of 50 birds from the same flock were collected. The caeca were removed and subsequently cultured according to the methods described in Chapter III.

RESULTS

The recovery rates of intestinal thermophilic Campylobacter, from cloacal swabs and caecal content birds from the three flocks are shown in Table 4.8.

Campylobacter were recovered from all the cloacal and caecal swabs of flocks A and B, and from 90% of cloacal swabs and 96% of caecal contents from flock C. This difference of rates of recovery in flock C was not statistically significant.

The 100% recovery rate from cloacal swabs from the young birds (flocks A and B) was statistically different ($P < 0.005$) from the 90% recovery from the older birds (flock C). However, the 100% recovery rate of the caecal content from the younger group of birds (41 days old) compared with 96% from the older birds (60 days old) was not significantly different ($0.05 < P < 0.10$).

TABLE 4.8 : RECOVERY OF CAMPYLOBACTER FROM CLOACAL SWABS AND CAECAL CONTENTS OF CHICKENS

Poultry Flock	Age (days)	Infectious agent	Cloacal swabs		Caecal swabs	
			No. of swabs inoculated	No. of swabs positive	No. of swabs inoculated	No. of swabs positive
A	41	*C. jejuni*	50	50	50	50
B	41	*C. jejuni*	50	50	50	50
C	60	*C. coli*	50	45 (90%)	50	48 (96%)
TOTAL			150	145 (96.7%)	150	148 (97.7%

DISCUSSION

The overall results obtained from this study indicate that either cloacal swabs or culture of caecal contents can be used satisfactorily to detect poultry flocks infected with intestinal thermophilic Campylobacter. Although the use of cloacal swabs is slightly less sensitive than the culture of caecal contents, it is the only practical method for sampling poultry flocks at the farm level. These results are also in agreement with the findings of Leuchtefeld et al (1981) in relation to turkey flocks, but are apparently contrary to the results of Leuchtefeld and Wang (1980), obtained from wild ducks. This apparent contradiction may be related to the different ages of the ducks examined, and the time at which they had first become infected. As will be discussed in Chapters VI and VIII, poultry infected with C. jejuni, gradually eliminate infection, and in the latter stages of infection, isolates can be obtained from the caeca but not from cloacal swabs. This phenomenon may also account for the higher rate of recovery from the caeca compared with the cloaca in the older birds in flock C compared with the young birds in flocks A and B. However, one must be cautious in making such an assumption in light of the different species of Campylobacter isolated from flocks A and B as compared with flock C.

CONCLUSIONS

1. C. coli and C. jejuni survive for at least four times as long, in either faeces or on swabs, at $4^{o}C$ compared with $25^{o}C$.

2. Individual pigs and birds from herds and flocks of pigs and poultry infected with C. coli and C. coli and C. jejuni respectively, can be readily identified by the use of rectal or cloacal swabs without any form of transport medium, providing such swabs are held at $4^{o}C$ and cultured within six hours.

3. The survival of C. coli in faeces on swabs can be extended to 48 h if a transport medium (Amies transwabs) is used and the swabs are kept at $4^{o}C$.

4. In pigs the greatest numbers of C. coli occur in the distal G.I.

tract (caecum, colon and rectum) and the culture of rectal swabs gives an accurate assessment of the prevalence of G.I. tract infection by C. coli.

5. In pigs, C. jejuni occurs only in the proximal portions of the intestinal tract and such infections are uncommon.

6. Cultures of C. jejuni can be preserved for more than 24 months if kept in FBP broth at -70°C.

7. C. jejuni can be isolated from cloacal swabs from chickens, when the swabs are premoistened with FBP broth, for 24 h if kept at 25°C and for 96 h if kept at 4°C.

8. The culture of cloacal swabs from chickens gives a satisfactory assessment of the prevalence of caecal infection by either C. jejuni or C. coli.

CHAPTER V

THE PREVALENCE OF INTESTINAL CAMPYLOBACTER IN PIGS AND THEIR DISTRIBUTION WITHIN THE GASTRO-INTESTINAL TRACT

INTRODUCTION

From the review of the literature (Chapter II), there appears to be general agreement, that in many countries thermophilic Campylobacter, and in particular C. coli, are endemic at high rates of prevalence in healthy pigs by the time they reach the age of slaughter (four to six months old). Although Smibert (1978) has stated that C. fetus subsp. jejuni is part of the normal faecal flora of young pigs, but is less common in older pigs, there is a lack of information on the degree of endemicity of Campylobacter in young and old pigs.

Information was lacking on the prevalence of intestinal Campylobacter in pigs in New Zealand. Thus the basic objectives of the investigations outlined in this Chapter were:

1. To determine the prevalence of infection in three age groups of pigs; suckling piglets (less than five weeks old), weaners and growers (five to 20 weeks of age), and mature breeding females (more than 10 months of age).

2. To extend the work reported in Chapter IV, Part 2, on the rate and levels of infection of different parts of the G.I. tract of pigs, by thermophilic Campylobacter.

In order to achieve the objectives, two surveys were undertaken; a farm survey based on the examination of rectal samples from live pigs, and an abattoir survey of rectal samples and cultural examination of the G.I. tract soon after slaughter.

MATERIALS AND METHODS

A total of 247 pigs of different ages from 20 different piggeries, were examined in both the farm and the abattoir surveys.

Farm Survey

One hundred and forty four pigs (144) from 4 days to 3 years old were examined from three farms (piggeries) by taking rectal swabs (see Chapter III and Chapter IV, Parts 1 and 2). Of these pigs, 64 were suckling piglets 4 days to 28 days old, 60 weaners and growers 6 to 20 weeks of age and 20 sows between one and three years old.

Table 5.1 shows the type, the age and the number of pigs examined in each of the three piggeries, during the course of the farm survey.

TABLE 5.1 : PIGS EXAMINED DURING THE FARM SURVEY

Piggery	Suckling Piglets 4 to 28 Days	Weaners and Growers 6 to 20 Weeks	Sows 1 to 3 Years
No. 1	64	30	15
No. 2	-	15	5
No. 3	-	15	-
Total	64	60	20

All three piggeries consisted of enclosed units with concrete floors and the pigs were kept inside at all times. Piggery No. 1 (National Pig Research Centre) was the largest with 172 breeding sows. Piggery No. 2 (Massey University Pig Unit) had 56 sows, while Piggery No. 3 was a private unit with 50 sows.

Potential breeding stock, in all three units, were kept together for up to 10 days before mating and then transferred to individual pens, where they remained for the whole of their productive life. The weaners and growers were kept in groups of 15 - 20 in pens in rooms separated from the sows and the suckling piglets. The general standards of hygiene were good in Piggery No. 1 and No. 2, but were of a slightly lower standard in Piggery No. 3.

All 64 piglets examined, were from two rooms in Piggery No. 1. Sows from this unit either mothered their own litters of 8 to 10 piglets or two sows which had farrowed at similar times, shared the mothering of their 15 to 20 combined offspring.

Table 5.2 lists the sows and piglets examined, the room in which they were housed, the relationship of the piglets to the sows, and the age of the piglets at the time of examination. Four groups of sixteen piglets of each different age group were examined (4 - 5 days, 10 - 14 days, 21 days and 28 days old).

TABLE 5.2 : SOWS AND PIGLETS EXAMINED FROM PIGGERY NO. 1

Sow No.	Piglet Nos	Age of Piglets (Days)	Room Housed
S 1	1 - 8	4 - 5	1
S 2	9 - 16	4 - 5	1
S 3	17 - 24	10 - 14	1
S 4	25 - 32	10 - 14	1
S 5) S 6)	*33 - 40	21	2
S 7) S 8)	*41 - 48	21	2
S 9	49 - 56	28	2
S 10) S 11)	*57 - 64	28	2
S 12	-		1
S 13	-		1
S 14	-		2
S 15	-		2

* = Combined litters and dams

Abattoir Survey

One hundred and three (103) pigs from 17 piggeries were examined at a local abattoir. Cultural examinations were carried out on rectal swabs taken immediately after electrical stunning of the pigs and before they were slaughtered, and of swabs taken from various

sites of the G.I. tract immediately after evisceration of the carcases.

Sixty rectal swabs, from groups of five to eight pigs from ten different piggeries were examined. Rectal swabs and the complete G.I. tracts of a further 43 pigs from seven different piggeries were also examined. In all cases swabs were taken from the middle of the duodenum, the terminal ileum, the caecum and colon (see Chapter IV). Swabs from the middle of the jejunum were also examined from 30 pigs and up to five colonies of Campylobacter isolated from each intestinal site of these 30 pigs were subjected to detailed examination. (The results obtained from 13 of these pigs have been recorded and partially discussed in parts 1 and 2 of Chapter IV).

RESULTS

Farm Survey

Table 5.3 shows the prevalence of infection in the three different age groups (piglets, weaners and growers, and sows), examined from the three piggeries in the farm survey.

Twenty three (35.9%) of the 64 piglets, 54 (90%) of the 60 weaners and growers, and 17 (85%) of the 20 sows were infected. No difference was observed between the rate of infections of the weaners and growers from piggery No. 1 and the combined rate of infection of the weaners and growers from the other two piggeries.

The prevalence of infection in weaners and growers was approximately two and a half times greater than the rate in piglets. This difference was statistically significant ($P < 0.005$). There was no significant difference between the infection rate of weaners and growers and the sows.

Table 5.4 shows the results obtained during the investigation of the four different age groups of suckling piglets, examined from piggery No. 1. All the four age groups were infected, and when sows were infected their piglets were also infected. Only sow S 2 was uninfected and it is interesting to note that intestinal Campylobacter were not isolated from the eight piglets examined from this sow.

TABLE 5.3 : PREVALENCE OF INTESTINAL CAMPYLOBACTER IN PIGLETS, WEANERS AND GROWERS, AND SOWS (FARM SURVEY)

No. of Piggery	Piglets (4 - 28 days old)			Weaners and Growers (6 to 20 weeks old)			Sows		
	No. examined	No. infected	(%)	No. examined	No. infected	(%)	No. examined	No. infected	(%)
1	64	23	35.9	30	27	90.0	15	14	93.3
2	-	-		15	15	100.0	5	3	60.0
3	-	-		15	12	80.0	-	-	
Total 3	64	23	35.9	60	54	90.0	20	17	85.0

TABLE 5.4 : PREVALENCE OF INTESTINAL CAMPYLOBACTER IN PIGLETS OF DIFFERENT AGES

Age	Sow identification No.	Piglet identification No.	Infection rate/ litter	Infection rate/age group (%)
4 - 5 days	(S1)	1, 2, (3), (4), 5, 6, 7, (8).	$^{3}/_{8}$	$^{3}/_{16}$ (18.8)
	S2	9, 10, 11, 12, 13, 14, 15, 16.	$^{0}/_{8}$	
10-14 days	(S3)	17, 18, (19), (20), 21, (22), 23, 24.	$^{3}/_{8}$	$^{5}/_{16}$ (31.3)
	(S4)	25, 26, 27, (28), 29, (30), 31, 32.	$^{2}/_{8}$	
21 days	(S5) + (S6)	33, 34, 35, 36, (37), (38), 39, 40.	$^{2}/_{8}$	$^{7}/_{16}$ (43.8)
	(S7) + (S8)	(41), (42), (43), (44), 45, (46), 47, 48.	$^{5}/_{8}$	
28 days	(S9)	(49), 50, 51, (52), 53, 54, (55), (56).	$^{4}/_{8}$	$^{8}/_{16}$ (50.0)
	(S10) + (S11)	(57), 58, (59), (60), (61), 62, 63, 64.	$^{4}/_{8}$	

( ) = Infected pig

Figure 5.1 illustrates the prevalence of infection in the different age groups of piglets. The overall prevalence in the youngest group (4 - 5 days) was 19%, in the 7 - 14 day olds 31%, in the 21 day olds 44%, and in the 28 day olds 50%. None of these differences were significant.

Abattoir Survey

Table 5.5 shows the prevalence of intestinal Campylobacter, based on the examination of rectal swabs, in 103 pigs from 17 different piggeries examined during the course of the abattoir survey.

Campylobacter were isolated from 92 (89.3%) of the 103 pigs examined and pigs from all 17 piggeries were infected, and prevalence rates varied from 60 to 100% with a median rate of 85.7%.

There was no significant difference between the prevalence of infection of the 60 weaners and growers (6 to 20 weeks old) from the three piggeries in the farm survey (90%) and the 103 pigs from the 17 piggeries in the abattoir survey (89.3%).

Table 5.6 and Figure 5.2 show the rates of isolation of Campylobacter from the G.I. tract of the 43 pigs examined, and Table 5.7 the comparative levels of colonisation of the G.I. tract at the different sites examined of the 30 pigs investigated in greater detail.

It can be seen from the results presented in Table 5.6 that Campylobacter were isolated from the caeca of 28 of the 30 pigs, and also from the colon and rectum of 27 of these 28 infected pigs. The greatest number of organisms were found in the caeca, with slightly lower numbers in the colon and rectum. The overall prevalence and level of infection of the small intestine was very much less than the large intestine. In most cases, where infection of the small intestine was demonstrated, less than ten colonies of Campylobacter were isolated from any one site.

The mean number of Campylobacter per gram of colonic content from 14 pigs examined (two pigs per piggery) was $10^{3.59}$ with a range of $10^{1.70}$ to $10^{5.63}$. Counts of less than $10^{3.0}$ were recorded for five pigs, counts of between $10^{3.0}$ and $10^{5.0}$ from seven, and counts of more

FIGURE 5.1 : PREVALENCE OF INTESTINAL THERMOPHILIC CAMPYLOBACTER IN DIFFERENT AGE GROUPS OF PIGLETS

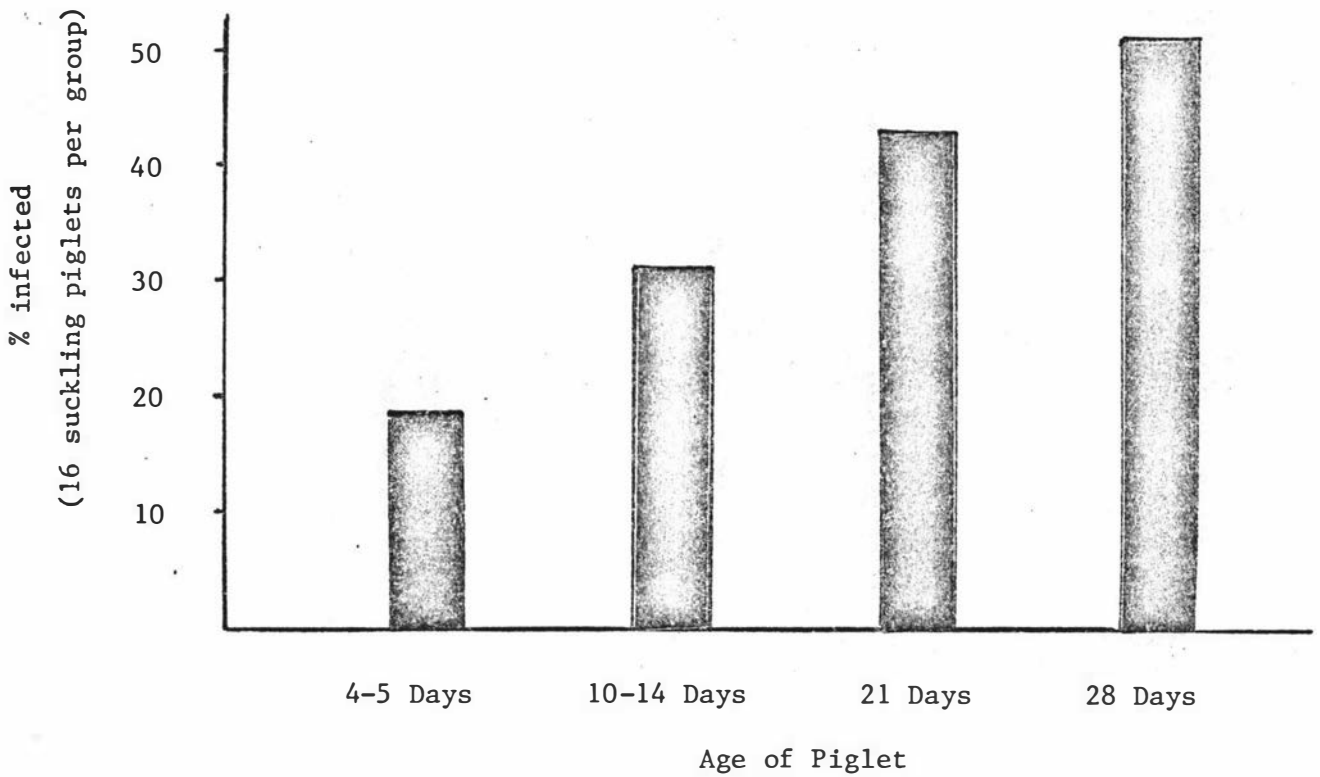


TABLE 5.5 : PREVALENCE OF INTESTINAL CAMPYLOBACTER IN PIGS SUBMITTED FOR SLAUGHTER

(Based on Rectal Swabs)

No. of Piggery	No. of pigs examined	No. of positive pigs	% of positive pigs
4	8	8	100
5	8	7	87.5
6	8	8	100
7	6	6	100
8	5	4	80
9	5	3	60
10	5	3	60
11	5	4	80
12	5	5	100
13	5	4	80
14	7	7	100
15	6	6	100
16	8	7	87.5
17	7	6	85.7
18	5	5	100
19	5	4	80
20	5	5	100
Total 17	103	92	Mean 89.3 (range 60-100%)

TABLE 5.6 : ISOLATION RATES OF INTESTINAL THERMOPHILIC CAMPYLOBACTER FROM DIFFERENT SITES IN THE G.I. TRACT OF 43 PIGS

(*C. coli* unless otherwise specified)

No. of piggery	No. of pigs examined	Sites Cultured						Numbers (Log_{10}) of Campylobacter/gram of colonic content (n = 14)
		Duodenum	Middle of jejunum	Terminal ileum	Caecum	Middle of colon	Rectal swabs	
14[a]	7	3(3)[b]	ND	4(1)[b]	7	7	7	4.46 4.96
15[a]	6	1(1)[b]	ND	1	6	6	6	2.32 2.70
Sub total	13	4/13	ND	5/13	13/13	13/13	13/13	
16	8	2	3(1)[b]	4	7	7	7	2.34 3.79
17	7	1	1	1	6	6	6	3.69 3.85
18	5	1	1	2	5	5	5	3.30 5.63
19	5	1	-	1	5	4	4	1.70 2.86
20	5	(1)[c]	2	2	5	5	5	3.57 5.15
Sub total	30	6/30	7/30	10/30	28/30	27/30	27/30	
Overall Rate	43	10/43	7/30	15/43	41/43	40/43	40/43	Mean 3.59 (1.70 - 5.15)
%		23.3%	23.3%	34.9%	95.35%	93.0%	93.0%	

[a] The results from piggeries Nos. 14 and 15 have also been shown in Table 4.4.

[b] No. of isolates of *C. jejuni*.

[c] No. of isolates of *C. fetus* subp. *fetus*

FIGURE 5.2 : ISOLATION RATES OF INTESTINAL THERMOPHILIC CAMPYLOBACTER FROM DIFFERENT SITES IN THE G.I. TRACT OF 43 PIGS

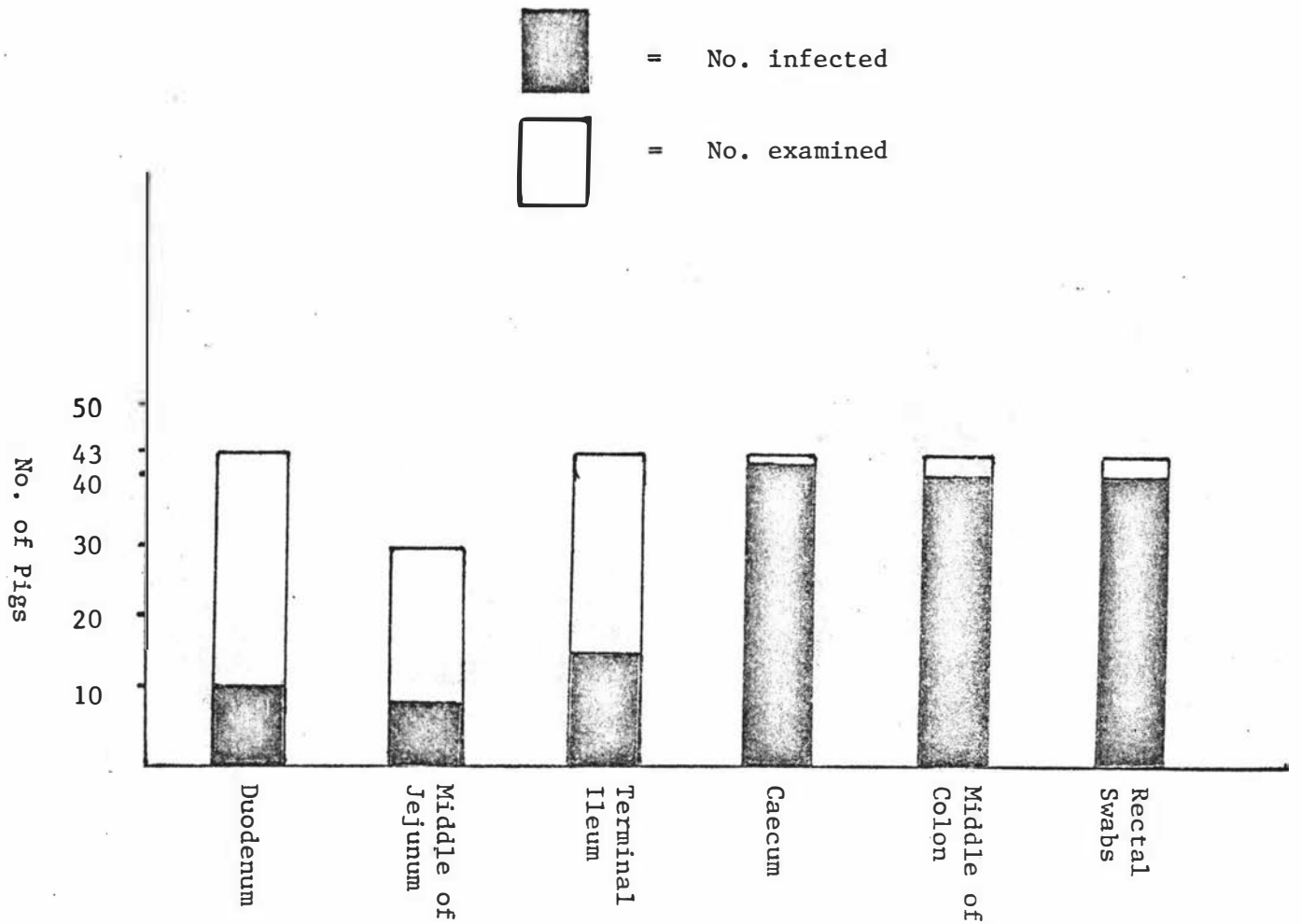


TABLE 5.7 : COMPARATIVE LEVEL OF CAMPYLOBACTER INFECTION AT DIFFERENT SITES OF THE G.I. TRACT OF 30 PIGS

No. of piggery	No. of pigs examined	Middle of duodenum	Middle of jejunum	Terminal ileum	Caecum	Colon	Rectum	Number (Log_{10}) of Campylobacter/gram of colonic content
16	1	+	+	+	++++	++++	++++	
	2	-	-	-	++++	+++	+++	2.34
	3	-	-	-	++	+++	++	
	4	+	++[a]	++	++++	++++	++++	3.74
	5	-	-	+	++	+	++	
	6	-	-	-	++++	++++	+++	
	7	-	+	++	+++	+++	+++	
	8	-	-	-	-	-	-	
17	9	-	+	-	+++	++	++	
	10	-	-	-	++++	++++	+++	3.69
	11	-	-	-	-	-	-	
	12	-	-	-	++	++	++	
	13	-	-	-	++++	+++	+++	
	14	-	-	+	++++	++++	+++	3.85
	15	+	-	-	+++	+++	+++	
18	16	-	-	-	+++	+++	++	
	17	-	-	++	++++	+++	+++	3.30
	18	+	+	++	++++	++++	+++	5.63
	19	-	-	-	+++	+++	++	
	20	-	-	-	++++	+++	+++	

(To be cont.)

TABLE 5.7 (cont)

No. of piggery	No. of pigs examined	Middle of duodenum	Middle of jejunum	Terminal ileum	Caecum	Colon	Rectum	Number (Log_{10}) of *Campylobacter*/gram of colonic content
19	21	-	-	-	++	+	+	1.70
	22	+	-	-	++	+	++	
	23	-	-	-	+	-	-	
	24	-	-	+	++	++	+	2.86
	25	-	-	-	++	++	++	
20	26	-	-	-	+++	+++	++	
	27	+[b]	-	-	++++	++++	+++	3.57
	28	-	+	++	++++	++++	++++	
	29	-	-	-	++++	+++	++	
	30	-	+	+	++++	++++	++++	5.15
Totals	30	6/30	7/30	10/30	28/30	27/30	27/30	3.59 (1.70 - 5.13)

[a] Two isolates were *C. jejuni*

[b] One isolate was *C. fetus* subsp. *fetus*

\+ = < 10 colonies/plate

++ = 11 - 50 colonies/plate

+++ = 51 - 200 colonies/plate

++++ = > 200 colonies/plate

than $10^{5.0}$ per gram of colonic content from only two (see Tables 5.5 and 5.6).

Species Identification of the Isolates from the Farm and the Abattoir Surveys

Two hundred and fifty three colonies of Campylobacter were collected from the 28 infected G.I. tracts of the 30 examined, and subjected to detailed tests of identification. Of the 253 colonies, 250 were C. coli, two were C. jejuni and one was C. fetus subsp. fetus. Both the isolates of C. jejuni and the C. fetus subsp. fetus were associated with concurrent infection with C. coli. The isolates of C. jejuni were recovered from the middle of the jejunum of the same pig (No. 4), while three other colonies from the same site were identified as C. coli. Thirteen more colonies examined from the distal part of the G.I. of the same pig were all identified as C. coli. The isolate of C. fetus subsp. fetus was recovered from the duodenum of pig No. 27 and was the only species of Campylobacter isolated from the whole proximal part of the G.I. tract of this pig. Another seven isolates examined from the distal part of the intestine of pig No. 27 were all identified as C. coli.

Fifty of the 52 infections due to Campylobacter detected in the 60 pigs, which were examined only by means of rectal swabs, in the abattoir survey, were associated with C. coli. Subcultures of one of these isolates of C. coli revealed a few colonies resistant to nalidixic acid, (see Plate 5.1). Subsequent examination by restriction endonuclease DNA analysis of both the resistant and non resistant colonies indicated that they had a similar pattern, indicating that both colony types were C. coli (see Chapter X).

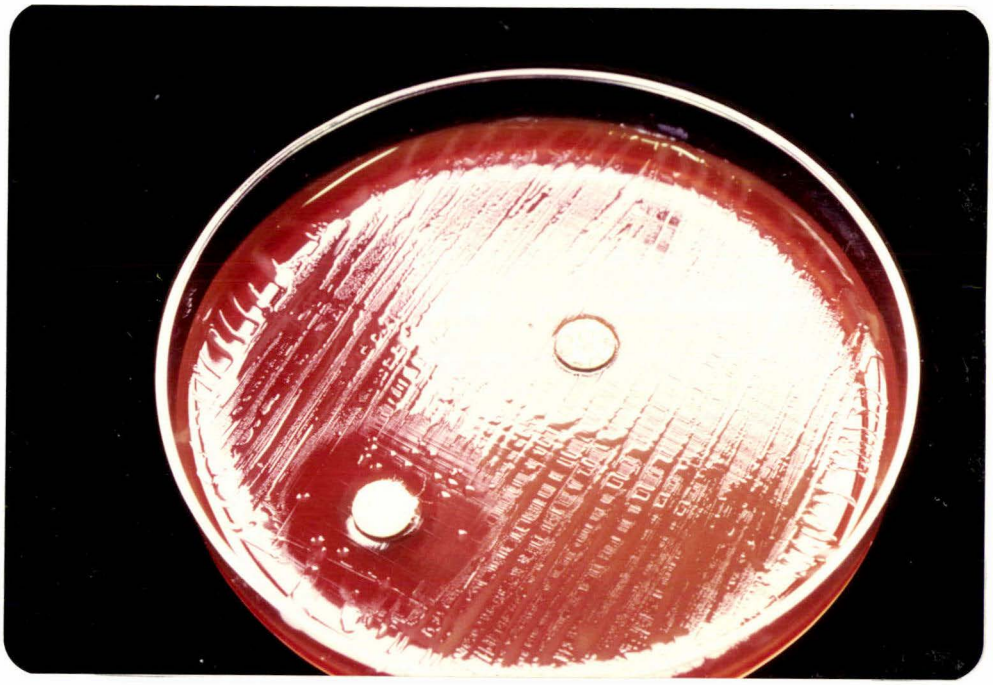
One other pig from this series of 60, was apparently infected by only C. jejuni and one was infected with a NARTC (C. laridis).

The 64 isolates examined from infected pigs from the farm survey, were all identified as C. coli.

DISCUSSION

The overall results clearly demonstrated that there was a high prevalence of infection of C. coli in pigs over six weeks of age from

PLATE 5.1 : DEVELOPMENT OF RESISTANCE TO NALIDIXIC ACID BY C. COLI ON SUBCULTURE



all the 20 piggeries examined. Although 18 of the piggeries from which pigs were examined, were situated within a radius of 100 km from Massey University, two piggeries (Nos 4 and 5) were from the northern and eastern parts of the South Island (Nelson and Christchurch). It would therefore seem reasonable to assume that such high rates of infection of pigs with C. coli are typical of the overall situation in New Zealand.

The 89.3% prevalence rate of intestinal Campylobacter recorded in pigs submitted for slaughter, is similar to the results recorded from other countries (Deas 1960, Oesteron 1980, Luechtefeld and Wang 1982, Tuefel 1981, Stern 1981, Svedhem and Kaijser 1981, Stich Groh 1982 and Munroe et al 1983), where prevalence rates from 55% to 95% have been recorded. However, Prescott and Bruin-Mosh (1981) recorded a prevalence rate of only 1%.

The number of Campylobacter obtained per gram of colonic content from 14 pigs, is very similar to the findings of Teufel (1981), who reported counts of less than 10^3 in 30% of pigs, 10^3 to 10^5 in 55% and counts of more than 10^5 in 15%.

Although the present work did not include an assessment of the contamination of pig carcases, it might be reasonable to assume that the rate of contamination of pig carcases could be at least as great in New Zealand as that recorded in other countries and outlined in Chapter II.

The high prevalence rate (85%) recorded in the sows of piggery Nos 1 and 2, is considerably higher than that recorded by Smibert (1978). However, this high prevalence in sows would account for the high levels of infection by C. coli in piglets and growers. Suckling piglets probably become infected by the inadvertent ingestion of maternal faeces from the infected sows and with increasing age the chance of infection will increase. The only subgroup of piglets (Nos 9 to 16) which remained Campylobacter free, were being suckled by an uninfected sow (S 2). The most convincing evidence that suckling piglets became infected from their dams, is that in most cases, the suckling piglets were colonized with the same Campylobacter BRENDA type as their dams (see Chapter X).

The finding that piglets, only 4 to 5 days old, were infected is of interest. It indicates, in view of the high numbers of organisms recovered, that the incubation period may be shorter than four days. Prevalence rates of 90% in weaners and growers could be explained on the basis of an incubation period for C. coli in pigs of less than four days, a prevalence rate of 50% in piglets at the age of four weeks, and the practice of mixing weaners from different sows together.

Further information on the distribution of Campylobacter in the G.I. tract of pigs, recorded in these studies, confirms the results recorded in part 2 of Chapter IV and the previous finding that C. jejuni infections of pigs are uncommon. These results also indicate that studies on C. jejuni infection of pigs are better based on the examination of swabs of small intestinal content rather than on rectal swabs. None of the five pigs shown to be infected with C. jejuni by examination of small intestinal swabs, was detected by examination of rectal swabs.

The recovery of C. laridis from one pig is apparently the first reported isolation of this species from pigs, but also indicates that C. laridis infection of pigs is rare. It is interesting to note that the piggery from which this pig originated was from the Foxton region on the coast, which was an area where seagulls were found to be infected with C. laridis (see Chapter VII). However, C. laridis was not isolated from other pigs in the area.

CONCLUSIONS

1. Infection by C. coli is endemic in all piggeries in New Zealand.

2. The majority of pigs over six weeks of age are infected with C. coli.

3. Infection of pigs by C. jejuni is uncommon.

4. The major source of infection of C. coli for newborn piglets, is their infected dams.

5. In young pigs, the time between infection and excretion of *C. coli* in their faeces, is less than four days.

6. Most pigs infected with *C. coli*, excrete between 10^2 and 10^5 organisms per g of faeces.

CHAPTER VI

THE PREVALENCE OF INTESTINAL THERMOPHILIC CAMPYLOBACTER IN POULTRY AND CARCASES AND EDIBLE VISCERA

INTRODUCTION

The basic objectives of this part of the work were:

1. To determine the prevalence of intestinal thermophilic Campylobacter in poultry flocks of broilers and layers.

2. To determine the duration of infection in affected birds.

3. To determine both the prevalence and level of contamination in processed carcases and edible viscera derived from infected broiler flocks.

4. To determine the prevalence of contamination of chilled chicken wings sold in a local supermarket supplied by a poultry company known to process infected birds.

To achieve these objectives, surveys were carried out on poultry farms, at a poultry processing plant and at a local supermarket.

MATERIALS AND METHODS

Farm Survey

Source of poultry: A total of 480 birds from 39 poultry flocks, raised by 26 different farmers, were examined for intestinal thermophilic Campylobacter by taking cloacal swabs at the farm. Of these birds, 390 were broilers from 33 flocks on 22 different farms. Forty (40) were young birds from two breeding flocks from the same farm and the remaining 50 birds were adult layers. Of these latter birds, 40 were from three commercial layer flocks on two farms and 10 from a small household flock of 12 birds.

Table 6.1 lists the poultry farms and the flocks which were

sampled, the number of samples taken from each flock, the age and the total number of birds in each flock. With the exception of broiler farms 21 and 22, from which three flocks were sampled, no more than two flocks were examined from any farm.

The broiler flocks were under the supervision of two of the major New Zealand poultry companies (Company A and B). The companies supplied the farmers with day-old chickens, food, advice and general supervision and slaughtering and processing facilities. The poultry farmers raised the broilers in their own facilities and they were paid according to the performance of their flocks. Twenty seven flocks from 20 farms, and six flocks from two farms, were investigated from Company A and B respective (see Table 6.1).

The two young breeding flocks were under the supervision of Company A, and these birds were intended for use as future breeding stock. The layers were kept in individual cages (batteries) while the laying birds from the small household flocks were kept on litter. The three commercial layer flocks were independent of the poultry Companies A and B.

All the birds from the broiler flocks, with the exception of one flock (No. 9) were between 28 and 43 days old when they were sampled and subsequently slaughtered between 40 to 44 days of age. The birds in the other flock were 53 days old when they were sampled and they were slaughtered at the age of 60 days.

Repeated sampling of infected flocks: Young birds from breeding flock No. 23a and the household layer flock No. 26 were found to be infected with Campylobacter. As they were not to be slaughtered, they were re-examined four times at five-weekly intervals by taking 20 and 12 samples respectively.

Examination of water supplies: Water samples from all farms, with the exception of farms 21 and 22, were collected and cultured at the same time that samples were collected from the birds (the techniques used are described in Chapter III). Table 6.1 indicates whether or not the water supply to each farm was chlorinated.

TABLE 6.1 : THERMOPHILIC CAMPYLOBACTER SURVEY:

POULTRY FARMS AND FLOCKS SAMPLED

BROILERS

Farm No.	Flock No.	No. of samples taken from each flock	Age of birds at time of sampling (days)	Approximate No. of birds in flock
1	1	10	35	12,500
2*	2	10	38	12,500
3*	3a	10	43	12,500
3*	3b	20	35	25,000
4*	4	10	28	12,500
5	5	20	37	25,000
6*	6	20	30	25,000
7	7	20	39	
8	8	20	33	
9	9	20	53	
10	10	10	32	12,500
11	11	10	30	
12	12a	10	38	
	12b	10	38	
13*	13a	10	37	
	13b	10	37	
14	14	10	30	
15	15	10	33	
16	16	10	28	
17*	17a	10	30	
	17b	10	33	
18	18a	10	37	
	18b	10	37	
19	19a	10	40	
	19b	10	40	
20	20a	10	28	
	20b	10	28	
21	21a	10	32	
	21b	10	32	
	21c	10	35	
22	22a	10	30	
	22b	10	30	
	22c	10	32	
22	33	390		TOTAL

(To be cont.)

TABLE 6.1 (Continued)

BREEDING FLOCKS

	Farm No.	Flock No.	No. of samples taken from each flock	Age of birds at time of sampling (weeks)	Approximate No. of birds in flock
	23*	23a	20	9	400
		23b	20	10	2,500
TOTAL	1	2	40		

LAYERS

	Farm No.	Flock No.	No. of samples taken from each flock	Age of birds at time of sampling (weeks)	Approximate No. of birds in flock
	24*	24	18	50	4,000
	25*	25a	10	12	2,500
		25b	10	24	2,500
	26*	26	10	52	
OVERALL TOTAL	26	39	480		

Farms Nos 1 to 20 and Nos 21 and 22 were from poultry Companies A and B respectively.

* Farms with chlorinated water supplies.

Slaughterhouse Survey

Source and type of samples: Birds from broiler flock No. 5 and 8 (flocks A and B in Chapter IV) which had been found to be 100% infected with C. jejuni and from broiler flock No. 9 (Flock C, Chapter IV) which was 85% infected with C. coli were examined on three different occasions at the slaughterhouse, four, eight and seven days respectively after samples had been taken at the farm. Five pairs of caeca, five carcases and five packets of giblets (liver, gizard, heart) were collected from each of the three flocks immediately after their slaughter (see Chapter III) and they were subjected to the following examinations:

Carcases and edible viscera (giblets) - the prevalence and levels of contamination were determined by using a whole sample washing technique (see Chapter III).

Caecal contents (Enumeration studies) - these studies were designed to determine whether, or not, there were differences in the level of infection between the two caeca from the same bird. Both caeca were removed from five birds from flock A. Each caecum was placed in an individually labelled container and then the ten separate containers were divided into two groups of five (Aa and Ab). Each group consisted of one caecum from each of the five different birds. Enumeration studies were carried out on contents of individual caecal contents.

Survival of C. jejuni and C. coli in caecal contents kept at 25°C and 4°C: The contents of one caecum from each of five birds from flock B and five from flock C were individually kept in capped universal bottles at 25°C. The contents of the other caecum from each of the five birds, were kept in a similar manner at 4°C. The caecal contents were subcultured by dipping a cotton sterile swab in the material, mixing it in 0.5 ml sterile saline and then inoculating the selective medium (see Chapter III). The caecal contents kept at 25°C were subcultured daily, while those kept at 4°C were subcultured every two days for the first 10 days and subsequently every five days. After two consecutive negative results, no further examinations were carried out.

Supermarket Survey

Source of chicken wings: Fifty packets of chilled chicken wings were collected approximately three months after the completion of the Farm Survey, from a local supermarket during a period of five weeks (10 packets per week). These samples were examined by the method described in Chapter III.

The supermarket was supplied by the poultry Company A at biweekly intervals (Mondays and Thursdays) with fresh poultry carcases in boxes of 12. Boxes were received the same day that the birds were slaughtered. Supermarket personnel cut the carcases into pieces (wings, legs, breasts, etc), both on the day of arrival and the next day (carcases not cut up on the day of arrival were kept overnight in the chiller). Cut pieces were immediately packed and presented for sale in a chiler cabinet.

Ten packets were collected each week (Fridays) making sure that the date on the packets was either the same as the day of collection or that of the previous day (Thursday).

From each batch, six packets of chicken wings were examined immediately after the collection (1 to 2 days' post processing) while the remaining four packets were kept at $4^{o}C$. When the first six packets of chicken wings produced no growth of Campylobacter after 24 h, the packets which were kept at $4^{o}C$ were examined immediately. When, however, the first six packets produced positive results, two of the packets which were held at $4^{o}C$ were not examined until four days after collection and the remaining two packets until eight days after collection.

RESULTS

Farm Survey

Table 6.2 shows the prevalence of Campylobacter infection in the broiler, breeding and layer flocks identified as positive during the course of the Farm Survey.

From the total of 39 flocks from 26 different farms examined, only 11 (28.2%) flocks and 8 (30.8%) farms were infected respectively. Both the flocks of young breeding birds were infected. There was no significant difference in the rate of flock infection between broilers

TABLE 6.2 : PREVALENCE OF INFECTION WITH INTESTINAL THERMOPHILIC CAMPYLOBACTER IN POULTRY FLOCKS

(FARM SURVEY)

Type of Bird	Farm No.	Flock No.	No. of birds examined	No. of birds infected	Rate of infection (%)	Species isolated	
						C. jejuni	C. coli
BROILER	5	5	20	20	100	20	-
	8	6	20	20	100	20	-
	9	9	20	17	85	-	17
	12	12a	10	10	100	8	2
		12b	10	10	100	9	1
	19	19a	10	10	100	10	-
		19b	10	10	100	10	-
Ratio of infected to non-infected	5/22	7/33	100/390	97	97.9	77	20
YOUNG BREEDING STOCK	23	23a	20	16	80	16	-
		23b	20	14	70	14	-
Ratio of infected to non-infected	1/1	2/2	40/40	30	75	30	0
LAYER	25	25b	10	7	70	7	-
	26	26	10	9	90	9	-
Ratio of infected to non-infected	2/3	2/4	20/52	16	80	16	0
Overall ratio of infected to non-infected	8/26	11/39	160/480	143		123(86%)	20(14%)

and layers.

When a poultry farm (see farms Nos. 12, 19 and 23) was infected, all flocks on the farm usually were infected (see flock Nos 12a, 12b, 19a, 19b and 23a, 23b). An exception was noticed with the layer farm No. 25 where only one of its two flocks was infected (No. 25b).

The prevalence of infection within an infected broiler flock appeared to be almost one hundred percent (average 97.9%). All samples from six of the flocks were positive and from only one flock was only 85% of the samples positive. This latter flock comprised the oldest broiler birds examined and they were infected with C. coli. The average prevalence of infection in the infected breeding and layer flocks, which were older than broilers, was 75% and 80% respectively. This was a statistically significant ($P < 0.005$) lower rate compared with the broilers. No significant differences in rates of infection were observed in the positive breeding and layer flocks.

The predominant isolate was C. jejuni. From the 143 Campylobacter isolates, 123 (86%) were C. jejuni and only 20 (14%) were C. coli. C. jejuni was the only species isolated from eight (72.7%) of the 11 flocks and C. coli the only isolate from one (9.1%) flock. Two (18.2%) flocks (12a, 12b) from the same farm (farm 12), had dual infections with C. jejuni and C. coli, but in both these flocks, C. jejuni comprised 17 of the 20 isolates examined.

Table 6.3 shows the results obtained from the infected breeding flock No. 23a and household layer flock No. 26 which were subjected to repeated sampling at intervals of five weeks over a period of 20 weeks.

A considerable reduction in the prevalence of infection was observed in both infected flocks, five weeks after their first examination. The reduction was greater (50%) for the young breeding flock and this difference was statistically significant ($P < 0.005$). The household layers had a lower (32%) reduction in the prevalence of infection, but this decline was not significant.

No Campylobacter were isolated from the household layer flock on

TABLE 6.3 : PREVALENCE RATES IN TWO INFECTED FLOCKS RE-EXAMINED AT FIVE-WEEK INTERVALS

Type of flock	Prevalence at different times			
	Week 0	Week 5	Week 10	Week 15
Young breeding flock (No. 23a)	16/20 (80%)	6/20 (30%)	ND*	ND*
Household layer flock (No. 26)	9/10 (90%)	7/12 (58%)	0/12 (0%)	0/12 (0%)

ND* = Not done. Flock No. 23a could not be sampled after the second examination because it developed Mycoplasmosis and was destroyed.

the third and fourth examinations, 10 and 15 weeks after the first examination. The young breeding flock could not be followed after the second examination because the birds developed mycoplasmosis and they were destroyed.

No Campylobacter were isolated from any water sample.

Slaughterhouse Survey

Table 6.4 shows the results obtained from the slaughterhouse survey. The carcases, packets of giblets and the caeca were all contaminated, irrespective of whether they were derived from the 100% infected flocks (A and B) or from the 90% infected flock (C). The mean contamination levels of the carcases from flocks A and B was equal or greater than 10^6 organism per carcase, while that of flock C was less than 10^6 ($10^{5.62}$). Levels of contamination of the giblets showed a similar correlation.

The mean counts of Campylobacter per gram of caecal content from birds from both A and B flocks were greater than 10^8 per g ($10^{8.23}$ for A and $10^{8.56}$ for B). The mean count of Campylobacter from the caeca of birds from flock C was only $10^{7.36}$ per gram of caecal content which was significantly lower from the mean counts of any of the other two flocks ($P < 0.001$). It was also noted that the range of counts of Campylobacter per g of caecal content was less in birds from both flocks A and B (less than $10^{0.35}$) compared with birds from flock C ($10^{1.31}$).

Table 6.5 shows the number of organisms per g of caecal content from each caeca from five birds from flock A. The mean number for group Aa was $10^{8.16}$ and for group Ab was $10^{8.29}$ and no significant difference was demonstrated between the level of infection in differenct caeca from the same bird.

Figures 6.1 and 6.2 demonstrate the survival of C. jejuni, and C. coli in caecal contents at 25°C and 4°C respectively. At 25°C the mean survival of C. jejuni was 10 days (range 7 to 13 days) while C. coli had a mean survival of 7.8 days (range 6 to 11 days). The differences were not significant. At 4°C the mean survival of C. jejuni was 64 days (range 45 to 100 days) and that of C. coli 56

TABLE 6.4 : INFECTION RATES OF BIRDS AND CONTAMINATION RATES BY C. JEJUNI OR C. COLI OF CARCASES, GIBLETS AND CAECA OF BIRDS FROM THREE INFECTED BROILER FLOCKS (SLAUGHTERHOUSE SURVEY)

Flock	Bird Age (days)	Infection rate from farm survey (%)	Carcase		Edible viscera		Caecal content (/g content)	
			Rates of isolation	Log_{10} mean levels (range)	Rates of isolation	Log_{10} mean levels (range)	Rates of isolation	Log_{10} mean levels (range)
A (No 5)	41	100% C. jejuni	5/5	6.14 (5.65-6.33)	5/5	4.48 (4.04-4.70)	5/5	8.23 (8.08-8.42)
B (No 8)	41	100% C. jejuni	5/5	6.00 (5.65-6.48)	5/5	4.26 (3.60-4.95)	5/5	8.56 (8.45-8.81)
C (No 9)	60	85% C. coli	5/5	5.62 (4.83-6.35)	5/5	4.01 (3.56-4.22)	5/5	7.36 (6.78-8.09)
			15/15	5.92 (4.83-6.48)	15/15	4.25 (3.56-4.95)	15/15	8.05 (6.78-8.81)

TABLE 6.5 : COMPARISON OF NUMBER OF CAMPYLOBACTER IN EACH CAECUM FROM THE SAME BIRD

Bird Number	Group Aa[a]	Group Ab
1	8.18	8.18
2	8.00	8.34
3	7.95	8.25
4	8.32	8.49
5	8.36	8.20
TOTAL	8.16	8.29
S.E.	±0.07	±0.05

[a] Although each caecum was examined separately, a record was not kept from which side they originated. See page 114 for the explanation.

FIGURE 6.1 : SURVIVAL OF C. JEJUNI AND C. COLI IN CAECAL CONTENTS, (5 BIRDS INFECTED WITH EACH SPECIES) STORED AT 25°C.

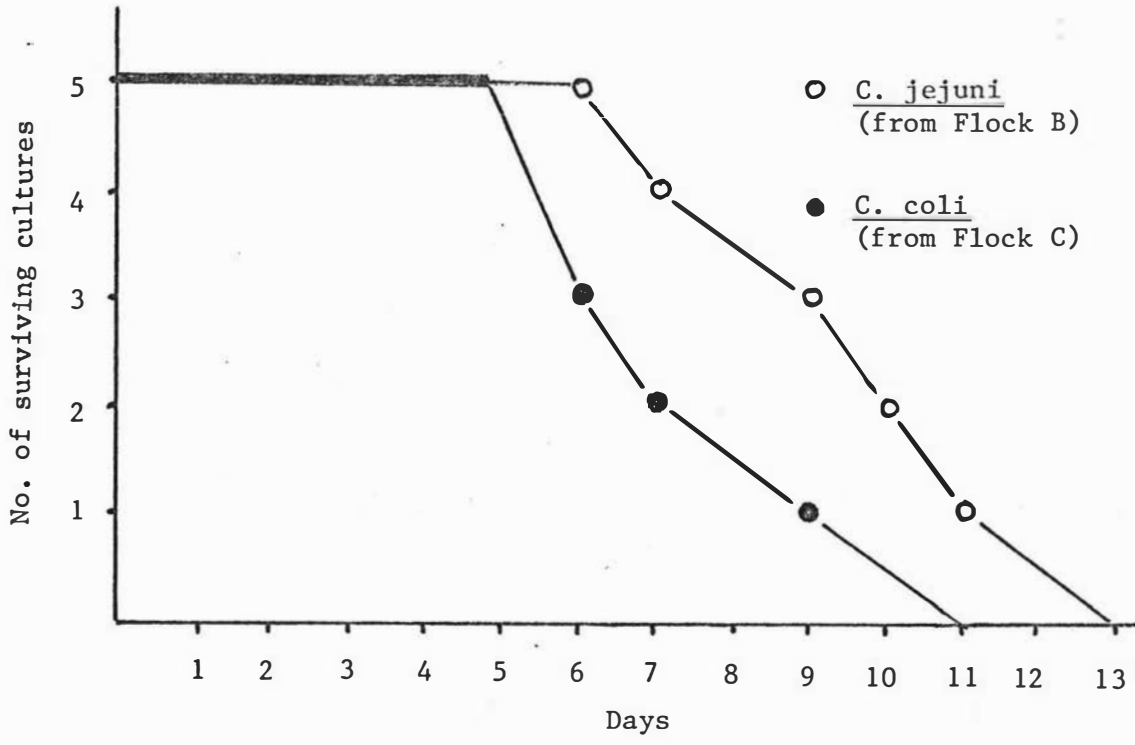
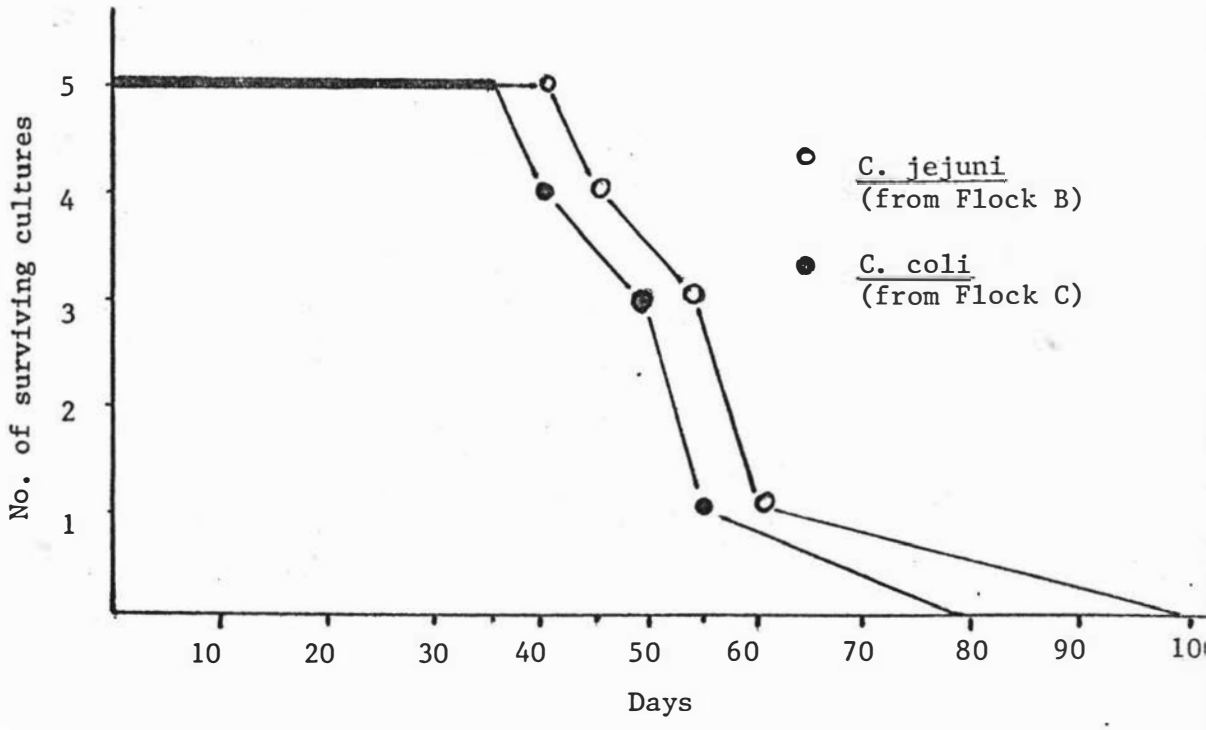


FIGURE 6.2 : SURVIVAL OF C. JEJUNI AND C. COLI IN CAECAL CONTENTS, (5 BIRDS INFECTED WITH EACH SPECIES) STORED AT 4°C.



days (range 40 to 80 days). These differences also were not significant.

Supermarket Survey

Table 6.6 summarised the results of the Supermarket Survey. Two of the five batches of chilled chicken wings examined were contaminated with Campylobacter and all the packets within such a batch were contaminated (one batch with C. jejuni and the other with C. coli).

The mean C. coli counts of the fresh (1 to 2 days old) per chilled wing and per millimeter of liquid present in the packets of these wings were respectively $10^{3.85}$ organisms (range $10^{3.54}$ to $10^{4.25}$) and $10^{2.92}$ (range $10^{2.63}$ to $10^{3.30}$). After four days storage at 4°C, the mean numbers of C. coli per wing and per millimeter of liquid had decreased by a similar amount of $10^{0.49}$. This reduction was not significant. Four days later (eight days of storage at 4°C), the numbers of C. coli declined by a further $10^{.53}$ and $10^{.63}$ per wing and per millimeter of liquid respectively. These reductions also were not significant. However, the overall reduction in number, of $10^{1.02}$ ($10^{0.49} + 10^{0.53}$) and $10^{1.12}$ ($10^{0.49} + 10^{0.63}$) per wing and per millimeter of liquid present in the packets of these wings respectively, between the initial examination (1 to 2 days' storage) and the final examination after eight days' storage at 4°C for both (wings and liquid) were significant ($P < 0.001$).

The results obtained from the survival of C. jejuni either on the wings kept in packets at 4°C, or in the liquid which was present in each packet of chilled chicken wings, were similar to the results obtained from the batch of chicken wings which was contaminated with C. coli. Although the initial levels of C. jejuni were lower than the levels of C. coli ($10^{3.14}$ per wing and $10^{2.29}$ per millimeter of liquid) the reduction in counts with time were similar. After four days' storage at 4°C, the mean counts per wing and per millimeter of liquid were reduced by $10^{.54}$ and $10^{.47}$ respectively. Four days later (eight days' storage at 4°C), the counts were further reduced by $10^{0.51}$ and $10^{0.72}$ respectively. The differences were not significant. However, the overall reductions of $10^{1.05}$ ($10^{0.54} + 10^{0.51}$ and $10^{1.19}$ ($10^{0.47} + 10^{0.72}$) between the initial counts (1

TABLE 6.6 : MEAN COUNTS (Log_{10}) AND RANGE OF CAMPYLOBACTER FROM 20 PACKETS OF CONTAMINATED CHILLED CHICKEN WINGS[a] FROM 50 OBTAINED FROM A LOCAL SUPERMARKET[b] (10 PACKETS EXAMINED EACH WEEK[c])

	TIME EXAMINED AFTER BIRDS PROCESSED								
	1 - 2 Days			4 Days			8 Days		
	No. of packets positive	Mean counts per wing (Range)	Mean counts per ml of liquid in the packet (Range)	No. of packets positive	Mean counts per wing (Range)	Mean counts per ml of liquid in the packet (Range)	No. of packets positive	Mean counts per wing (Range)	Mean counts per ml of liquid in the packet (Range)
C. coli	6	3.85 (3.54-4.25)	2.92 (2.63-3.30)	2	3.365 (3.34-3.39)	2.43 (2.43-2.43)	2	2.835 (2.78-2.89)	1.80 (1.70-1.90)
C. jejuni	6	3.14 (2.70-3.35)	2.29 (1.76-2.70)	2	2.60 (2.44-2.76)	1.82 (1.79-1.85)	2	2.09 (2.00-2.18)	1.10 (0.80-1.40)
	12	3.50 (2.70-4.25)	2.605 (1.70-3.30)	4	2.98 (2.44-3.39)	2.125 (1.79-2.43)	4	2.46 (2.00-2.89)	1.45 (0.80-1.90)

a Based on whole washing of four chicken wings from each packet of six with a detection level of $> 10^2$ cells per wing.

b The supermarket was supplied by the same poultry company (Company A).

c The ten packets from each week were from the same batch of chickens, but each week represented a different batch.

to 2 days storage) and the final counts after eight days storage at $4^{o}C$ for both wings and liquids, were significant ($P < 0.001$).

The wings, after eight days storage at $4^{o}C$, developed a slight green discolouration and mildly unpleasant odour.

There was an almost linear and comparable reduction with the time of storage at $4^{o}C$, in the counts of either C. jejuni or C. coli on the chicken wings and in the liquid present in the packets of wings. This is demonstrated by Figure 6.3. It can be seen from this Figure and from Table 6.6 that there was an approximately tenfold reduction in the number of organisms on both the surface of the wings and in the fluid in the packet during the eight day period of study. There was also an approximate tenfold difference, at any time, between the counts on the surface of the wings and the fluid in the packet.

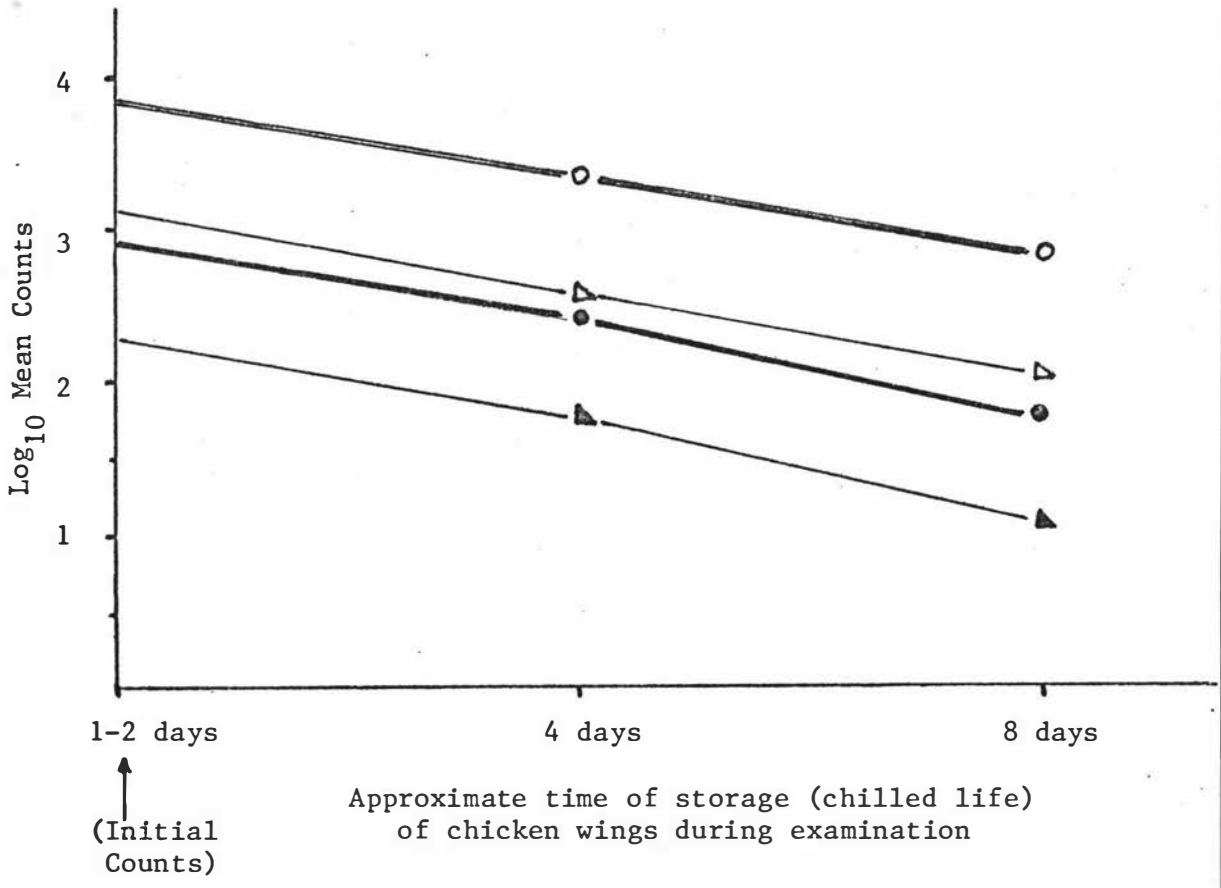
DISCUSSION

The findings that some flocks are infected while others are not, indicates that Campylobacter are not an inevitable component of the gut flora of poultry. This finding is in agreement with recent observations of others (Cruickshank et al 1982, Mehle et al 1982, Oesteron and Becker 1982, Munroe et al 1983, Prescott and Gellner 1984). The present recorded prevalence rate in broiler flocks is within the range of 10% to 70% recorded by these other workers. All these findings indicate that Campylobacter infections in poultry may be able to be controlled by appropriate methods of husbandry, including strict adherence to barrier maintenance.

The finding that the layer flocks were more commonly infected than the broiler flocks may not reflect the true circumstances. First, only a small number of layer flocks were examined compared with the number of broiler flocks. Secondly, birds in one of the infected layer flocks (household layer flock) had access to the backyard of the house, and presumably greater contact with general environmental sources of Campylobacter, compared with the commercial flocks (broilers and layers), which were relatively insulated from the general environment.

FIGURE 6.3 : EFFECT OF STORAGE AT 4°C ON THE SURVIVAL OF C. COLI AND C. JEJUNI ON CHICKEN WINGS AND LIQUID FROM 20 PACKETS

(Each initial count was based on samples of six and subsequent counts on samples of two)



Mean counts of C. coli per chicken wing

Mean counts of C. coli per millimeter of liquid present in the packet

Mean counts of C. jejuni per chicken wing

Mean counts of C. jejuni per millimeter of liquid present in the packet

In spite of these reservations, it is believed that the prevalence of infection in older laying birds is likely to be less than in younger broilers. Unlike broilers, laying birds are normally kept off the ground in individual cages and are likely to be less exposed to contact with infected fomites or direct contact with infected birds. However, there is usually a greater degree of human activity in a shed containing laying birds compared with one containing broilers. From the work on experimental transmission discussed in Chapter VIII, the spread of Campylobacter infection in birds kept in individual cages would be expected to be slower than in birds kept together as a flock.

The prevalence of an infection is proportional to the product of the incidence and duration of the infection (MacMahon and Pugh 1970). Thus cross-sectional surveys, such as those described in this chapter, which only measure prevalence, are more likely to detect infection when the duration of infection is long compared with an infection of shorter duration. As discussed in Chapter VIII, the duration of Campylobacter infection in poultry is in the region of 18 weeks, but elimination of infection starts approximately seven weeks after infection. Thus, if infection occurs early in life, the prevalence in older laying birds (more than 18 weeks old) may be less than in younger broilers. This hypothesis tends to be supported by the finding that 100% infection rates were recorded in six of the infected flocks of young birds less than six weeks old. A lower prevalence of infection by C. jejuni was recorded in breeding flocks (Nos 23a and 23b), the layer flocks (Nos 25b and 26) and broiler flock No. 9, (which was infected with C. coli) where all birds were more than eight weeks old. In flocks 23a and 26, which were examined at five-week intervals, a progressive decrease in prevalence was demonstrated and total elimination occurred in flock 26. Between the first and second examinations, a greater reduction was observed in the prevalence of infection of flock No. 23a compared with flock No. 26. However, the initial prevalence in flock 23a was only 80% compared with a 90% prevalence in flock 26. This was assumed to indicate the elimination of infection was more advanced in flock 23a when first examined.

However, the data obtained from the cross-sectional surveys of

older birds could have been detecting either the beginning or the end of an epidemic.

The results presented, indicate that all ages of poultry (young and old birds) can be infected, but the time at which each flock became infected was unknown. Unlike Salmonella infections, Campylobacter infections are not transmissible by the trans-ovarian route or by contamination of egg shells after they have been laid (Cruickshank et al 1982). However, it is probable that the broiler flocks became infected relatively early in their life as up to 100% infection rates had been established by six weeks of age. It would be unrealistic to assume that all the positive broiler flocks became so extensively infected only a few days before they were examined. Contaminated water could theoretically act as a point source of infection for such a sudden epidemic, but no contamination of the water supplies could be demonstrated. Theoretically, a massive contamination of the water supplies could have happened a few weeks before the birds were examined, but were free of contamination by the time they were examined, but this is considered an unlikely explanation for all poultry infections. Although food was not examined, it was not thought to be a likely point source of a sudden epidemic. It is considered more probable that one or more infected young birds were the source of a propagating epidemic. The high density of birds within broiler flocks would provide ideal conditions for such a rapid spread of Campylobacter infection, particularly if the infective dose is low as is indicated by the work reported in Chapter VIII.

When an infected broiler flock was detected, all other flocks examined from the same farm were also infected. This however, was not the case with the farm with two flocks of layers, in which only one of the two flocks was infected. It is believed that these circumstances indicate that cross transmission may be associated with the transference of infected material on the footwear of workers or by rodents. Such a mode of transmission would create a greater risk to broilers on litter, over which people would walk, compared to layers kept in individual cages above ground level. Also infected rats on a property might act as a reservoir of infection and a mode of transmission between flocks (see Chapters VIII and X).

Campylobacter jejuni has been the only species recovered from poultry by most investigators (Cruickshank et al 1982, Mehle et al 1982, Munroe et al 1983, Prescott and Gellner 1984). However, the 14% recovery rate of C. coli reported in this thesis is similar to that reported by Skirrow and Benjamin (1982b), who found that 20% of poultry isolates were C. coli (the remainder were C. jejuni), and the results reported by Rosef and Yndestad 1982)

The lower rates of isolation of C. coli compared with C. jejuni from flocks with dual infection (see flock Nos 12a, 12b) is interesting. Although there could be several reasons for these differences, they might be due to C. coli being less infective for poultry than C. jejuni. (Some experimental evidence to support this hypothesis is reported in Chapter VIII). This hypothesis could account for the finding that the flock infected with C. coli only (No. 9) had an 85% prevalence of infection in birds of 53 days old, while all the younger broiler flocks infected with C. jejuni had a 100% infection rate.

An alternative hypothesis to those which have been discussed, is that the flocks became infected with C. coli subsequent to C. jejuni becoming established, and that sufficient time had not elapsed for C. coli to reach a similar level of infection.

The way in which poultry are handled after slaughter, including passage through a scalding tank, being mechanically plucked and eviscerated and finally being subjected to the spin washer and the spin chiller, is conducive to cross contamination (Cunningham 1982). Morris and Wells (1970) showed that the prevalence of contaminated broiler carcases (before and after evisceration) was similar to the prevalence of infection in birds submitted for slaughter. Galton et al (1955) demonstrated an increased rate of contamination of carcases compared with live birds. Passage through continuous counter-flow chillers, reduces the total bacterial counts of eviscerated chickens but has no significant effect on contamination with Salmonellae (Surkiewicz et al 1969). Wempe et al (1983) found that the outflow water from spin chillers was always contaminated with Campylobacter when infected flocks were processed. It was not surprising therefore that all the carcases and edible viscera from infected flocks A and B and the C flock were contaminated, although not all the birds from flock C were

infected. Similar findings have been reported by Mehle *et al* (1982) who found an approximately 99% contamination rate of carcases from 19 broiler flocks which were 100% infected with *Campylobacter*.

The mean count of $10^{5.92}$ *Campylobacter* per carcase (range $10^{4.82}$ to $10^{6.48}$) recorded in this study is higher than that reported by Shanker *et al* (1982) of 8.6×10^4 who used a similar whole washing technique. Shanker's investigation was based on the results from four different slaughterhouses. In one slaughterhouse, the ten positive poultry carcases examined by Shanker, had a range of contamination from 1.2×10^4 to 4.8×10^6, which was similar to the results reported in this thesis from the 15 positive carcases examined. Shanker does not record the time after slaughter, at which carcases were examined. It is probable that levels of contamination would be less in birds first processed, compared with those processed at the end of the run. In the present investigation, carcases were examined two to three hours after the beginning of the slaughter and processing.

Another factor which is likely to affect the level of contamination of poultry carcases is the temperature of the scalding water. Kinde *et al* (1983) reported, with insufficient explanation, that scald water temperatures of 60°C for 90 seconds resulted in a higher prevalence of *C. jejuni* on chicken carcases in comparison with scald temperatures of 53°C and 49°C. These results (Kinde *et al* 1983) are in apparent conflict with the findings of Doyle and Roman (1981) who found that the thermal inactivations (D-values) of five strains of *C. fetus* subsp. *jejuni* under laboratory conditions, at 48°C ranged from 7.2 to 12.8 minutes, while at 55°C ranged from only 0.74 to 1.00 minute. High temperature scalding (60°C) facilitates defeathering, but also removes the surface layers (epidermis) of the skin (Bremner 1977), making it moist and sticky (Mountney 1966), and presumably rough in texture. Lower scalding temperatures (49°C to 53°C) leave the skin intact (Mountney 1966). Thomas and McMeekin (1981), using electron microscopy, showed that *Salmonellae* attached better, and in greater numbers, to chicken skin which is crushed and rough, compared with less rough and undamaged skin. It is the present author's opinion that a rough, moist and sticky chicken skin, produced by a higher scald temperature (60°C), provides better opportunities for *Campylobacter* to attach to its surface, than the smoother skin surface produced by lower temperatures. In spite of higher temperatures inactivating more organisms,

it is assumed that this effect is more than counteracted by the greater attachment of organisms, subsequently contaminating the carcase, to roughened skin, resulting from scalding carcases in water at 60°C compared with water at 49°C to 53°C. This hypothesis would account for both the results recorded in this thesis and those recorded by Kinde et al (1983).

The higher contamination levels of carcases and giblets from birds from flocks A and B compared with flock C, although not significant, are believed to be associated with the 100% infection rate of the former flocks compared with the relatively lower rate of the latter flock (85%). Further support for this hypothesis is provided by the fact that the counts per gram of caecal content from the first two flocks were significantly higher than the counts from the latter flock and thus the expected degree of contamination would be less. Feathers collected from the plucking machines during the slaughter of infected flocks have been reported to be highly contaminated (Wempe et al 1983), but the primary source of any contamination is likely to be from the G.I. tract. Thus the level of G.I. infection will affect the level of contamination of feathers and other by-products.

The finding that no differences were observed in the counts between the two caeca from the same birds was not unexpected. On the basis of these results, counts based on the caecal contents for either caecum will give an accurate assessment of the degree of infection. Thus subsequent studies on flocks B and C were based on caecal counts from only one caecum.

The demonstration of over 10^8 cfu of Campylobacter per gram of caecal content is in agreement with the findings of Grant et al (1980). These investigators reported a mean level of Campylobacter of 4.4×10^6 per gram of rectal content. However, they emphasised that their technique (filtering) was holding back approximately 99% of Campylobacter. These results concerning the numbers of Campylobacter in caecal contents of infected chickens are supported by the results from the experimental infection reported in Chapter VIII. However, this latter work showed that the number of organisms in the faeces were less than in the caecal content.

The differences in the survival of C. jejuni and C. coli in caecal

contents at 25°C and 4°C was to be expected in light of the work reported in Chapter IV. The slight and insignificant difference in the survival of C. jejuni compared with C. coli at both temperatures, is likely to be associated with the higher numbers of C. jejuni in the caecal content (flock B versus flock C). In this respect, it is interesting to note that Savopoulou (1980) reported that C. fetus subsp. jejuni survived longer in diarrhoeic faeces from humans at 4°C if the initial level of infection was high, compared with faeces where the initial counts were lower.

Other workers have failed to demonstrate such long times of survival for Campylobacter in faeces or caecal content, as reported in this chapter. This includes the work of Blaser et al (1980d) and Savopoulou (1980) on the survival in human faeces, the work of Grant et al (1980) on chicken rectal contents and the work of Luechtefeld et al (1981) on turkey caecal contents. Blaser et al (1980d) failed to demonstrate survival of organisms for more than 22 days at 4°C and Luechtefeld et al (1981) for more than 9 days at 25°C. One of the reasons which might account for the longer survival of both C. jejuni and C. coli reported in these results, may be associated with the fact that the caecal contents were stored in closed universal bottles. Thus the material would be less likely to dry out compared with faecal material directly exposed to the air (see also the discussion of part 1, Chapter IV). Another factor could be the coarser particulate nature of human faeces and chicken rectal content compared with caecal faeces. This could affect the penetration of faeces by air as well as the evaporation of water, both of which are known to adversely affect the survival of Campylobacter. Although this argument would not account for the lower number of organisms reported from the caecal content from turkeys, the mean number of C. jejuni subsp. jejuni in turkey caecal content recorded by Luechtefeld et al (1981) was only 2.7×10^6 per g of material, while the chicken flocks B and C examined in the present study had mean values of $10^{8.56}$ (3.63×10^8) and $10^{7.36}$ (2.29×10^7) respectively. This 10 to 100 fold greater level of infection would increase the duration of recovery of both C. jejuni and C. coli.

This rather lengthy discussion on the survival of Campylobacter in caecal contents, was thought to be appropriate, because of its implications in relation to environmental contamination and possible sources of infection for both poultry and other animals including

humans. The results tend to indicate that in cold and humid conditions, prolonged environmental contamination is likely to occur. This could be particularly relevant to poultry shed management and hygiene, particularly in relation to shed cleaning and disinfection between different batches of broiler chickens.

In a limited study of this problem, Cruickshank et al (1982) failed to report cross infection following normal cleaning and disinfection of the poultry houses, which had been previously inhabited by infected chickens. The present author noted that two flocks of three-week-old birds did not become infected after being transferred to poultry houses previously inhabited by infected birds (the dual infected flocks No. 12a and 12b). The usual practice with the broiler flocks studied, was that sheds were left empty for six weeks before being restocked. This allowed time for cleaning and disinfection and presumably the elimination of Campylobacter. Moreover, the temperature within poultry houses is not conducive to the survival of Campylobacter. Temperatures range from 35°C to 23°C in the first four to five weeks under the lamp, and are 20°C to 15°C away from them. Chickens older than four to five weeks are not given artificial heat, but the temperature in a properly operated house is normally around 12°C to 15°C (Spais 1979).

The results from the Supermarket Survey were to be expected from the results of the Farm and the Slaughterhouse Surveys. The positive and negative batches of chilled chicken wings reflect the occurrence of infected and non-infected flocks. The 100% contamination rate of the positive batches of chicken wings was to be expected in light of the results from the Slaughterhouse Survey.

These results clearly demonstrate that poultry are not only a potential source of C. jejuni, but also of C. coli, and that high contamination levels of poultry carcases and their freshly processed products by both species of Campylobacter, are to be expected.

The number of Campylobacter in ground beef liver, experimentally inoculated with different isolates of C. jejuni and C. coli and stored at 4°C, decreased only slightly over a period of 6 days, although the product showed signs of insipient spoilage. After approximately

three months' storage at -20°C, the numbers decreased by 10^2 to 10^3. The number of Campylobacter on chicken wings stored at -20°C (a similar experiment was not conducted at 4°C) decreased in number by $10^{0.5}$ to 10^2 after three months' storage (Hanninem 1981). Conversely Kinde et al (1983) found that the number of organisms chilled chicken wings naturally contaminated with C. fetus subsp. jejuni, was reduced by approximately $10^{0.7}$ and $10^{1.3}$ after storage at 4°C for three and six days respectively. The findings on the survival of C. jejuni and C. coli reported in this part of the thesis appeared to be intermediate between those of Hanninem (1981) and of Kinde et al (1983).

From the results obtained on the survival of C. jejuni and C. coli in caecal contents and on carcases, it appears that there is generally no difference in the times of survival of these two species outside the G.I. tract.

The good correlation between the mean counts of C. coli and C. jejuni per millimeter of liquid present in the packets of chicken wings and the mean total counts per wing, indicate that there is no difference between the ability of these two species to become detached from the surfaces of poultry carcases. It would also appear that by adjusting counts obtained per millimeter of liquid in the packet of chilled chicken wings by $10^{0.92}$, the numbers of C. jejuni or C. coli per wing can be calculated. This avoids the more time consuming technique of whole wing washing.

The shelf life of chicken carcases and other chicken by-products stored in the chillers is usually not more than eight to ten days (Panetsos 1978) during which time Campylobacter would be able to survive.

CONCLUSIONS

1. Thermophilic Campylobacter are common, but not obligatory, inhabitants of the intestinal tract of chickens.

2. In flocks which are infected (28%), the prevalence of infection in young birds (broilers) is almost 100%, while in older birds

(layers) the prevalence rates are lower.

3. Infection in some flocks has been shown to be self-limiting.

4. Eighty six per cent of isolates recovered from chickens were *C. jejuni* and 14% *C. coli*.

5. Within the G.I. tract, highest levels of infection became established in the distal parts ($>10^8$ organisms per g of caecal content).

6. Carcases and viscera derived from birds from infected flocks are highly contaminated with *Campylobacter* ($>10^4$ organisms).

7. Both *C. jejuni* and *C. coli* can survive on chilled chicken wings ($4^{\circ}C$) for more than the 'shelf life' of the product (8 - 10 days).

8. Both *C. jejuni* and *C. coli* are able to survive in caecal contents for more than 6 days at $25^{\circ}C$ and more than 40 days at $4^{\circ}C$. *C. jejuni* can survive for up to 100 days at $4^{\circ}C$.

CHAPTER VII

THE PREVALENCE OF INTESTINAL THERMOPHILIC CAMPYLOBACTER INFECTIONS IN WILD LIFE (GULLS, DUCKS, HOUSE SPARROWS AND RATS)

INTRODUCTION

Wildlife can act as reservoirs of infection for a variety of infectious diseases, for man and domestic stock. This can occur in two different epidemiological circumstances, the first of which is exemplified by the transmission of salmonellosis. Surveys on the prevalence of Salmonellae in seagulls have shown a much higher rate in birds feeding around effluent ponds compared with those away from human habitation (Fenlon 1981b, Butterfield et al 1983). In this example, the prevalence of infection in the gulls is a reflection of the degree of contamination of the environment which they inhabit.

The second way in which wildlife act as reservoirs of infection is by acting as strict maintenance hosts for specific agents and as direct sources of infection for man and domestic stock. For example, in New Zealand Leptospira interrogans serovar ballum is maintained only in mice, rats and hedgehogs (Hathaway 1981), which are the sole source of infection for other animals including man.

The work outlined in this chapter was undertaken to investigate whether or not, some species of wildlife were capable of maintaining intestinal thermophilic Campylobacter and were a potential source of infection for man and domesticated animals.

MATERIALS AND METHODS

Gulls

Location and species of gulls investigated: Seventy freshly void faeces from gulls were collected from the area of the rubbish tip of Palmerston North (P.N.), a city of 62,000 people, and a further 70 samples from the Manawatu River estuary at Foxton Beach.

Figure 7.1 shows the two locations from where the samples were collected.

The Southern black-backed gull (Larus dominacanus) was the only species of gull scavenging in large numbers on the rubbish tip throughout the year. When not feeding, these birds rest on the more level areas of the tip slightly away from the garbage. The same species of gull was also present, but in smaller numbers, at the estuary of the river.

It was noticed that the majority of the birds inhabiting the rubbish tip were of immature plumage, while on the estuary the majority of birds were of mature plumage.

Times and method of sampling, transportation and culture: The faeces from the rubbish tip area were collected during July - August 1982, by walking among gulls on their resting sites. The faeces from the estuary of the river were collected in May - June 1983. At low tide, the position of flocks on the sand near the water, were noted from several hundred meters away, and then the flock was approached and fresh faeces were individually collected in universal containers from these recently vacated sites.

The transportation and culture of the faeces collected, was similar to that as described in Chapter III, with the exception that a 30 µg nalidixic acid disc was placed on the surface of the selective plates immediately after culture. The position of the disc is shown in Figure 7.2. The disc was intended to cover part of the primary heavy inoculation from the suspended faeces and part of the subsequent three to four lines of secondary inoculation.

The addition of the nalidixic acid 30 µg disc was used to facilitate the recognition on the same plate, of both nalidixic resistant (C. laridis) and non resistant species of Campylobacter (C. jejuni/C. coli). If C. laridis was present, growth would be expected over the whole surface of the plate. If only C. jejuni or/and C. coli were present, no growth would be expected within the zone of inhibition of the disc. If however, both resistant and non-resistant species were present, this was indicated by differences

FIGURE 7.1 : LOCATIONS WHERE FAECES FROM GULLS WERE COLLECTED

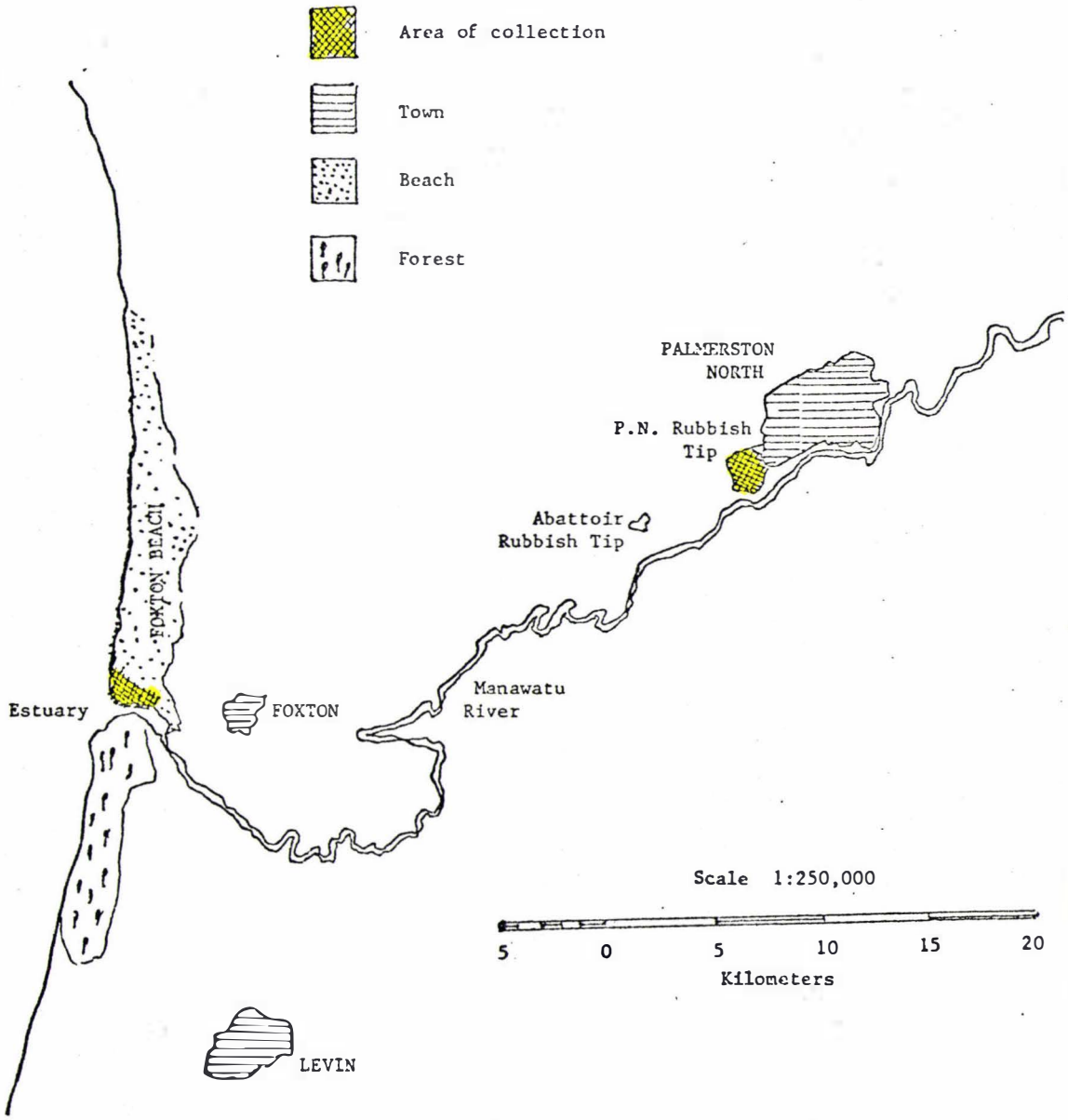
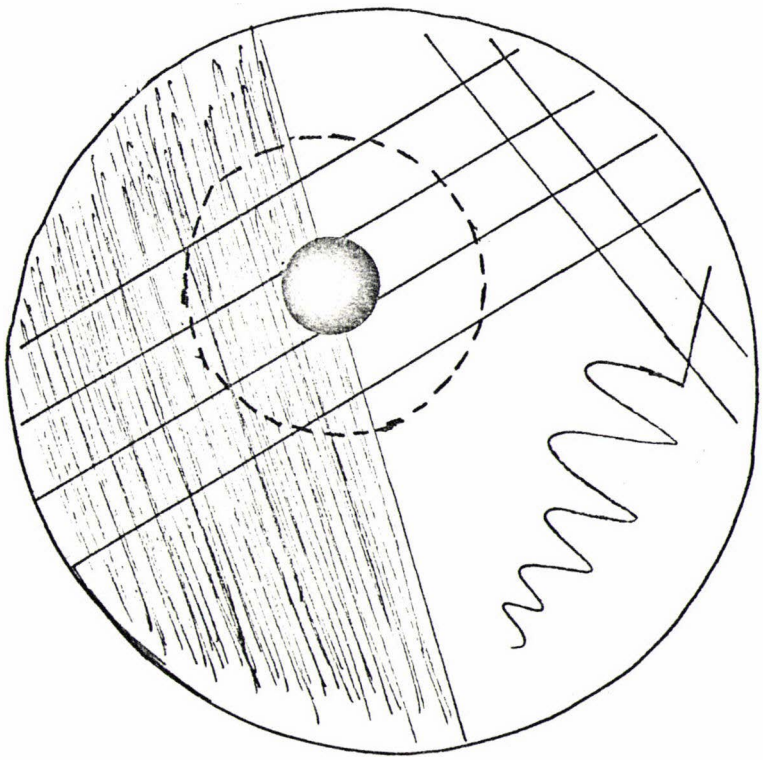


FIGURE 7.2 : POSITION OF THE 30 µg NALIDIXIC ACID DISC ON THE ANTIBIOTIC SELECTIVE MEDIUM AFTER FAECAL INOCULATION



Small circle (dark) = Nalidixic acid disc

Large circle = Periphery of the zone of inhibition produced by the disc.

in the density of the growth of Campylobacter around the disc compared with elsewhere on the plate.

Trial using selective plates with and without 30 µg nalidixic acid discs: In order to confirm the assumptions in the preceding paragraph, each of the first 33 faecal samples collected from the rubbish tip was cultured on selective plates with and without nalidixic acid discs.

From each plate with no disc, on which Campylobacter were identified, two colonies from opposite sides of the plate were taken for further study.

From each of the plates with the nalidixic acid 30 µg disc, either one or two colonies were examined in further detail. If there was total inhibition of growth around the disc, only one colony was examined from the periphery of the plate. If growth was present within the zone of inhibition, a colony was taken close to the disc. A second colony was taken only when there was a partial inhibition of growth. This second colony was taken from a site just outside the periphery of the activity of the disc on a line of inoculation which passed through the zone of inhibition without any colonies upon it within this zone (see Figure 7.3).

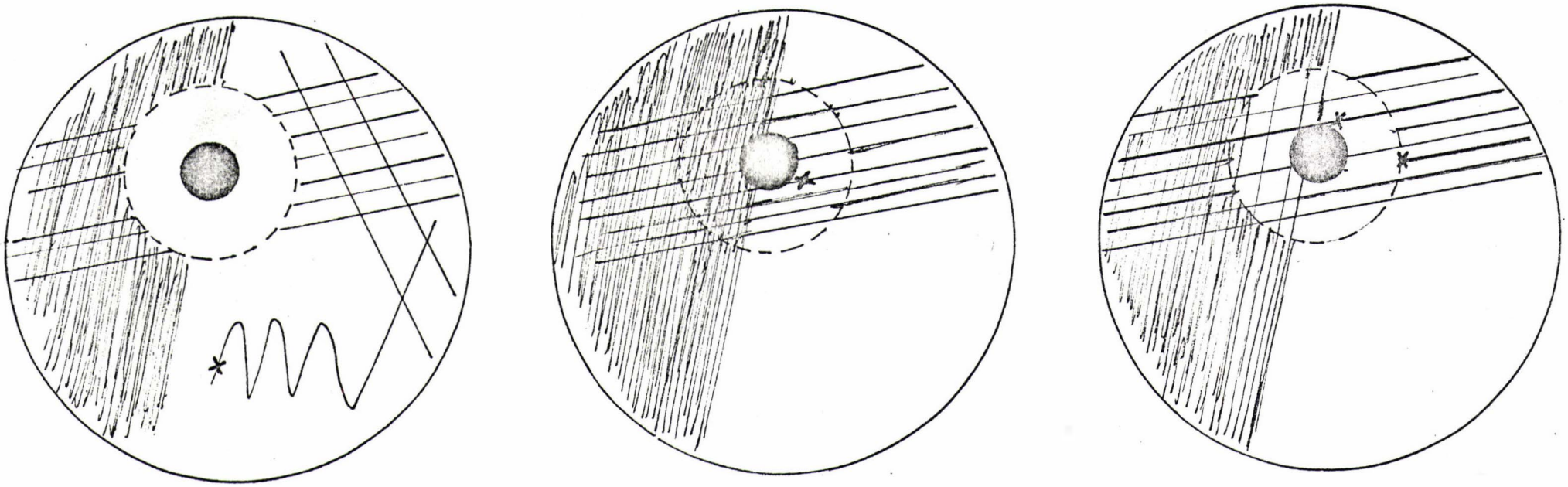
After the results of this technique had been evaluated (see next page), a 30 µg nalidixic acid disc was placed on all plates used to culture the faeces of gulls.

Enumeration of Campylobacter: Enumeration studies were carried out on ten of the 70 faecal samples collected from the rubbish tip (see Chapter III).

Ducks

Thirty one freshly void faecal samples were collected during October/December 1982 from the banks of three ponds which were inhabited by Mallard (Anas platyrhynhos) and Grey (Anas supersiliosa) ducks. One pond was within the city of Palmerston North (P.N.), one was 3 km away at Massey University and the third was on the outskirts of the city of Wanganui, 60 kms away from P.N. The ponds varied in

FIGURE 7.3 : METHOD OF SELECTION OF COLONIES FOR FURTHER INVESTIGATION



(a) Total Inhibition
One colony examined

(b) No Inhibition of Growth
One colony examined

(c) Partial Inhibition
Two colonies examined

x = site from which colony is examined.

size from approximately 0.25 to 0.75 hectares and the population of ducks on each pond varied from between 100 and 300 at the times of each visit. Of the 31 faeces collected, 13 were from the pond in Palmerston North, eight from the pond at Massey University and ten from Wanganui.

Sparrows

Thirty two freshly void faeces from house sparrows (Passer domesticus) were collected during a period of a few days in November 1982 from around the clinic area of the Faculty of Veterinary Science at Massey University. Faeces were collected on clean newspapers placed in areas frequented by sparrows, and samples were collected every 2 - 3 h and examined immediately (see Chapter III).

Rats

Caecal swabs taken from thirty adult rats (Rattus norvegicus) were cultured for intestinal thermophilic Campylobacter. The rats were shot during four night visits to a rubbish tip at an abattoir in June and July 1983. Further details have been described in Chapter III.

RESULTS

Gulls

Use of selective plates with and without 30 µg nalidixic acid disc: Table 7.1 gives the results obtained during this trial of 33 faecal samples from gulls.

The addition of the disc had no adverse effect on the recovery of intestinal thermophilic Campylobacter, and Campylobacter were detected in 22 of the 33 faeces examined, whether or not a disc was employed. On the selective plates with the disc, 16 isolates of C. jejuni, eight of C. laridis and one of C. coli were recovered from the 22 infected faeces, while from equivalent plates with no discs, only 15 C. jejuni, six C. laridis and one C. coli were recovered. The disc method showed that three of the infected faeces, (Nos 9, 10 and 20), contained both C. jejuni and C. laridis which was not determined from the plates without nalidixic acid discs. Faeces Nos 9 and 10 were identified by the non disc method to contain only

TABLE 7.1 : THE ISOLATION OF INTESTINAL THERMOPHILIC CAMPYLOBACTER FROM 33 FAECES OF GULLS CULTURED ON SELECTIVE PLATES WITH AND WITHOUT 30 µg NALIDIXIC ACID DISCS

No. of infected faecal sample	Selective plates with 30 µg Nalidixic acid disc			Selective plates without 30 µg Nalidixic acid disc			
	C. jejuni	C. laridis	C. coli	C. jejuni	C. laridis	C. coli	
1	+			+			
2		+			+		
3		+			+		
4	+			+			
5		+			+		
6	+			+			
7			+			+	
8	+			+			
9*	+	+		+			
10*	+	+		+			
11	+			+			
12	+			+			
13	+			+			
14		+			+		
15	+			+			
16	+			+			
17	+			+			
18	+			+			
19	+			+			
20*	+	+			+		
21		+			+		
22	+			+			
22	16	8	1	15	6	1	TOTAL

* dual infections detected by use of disc

C. jejuni and faeces No. 20, C. laridis.

Although the selective plates contained amphotericine B (see Chapter III) to control fungal growth, such contaminants remained a problem after incubation periods of 48 hours or greater.

Table 7.2 shows the prevalence of intestinal thermophilic Campylobacter in faecal samples from the rubbish tip in P.N. and the estuary of the Manawatu River at Foxton Beach. From each locality, Campylobacter were isolated from 41 (59%) of the 70 samples examined from each site. From the rubbish tip, twenty five (61%) of the 41 isolates were C. jejuni, 18 (44%) C. laridis and four (10%) C. coli. From the estuary 14 (34%) isolates were C. jejuni, 27 (66%) C. laridis and 7 (17%) C. coli. Six of the faecal samples taken from the rubbish tip and six from the estuary were found to contain both C. jejuni and C. laridis. C. laridis and C. coli were also isolated from a faecal sample from the estuary.

The differences in the prevalences of C. jejuni between the two locations were significant ($P < 0.05$), but there was no significant difference in the prevalence of C. laridis and C. coli in birds from the two sites.

Enumeration of Campylobacter: Table 7.3 shows the number and the species of thermophilic Campylobacter present in the faeces of infected gulls collected from the rubbish tip at P.N. Only seven of the ten faecal samples examined contained Campylobacter; four with C. jejuni (Nos 2, 3, 5 and 7), one with C. laridis (No. 1) and two with both C. jejuni and C. laridis (Nos 4 and 6). Infected faeces (Nos 4 and 6) produced two distinct kinds of colonies on the selective plates after 48 h incubation. One was large, flat and with an undulate edge with a tendency to spread. The other was smaller, umbonate and with an almost entire edge and with no tendency to spread. The former colonies were identified as C. jejuni and the latter as C. laridis. By counting each type of colony separately from the faeces containing two species of Campylobacter (Nos 4 and 6), it was possible to determine the number of both C. jejuni and C. laridis, as well as the total number of thermophilic Campylobacter (see Table 7.3).

The overall mean number of Campylobacter per gram of gull faeces

TABLE 7.2 : PREVALENCE OF INTESTINAL THERMOPHILIC CAMPYLOBACTER IN THE FAECES OF BLACK BACKED GULLS

Species	Rubbish Tip, P.N. (n = 70)		Foxton Beach, Manawatu Estuary (n = 70)	
	Prevalence	Percentage of positive samples	Prevalence	Percentage of positive samples
C. laridis (NARTC)	26% (18)	44%	39% (27)	66%
C. jejuni	36% (25)	61%	20% (14)	34%
C. coli	6% (4)	10%	10% (7)	17%
Overall prevalence	59%[a] (41)	100%	59%[b] (41)	100%

( ) = number of positive samples

a Six gulls had dual infection with *C. laridis*/*C. jejuni*

b Six gulls had dual infection with *C. laridis*/*C. jejuni* and one with *C. laridis*/*C. coli*

TABLE 7.3 : NUMBER AND SPECIES OF THERMOPHILIC CAMPYLOBACTER ISOLATED FROM INFECTED FAECES OF GULLS COLLECTED FROM THE RUBBISH TIP

No. of infected faeces	Number/g faeces (Log_{10})		
	C. jejuni	C. laridis	Total
1	-	+ (2.60)	2.60
2	+ (3.04)	-	3.04
3	+ (2.06)	-	2.06
4	+ (2.48)	+ (2.00)	2.60
5	+ (5.08)	-	5.08
6	+ (3.46)	+ (2.85)	3.57
7	+ (3.60)	-	3.60
Means	6 (3.29)	3 (2.48)	7 (3.22)

was $10^{3.22}$ (range $10^{2.06}$ to $10^{5.08}$) while that of C. jejuni and C. laridis was $10^{3.29}$ and $10^{2.48}$ respectively.

Development of resistance to nalidixic acid by C. jejuni and C. coli: Three cloned colonies of C. jejuni and two of C. coli, of more than 150 examined from gulls, were found to develop resistance to nalidixic acid on subculture. These colonies on several subsequent subcultures remained resistant to nalidixic acid. Those which were originally classified as C. jejuni, remained hippurate positive while those which were classified as C. coli remained hippurate negative. Resistant and non-resistant colonies from the original isolates were shown to have identical DNA patterns and it was assumed that they were variants of the same strain (see Chapter X).

Ducks

Thermophilic Campylobacter were isolated from nine (29%) of the 31 duck faeces examined. Seven of these isolates were C. jejuni and two C. coli. Four isolates (two C. jejuni and the two C. coli) were recovered from the 13 faeces collected from the banks of the pond in P.N., three were from the eight faeces taken from the pond at Massey University and the remainder two isolates from the ten faeces from the pond in Wanganui. There were no significant differences in the rate of isolation of Campylobacter from the three localities.

House Sparrows

No isolates of Campylobacter were found from the 32 faecal samples from sparrows.

Rats

Eighteen (60%) of the 30 rats (caecal swabs) examined were infected with C. jejuni. No other species of thermophilic Campylobacter were isolated. Five of the ten caecal contents used for the enumeration of thermophilic Campylobacter were infected. The mean number of C. jejuni per gram of caecal content was $10^{4.88}$ (range $10^{4.0}$ to $10^{6.88}$).

During identification procedures, subcultures of two of the 18 isolates developed some colonies resistant to nalidixic acid. These colonies, after a further subculture remained fully resistant to

nalidixic acid, but remained hippurate positive. They were considered variants of the original C. jejuni isolates and subsequent DNA analysis of the resistant and the non-resistant colonies, indicated that they had similar patterns (see Chapter X).

The overall prevalence of thermophilic Campylobacter in the various species of wildlife examined are summarised in Table 7.4.

DISCUSSION

The comparison of the two methods of isolation - one using a selective plate with a 30 µg nalidixic acid disc and the other without a disc, showed them to be equally accurate in detecting the overall prevalence of intestinal thermophilic Campylobacter in faeces from gulls. The former however, was clearly more sensitive in detecting dual infections,than the latter.

The addition of the 30 µg nalidixic acid disc on the selective plate could theoretically inhibit the growth of C. jejuni and C. coli. This could alter the isolated rate if during the plating out procedure, these organisms were limited to the area of the disc. This would be however, a most unlikely circumstance. The 13 (16%) dual infections reported in this work, is almost double the rate (9.5%) recorded by Kapperud and Rosef (1983) who used more conventional methods and claimed to have examined in detail, all suspect Campylobacter colonies growing on the plates.

The high prevalence (59%) of Campylobacter in southern black-backed gulls is in agreement with the findings of other investigators who reported rates of isolation of Campylobacter in gulls in Scotland from 20 to 70% (Fenlon 1981a) and in Norway up to 63% by Kapperud and Rosef (1983). However, any direct comparison is difficult because the species of gulls involved were different. However, Fenlon et al (1982) reported a prevalence rate of 55% from 167 herring gulls (L. argentanus) and greater black-backed gulls (L. ichthyaetus). The later European species is closely related to the Southern black-backed gull (L. dominicanus).

Fenlon (1981a) stated that unlike Salmonellae, the presence of

TABLE 7.4 : PREVALENCE OF CAMPYLOBACTER IN FAECES FROM WILD LIFE

Species	No. examined	No. positive	Prevalence C. jejuni	Prevalence C. coli	Prevalence NARTC	Overall Prevalence
Black backed gulls	140	82	28% (39)	8% (11)	32% (45)	59%
Ducks	31	9	23% (7)	6% (2)	-	29%
House Sparrows	32	nil	-	-	-	-
Rats[a]	30	18	60% (18)	-	-	60%

( ) = Number of positive samples

[a] = Caecal contents, not faeces

Campylobacter in the faeces of gulls does not seem related to the birds' feeding habits. The identical prevalence of Campylobacter obtained from the southern black-backed gulls, from two different localities, the rubbish tip and the estuary, where their food would have been different, is in agreement with Fenlon's (1981a) statement. However, it is possible that the differences in feeding habits may alter the relative proportions of C. jejuni, C. laridis, and C. coli in the G.I. tract. Such differences, if they occur, could be obscured if results from birds from different localities are not analysed separately.

Within the limitations of a relatively small survey, the results indicated that the prevalence of C. jejuni was significantly higher in gulls on the rubbish tip than on the estuary of the river at Foxton Beach. (Similar significant differences were not demonstrated for C. laridis and C. coli). This apparent difference associated with locality could have been confounded by age, as the birds on the rubbish tip were immature and the majority at the estuary were adult. These observations are in agreement with the findings of Vernon (1972). This author stated that "there is a marked increase in the number of first year birds in flocks of black-backed gulls as one goes inland from the coast in most habitats, but in urban habitats immatures tend to be present in higher numbers irrespective of distance from the sea, though they are noticeably absent from dockland areas."

If the age of the gulls was the factor associated with the prevalence of C. jejuni, then there could be a similarity to chickens in which lower prevalence rates occur in older birds. In this respect, it is interesting to note that chickens dosed orally with C. jejuni isolated from the faeces of gulls remained infected for up to 16 weeks after which infection was eliminated (see Chapter VIII). If C. jejuni infection was also self-limiting in gulls, a lower prevalence would be expected in older birds. Similar comparisons between gulls and poultry and infection with C. laridis and C. coli cannot be made, as isolates of these species from gulls failed to infect chickens (see Chapter VIII) and may in fact be the same species (see Chapter X).

The locality of the gulls could affect the prevalence of C. jejuni and C. laridis and C. coli in two ways:

1. Campylobacter jejuni and C. coli are known to be less halophilic than C. laridis (Skirrow and Benjamin 1980a, Benjamin et al 1983). Thus the saline environment of the estuary might favour survival and transmissability of C. laridis compared with the other two species.

2. The muscle of marine fish contains trimethylamine N-oxide hydrochloride (TMAO), (Connell 1980). Benjamin et al (1983) suggested that TMAO would favour the growth of C. laridis in the intestine of the fish eating birds. They showed that C. laridis grows anaerobically in media containing TMAO, while this compound inhibits the growth of C. jejuni and C. coli.

C. coli and C. laridis have been shown to be more closely related to each other than to C. jejuni (Benjamin et al 1983). Work presented later in this thesis (see Chapter X) shows that the isolates of C. coli from gulls have the same biochemical characteristics as C. laridis while isolates of C. coli from other sources are quite different. For example C. coli from gulls consistently grow in the presence of 1.5% NaCl and TMAO (see Chapter X). These biochemical characteristics of isolates of C. coli and C. laridis from gulls are further characteristics which might be associated with species isolated from fish eating animals from a marine environment.

Evidence is presented in Chapter X, which indicates that isolates of 'C. coli' from gulls are different from isolates of C. coli from other species of animal. Furthermore, it is believed that these isolates of 'C. coli' from gulls, would be more correctly classified as C. laridis. Until the data presented in Chapter X are discussed, the classification of C. coli and C. laridis is based on conventional taxonomic criteria.

The high prevalence rates of C. laridis in gulls compared with other species investigated confirms the work of others (Skirrow and Benjamin 1980a, Fenlon 1981a, Fenlon et al 1982 and Kapperud and Rosef 1983). However these investigators demonstrated a higher prevalence of C. jejuni than C. laridis, which was the reverse of

the situation reported in this thesis (45 C. laridis compared with 39 C. jejuni). Recently Fricker et al (1983) reported higher isolation rates of C. laridis in gulls compared with C. jejuni using both direct culture, and culture after enrichment, on different types of selective media.

The distance between the Manawatu estuary and the Palmerston North rubbish tip is just over 30 km and previous investigations of gulls by the Wildlife Services of the Department of Internal Affairs has demonstrated movement of birds from the estuary to the Palmerston North region (R.O. Cossee, pers. comm). Presumably at some stage the direction of movement of the gulls is reversed. In addition, based on BRENDA typing, (see Chapter X), it was found that a number of faecal samples taken from the two locations contained identical BRENDA types. Thus the two populations cannot be considered to be entirely separate.

The low mean count of thermophilic Campylobacter obtained from the faecal samples of gulls examined, indicates that these birds have a lower level of infection compared with other species of animals and birds examined during the course of this work. The low numbers of Campylobacter in the faeces of gulls and the high prevalence of infection found in these birds may also indicate that the infectivity of these organisms for gulls may be relatively high.

The 29% isolation rate of thermophilic Campylobacter from the relatively small number of duck faeces examined, is similar to the findings of Luechtefeld et al (1980), who recorded a 35% prevalence rate from the caecal contents of 445 wild ducks. The results also demonstrate that wild ducks, like chickens, can be infected with both C. jejuni and C. coli.

The failure to isolate thermophilic Campylobacter from 32 freshly void faeces of house sparrows (Passer domesticus) is in agreement with the findings of Kapperud and Rosef (1983) who also failed to isolate Campylobacter from nine cloacal swabs from seven house sparrows and two tree sparrows (P. montanus). However Smibert (1969) isolated thermophilic Campylobacter from the intestinal content of 11 of 15 sparrows caught in and around buildings in which sheep, infected with

similar organisms, were housed. The sparrows examined during this study were also from an area where infected stock were likely to have been present.

Experimentally infected chickens had higher numbers of thermophilic Campylobacter in their caecal contents than from contents taken from other sites in the G.I. tract and the infection persisted for longer in the caeca than in other sites (see Chapter VIII). Luechtefeld et al (1980) also reported higher isolation rates from the caeca of wild ducks than from the cloacae. It is interesting to speculate whether the poor development of caeca in house sparrows (P. domesticus) is associated with their apparent lower susceptibility to infection.

The isolation of C. jejuni from wild rats (Rattus norvegicus) is another example of the wide range of free-living animals and birds which are susceptible to infection with intestinal thermophilic Campylobacter. The high prevalence rate (60%) may be associated with the high population density of rats observed on the particular rubbish tip investigated and the known habit of coprophagy among rats.

It is interesting that based on BRENDA analysis (Chapter X), 15 of the 18 isolates recovered from rats had identical DNA patterns. This observation suggests a possible difference in infectivity between strains, a finding which was subsequently confirmed by trials in laboratory rats (see Chapter VIII). It is also of interest that none of the rats examined were infected with C. laridis in spite of the rubbish tip being frequented by gulls. In later experimental work (Chapter VIII), it was not possible to infect rats with C. laridis.

The in vitro development of nalidixic acid resistant by both C. jejuni and C. coli (see Chapter X), is of interest. This phenomenon has been recorded with C. jejuni (Benjamin et al 1983), but similar cases have not been reported for C. coli. In the light of this work, it is believed that the unknown biotype of Campylobacter reported by Fenlon et al (1982) as being hippurate negative, but resistant to nalidixic acid, was probably C. coli. Isolates of C. jejuni which develop nalidixic acid resistant can still be

differentiated from C. laridis on the basis of hippurate hydrolysis, but this criterion cannot be used to differentiate C. coli from C. laridis, as both fail to hydrolyse hippurate. It is unfortunate the attempts to carry out tests for H_2S production in an iron-metabisulfite pyruvate medium (FBP medium) as recommended by Skirrow and Benjamin (1980b) were unsuccessful. This test allows the separation of C. jejuni into two biotypes and provides a further criterion for the differentiation of C. coli and C. laridis (see Chapter II). It appears that some other workers have had similar difficulties (Skirrow, pers. comm).

A minor technical problem which occurred during the course of this work was fungal contamination of plates inoculated with faeces from seagulls and rats. This occurred in spite of the inclusion of amphotericin B in the media. Fricker et al (1983) suggested that actidione (cyclohexamide), which is included in Butzler's medium (Lauwers et al 1978) and Preston's medium (Bolton and Robertson 1982), was not as effective as amphotericin B, which is contained in the Campy-BAP Blaser's medium (Blaser et al 1978), for the control of yeasts in the faeces of gulls. The same authors found Butzler medium unsuitable for the isolation of Campylobacter from seagulls because it suppressed the growth of the NARTC (C. laridis) and C. coli. (This finding is a further evidence of the similarity between C. laridis and C. coli from gulls.) Until a more effective mycostatic agent for inclusion in culture media is developed, it would appear that in spite of its limitations, amphotericin B remains the agent of choice.

CONCLUSIONS

1. Populations of Southern black-backed gulls (L. dominicanus), wild ducks (Mallard (A. platyrynchos) and Grey ducks (A. supersiliosa)) and rats (R. norvegicus) are endemically infected with intestinal thermophilic Campylobacter. Such infection was not demonstrated in house sparrows (P. domesticus).

2. Endemic infection in gulls is associated with C. laridis, C. jejuni and C. coli.

3. Although the overall prevalence of infection in gulls is not influenced by their habitat, differences in habitat apparently affect the relative proportions of the species of Campylobacter isolated. C. laridis is the predominant species isolated from the faeces of gulls from an esturine environment and C. jejuni from those from an inland rubbish tip.

4. Gulls compared with other animals examined (pigs, poultry and rats) excrete lower numbers of thermophilic Campylobacter (mean $10^{3.2}$/g) in their faeces.

5. Infection in wild ducks is associated with C. jejuni and C. coli.

6. Infection in rats is associated with C. jejuni only.

7. When attempting to culture Campylobacter from the faeces from gulls or rats, a mycostatic agent needs to be included in the selective media to inhibit growth of fungi.

CHAPTER VIII

STUDIES OF EXPERIMENTAL INFECTION

INTRODUCTION

As a consequence of the studies reported in the preceding chapters, a number of problems became apparent, which required experimental investigation.

In Chapter VI, infection rates of up to 100% by *C. jejuni* were reported in broiler flocks less than six weeks of age. This finding indicated that the infective dose of this organism for poultry might be low and/or large numbers of organisms were shed in the faeces of infected birds. Work by other investigators, and that reported in Chapters IV and VI, has provided information on the duration of survival of thermophilic *Campylobacter* in faeces which could be another factor affecting the spread of infection in a flock.

Although infection of poultry by *C. jejuni* is self-limiting (see Chapter VI), the duration of infection had not been determined.

The reason for the prevalence of *C. coli* infection in poultry being less than the prevalence of *C. jejuni*, might be due to the infectivity of *C. coli* being less than *C. jejuni*.

In the previous chapters, it has been shown that some animals were infected with a particular species of thermophilic *Campylobacter* almost exclusively (pigs with *C. coli*), while others, such as poultry and ducks, were capable of being infected predominantly with *C. jejuni*, and with *C. coli* only to a lesser extent. *C. laridis* appeared to be exclusively confined to gulls, but concurrent infection with either *C. jejuni* or *C. coli* frequently occurred. These findings raise questions concerning the host specificity of thermophilic *Campylobacter* and the degree to which they have become host adapted.

These questions and hypotheses were investigated in a series of experiments, the results of which are reported in this chapter.

MATERIALS AND METHODS

Experimental Animals

Chickens: Birds were one, three and eight weeks of age at the commencement of the experiments.

The three and eight-week-old birds were obtained from flocks known to be free of infection with intestinal Campylobacter (poultry company A, Chapter VI). The flocks were free of overt disease and had not been subjected to any form of medication. The one-week-old birds were received as day-old chicks directly from the hatchery of poultry company A.

The chickens were collected at least seven days before the experiments were undertaken and cloacal swabs from each chicken and pooled faeces were cultured at least three times, including the day of their arrival and the morning before the experiment was commenced, in order to confirm that they were free of infection with Campylobacter.

Laboratory rats: Animals were 30 to 45 days of age at the commencement of the experiments. The rats used for the experiments, their dams and representative numbers of rats from the whole colony, were cultured repeatedly to confirm that the colony was free of intestinal Campylobacter.

Housing

Birds: For the duration of the experiment, birds were kept individually in wire cages which were divided into two compartments by a double hard cardboard partition approximately 6 to 8 cm apart. Each cage was placed above a metal tray which was covered with sterile newspapers which were changed every week. The cages were positioned in two parallel lines approximately 75 cm above the floor and they were separated by a corridor approximately 1 m wide. Individual cages in the same line were approximately 25 to 30 cm

from each other and also separated by a cardboard partition. Plate 8.1 shows an individual cage divided into two, and plate 8.2 shows part of the view of the room with the two lines of cages (both photographs were taken while an experiment was in progress).

The feed provided for the chickens was a commercial high energy chick mash[a] which was stored and sterilised in metal containers autoclaved at 121°C for 15 minutes. For each chicken, food and tap water was supplied in individual metal containers which were filled every two days when the experimental birds were less than 3 weeks of age, and once each day for older birds.

Plastic gloves were worn when handling or feeding birds. These were dipped and washed in 10% Medol disinfectant[b] immediately after handling any bird, or the other contents of the cage.

Room temperatures were maintained at approximately 35°C to 25°C for the one to three-week-old birds and at about 15 to 20°C for older chickens.

Between each series of experiments, the room was cleaned and disinfected and fumigated by the addition of 500 ml of formalin to 250 g of potassium permanganate which was recommended procedure for a room of approximately 27 m^3 (Blackmore 1976). The cages, feeding containers and metal trays were washed with water under pressure and autoclaved at 121°C for 30 minutes.

The room remained locked and only the author had access to it. In front of its entrance a tray containing 10% Medol was provided to disinfect footwear. The windows of the room were fitted with a fine wire mesh to prevent the entry of flies and other insects.

Rats: The rats while on experiment, except for those involved in studies of natural transmission, were kept individually in conventional rat cages.

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a Farm Products Co-operative (Manawatu) Limited, New Zealand.

b Gibco New Zealand Ltd., 18 - 24 Botha Road, Penrose, Auckland, 6. P.O. Box 12-502, Penrose.

PLATE 8.1 : CAGE WITH TWO EXPERIMENTAL CHICKENS SEPARATED BY DOUBLE CARDBOARD PARTITION

PLATE 8.2 : VIEW OF LINES OF CAGES WITH EXPERIMENTAL CHICKENS

Animals were fed a commercially manufactured pellet.[a] Food was not sterilised, but the bags of food were kept for at least 20 days in a dry, warm place before being fed. Thus any possible initial contamination with Campylobacter should have been eliminated.

Preparation of Cultures

The strains and species of intestinal thermophilic Campylobacter used in the series of experiments were kept at -70°C (see preservation of cultures, Chapter III), for approximately six to 12 months. The growth from an individual colony from each strain was inoculated on five blood agar plates. After an incubation period of 20 to 24 h at 42°C in microaerophilic conditions (see Chapter III), the growth from four plates was harvested in 3 to 4 ml of FBP broth. The suspension of approximately 10 ml, was concentrated in a universal container and mixed using a Vortex mixer. Subsequently, in order to assess the cfu of Campylobacter per millimeter of harvest, tenfold dilutions in PBS were plated out and colonies counted (see Chapter III). Within 24 to 30 h the results from the viable counts were known, while the original harvest was kept at 4°C. It had been shown previously (see Chapter IV) that holding a culture for this time and temperature did not affect the viability of a strain of C. jejuni in FBP broth. Based on the results of the viable counts, the suspension was diluted with PBS to produce a final suspension containing the desired number of organisms. Viable counts were also carried out on the remaining suspension immediately after animals had been inoculated.

The growth of the fifth plate was used for BRENDA analysis (see Chapter III) of the challenging strain. Subsequent isolations made at various intervals from the experimentally infected animals were also analysed by BRENDA and their patterns were compared with that of the original challenging strains.

The strains of Campylobacter used to challenge animals had usually been subjected to four to five subcultures before being used.

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[a] Farm Products Co-operative (Manawatu) Limited, New Zealand.

Method of Dosing

Chickens: An inoculum of 2 ml was introduced directly into the crop of the experimental chickens by means of a plastic tube attached to a syringe, using a similar technique to that described by Blackmore and Lucas (1965) for budgerigars. The only exception was that the polythene tube was longer (approximately 9 cm) and the hypodermic needle (14 gauge) was curved and cut down to only 4.5 cm in length, to adapt the technique to birds larger than a budgerigar. The technique is illustrated in plate 8.3. The tube was confirmed to be in the oesophagus and subsequently in the crop by palpation of the neck.

Rats: Each rat was dosed with 0.5 ml of the inoculum, using a 1 ml disposable plastic syringe and a cut down hypodermic needle. The inoculum was given slowly in the caudal region of the pharynx. The animals swallwed the inoculum without any obvious spillage.

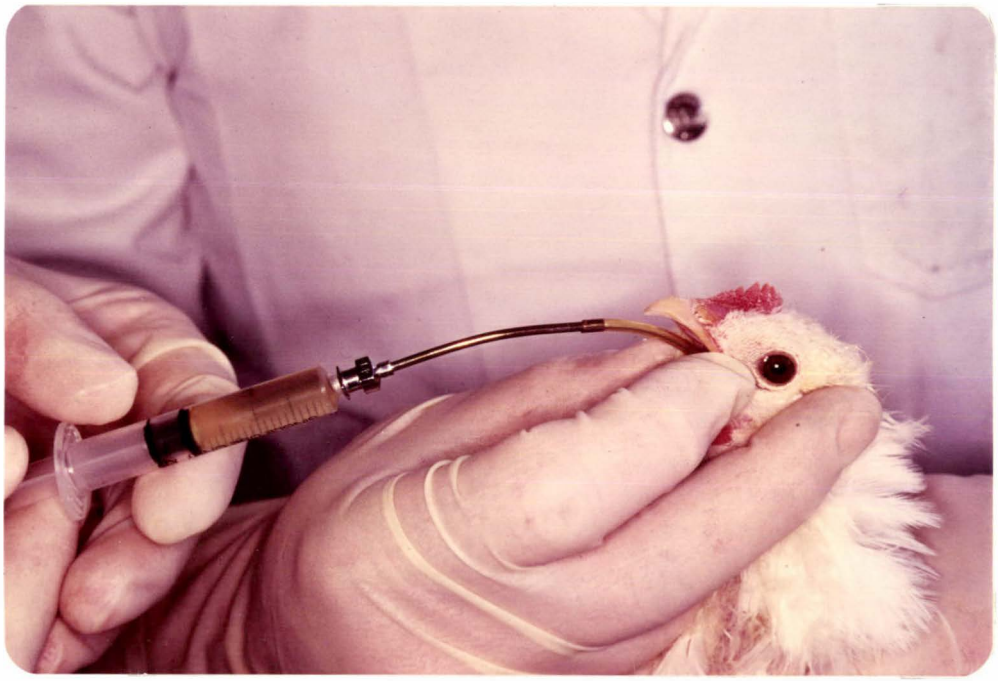
Collection of Fresh Faeces from Chickens for Direct Culture and Enumeration Studies

Daily, during the first 10 days post inoculation, approximately 2 g of fresh faeces were collected individually in preweighed sterile universal containers from the floor of the cage of each inoculated and non-inoculated control chicken. Subsequently faeces were collected two weeks post inoculation and thereafter at weekly intervals.

Direct culture of faeces: Direct cultures were carried out on all faecal samples by dipping and rotating a cotton wool swab in the material. Subsequent techniques were similar to those previously described (see Chapter III). Examinations were discontinued after two consecutive negative results (in most cases, this was based on culture of caecal faeces).

Enumeration studies of faeces: After swabs had been taken from faeces for direct culture, the remaining faeces in the universal containers were used for enumeration studies (see Chapter III), which were undertaken at 2, 4, 6, 8, 10 and 14 days' post inoculation and subsequently at weekly intervals.

PLATE 8.3 : INOCULATION BY THE ORAL ROUTE OF AN EXPERIMENTAL CHICKEN ONE WEEK OLD



The direct cultures and the enumeration studies were based mainly on the total faeces void at one time. However, it was soon appreciated that 'caecal faeces', which are chocolate in colour and very fine and semisolid on consistency and excreted one to two times per day (Duke 1977), contained more organisms than the 'normal' non-caecal faeces, which are excreted up to 20 and 25 times per day.

On the basis of this observation, 'normal faeces' and 'caecal faeces' were collected separately at the same time whenever possible and cultured and enumerated separately.

Collection of Faeces from Rats for Direct Culture and Enumeration Studies

One or two fresh faecal pellets were collected directly from a defecating rat while it was held by the tail over a clean paper towel. Subsequently the pellets from each rat were placed in individual sterile bijou bottles.

Direct culture of faecal pellets: Faeces were cultured daily for the first 10 days post inoculation. The pellets were suspended in 1 ml of sterile saline using a cotton swab. The subsequent steps were similar with that of culturing intestinal and faecal swabs reported in Chapter III.

Enumeration studies on faecal pellets: They were undertaken at 2, 4 and 10 days post inoculation and were continued thereafter at weekly intervals until two consecutive negative results were obtained (detection level 10^2:2 cfu of Campylobacter/g of faeces). Direct culture of faeces was reintroduced 48 h after an enumeration count was negative and were carried out at weekly intervals until two consecutive negative results were obtained.

Autopsy Procedures for Chickens

Chickens were killed by dislocation of the neck and they were subject to standard necropsy procedures. The liver with gall bladder attached, the spleen, the heart and part of the kidneys were removed separately and placed in sterile petri dishes. The G.I. tract, excluding the oesophagus and crop, but including the cloaca was removed in one portion. The crop was removed separately.

Culture of Liver, Gall, Spleen, Heart and Kidney from Chickens

Approximately one-fifth of the liver, the spleen, heart and part of the kidneys were dipped in 70% alcohol and immediately 'flamed off' and placed in separate γ radiated sterile polythene bags[a] containing 5 ml of sterile saline. The bags were placed in a Colworth Stomacher 400[b] and macerated for two minutes. After maceration, a cotton swab was dipped in the suspension and directly cultured on to the antibiotic selective agar medium as previously described in Chapter III. Portions (0.5 ml) from each tissue suspension were placed in MacCartney bottles containing 5 ml of the selective enrichment medium described by Fitzgeorge et al (1981) incubated anaerobically for 18 - 24 h. Subcultures from the enrichment medium were made on the antibiotic selective agar medium.

The gall was removed from the gall bladder in a 2 ml disposable sterile syringe. 0.5 ml was plated directly on the antibiotic selective medium using a sterile glass rod, and the remainder (0.5 to 1 ml) was added to the enrichment selective broth. Subsequent incubation and subcultures were similar to that of the other tissues.

Collection of Intestinal Contents, including that from the Crop, and Glandular and Muscular Stomachs, for Culture and Enumeration Studies

Segments of 10 to 15 cm were removed from the duodenum, middle jejunum, terminal ileum and colon. One caecum was also removed. Their contents were expressed into individual universal containers. The crop, glandular and muscular stomachs were opened and their contents were collected on cotton swabs in separated universal containers.

Direct culture of intestinal content: The content from intestinal segments was mixed with a cotton swab which was then used to inoculate the antibiotic selective plates (see Chapter III).

Enrichment culture of intestinal contents: Approximately 0.1 g of each sample was transferred in 5 ml of the enrichment broth of

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a Stomacher '400' bags, Medical DDS Ltd., P.O. Box 205, Wellington, New Zealand.

b A.T. Seward, VAC House, Blackfriars Road, London SE19VG, Great Britain.

Fitzgeorge et al (1981) and incubated and subcultured as previously reported. When birds were destroyed which had either ceased to excrete Campylobacter or their faecal counts were lower than $10^{3.0}$ per g, an enrichment cultured technique on their intestinal content was carried out.

Enumeration studies on intestinal contents: Approximately 2 g of well mixed separate samples of the contents from the crop, glandular and muscular stomachs, and from other parts of the intestine, were transferred to preweighed sterile universal containers, which were reweighed and enumeration studies were undertaken by methods already described (see Chapter III).

Rechallenge of Culturally Negative but Previously Infected Chickens

A number of chickens which had apparently eliminated their experimental infection were rechallenged either by direct dosing or by direct contact with other infected birds.

Direct rechallenge: Each bird to be rechallenged received between 5×10^6 to 1×10^9 organisms. Reinfected chickens were examined by direct culture and the number of organisms in their faeces were enumerated at similar intervals to those already described. Rechallenged chickens, which did not shed any Campylobacter in their faeces for two weeks post inoculation, were considered to have resisted reinfection.

Natural experimental rechallenge and transmission trial: Ten birds were placed in a cage measuring 1 x 1 x 0.6 m with a wire floor which allowed faeces to be collected on a tray beneath the cage. Food and water containers were common to all birds. Four birds were between four and eight months of age and all have been previously infected, and eliminated infection caused by C. jejuni (strain 251). One 3-month-old bird had been previously unsuccessfully challenged with 5×10^2 organisms of the same strain. The five other birds were three weeks old and had never been exposed or infected. All birds were individually marked with leg rings.

Five $\times 10^6$ cfu of the strain of C. jejuni (No. 251) which had been eliminated from chickens previously experimentally infected, was given by crop inoculation to one of the five 3-week-old chickens in the cage. Cloacal swabs were collected and cultured daily for two weeks from

each chicken in the cage. All the chickens were destroyed 3 weeks after commencement of the experiment and caecal swabs were cultured.

EXPERIMENTAL DESIGN

Four experiments were undertaken.

Experiment I

Aim: To determine the infective dose for chickens of a strain of C. jejuni isolated from broilers when administered by the oral route.

Objectives:

1. To investigate the effect of the age of birds on their susceptibility to infection.
2. To determine the incubation period, duration of infection and levels of excretion.
3. To investigate the levels of infection in different parts of the G.I. tract.
4. To determine whether or not the cessation of excretion of Campylobacter in the faeces coincides with failure to recover Campylobacter from the caeca.
5. To determine whether or not chickens which eliminated the infection become reinfected, either by direct oral dosing or by contact with infected chickens.

In order to investigate the objectives 1 to 4, a total of 36 chickens, 16 of 3 weeks of age and 20 of 8 weeks of age were challenged with different numbers of C. jejuni isolate No. 251. Another eight chickens remained as controls (see Table 8.1). Objective 5 was determined by the rechallenge of previously infected birds by the method described in the preceding page.

Experiment II

Aim: To determine the infectivity for chickens of C. coli isolated from broilers, and to compare the results with those related to C. jejuni from Experiment I. A total of 19 chickens of 3 weeks

of age were challenged, either with C. coli isolate No. 241 or with C. jejuni isolate No. 251. Table 8.2 shows the number of chickens used and the dose of each organism given.

Experiment III

Aim: To determine whether or not various isolates of Campylobacter from gulls including C. jejuni, C. laridis and C. coli and an isolate of C. jejuni from rats were capable of infecting chickens.

Objectives:

1. To determine the duration and levels of excretion.
2. To determine the ability of the chickens which had eliminated the infection to become reinfected with higher doses than the original challenging doses.

A total of 22 chickens of one week of age were challenged with doses varying from 2×10^6 to 5×10^8 (see Table 8.3). Direct rechallenge doses varied from approximately 1×10^8 to 1×10^{10}.

Experiment IV

Aim: To determine the infectivity of a number of thermophilic Campylobacter isolated from various sources to laboratory rats.

Objectives:

1. To determine the duration and levels of excretion in infected rats.
2. To determine whether or not natural cross transmission occurs between rats.

Table 8.4 shows the various strains of thermophilic Campylobacter used to dose the laboratory rats.

TABLE 8.1 : EXPERIMENT NO. I: CHICKENS CHALLENGED WITH C. JEJUNI NO. 251 ISOLATED FROM BROILERS

Groups of Chickens	Age in Weeks	No. of Chickens Dosed	Dose/ Chicken	Control Chickens
A_2	3	4	5×10^5	G_1
B_2	8	4	5×10^5	G_2
A_1	3	4	5×10^3	G_3
B_1	8	4	5×10^3	G_4
C_2	3	4	5×10^2	G_5
D_2	8	6	5×10^2	G_6
C_1	3	4	5×10^1	G_7
D_1	8	6	5×10^1	G_8
Total		36		8

TABLE 8.2 : EXPERIMENT NO. II: CHICKENS CHALLENGED WITH C. COLI NO. 241 AND C. JEJUNI NO. 251 ISOLATED FROM BROILERS

Age of Challenging Chickens	Challenging Species	Isolate No.	No. of Chickens Dosed	Dose/ Chicken
3 weeks	C. coli	241	8 (Cc_1-Cc_8)	5×10^5
			5 (Cc_9-Cc_{13})	5×10^7
3 weeks	C. jejuni	251	6 (Cj_{14}-Cj_{19})	5×10^4
			19	

TABLE 8.3 : EXPERIMENT NO. III: CHICKENS CHALLENGED WITH VARIOUS STRAINS OF CAMPYLOBACTER ISOLATED FROM GULLS AND RATS

Source of Isolation	Challenging Species	Isolate No.	No. of Chickens Dosed	Dose/ Chicken
Gulls	C. laridis	812	2	2.3×10^7
		820	2	1.6×10^7
		847	2	5×10^8
Gulls	C. coli	816	2	2×10^7
		877	2	1.3×10^7
		889	2	5×10^8
Gulls	C. jejuni	822	2	2×10^7
		888	2	6.7×10^6
		891	2	5×10^7
Rats	C. jejuni	R_{18}	4	2×10^6
			22	

TABLE 8.4 : EXPERIMENT NO. IV: RATS CHALLENGED WITH VARIOUS STRAINS OF CAMPYLOBACTER ISOLATED FROM RATS, CHICKENS, GULLS AND PIGS

Challenging Species	Source of Isolate	Isolate No	No. of rats Dosed	Dose/Rat
C. jejuni	Rats	R_{18}	5	5×10^7
			5	2×10^6
			5	2×10^5
			5	2×10^4
		R_2	5	2×10^8
C. jejuni	Chickens	251	5	2.5×10^8
			5	2.5×10^7
			5	2.5×10^6
			5	2.5×10^4
C. laridis	Gulls	820	4	9×10^8
C. laridis		847	4	7.3×10^8
C. laridis		877	4	6.6×10^8
C. coli	Pigs	319	4	5.5×10^8
			4	5.0×10^6
		320	4	4.7×10^8
			4	4.7×10^6

RESULTS

Experiment I

The effect of age on the susceptibility of three and eight-week-old birds to infection with C. jejuni: Table 8.5 indicates the results obtained from the oral inoculation of three and eight-week-old chickens with different numbers of organisms. The rates of infection appeared to be dose dependent and there were no significant age differences in susceptibility to infection. Doses of only 50 organisms failed to infect and the minimal infective dose was 5×10^2, but at this level of infection only three of the ten birds became infected, while when 5×10^5 organisms were administered, all eight birds challenged became infected.

Time elapsed after an oral challenge before C. jejuni is shed (defecated in faeces): Campylobacter appeared in the faeces of some infected birds when first examined at 24 h post inoculation and by 72 h post challenge, organisms were in the faeces of all infected birds (see Table 8.5). Organisms continued to be shed for several weeks without interruption (see further results, Table 8.6). The time of onset of shedding was also dose dependent. The birds of Groups A2 and B2 which were dosed with the higher dose (5×10^5) started to shed organisms sooner than others, 33 h (range 24 to 48 h), while the birds of Groups A1 and B1 dosed with 5×10^3 had a mean onset of shedding of 36 h (range 24 to 48 h). The longest mean time between inoculation and faecal excretion (48 h) was seen in Groups C2 and 2 which received the smallest infective dose (5×10^2). The mean onset of shedding in all groups was 39 h (range 24 to 72 h). However, these differences were not significant.

Control and Non-infected Chickens

The control chickens G_1, G_2 and G_3 of the A2, B2 and A1 groups took approximately 3.5 to 4 weeks to become cross-infected. Three ($A1_2$, $A1_3$, and $B1_2$) of the four non-infected chickens which had been challenged from groups A1 and B1 became cross-infected approximately 4 weeks later, while the remaining chicken, $A1_4$ and the control G4 remained uninfected up to the time of slaughter seven weeks later (see Table 8.6).

TABLE 8.5 : EXPERIMENT NO. I: INFECTIVITY OF C. JEJUNI NO. 251 FOR CHICKENS BY THE ORAL ROUTE

Chicken Group No.	Age in Weeks	Dose/ Chicken	Infection Rate	Time of onset of faecal excretion 24h	48h	72h*	Mean time (hours) of onset of excretion and (range)
A2	3	5×10^5	4/4	2	2	-	33 (24 - 48)
B2	8		4/4	3	1	-	
Sub Total			8/8 (100%)				
A1	3	5×10^3	1/4	1	-	-	36 (24 - 48)
B1	8		3/4	1	2	-	
Sub Total			4/8 (50%)				
C2	3	5×10^2	1/4	1	-	-	48 (24 - 72)
D2	8		2/6	-	1	1	
Sub Total			3/10 (33%)				
C1	3	5×10^1	0/4	-	-	-	-
D1	8		0/6	-	-	-	
Sub Total			Nil (0%)				
Overall Sub Totals	3		6/16 (37.5%)				39 (24 - 72)
	8		9/20 (45%)				

* No further infected animals were detected after 72 h.

The cross-infected chickens were slaughtered approximately three to four weeks after infection was detected and the results of enumeration studies on their intestinal contents, except from $A1_3$, $B1_2$ and G_1 from which only caecal swabs were taken, are shown in Table 8.8. The table also includes the findings from the examination of the entire G.I. tract of chickens $A1_4$ and G_4 which remained uninfected.

All the untreated controls (G_5 - G_8) and all except one of the birds in groups C_2, D_2, C_1 and D_1 which failed to excrete organisms after challenge by the oral route, (see Table 8.6) were destroyed two to three weeks after the commencement of the experiments. (The remaining uninfected bird ($C2_2$) was used for a later rechallenge experiment).

Levels and duration of excretion: Table 8.6 shows the levels of Campylobacter per g of faeces and the duration of infection based on the direct culture of 'normal' and 'caecal faeces' from all the infected birds.

In most of the infected chickens, the excretion level in the faeces reached its maximum value within four to six days (maximum eight days) post inoculation, (see $A2_1$, $A2_2$, $A2_4$, $B2_1$, $B2_2$, $B2_3$, $A1_1$, $B1_1$, $B1_3$, $B1_4$). During this period more than $10^{8.0}$ Campylobacter per g of 'normal faeces' were being excreted by individual chickens (see $A2_1$, $A2_2$, $A2_4$, $B2_1$, $B1_4$). Thereafter some chickens, particularly those of groups A2 and B2 which were given the higher dose (5×10^5) showed a considerable reduction in numbers of organisms excreted ($10^{2.00}$/g to $10^{4.00}$/g), during the next two to four days (8 to 10 days post inoculation). However, within 14 days post inoculation, the number of organisms in the faeces increased to $10^{7.00}$ to $10^{8.00}$ and most of them (see $A2_2$, $A2_3$, $A2_4$, $B2_3$, $B2_4$) continued to shed more than $10^{6.0}$ Campylobacter for up to five weeks post inoculation and some even for longer periods (see $B2_4$). Thereafter the number of Campylobacter in the faeces gradually decreased.

Chickens in groups B1, C2 and D2, except bird $A1_1$ of group A1, which received lower doses of 5×10^3 or 5×10^2, showed after an initial rise in numbers, a continual and gradual reduction in the numbers of Campylobacter in the faeces. They did not show the secondary increase in numbers which was observed in chickens receiving the higher dose.

TABLE 8.6 : EXPERIMENT I: LEVELS AND DURATION OF EXCRETION OF C. JEJUNI IN NORMAL (N) AND CAECAL (C) FAECES IN 3 AND 8-WEEK-OLD CHICKENS

Age	Bird No.	Dose/ Bird	Type of Faeces	2 d	4 d	6 d	8 d	10 d	2 w	3 w	4 w	5 w	7 w	8 w	9 w	11 w	12 w	13 w	14 w	15 w	16 w	17 w	18 w	19 w	Duration (weeks) of elimination of infection based on 'Normal faeces'	'Caecal faeces'
3w	$A2_1$	5×10^5	N	4.38	8.18	7.85	5.71	3.60	8.08	7.47	4.86	4.92	2.74	2.35	2.54	2.00	2.30	<2.0	-	-	-	-	-	*	14	18
			C							8.91			4.08			4.70		5.30	5.18	ND	4.52	<2.0	-			
3w	$A2_2$		N	4.85	8.11	7.51	4.00	4.93	5.92	6.83	6.80	6.98	5.23	3.95	3.95	-	-	-	*						11	13
			C													2.81	<2.0	-								
3w	$A2_3$		N	5.54	6.99	7.32	5.00	5.70	7.74	6.92	8.08	7.62	*													
			C									8.82														
3w	$A2_4$		N	2.93	8.48	8.94	5.60	7.37	7.49	6.11	8.48	7.41	ND	*												
			C																							
3w	G_1 (Control)									Cross Infected				*												
8w	$B2_1$		N	4.23	6.59	5.00	7.98	6.74	6.80	6.06	6.32	5.70	4.00	4.78	4.30	3.99	4.28		*							
			C								8.10	7.40		4.86												
8w	$B2_2$		N	5.32	7.02	7.18	6.36	8.04	5.98	4.75	2.60	<2.0	-	-	-	-	*								7	9
			C							8.55	4.56	3.12	2.0	2.0	-	-										
8w	$B2_3$		N	5.60	7.81	6.85	5.34	5.26	4.76	7.47	6.80	6.30	4.74	ND	*											
			C					6.54				6.81														
8w	$B2_4$		N	2.60	5.00	6.33	7.48	4.00	6.19	7.24	7.60	7.05	5.43	6.61	6.03	7.31	5.20		*							
			C												8.21											
3w	G_2 (Control)									Cross Infected			*													

(To be cont.)

Table 8.6 (cont)

Age	Bird No.	Dose/ Bird	Type of Faeces	2 d	4 d	6 d	8 d	10 d	2 w	3 w	4 w	5 w	7 w	8 w	9 w	11 w	12 w	13 w	14 w	15 w	16 w	17 w	18 w	19 w	Duration (weeks) of elimination of infection based on 'Normal faeces'	'Caecal faeces'
3w	$A1_1$		N	5.10	7.29	7.90	5.71	6.95	4.40	7.26	6.40	5.13	5.60	ND	ND	ND	-	<2.0	<2.0	-	-	-	-		15	17
			C						6.70	7.20	6.83	ND	5.54	3.40	5.38	4.50	2.18	5.18	5.51	ND	2.00	-	-			
3w	$A1_2$	5×10^3	C	remained uninfected							cross infected			*												
3w	$A1_3$		C	remained uninfected							cross infected			*												
3w	$A1_4$		C	remained uninfected									*													
	G_3 (Control)										cross infected		*													
8w	$B1_1$		N	4.71	5.80	5.30	4.54	4.15	3.48	2.65	3.60	2.18	<2.0	-	-	-	*								8	11
			C									4.60	4.70	4.70	2.44	-										
8w	$B1_2$		C	remained uninfected							cross infected		*													
8w	$B1_3$		N	2.78	7.40	6.54	7.30	5.35	5.29	5.40	3.70	3.58	-	3.18	<2.0	-	-	-	-	-					11	14
			C						7.34						3.42	2.40	2.44	<2.0	-	-					
8w	$B1_4$		N	3.56	6.52	8.25	5.95	6.00	4.70	3.00	2.85	2.51	<2.0	-	-	*									8	8
			C						6.89	5.67			<2.0	-	-											
3w	G_4 (Control)												* Not cross infected													

(To be cont.)

Table 8.6 (cont)

Age	Bird No.	Dose/ Bird	Type of Faeces	2 d	4 d	6 d	8 d	10 d	2 w	3 w	4 w	5 w	6 w	7 w	8 w	Duration (weeks) of elimination of infection based on 'Normal faeces'	Duration (weeks) of elimination of infection based on 'Caecal faeces'
3w	$C2_1$		N			remained uninfected				*							
			C														
3w	$C2_2$		N			remained uninfected				*							
		5×10^2	C														
3w	$C2_3$		N			remained uninfected				*							
			C														
3w	$C2_4$		N	2.35	5.68	7.32	ND	6.50	6.11	5.28	*						
			C							ND							
3w	G_5	(Control)								* not cross infected							
8w	$D2_1$		N	-	4.47	5.99	ND	6.87	6.72	6.00	3.78	<2.00	-	-		6	7
			C							ND	2.70	<2.00	<2.00	-			
8w	$D2_2$		N			remained uninfected				*							
		5×10^2	C														
8w	$D2_3$		N	-	2.87	4.13	ND	5.92	5.88	4.00	2.38	2.10	-	-		6	7
			C							ND	4.18	4.20	2.35	-			
8w	$D2_4$ to $D2_6$		N			remained uninfected				*							
			C														
3w	G_6	(Control)								* not cross infected							

(To be cont.)

Table 8.6 (cont)

Age	Bird No.	Dose/ Bird	Type of Faeces	2 d	4 d	6 d	8 d	10 d	2 w	3 w	4 w	5 w	6 w	7 w	8 w	Duration (weeks) of elimination of infection based on 'Normal faeces'	'Caecal faeces'
3w	Cl_1 to Cl_4	5×10^1	N							*							
			C				All remained uninfected										
3w	G_7 (Control)		N							*							
			C							not cross infected							
8w	Dl_1 to Dl_6	5×10^1	N							*							
			C				All remained uninfected										
3w	G_8 (Control)		N							*							
			C							not cross infected							
															Mean and (Range)	9.6(6-15)	11.6(7-18)

< 2.00 = Culture of 100-fold dilution failed to reveal growth. Direct culture of the faeces was positive

* Bird destroyed (see also Table 8.8)

Birds $A2_1$ and Bl_4 found infected on examination post mortem (see Table 8.8)

All but one infected chicken ceased shedding Campylobacter in their 'normal faeces' one to four weeks before the 'caecal faeces' (see chickens $A2_1$, $A2_2$, $B2_2$, $A1_1$, $B1_1$, $B1_3$, $D2_1$, $D2_3$. Only chicken $B1_4$ appeared to cease shedding Campylobacter in both its 'normal' and 'caecal faeces' simultaneously. The mean elimination time of infection (duration) based on the direct culture of the 'normal' and 'caecal faeces' was 9.6 weeks (range 6 to 15 weeks) and 11.6 weeks (range 7 to 18 weeks) respectively.

Table 8.7 presents a summary of the number of organisms in 'normal', and in 'caecal faeces'. The mean counts of C. jejuni obtained from the 'caecal faeces' were $10^{5.20}$ cfu per g of faeces, while that of the 'normal faeces' were $10^{2.83}$ cfu per g of faeces. This was a significant difference ($P < 0.001$).

Plate 8.4 shows the DNA 'fingerprints' of the challenging strain No. 251 and a number of isolates recovered at various intervals from birds infected with this strain. All had identical patterns.

Number of C. jejuni/g of content from the G.I. tract: Table 8.8 shows the number of C. jejuni in the gut contents from different parts of the G.I. tract of the 14 birds which were destroyed. (Eleven birds had been infected by stomach tube, and three became accidentally infected (G_2, G_3 and $A1_2$).) Although organisms were found at all levels, the greatest number were in the lower G.I. tract, especially the caeca. (See also figure 8.1.)

Two chickens ($B1_4$ and $A2_1$) of the five ($B1_4$, $A2_1$, $A2_2$, $B1_1$, $B2_2$) which were destroyed (see Table 8.8) after no further isolates were recovered from either 'normal' or 'caecal faeces' (see Table 8.6) were found post-mortem to have low numbers of organisms in their caeca. A few colonies of C. jejuni were recovered from bird $B1_4$ by the use of enrichment cultural procedures, while organisms were isolated from bird $A2_2$ ($10^{2.00}$ cfu/g of caecal content) by both direct and enrichment procedures.

Culture of Liver, Spleen, Heart and Kidney Tissues and Gall

C. jejuni was isolated from the gall of two (14.3%) chickens

TABLE 8.7 : EXPERIMENT NO. I: COMPARATIVE LEVELS OF C. JEJUNI IN THE 'NORMAL' AND 'CAECAL' FAECES OF CHICKENS AT DIFFERENT TIMES POST INFECTION

Log_{10} counts/g of faeces

Chicken No.	'Normal faeces'	'Caecal faeces'	Time of enumeration post challenge (weeks)
$A2_1$	7.47	8.91	3
	2.74	4.08	7
	2.00	4.70	11
	<2.00	5.30	13
	-	5.18	14
$A2_2$	-	2.81	11
$A2_3$	7.62	8.82	5
$B2_1$	6.32	8.10	4
	5.70	7.40	5
	4.78	4.86	8
$B2_2$	4.75	8.55	3
	2.60	4.56	4
	<2.00	3.12	5
$B2_3$	5.26	6.54	1.5 (10 days)
	6.30	6.81	5
$B2_4$	6.03	8.21	9

(To be cont)

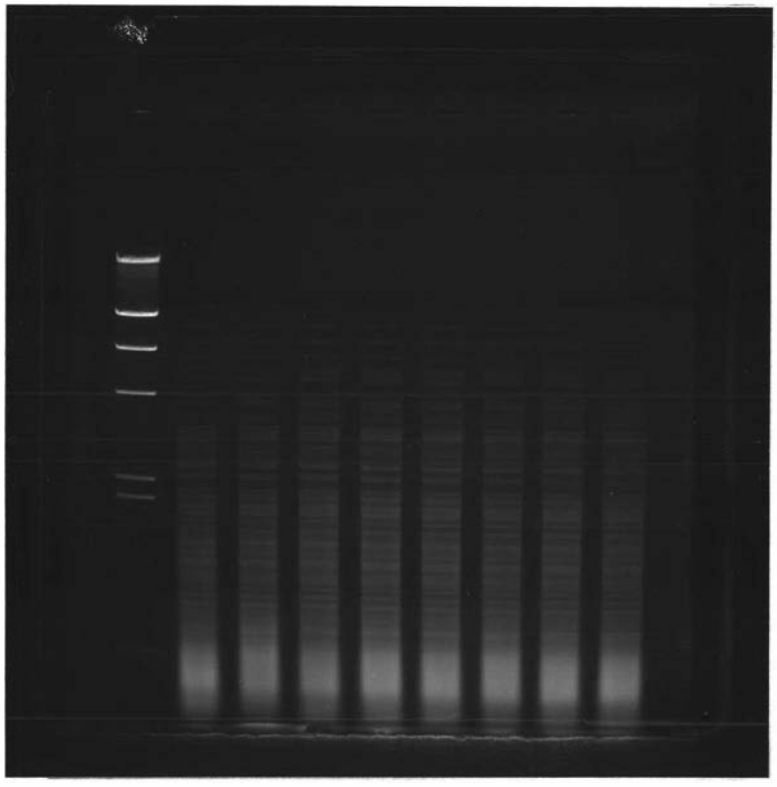
Table 8.7 (cont)

Log_{10} counts/g of faeces

Chicken No.	'Normal faeces'	'Caecal faeces'	Time of enumeration post challenge (weeks)
$A1_1$	4.40	6.70	2
	7.26	7.20	3
	6.40	6.83	4
	5.60	5.54	7
	-	2.18	12
	<2.00	5.18	13
	<2.00	5.51	14
	-	2.00	16
$B1_1$	2.18	4.60	5
	<2.00	4.70	7
	-	2.44	9
$B1_3$	5.29	7.34	2
	<2.00	3.42	9
	-	2.40	11
	-	2.44	12
$B1_4$	4.70	6.89	2
	3.00	5.67	3
$D2_1$	<2.00	2.70	5
$D2_3$	2.38	4.18	4
	2.10	4.20	5
	-	2.35	6
Means of infected faeces	2.83	5.20	7.01

* Culture of 100 fold dilution failed to reveal growth but direct culture of the faeces was positive. Thus there were <2.00 organisms/g of faeces.

PLATE 8.4 : STABILITY OF DNA 'FINGERPRINT' OF C. JEJUNI (251) DURING EXPERIMENTAL TRANSMISSION



1 2 3 4 5 6 7 8 9

Lane 1 is the reference bacteriophage C1857 S7 (lambda).

Lane 2 is the original challenge strain. The others (3 - 9) are isolates recovered from different infected chickens.

TABLE 8.8 : EXPERIMENT NO. I: NUMBERS OF C. JEJUNI/G OF GUT CONTENT AT DIFFERENT LEVELS OF G.I. TRACT AT VARIOUS TIMES POST INFECTION[a]

Chicken No	Crop	Glandular Stomach	Muscular Stomach	Duodenum	Middle Jejunum	Terminal Ileum	Caecum	Colon	'Normal faeces'	Time of slaughter Post infection (weeks)
G_2[b]	4.43	3.58	3.57	3.53	3.72	3.95	7.26	6.29	6.25	3.5
G_3[b]	5.08	4.52	<2.00	1.70	2.18	6.84	8.23	6.75	ND	4
Al_2[b]	4.51	3.08	3.34	3.83	3.91	4.27	7.62	6.65	5.04	4
$C2_4$	3.89	5.40	4.03	4.51	4.88	5.02	8.02	5.88	5.28	4
$A2_3$	4.27	3.08	2.85	3.93	3.73	2.95	8.60	6.94	6.83	7
Al_4	-	-	-	-	-	-	-	-	-	7
G_4	-	-	-	-	-	-	-	-	-	7
$A2_4$	4.57	3.52	3.08	4.32	3.97	4.75	7.53	6.38	6.53	8
$B2_3$	4.08	6.13	3.04	3.34	2.65	2.74	6.03	5.32	5.51	9.7
Bl_4	-	-	-	-	-	-	+[c]	-	-	11.5
Bl_1	-	-	-	-	-	-	-	-	-	12.2

(To be cont.)

Table 8.8 (Cont)

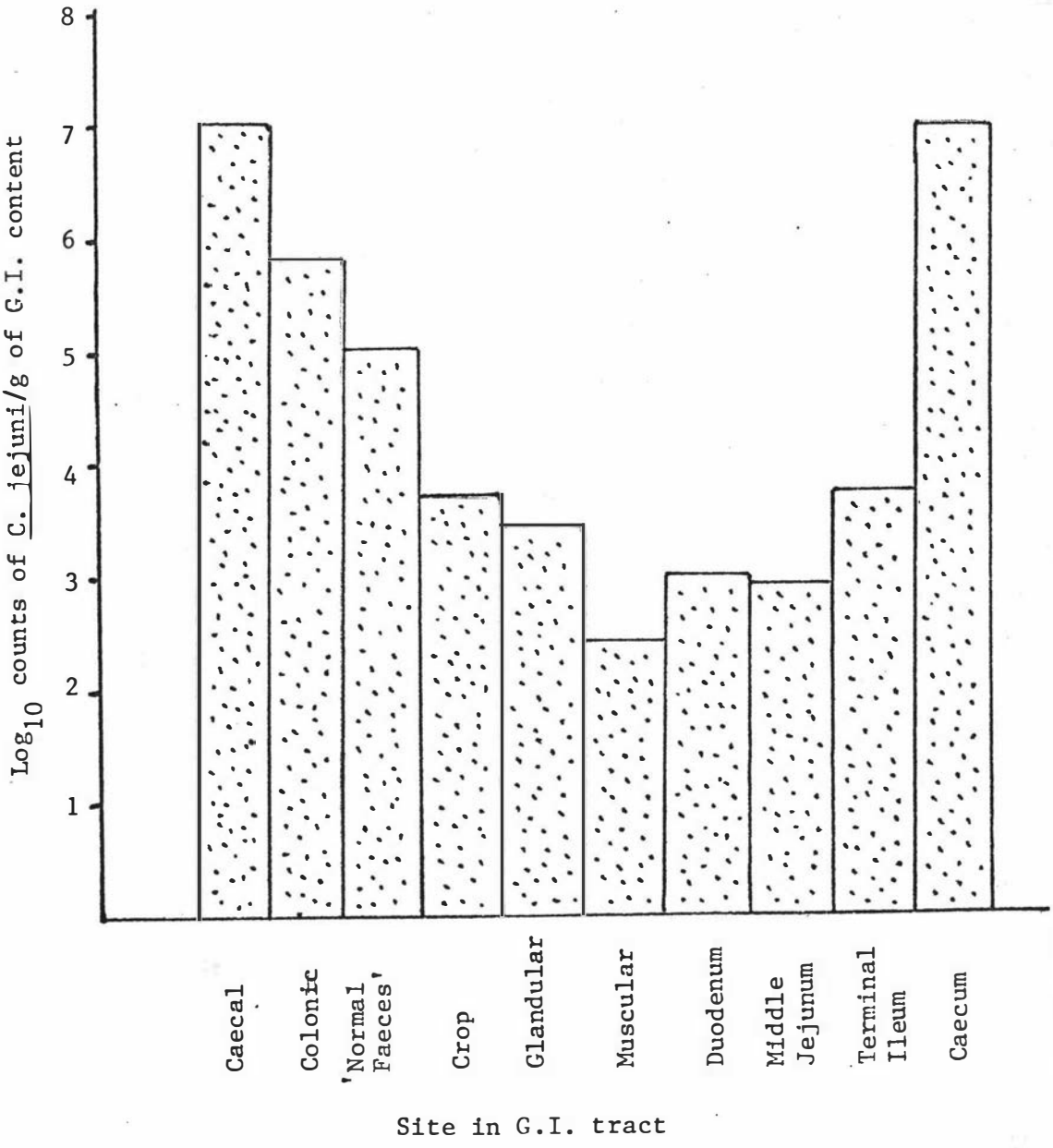
Chicken No	Crop	Glandular Stomach	Muscular Stomach	Duodenum	Middle Jejunum	Terminal Ileum	Caecum	Colon	'Normal faeces'	Time of slaughter Post infection (weeks)
$B2_2$	-	-	-	-	-	-	-	-	-	12.2
$B2_1$	2.72	1.39	1.39	2.00	1.70	2.86	6.40	5.48	ND	13.2
$A2_2$	-	-	-	-	-	-	-	-	-	14
$B2_4$	ND	3.68	3.56	3.34	2.74	3.44	8.66	7.57	6.59	14.3
$A2_1$	-	-	-	-	-	-	2.00	-	-	19
Means	3.73	3.44	2.66	3.05	2.95	3.68	7.04	5.73	5.06	10.8

a Eleven birds were infected by oral inoculation and three by cross infection. Birds $A1_4$ and G_4 did not become infected.

b Cross infected 3.5 - 4 weeks after the beginning of the experiment and slaughtered 3.5 - 4 weeks post cross infection.

c Positive on direct culture only

FIGURE 8.1 : EXPERIMENT NO. I: NUMBERS OF C. JEJUNI/G OF GUT CONTENT AT DIFFERENT LEVELS OF G.I. TRACT OF CHICKENS* AT VARIOUS TIMES POST INFECTION



* Fourteen chickens slaughtered at a mean time approximately 11 weeks post infection.

($B2_1$ and $B2_4$) of the 14 examined, after enrichment procedures. C. jejuni was not recovered from tissues from any other chicken.

Direct rechallenge dosing: Of the two rechallenged chickens ($A1_1$ and $B1_3$) which received 5×10^6 cfu of C. jejuni by the oral route, chicken $A1_1$ remained uninfected while chicken $B1_3$ started shedding Campylobacter 72 h post dosing. The counts from the 'caecal faeces' were usually several hundred times greater than those from the 'normal' faeces'. The highest counts of C. jejuni in the 'normal faeces' were obtained approximately 10 days post challenge, and those from the 'caecal faeces' between 8 and 14 days, (no counts of caecal faeces were undertaken earlier than 8 days post rechallenge). C. jejuni was not detected in 'normal and 'caecal faeces' by 18 and 28 days respectively post rechallenge. A second rechallenge of $B1_3$ with 5×10^6 cfu of the same strain (251) of C. jejuni one week after it had apparently ceased shedding Campylobacter in its 'caecal faeces', failed to cause reinfection. Figure 8.2 demonstrates the progression of the first rechallenge infection of chicken $B1_3$.

Rechallenged and cross-transmission trial: Figure 8.3 demonstrates the results of the trial. A 3-week-old chicken (F_1), orally dosed with strain 251 and previously free of any Campylobacter infection, started to shed organisms 24 h post inoculation. All the previously non-infected chickens (F_2 to F_5) of the same age which were in contact with F_1 but not dosed, became infected. Birds F_2 and F_5 started shedding Campylobacter at 48 h, F_4 at 72 h and F_3, 5 days after the beginning of the experiment. None of the four chickens ($A1_1$, $B1_3$, $D2_1$ and $D2_3$) (see Table 8.6), which had previously been infected with strain 251, became reinfected by contact with birds F1 - F5. The three-month-old chicken ($C2_2$) which had previously been dosed with the lower infective dose (5×10^2), but which did not become infected (see Table 8.6), now became infected after contacting birds F_1 - F_5 and organisms were recovered from its faeces four days after the start of the experiment.

The mean time for the detection of organisms in the five cross-infected chickens was 76.8 hours (range 48 to 120 h), after the oral dosing of chicken F_1.

FIGURE 8.2 : EXPERIMENT NO. I: COMPARATIVE LEVELS OF C. JEJUNI IN THE 'NORMAL AND CAECAL FAECES' OF CHICKEN NO. Bl_3 RECHALLENGED ORALLY WITH THE SAME STRAIN OF C. JEJUNI NO. 251

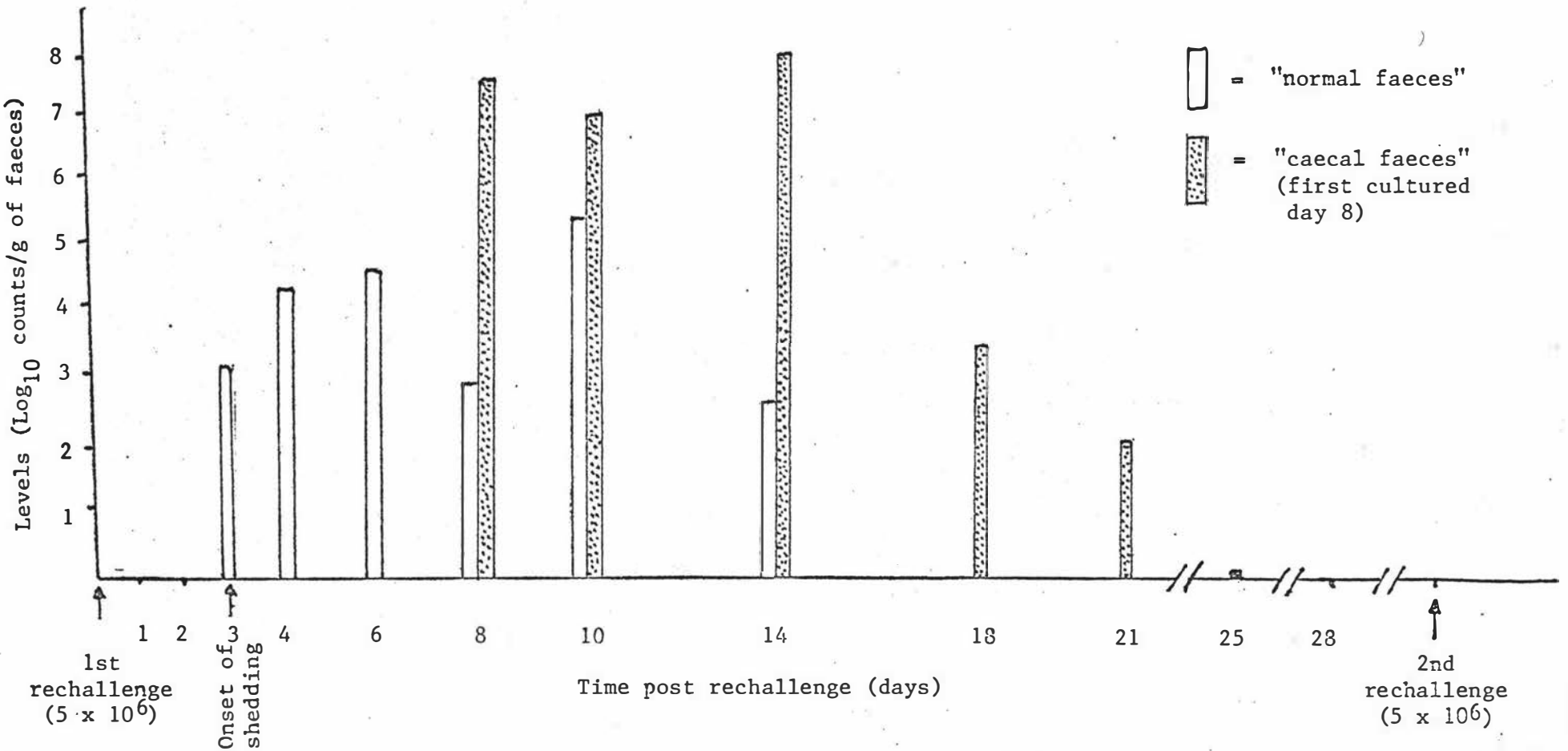


FIGURE 8.3 : EXPERIMENT I: DETECTION OF C. JEJUNI ON CLOACAL SWABS IN THE RECHLLENGE TRIAL OF CHICKENS BY C. JEJUNI NO. 251

		Housed in the same cage	Age	Days Post Inoculation														
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	21
In contact with F_1	Previously infected and eliminated infection*	$A1_1$	8 m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		$B1_3$	8 m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		$D2_1$	4 m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		$D2_3$	4 m	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	"Control" not previously infected with the minimal infective dose (5×10^2)*	$C2_2$	3 m	-	-	+	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
		F_1**	3 w	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
In contact with F_1	Not previously infected	F_2	3 w	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
		F_3	3 w	-	-	-	-	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
		F_4	3 w	-	-	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
		F_5	3 w	-	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Mean and (range) of onset of shedding = 62.4 h (24 - 120 h)

* See Table 8.6

** Dosed orally with 5×10^6

\+ = < 10 Campylobacter colonies/plate

++ = 11 - 50 Campylobacter colonies/plate

+++ = 51 - 200 Campylobacter colonies/plate

++++ = > 200 Campylobacter colonies/plate

Three weeks after the commencement of the rechallenge and cross transmission trial, all ten birds of the trial were destroyed. Caecal swabs from all the four non-infected chickens were confirmed as being negative for Campylobacter, while those from the remaining chickens were all positive for Campylobacter.

Experiment II

Infectivity of c. coli (chicken isolate) for chickens: Table 8.9 compares the rates of infection and the time at which organisms were detected in the faeces of chickens dosed with C. coli and C. jejuni.

The infectivity of C. coli was found to be less than that of C. jejuni. Only two of the eight chickens challenged with 5×10^5 cfu of C. coli became infected, while five of six chickens challenged each with 5×10^4 cfu of C. jejuni became infected, but the higher dose of 5×10^7 cfu of C. coli caused infection in all five chickens. The mean time for C. coli to be detected in the 'normal faeces' of infected chickens was greater than in birds dosed with C. jejuni.

Between 10 to 15 days post inoculation all birds, except four infected with C. coli (chickens Cc_4 and Cc_9, Cc_{11}, Cc_{13}), were destroyed. No gross lesions were observed in any of the birds and caecal cultures from the birds considered to be uninfected, failed to reveal any thermophilic Campylobacter.

The number of organisms recovered from the intestinal content of chickens Cc_5 and Cc_{12}, which had been dosed with 5×10^5 and 5×10^7 cfu of C. coli respectively, are shown in Table 8.10 and Figure 8.4. The results were similar to those obtained in Experiment I, using C. jejuni, except that the numbers recovered were lower.

Table 8.11 shows the results from enumeration studies and direct culture of faeces of the four surviving chickens.

Excretion of C. coli in 'normal faeces' of some individual birds appeared to reach a maximum of approximately 5×10^6 organisms/g, between four days and two weeks post inoculation and thereafter the numbers gradually

TABLE 8.9 : EXPERIMENT II: INFECTIVITY OF C. COLI NO. 241 AND C. JEJUNI NO. 251, ISOLATED FROM CHICKENS, FOR 3 WEEKS OLD CHICKENS, BY THE ORAL ROUTE

Challenging Species	Chicken Nos	Dose/ Chicken	Infection rate (%)	Numbers of chickens beginning shedding the organism at				Mean time of onset of shedding and (range)
				24h	48h	72h	96h*	
C. coli	Cc_9 - Cc_{13}	5×10^7	5/5 (100%)	1	4	-	-	43.2 (24 - 48)
C. coli	Cc_1 - Cc_8	5×10^5	2/8 (25%)	-	1	-	1	72.0 (48 - 96)
C. jejuni	Je_{13} - Je_{19}	5×10^4	5/6 (83.3%)	2	3	-	-	38.4 (24 - 48)

* No further infected animals were detected after 72 h.

TABLE 8.10 : EXPERIMENT NO. II : COMPARATIVE LEVELS OF C. COLI IN THE CONTENTS OF THE G.I. TRACT OF CHICKENS DOSED ORALLY WITH STRAIN 241

		Log_{10} counts/g of G.I. Content									
Chicken No.	Dose	Caecum	Colon	Normal Faeces	Crop	Glandular Stomach	Muscular Stomach	Duodenum	Middle Jejunum	Terminal Ileum	Time of Slaughter Post Challenge
Cc_5	5×10^5	7.30	6.41	5.90	3.04	2.95	2.00	2.30	4.89	5.38	12 days
Cc_{12}	5×10^7	7.78	7.00	6.30	3.38	3.30	- *	2.60	6.20	6.74	10 days
Means		7.54	6.70	6.10	3.21	3.13	1.00	2.45	5.55	6.06	11 days

* No enrichment was undertaken.

FIGURE 8.4 : EXPERIMENT NO. II: COMPARATIVE LEVELS OF C. COLI IN THE CONTENTS OF THE G.I. TRACT OF CHICKENS DOSED ORALLY WITH STRAIN 241

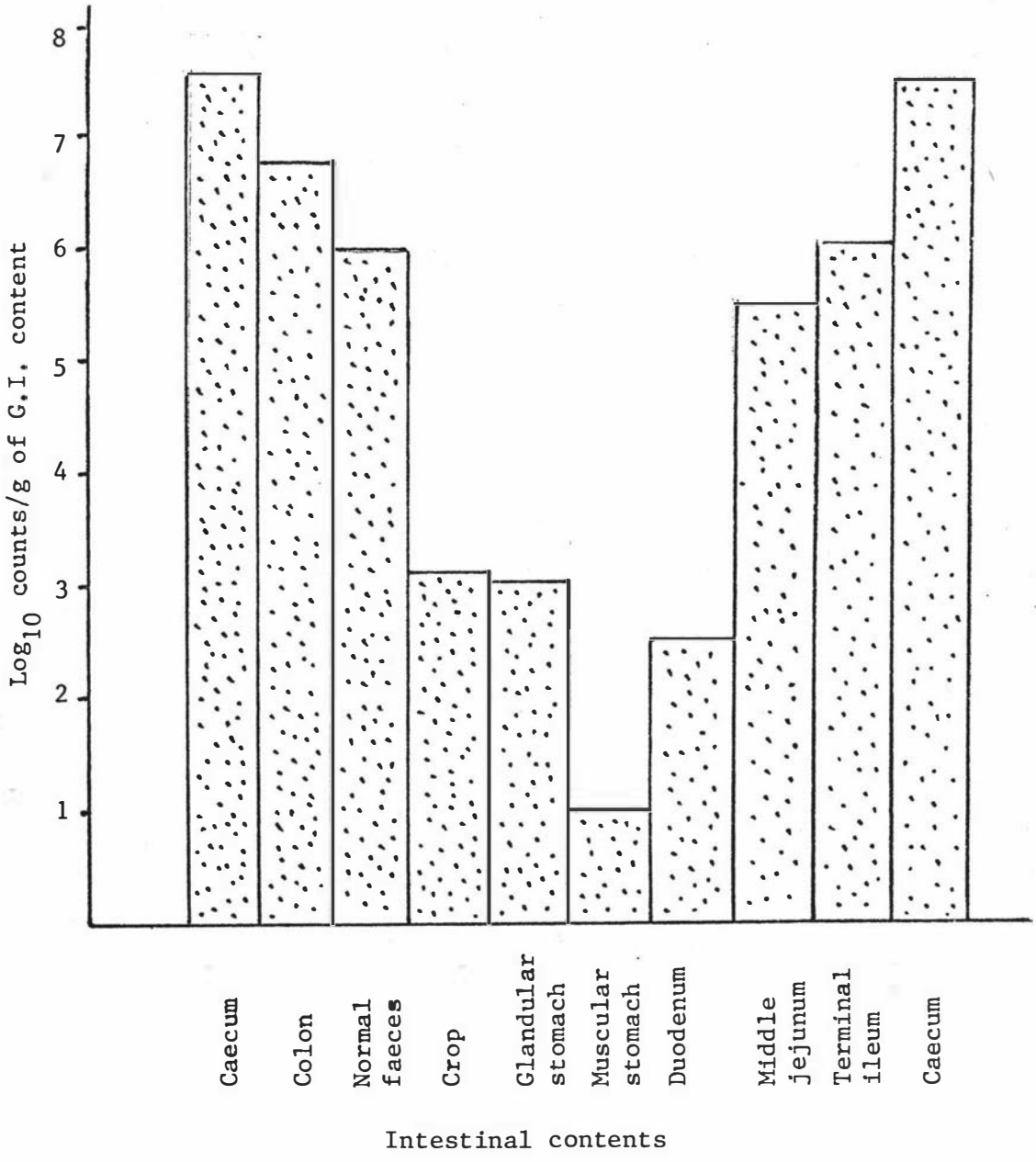


TABLE 8.11 : EXPERIMENT NO. II: LEVELS AND DURATION OF INFECTION BY C. COLI IN 3-WEEK-OLD CHICKENS (STRAIN NO. 241 ISOLATED FROM CHICKENS)

Results of direct culture and number/g of faeces (log_{10} counts)

Chicken No.	Type of faeces	Time Post Inoculation														
		1 day	2d	3d	4d	8d	14d	18d	4w	7w	10w	11w	13w	16w	17w	18w
Cc_4	Normal	-	-	-	3.95	4.30	4.90	4.38	+++	++	2+	3.34	-	-	-	ND
	Caecal											6.30	++	+	-	-
Cc_9	Normal	-	+	+	4.08	5.90	6.71	6.60	5.20	++++	++++	+++	ND	-	ND	ND
	Caecal												++	++	+	-
Cc_{11}	Normal	-	+	+	6.45	4.70	ND	5.95	++++	++++	+++	-	-	-	-	-
	Caecal											++	-	-	-	-
Cc_{13}	Normal	+	+	+	6.69	5.58	5.20	4.20	++++	+++	+++	-	+	ND	++	ND
	Caecal												+	ND	-	++
	Mean				5.54	5.12	5.60	5.28								

\+ = < 10 C. coli colonies/plate
++ = 11 to 50 C. coli colonies/plate
+++ = 51 to 200 C. coli colonies/plate
++++ = > 200 C. coli colonies/plate
ND = Not done.

decreased. These lower levels were similar to those recorded from the lower dose rates of C. jejuni in experiment No. I (see Table 8.6).

'Caecal faeces' remained infected with C. coli for a longer time than the 'normal faeces'. Chickens Cc_4, Cc_9 and Cc_{11} ceased shedding the organism in their 'caecal faeces' at 17, 18, and 13 weeks post inoculation (mean duration of infection 16 weeks). The same birds, based on their cultures of 'normal faeces', had a mean duration of infection of 13.3 weeks. Bird Cc_{13} continued to shed C. coli for more than 18 weeks (see Table 8.11).

A number of isolates recovered at various intervals from the chickens infected with C. coli had identical DNA patterns to the original strain with which they were challenged (see Plate 8.5).

Experiment III

Infectivity of various isolates from gulls and rats for chickens: Table 8.12 shows the ability of these isolates to infect chickens and plates 8.6 and 8.7 demonstrate the stability of their DNA fingerprint during the course of the experiment.

Neither the three strains of C. laridis, nor the three strains of C. coli isolated from seagulls were infective for the 12 chickens, even when high doses were used (1.3×10^7 to 5×10^8 cfu/chicken). However, one of the two chickens (816_1) challenged with a seagull strain of C. coli (816) and the two chickens (822_1 and 822_2) challenged with another seagull strain of C. jejuni (822), became accidentally infected with the chicken C. jejuni strain 251 five to nine days post challenge.

This cross infecting strain (251) was an isolate of C. jejuni from a chicken from the rechallenge and cross transmission trial in Experiment No. 1 which was being carried out at the same time and in the same room. The precise identity of this strain was confirmed by the BRENDA analysis of a number of Campylobacter colonies obtained from chickens 816_1 and 822_1, 822_2, (see Plates 8.8 and 8.9).

Two isolates of C. jejuni from seagulls infected all four chickens which were given doses similar to those used for C. laridis and C. coli (6.7×10^6 to 5×10^7 cfu/chicken). A

TABLE 8.12 : EXPERIMENT NO. III: INFECTIVITY BY THE ORAL ROUTE OF VARIOUS SPECIES OF CAMPYLOBACTER FROM GULLS AND RATS FOR ONE WEEK OLD CHICKENS

Challenging Species	Source of Isolate	Isolate No.	Dose/ Chicken	Infection Rate	Numbers of chickens beginning shedding organisms at 24 h	48 h**
C. laridis	Gulls	812	2.3×10^7	0/2	Not infected	
		820	1.6×10^7	0/2		
		847	5×10^8	0/2		
C. coli	Gulls	816	2×10^7	0/2*	Not infected	
		877	1.3×10^7	0/2*		
		889	5×10^8	0/2		
C. jejuni	Gulls	822	2×10^7	0/2	Not infected	
		888	6.7×10^6	2/2	2	
		891	5×10^7	2/2	2	
C. jejuni	Rats	R18	2×10^6	4/4	3	1

Mean time of onset of shedding and (range)

27 h (24 - 48 h)

* Became accidentally infected with C. jejuni 251

** No further infected animals were detected after 48 h

PLATE 8.5 : STABILITY OF DNA 'FINGERPRINT' OF C. COLI (241) DURING EXPERIMENTAL TRANSMISSION

Lane 1 is the bacteriophage C1857 S7.

Lane 2 is the challenge strain.

Lanes 3 - 6, isolates recovered from different infected chickens.

PLATE 8.6 : STABILITY OF DNA 'FINGERPRINT' OF C. JEJUNI (888)

Lane 1 is the challenge strain.

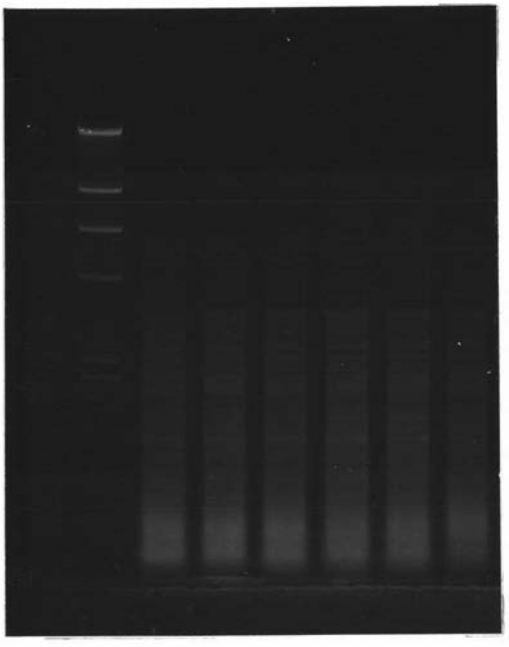
Lanes 2 - 5, isolates recovered from the two infected chickens.

PLATE 8.7 : STABILITY OF DNA 'FINGERPRINT' OF C. JEJUNI (R18)

Lane 1 is the challenge strain.

Lanes 2 - 5, isolates recovered from different infected chickens.

PLATE 8.5



1 2 3 4 5 6 7

PLATE 8.6

1 2 3 4 5

PLATE 8.7

1 2 3 4 5

PLATE 8.8 : DIFFERENCE BETWEEN THE DNA 'FINGERPRINT' OF CHALLENGE STRAIN 816 OF C. COLI AND THE ISOLATES OF C. JEJUNI 251 RECOVERED FROM THE INFECTED CHICKEN

Lane 1 is the bacteriophage C1857 S7.

Lane 2, challenge strain of C. coli (816).

Lanes 3 - 9, isolates of C. jejuni (251) recovered.

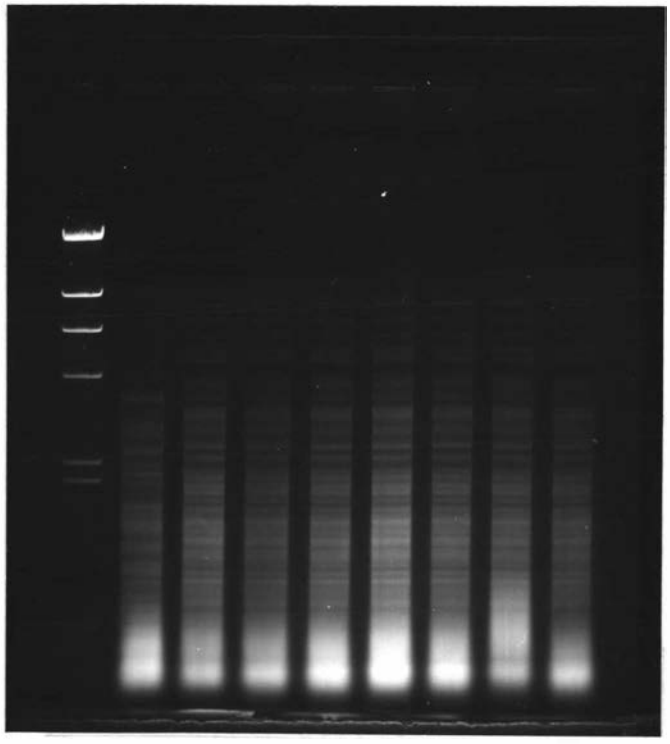
PLATE 8.9 : DIFFERENCE BETWEEN THE DNA 'FINGERPRINT' OF CHALLENGE STRAIN 822 OF C. JEJUNI AND THE ISOLATES OF C. JEJUNI 251 RECOVERED FROM THE INFECTED CHICKENS

Lane 1 is the bacteriophage C1857 S7.

Lane 4, challenge strain of C. jejuni (222).

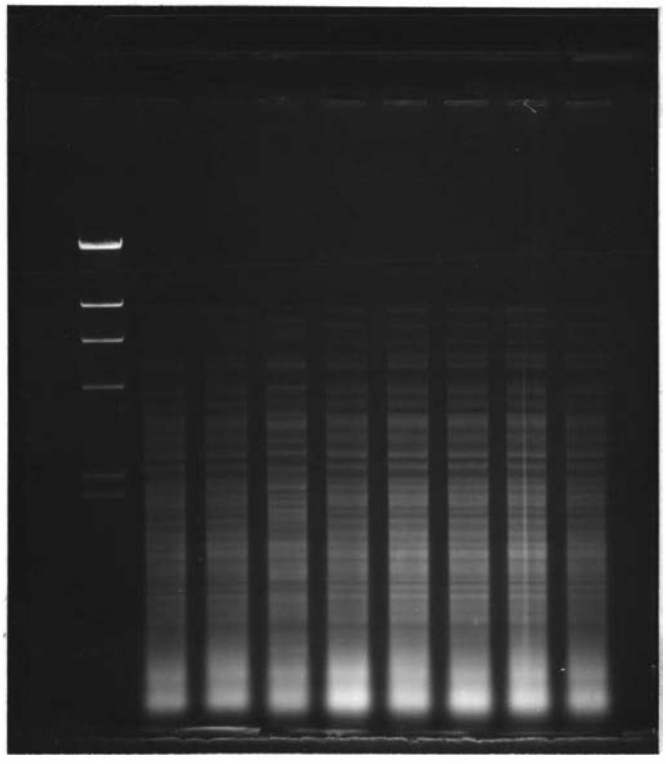
Lanes 2 - 3 and 5 - 9, isolates of C. jejuni (251) recovered.

PLATE 8.8



1 2 3 4 5 6 7 8 9

PLATE 8.9



1 2 3 4 5 6 7 8 9

third C. jejuni isolate obtained from a seagull failed to infect the two chickens at the dose 2 x 10^7 cfu/chicken. All the four chickens challenged with the C. jejuni R18 isolated from rats became infected. Within 24 h post inoculation, the birds started shedding Campylobacter in their faeces. One bird started to shed organisms 48 h post inoculation (mean time before shedding organisms 27 h).

Levels and duration of excretion: Table 8.13 and Figure 8.5 show the comparative levels and duration of infection based on the examination of 'normal' and 'caecal faeces'. It was clearly demonstrated again that the excretion levels of C. jejuni in the 'caecal faeces' were approximately 100 to 1,000 times higher than in 'normal faeces'. The mean excretion levels of C. jejuni per g of 'normal faeces' approached $10^{6.00}$ cfu at 48 h post inoculation and by the fifth day the number reached a mean maximum level of $10^{7.00}$ cfu and thereafter gradually declined. The mean counts for the 'caecal faeces' rose to $10^{8.30}$ cfu/g at the fifth day post inoculation and remained just below $10^{8.00}$ cfu/g of caecal faeces for approximately six weeks' post challenge. Thereafter the levels declined steadily.

The mean duration of excretion of all isolates of C. jejuni in 'normal faeces' was 10 weeks (range 8 to 14) and in 'caecal faeces' 13.3 weeks (range 10 to 16 weeks).

Intestinal counts: Enumeration studies were carried out on chickens $R18_1$ and $R18_4$ which were slaughtered five weeks post inoculation. Apart from the contents of the muscular stomach, no organisms were recovered from the G.I. tract proximal to the caecum. Glandular stomach content from chicken $R18_1$ was positive by direct culture (negative at dilution of 10^2:2/g content) while that of $R18_4$ had $10^{1.41}$ cfu/g content. The caecal and rectal contents of both chickens contained large numbers of Campylobacter as shown below.

$R18_1$	/g caecal content	$10^{7.30}$
	/g rectal content	$10^{5.69}$
$R18_4$	/g caecal content	$10^{8.70}$
	/g rectal content	$10^{6.00}$

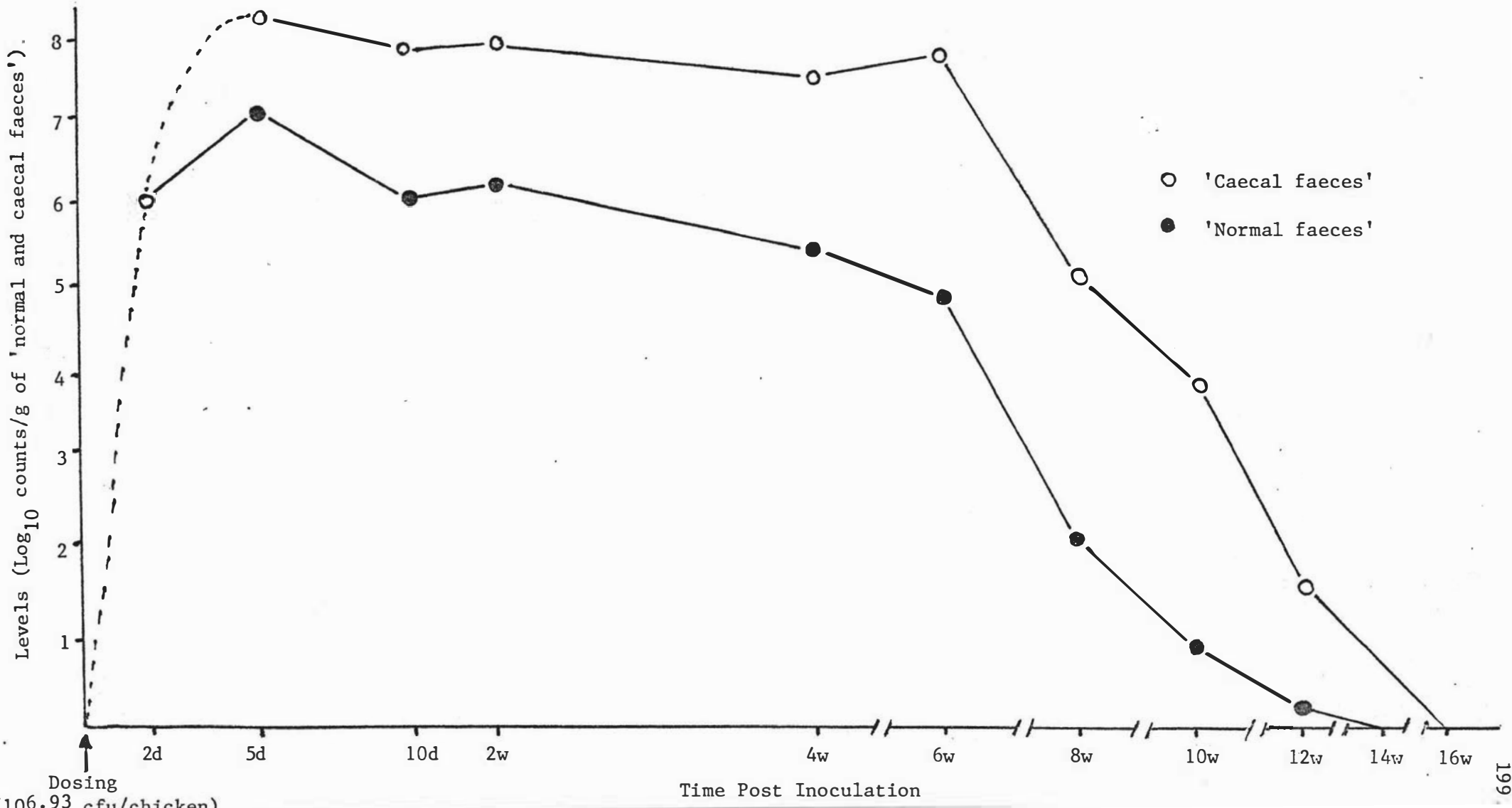
TABLE 8.13 : LEVELS AND DURATION OF INFECTION IN 1 WEEK OLD CHICKENS DOSED WITH VARIOUS STRAINS OF C. JEJUNI ISOLATED FROM GULLS AND RATS

C. jejuni Log_{10}/g of 'normal and caecal faeces'

Source of Isolate	Isolate No.	Chicken No.	'Normal'(N) 'Caecal'(C) Faeces	Time Post Inoculation 2 d	5 d	10 d	2 w	4 w	6 w	8 w	10 w	12 w	14 w	16 w	Duration of Excretion 'Normal' faeces	'Caecal' faeces
Gull	888	888_1	N	5.18	6.60	7.98	5.95	5.00	6.04	3.58	-	-	-	-	10	16
			C						8.20		6.32	3.04	1.70	-		
		888_2	N	5.81	7.27	5.95		5.11	4.70	-	-	-	-	-	8	12
			C					7.78	5.96	4.98	3.00	-	-	-		
Gull	891	891_1	N	6.60	6.00	5.70		6.16		4.18	2.78	-	-	-	12	14
			C		7.94	7.79	7.90	6.28		5.59	3.67	2.30	-	-		
		891_2	N	6.54	5.83	3.00		4.11	4.30	-	-	-	-	-	8	10
			C		7.48	7.79		7.30		2.48	-	-	-	-		
Rat	R18	$R18_2$	N	5.88	8.78	6.48		5.30	2.40	-	-	-	-	-	8	14
			C		9.48					7.70	6.56	2.60	-	-		
		$R18_3$	N	5.81	7.27	5.95		6.66	6.56	3.95	2.90	1.70	-	-	14	14
			C					8.41	8.15		3.90	2.30	-	-		
		Means	N	5.97	6.96	5.84	5.95	5.39	4.80	1.95	0.94	0.34	-	-		
			C		8.30	7.79	7.90	7.44	7.77	5.19	3.91	1.71	0.28	-	10.0	13.3
														Range	(8-14)	(10-16)

Empty space = Cultures Not Done

FIGURE 8.5 : EXPERIMENT III: COMPARATIVE MEAN LEVELS AND DURATION OF EXCRETION OF THREE STRAINS OF C. JEJUNI (TWO FROM GULLS AND ONE FROM RATS) IN THE NORMAL AND THE CAECAL FAECES OF EXPERIMENTALLY INFECTED 1 WEEK CHICKENS (6 CHICKENS)



Rechallenge: Two to three weeks after Campylobacter could no longer be demonstrated in 'caecal faeces', chickens 888_1, 888_2, 891_1, 891_2, $R18_2$, $R18_3$ were rechallenged with doses ranging from 10^8 to 10^9. All except one (891_1) resisted reinfection. Organisms could be demonstrated in the 'caecal faeces' of bird 891_1 for 17 days.

The uninfected chickens and the one which had been reinfected were slaughtered two and three weeks post rechallenge respectively. At this time, no organisms were recovered from the G.I. tract of any bird.

Experiment IV

Infectivity of various isolates of thermophilic Campylobacter originally isolated from rats, chickens, gulls and pigs to laboratory rats: Table 8.14 summarises the results of attempts to infect rats with the different isolates of Campylobacter. The results demonstrate that there were differences in the infectivity not only between the three species of Campylobacter, but also between the same species isolated from the same sources.

Infection was established in rats with four of the eight strains used the exceptions were C. jejuni (strain R_2) isolated from a rat, C. laridis (strain 820 and 847), and C. coli (strain 877) isolated from gulls. Dose rates of between 2.5×10^7 and 5×10^7 cfu per rat of C. jejuni (Strains 251 and R18) infected all the rats which were challenged. The minimal infective dose of C. jejuni was between 2.5×10^4 and 2×10^5 cfu, and that for C. coli approximately 10^7 cfu, which indicates a 100 to 1,000 times difference in infectivity of these two species of Campylobacter for rats. Similar differences between the infectivity of these two species of Campylobacter were recorded in chickens.

The onset of excretion of C. jejuni (strain R18) in faeces showed a dose dependent response and the higher the dose given, the shorter the time (see Table 8.14).

TABLE 8.14 : EXPERIMENT NO. IV: INFECTIVITY BY THE ORAL ROUTE OF VARIOUS SPECIES OF THERMOPHILIC CAMPYLOBACTER FOR LABORATORY RATS 30 TO 45 DAYS OF AGE

Challenging Species	Source of Isolate	Isolate No.	Dose/Rat	Infection Rate	Onset of faecal excretion (hrs) 24	48	72	96*	Mean time (hours) of onset of faecal excretion
C. jejuni	Rats	R_{18}	5×10^7	5/5	4	0	0	1	43.4 (24 - 96)
			2×10^6	4/5	1	1	1	1	60 (24 - 96)
			2×10^5	1/5	0	0	1	0	72
			2×10^4	0/5					
		R_2	2×10^8	0/5					
C. jejuni	Chickens	251	2.5×10^8	5/5					
			2.5×10^7	5/5					
			2.5×10^6	4/5					
			2.5×10^4	1/5					
C. laridis	Gulls	820	9×10^8	0/4					
C. laridis		847	7.3×10^8	0/4					
C. coli		877	6.6×10^8	0/4					
C. coli	Pigs	319	5.5×10^8	2/4					
			5×10^6	0/4					
		320	4.7×10^8	3/4					
			4.7×10^6	0/4					

* No further infected animals were detected after 96 h

Cross infection of rats: The results of this trial are shown in Tables 8.15 and 8.16, and Figures 8.6 and 8.7.

All the non-infected rats placed in contact with the animals infected four days previously, became infected within 96 h. The mean time before organisms were detected in their faeces was 62.4 h (range 24 to 96), (See Table 8.16).

Both the orally dosed (see Table 8.15 and Figure 8.6) and the naturally cross-infected rats (see Table 8.16 and Figure 8.7) developed faecal excretion rates of up to $10^{7.00}$ cfu/g of faeces. These high levels of excretion developed two weeks after contact with infected animals. There was then a decline in excretion rate to approximately $10^{5.50}$ cfu/g of faeces; this level remained stable for three to six weeks post contact. Only after this period did the mean numbers show a steady and slow decline. (The rats infected by direct oral dosage showed a more rapid decline.)

The mean duration of infection for the orally dosed and naturally cross-infected rats was 8.4 weeks (range 6 to 13) and 11 weeks (range 9 to 13) respectively. This difference was not significant. Plate 8.10 demonstrates the stability of C. jejuni R18 during the course of the cross-infection trial.

Table 8.17 and Figure 8.8 show the mean levels and mean duration of excretion which occurred in individual rats dosed with C. jejuni strains R18 (2×10^6/rat) and 251 (2.5×10^7/rat). Although the counts/g of faeces were generally lower than those dosed with 5×10^7 ($R18_1$ - $R18_5$) and those infected by contact (N_1 - N_5), (see Table 8.13 and Figure 8.7), the pattern of excretion was similar. The highest levels were obtained between 5 and 14 days and thereafter gradual elimination was observed. The mean duration of infection being 7 weeks (range 4 to 9 weeks) for the $R18_6$ - $R18_{10}$ and 7.8 weeks (range 4 to 11 weeks) for the rats 251_1 - 251_5.

No counts were carried out on faeces from rats infected with C. coli (strains 319 and 320). However, the direct culture of faeces indicated that the levels of excretion were generally lower than in rats infected with C. jejuni. All of these rats remained infected for two weeks post inoculation (see Table 8.18). By

TABLE 8.15 : EXPERIMENT NO. IV: LEVELS AND DURATION OF EXCRETION OF C. JEJUNI R18 IN FAECES OF RATS[a] CHALLENGED ORALLY (5×10^7 cfu/RAT)

(Cross Transmission Trial)

Rat No.	Log_{10} numbers of Organism/g of faeces at												Duration of Excretion (weeks)
	24 h	48 h	72 h	96 h	10 d	2 w	3 w	4 w	6 w	9 w	11 w	13 w	
$R18_1$	*	7.78	ND	7.60	7.81	7.30	5.40	5.11	4.94	2.60	1.70	-	13
$R18_2$	-	-	-	4.60	6.90	3.70	1.70	3.18	-	-	-	-	6
$R18_3$	*	7.70	ND	7.78	6.60	7.81	3.04	1.70	-	-	-	-	6
$R18_4$	*	7.30	ND	7.20	8.04	7.00	3.86	3.04	-	1.70	-	-	11
$R18_5$	*	6.81	ND	7.64	7.53	6.32	4.90	1.70	-	-	-	-	6
Means		7.40		6.96	7.38	6.43	3.78	4.36	0.99	0.86	0.34	-	8.4 (range 6-13)

a These rats were placed in the same cage with five non-infected rats (N_1 - N_5) 96 h post oral challenge (see Table 8.16).

* Number of organisms not counted but direct culture positive

ND Not done

TABLE 8.16 : EXPERIMENT NO. IV: LEVELS AND DURATION OF EXCRETION OF C. JEJUNI R18 IN FAECES OF RATS INFECTED BY CROSS TRANSMISSION BY CONTACT WITH RATS $R18_1$ - $R18_5$[a] (CROSS TRANSMISSION TRIAL)

Rat No.	Log_{10} numbers of Organisms/g of faeces post contact at													Duration of Excretion (weeks)
	24 h	48 h	72 h	96 h	10 d	2 w	3 w	4 w	6 w	9 w	11 w	13 w	15 w	
N_1	-	-	*	3.60	6.23	5.80	5.54	6.90	8.30	6.86	2.77	-	-	13
N_2	-	-	-	2.30	5.69	7.74	3.00	7.30	6.45	2.60	-	-	-	11
N_3	-	-	*	2.48	5.40	6.56	5.10	7.78	7.48	5.75	2.85	-	-	13
N_4	*	*	*	4.85	5.83	7.40	6.91	2.70	2.48	1.70	-	-	-	11
N_5	-	*	*	5.72	6.51	7.95	6.76	4.54	2.79	-	-	-	-	9
Means				3.79	5.93	7.09	5.46	5.84	5.50	3.38	1.12	-		11.4 (range 9-13)

[a] These rats were placed in the same cage with five non-infected rats (N_1 - N_5), 96 h post oral challenge (see Table 8.15).

* Number of organisms not counted but direct culture positive

FIGURE 8.6 : EXPERIMENT NO. IV: LEVELS AND DURATION OF EXCRETION OF C. JEJUNI R18 IN THE FAECES OF RATS DOSED ORALLY WITH STRAIN R18

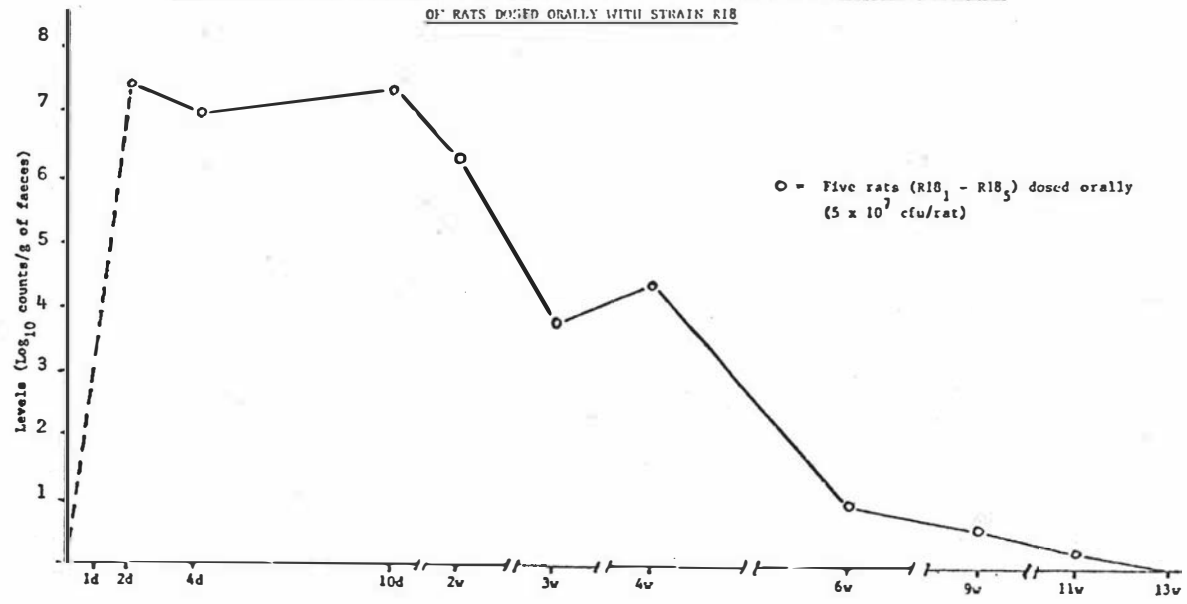


FIGURE 8.7 : EXPERIMENT NO. IV: LEVELS AND DURATION OF EXCRETION OF C. JEJUNI R18 IN FAECES OF RATS INFECTED BY CROSS TRANSMISSION BY CONTACT WITH INFECTED RATS $R18_1$ - $R18_5$*

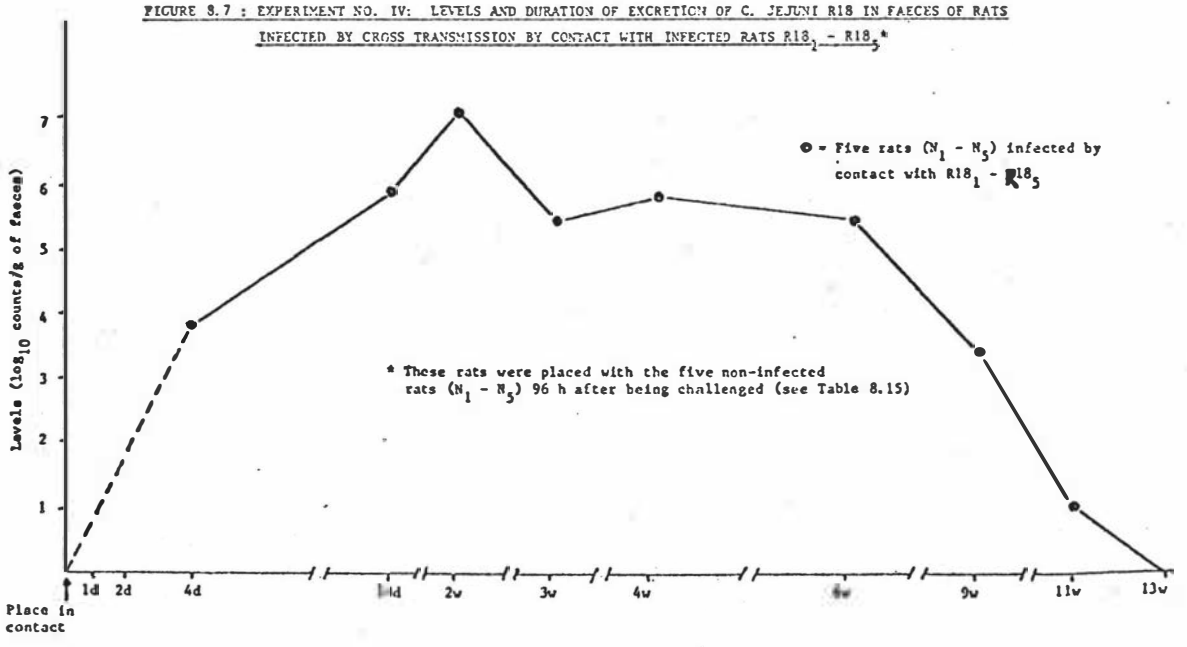
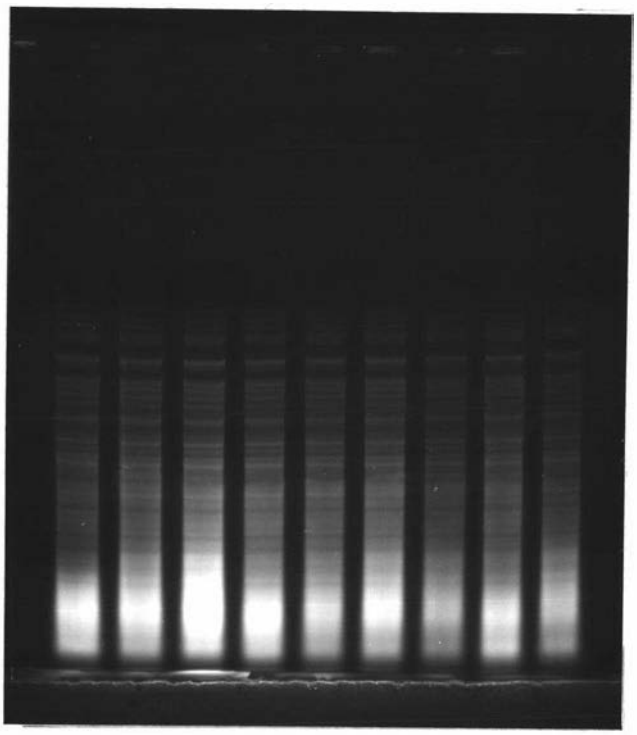


PLATE 8.10 : STABILITY OF THE DNA 'FINGERPRINT' OF C. JEJUNI (R18) DURING AN EXPERIMENTAL CROSS TRANSMISSION TRIAL



1 2 3 4 5 6 7 8 9

Lane 1 is the bacteriophage C1857 S7.

Lane 2, original challenge strain of C. jejuni (R18).

Lanes 3 - 9, isolates recovered from different infected rats in the trial.

TABLE 8.17 : LEVELS AND DURATION OF EXCRETION OF C. JEJUNI IN FAECES OF RATS DOSED ORALLY WITH STRAINS R18 AND 251 ISOLATED FROM RATS AND CHICKENS

Log_{10} numbers of organism/g of pellet faeces

Strain No	Dose/ rat	Rat No.	Time							Duration of Excretion (weeks)
			5 d	2 w	3 w	4 w	6 w	9 w	11 w	
R18	2×10^6	$R18_6$	3.90	3.86	4.90	4.70	3.23	-	-	9
		$R18_7$*	-	-	-	-	-	-	-	-
		$R18_8$	4.08	5.40	5.83	2.70	-	-	-	6
		$R18_9$	7.45	7.85	5.64	-	-	-	-	4
		$R18_{10}$	2.18	2.00	2.60	6.70	4.32	-	-	9
		Mean	4.40	4.78	4.74	3.53	1.83	-	-	7 (range 4-9)
251	2.5×10^7	251_1	6.63	7.60	5.34	3.15	1.70	-	-	9
		251_2	7.30	7.60	5.16	2.78	2.30	2.18	-	11
		251_3	4.95	6.70	4.34	2.48	-	-	-	6
		251_4	5.23	3.62	2.30	-	-	-	-	4
		251_5	3.00	5.18	3.04	2.78	1.70	-	-	9
		Mean	5.42	6.14	4.04	2.24	0.80	0.44	-	7.8 (range 4-11)
	Overall	Mean	4.91	5.46	4.39	2.89	1.32	0.22	-	7.4 (range 4-11)

* Did not become infected

FIGURE 8.8 : EXPERIMENT NO IV: COMPARATIVE LEVELS AND DURATION OF EXCRETION OF C. JEJUNI IN FAECES OF RATS DOSED ORALLY WITH STRAIN R18 and 251

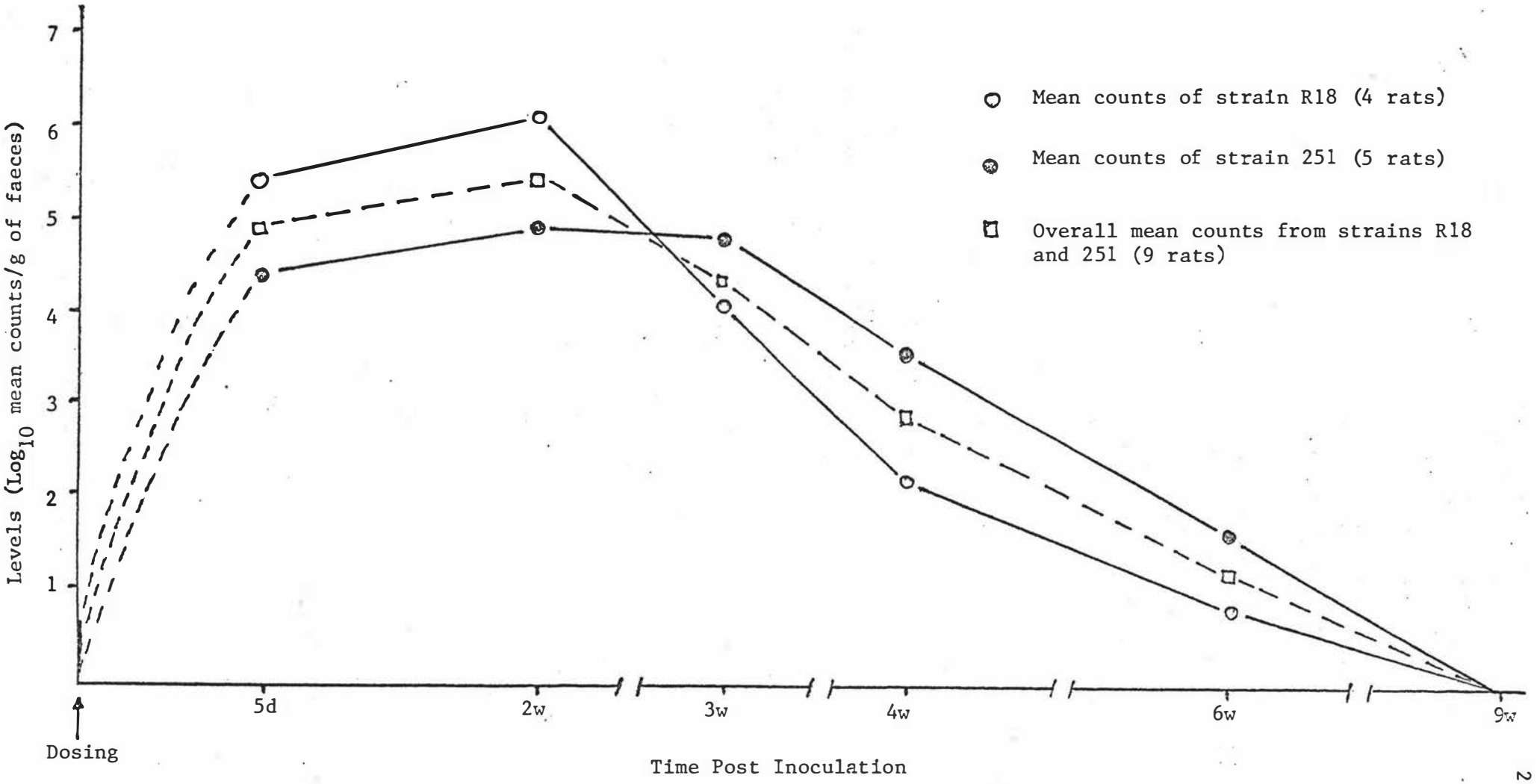


TABLE 8.18 : DURATION OF EXCRETION OF C. COLI IN THE FAECES OF RATS DOSED ORALLY WITH STRAINS 319 AND 320 ISOLATED FROM PIGS

Rat No.	Dose/rat	Time in weeks post inoculation (based on direct culture)					
		1	2	3	4	5	6
$R319_1$	5.5×10^8	-	-	-	-	-	-
$R319_2$		+	+	-	-	-	-
$R319_3$		-	-	-	-	-	-
$R319_4$		+	++	+	-	-	-
$R320_1$	4.7×10^8	+	++	++	+	-	-
$R320_2$		-	-	-	-	-	-
$R320_3$		+	+	-	-	-	-
$R320_4$		+	+	+	-	-	-

\+ < 10 Campylobacter colonies/plate

++ 11 - 50 Campylobacter colonies/plate

three weeks post inoculation, one rat from each group (319 and 320) ceased to shed Campylobacter and by four weeks post inoculation isolates could only be recovered from one rat. This rat eliminated the infection by the fifth week post inoculation. None of the infected animals showed any sign of overt disease or change in nature of the faecal pellets.

DISCUSSION

Although the experiments were not designed to investigate the pathogenesis of thermophilic Campylobacter infections in the animals studied, it is interesting to note that no gross or clinical signs of enteric disease were observed.

The lack of diarrhoea and other clinical signs is a finding that is in agreement with those of Butzler and Skirrow (1979) and Prescott et al (1982). The former authors' observations were of 25 conventional eight-day-old chickens placed in groups of five and individually dosed orally with 10^8 cfu of one of five human isolates of C. jejuni. Prescott et al (1982) based their observations on five-day-old gnotobiotic chickens challenged with two human isolates of C. jejuni. Their results are in apparent contradiction to the findings of Ruiz-Palacios et al (1981) who observed diarrhoea in 22 (88%) three-day-old chickens dosed intragastrically with 9 x 10^7 cfu of C. jejuni which had been isolated from a patient with sever dysentery. The mean onset of diarrhoea was 45 h (range 24 to 72 h) with a mean duration of 8 days, and seven (32%) of the infected chickens died between 4 and 7 days post infection. Both Butzler and Skirrow (1979) and Ruiz-Palacios et al (1981) were able to isolate Campylobacter from the heart blood of infected chickens. The former investigators isolated these organisms from approximately 50% of the 25 infected chickens which were sacrificed one week post challenge, and the latter investigators from all of the seven birds which died. Butzler and Skirrow (1979) demonstrated by electro-microscopy, Campylobacter within caecal tissue and Ruiz-Palacios (1981) penetration of the epithelial cells of the jejunum, ileum and caecum, 12 hours post inoculation by the organisms.

Bacteraemia in humans suffering with Campylobacter enteritis is probably limited to the early stage of the disease (Butzler and Skirrow 1979). Experimental infection of Rhesus monkeys with a human strain of C. jejuni resulted in a bacteraemia of two to three days duration (Fitzgeorge et al 1981). Blaser et al (1983) showed that adult SPF mice, dosed intragastrically with 10^8 cfu with isolates of C. jejuni from humans, developed a transient bacteraemia of up to 24 h duration without showing any signs of illness.

The failure by the present author to isolate any Campylobacter from the blood samples taken from chickens, at least 10 days post challenge or from tissue (heart, kidneys and liver) at least three weeks post challenge, is therefore not surprising. In an early report, Kölbl and Willinger (1967) recorded that they were unable to isolate any vibrios from the heart, kidney, liver and gall from 39 of 40 chickens dosed orally with a vibrio and slaughtered between 7 and 29 days post challenge. Enteric infection was demonstrated in all forty birds and in one of five, which were slaughtered 7 days post inoculation, vibrios were recovered from the liver and gall bladder.

The presence of C. jejuni (strain 251) in gall from two chickens may have been the result of either a transient bacteraemia in an early stage of the infection or to an ascending infection from the intestine. Gall has been shown to be the most suitable biological mileu of the human body for the survival of Campylobacter (Blaser et al 1980d) and organisms have been isolated from 16% and 17% of gall samples from slaughtered sheep and cattle respectively (Bryner et al 1972).

Although the number of chickens dosed with C. jejuni in the various subgroups of the three and eight-week-old chickens (Experiment I), was small (4 to 6 chickens/subgroup), the overall number of 16 younger and 20 older birds challenged and kept separately from each other, is considered a sufficiently large group to demonstrate gross differences in age susceptibility. The lack of significant differences in the susceptibility to infection in these two groups was not unexpected, particularly in the light of the results from the field work, where the infection rates between

broilers and layer flocks were not significantly different.

It has been demonstrated that the infectivity of C. jejuni and C. coli is dose dependent and this observation is in agreement with that made by Blaser et al (1983), regarding various isolates of C. jejuni from humans, which were given intragastrically to adult mice.

The greater infectivity of C. jejuni compared with that of C. coli observed in both the experimental chickens and the rats together with the general inability of all the strains of C. laridis and C. coli isolated from gulls to infect these animals, is compatible with the observations reported in Chapters VI and VII in this thesis. In this work, more poultry flocks were infected with C. jejuni than C. coli and the prevalence of C. jejuni within infected flocks was always higher than that of C. coli species. More ducks were infected with C. jejuni than C. coli. Rats were found to be infected only with C. jejuni, and C. laridis was only isolated from gulls.

The apparently higher infectivity of C. jejuni for many species of animal might explain the higher recovery of C. jejuni compared with other species of Campylobacter from a variety of other animals examined by several other investigators (see Chapter II).

In this work, the minimal infective doses of C. jejuni and C. coli for chickens, has been demonstrated to be from 5×10^2 and 2.5×10^5 organisms. Ruiz-Palacios et al (1981) reported that the minimal infective dose of a strain of C. jejuni isolated from humans for three-day-old chickens to be nine organisms only. However, this latter work is open to some criticism. One of the 86 chickens used was found to be infected before the investigation was started. Although this bird was removed, the others were kept in groups of ten, and natural transmission could have occurred between birds which were infected before the experiment commenced and were not at that time excreting organisms in their faeces. As the birds were only three days old, it would not have been possible to keep them for sufficient time before the experiment started, to ensure that they were not incubating an infection. Ruiz-Palacios et al (1981) recorded themselves, that cross-infection between groups of infected

and non-infected chickens, could not be detected for at least three days.

A human volunteer (Robinson 1981) was able to infect himself by ingesting 5×10^2 C. jejuni suspended in 180 millimeters of pasteurised milk. (There is no information regarding the infectivity of C. coli for humans.) As the infectivity of C. jejuni for both humans and chickens, and the relative proportion of isolates of C. jejuni and C. coli from both humans and chickens, are similar, it may be speculated that the differences observed between infectivity of C. jejuni and C. coli for chickens is similar for humans.

Two particular interesting observations arising from the work reported in this chapter are that:

1. The onset of excretion of C. jejuni in faeces is earlier than C. coli in both chickens or rats, and

2. The onset of excretion of both species is earlier in chickens compared with rats.

It is the author's opinion that these differences are associated with both the higher infectivity of C. jejuni compared with that of C. coli, and with the higher body temperature of chickens compared with rats.

A possible explanation for chickens excreting both C. jejuni and C. coli more rapidly in their faeces than rats, is the higher body temperature of 41.7°C (range 40.6°C to 43°C) of chickens (Anderson 1977), compared with only 37.5°C for rats (Blackmore and Keeley 1972). The higher intestinal temperature of chickens might provide a more optimal in-vivo temperature for both C. jejuni and C. coli than that of the intestine of a rat. This more favourable environment could result in faster generation times and a more rapid onset of excretion of organisms in the faeces.

In Chapter II it was reported that in humans, for reasons which were not known, the incubation period of Campylobacter enteritis is

longer than with most other bacterial enteric infections. The hypothesis postulated in the preceding paragraph could explain the comparatively long incubation period of Campylobacter enteritis in humans. For example, mesophilic organisms such as Salmonellae and Shigellae have an optimum temperature for growth of 37°C, similar to that of the human body temperature. As the result of the less favourable human intestinal temperature, compared with birds, thermophilic Campylobacter might have slower generation times than do the other common mesophilic enteric organisms.

An additional factor which might influence the incubation period, is that humans might consume with their foods lower numbers of C. jejuni or C. coli compared with Salmonellae. At ambient temperatures, the number of Salmonellae on a variety of foods will increase while the number of Campylobacter will decrease. As already discussed, the lower the infective dose, the longer the incubation period.

Cross transmission between infected and non-infected chickens in the same cage within 24 h has been previously demonstrated (Kŏlbl and Willinger 1967). The lack of transmission to control chickens, separated by only a simple cardboard partition from infected birds excreting up to 10^8 cfu of C. jejuni/g of faeces, indicates that the infection does not appear to be transmitted by the respiratory route. This finding indicates that it should be possible to maintain commercial flocks of poultry free of infection with Campylobacter by relatively simple barrier maintenance.

The natural elimination of the intestinal infection in chickens and their resistance to reinfection indicates the development of some form of protective mechanism. This mechanism is considered to be more likely some form of immune response than related to changes in the intestinal microflora and a form of competitive exclusion. The reasons to support this hypothesis are that birds of different age, and therefore with assumed different intestinal flora were equally susceptible to infection, and the same degree of resistance to reinfection occurred in birds of different age. In retrospect it is unfortunate that the chickens which developed resistance to reinfection with the same strain were not rechallenged with a different BRENDA

type of the same species.

The finding that the counts from the 'caecal faeces' and the caecal contents were always significantly higher than the counts from the 'normal faeces' or from other sites in the G.I. tract, indicates that the predilection site for both C. jejuni or C. coli is the caeca. Other important sites of infection appear to be the ileum and the colon. These findings are in agreement with the histo-immunological observations of Ruiz-Palacios et al (1981). these workers reported a moderate infiltration of mononuclear cells in the ileum and the caecum and specific immuno-fluorescence to C. jejuni was seen in phagocytic cells in the lamina propria of the distal portion of the jejunum, the ileum and the proximal portion of the colon of chickens, 12 to 48 h post challenge with C. jejuni.

The presence of C. jejuni and C. coli in gradually diminishing numbers from the crop to the glandular and muscular stomach is not thought to be due to active colonization and multiplication of organisms at these sites. It is believed that these results are a reflection of the consumption of food and water contaminated with infected faeces and the passive and transient presence of organisms. Antiperistaltic movements of the proximal part of the G.I. tract (Sturkie 1965) could also result in temporary contamination of the muscular and glandular stomachs.

During the course of these experiments, the various isolates used to infect experimental animals were subjected to BRENDA typing before, during and at the termination of experiments. The results of this technique provided strong evidence that inadvertent cross-contamination had not occurred, except on one occasion. It was also noted that the DNA patterns of the various isolates used, did not change over a period of several months during which these in-vivo experiments were conducted. A similar in-vitro stability of isolates has also been demonstrated (Kakoyiannis et al 1984).

CONCLUSIONS

1. C. jejuni is from 100 to 1,000-fold more infective for chickens than C. coli, the infective doses ranging from $5 \times 10^2 - 5 \times 10^5$ and $5 \times 10^5 - 5 \times 10^7$ organisms respectively.

2. The time between inoculation of chickens with either C. jejuni or C. coli, and the detection of organisms in the faeces is dose dependent. The higher the dose, the shorter the time before organisms appear in the faeces.

3. The maximum numbers of C. jejuni and C. coli which can be detected in the faeces of infected chickens, occur four to ten days after oral inoculation and are in the region of 10^8 cfu/g faeces.

4. Enteric infection of chickens, by both C. jejuni and C. coli, is self-limiting and is of seven to 18 weeks duration. Such infection is eliminated from the proximal portions of the G.I. tract before the distal parts.

5. The caeca of chickens are the predilection sites for both C. jejuni and C. coli and the highest number of organisms are found in the caecal contents and 'caecal faeces'. Thus culture of 'caecal faeces' is a more sensitive technique for detecting infected birds compared with culture of 'normal faeces'.

6. The majority of birds which were previously infected, are resistant to reinfection by the same strain of organism.

7. Both C. jejuni and C. coli are from 100 to 1,000-fold less infective for rats than for chickens.

8. The pattern for infection of C. jejuni and C. coli in rats is very similar to that in chickens. Maximum numbers of up to 10^7/g faeces occur in faeces between five and 14 days post challenge and infection is self-limiting within a period of three to 14 weeks.

9. Strains of C. jejuni and C. coli, isolated from the same or a different species of animal, have major differences in their infectivity for chickens and rats.

10. C. laridis, and isolates of C. coli from gulls, are not infective for either chickens or rats at dose rates of up to 10^9 cfu.

11. There was no evidence of any change in the BRENDA pattern of any isolate during the course of any transmission experiment.

CHAPTER IX

INTESTINAL THERMOPHILIC CAMPYLOBACTER IN HUMANS

INTRODUCTION

Campylobacter infections in humans have been discussed in Chapter II. In New Zealand, human *Campylobacter* infection was declared a notifiable disease in 1980 (Anon 1980). The first figures were reported in the monthly Notifiable Disease Report issued by the Department of Health in July 1980. However, a number of medical laboratories throughout the country had been attempting to isolate *Campylobacter* from faecal samples since the publication of Skirrow (1977).

The first report of the isolation of *C. fetus* subsp. *jejuni* from faeces of patients in New Zealand was received by the National Health Institute (NHI), which is the country's reference laboratory, in December 1977 (Cawley 1982). In 1979 Brougham and Meech (1979) described a case of acute diarrhoeal disease in a 24-year-old male due to *C. jejuni*. Watson et al (1979) reported the isolation of *C. jejuni* from the faeces of five (7.8%) of 64 and one (1.7%) of 58 domiciliary and hospitalised patients respectively, all of whom were suffering from acute diarrhoea. Cawley (1982) compared the number of isolates of *Campylobacter* received by the NHI between January 1979 and March 1981 with those of *Salmonella* and *Shigella*, and found that more isolates of *C. jejuni* were received than *Shigella*, but less than *Salmonella*. By 1984 the official notifications of *Campylobacter* infection were considered greater than the notifications of either Salmonellosis or Shigellosis (Anon 1984).

The objectives of the work outlined in this chapter were:

1. To determine the relative importance of the different species of thermophilic *Campylobacter* associated with clinical *Campylobacter* infection in humans.

2. To obtain preliminary information on sources of human infection by the use of BRENDA analysis. (This work is described in greater detail in Chapter X).

3. To investigate the public health significance of the disease in humans in New Zealand since it became notifiable in 1979.

MATERIALS AND METHODS

Collection and Identification of Campylobacter Isolated from Cases of Human Clinical Infection

Three hundred and sixty two Campylobacter isolates from cases of human clinical enteritis were received at Massey University from various laboratories throughout New Zealand between May 1982 and February 1984. Table 9.1 shows the laboratories from where the Campylobacter isolates were received.

TABLE 9.1 : CAMPYLOBACTER ISOLATES FROM HUMAN ENTERITIS CASES RECEIVED AT MASSEY UNIVERSITY BETWEEN MAY 1982 AND FEBRUARY 1984

Laboratories	1982	1983	1984	Sub Totals
National Health Institute (NHI)	119	139	-	258
Hamilton Medical Laboratory (HML)	38	44	-	82
Palmerston North Medical Laboratory (PNPL)	3	3	-	6
Christchurch Public Health Laboratory (CPHL)	-	3	5	8
Dunedin Public Health Laboratory (DPHL)	-	-	8	8
TOTAL				362

Of the 258 isolates received from the NHI, 119 were from the Wellington Health District (Wellington Public Health Laboratory), while the remaining 139 isolates were from other places in New Zealand.

The way in which isolates were received, subcultured and identified to a species level, have been outlined in Chapter III.

Direct Investigation of an Outbreak of Campylobacter Enteritis in a Family

Recently, (September 1984), five faecal samples were received from a medical practitioner, from a family of five members; a 32-year-old mother (sample No. 1), her three female young children aged eight (sample No. 2), four (sample No. 3) and three (sample No. 4) years old and the 34-year-old father (sample No. 5). The mother had consulted her medical practitioner four days earlier and was tentatively diagnosed as a case of Campylobacter enteritis.

The samples which were kept at approximately 10°C, were received on the same day as they were collected. Faecal samples Nos 2 to 5 were well formed with no gross signs of blood or mucous. Sample No. 1 was slightly less well formed and contained small amounts of blood. Faecal swabs were cultured immediately while enumeration studies on the remaining 1 to 2 g of each sample, which kept at approximately 3°C, were carried out 36 h later.

Typing of Campylobacter Isolates by Bacterial Restriction Endonuclease DNA Analysis (BRENDA)

Table 9.2 shows the number of Campylobacter isolates examined by the BRENDA technique.

TABLE 9.2 : CAMPYLOBACTER ISOLATES TYPED BY BRENDA ANALYSIS

Species	No. of isolates	No. of Patients	Source
C. jejuni	311	310[a]	Human enteritis cases received from NHI and other laboratories
	15	4	Multiple isolates from the family outbreak
	1	1	Type strain NCTC 11168 from a case of human enteritis
Sub total	327	315	
C. coli	25	23[a]	Human enteritis cases received from NHI
TOTALS	352	338	

[a] Three patients sampled twice (see results and Table 9.3)

Age Distribution of Patients with Campylobacter Enteritis

In order to obtain information on the age distribution of people affected with Campylobacter enteritis, the overall group of 359, from whom isolates were obtained, were classified in nine different age groups: less than 1 year, 1 to 4, 5 to 14, 15 to 24, 25 to 34, 35 to 44, 45 to 54, 55 to 64, and 65 years and over.

Monthly Distribution of Notified Campylobacter and Salmonella Enteritis Cases

Data were analysed from the monthly bulletin issued by the Department of Health between July 1980 and July 1984.

Campylobacter Enteritis Rates/100,000 N.Z. Population in Health Districts from 1.7.82 to 31.6.83

The source of data for this analysis was as described above.

RESULTS

Identification of Campylobacter Isolates from Human Cases of Clinical Enteritis

Table 9.3 shows the different species of thermophilic Campylobacter identified from the 362 isolates examined from 359 patients. Six samples were received from three patients who had been resampled twice, two of whom were infected with C. coli and one with C. jejuni. Resampling of the two patients infected with C C. coli was carried out 5 to 6 weeks after the first sample had been taken, while the patient infected with C. jejuni was resampled three days later. Only one isolate from each of these patients was included in the estimation of the relative proportions of the species infecting the 359 patients.

Of the 359 isolates of Campylobacter, 335 (93.3%) were C. jejuni, 23 (6.4%) C. coli and 1 (0.3%) C. fetus subsp. fetus.

The proportion of the C. coli isolates received from the HML, was three times greater (13.4%) than those from the rest of the country (4.3%).

TABLE 9.3 : SPECIES IDENTIFICATION OF CAMPYLOBACTER ISOLATES FROM HUMANS RECEIVED BY MASSEY BETWEEN MAY 1982 AND FEBRUARY 1984

	C. jejuni	C. coli	C. fetus subsp. fetus	Totals
NHI	244 (1)[a]	11 (1)[a]	1	256 (2)[a]
HML	71	11 (13.4%)	-	82
PNML	6	-	-	6
GPHL	8	-	-	8
DPHL	6	1 (1)[a]	-	7 (1)[a]
Total	335 (93.3%)	23 (6.4%)	1 (0.3%)	359 (362)

[a] = the number of isolates examined twice from the same patient on two occasions

The isolate of C. fetus subsp. fetus was initially reported as C. coli, but it grew at both 42°C and 25°C, was hippurate negative, sensitive to cephalothin and resistant to 30 µg nalidixic acid discs. It was also catalase and oxidase positive and it grew in the presence of 1% glycine. It produced H_2S in TSI Agar detected with lead acetate strips. After further inquiries it was found to have been isolated from the blood of an elderly hospitalised patient diagnosed as a case of subacute bacterial endocarditis.

Investigation of the Outbreak of Campylobacter Enteritis in a Family

Thermophilic Campylobacter were isolated from all the family members except the father (sample No. 5). Sixteen colonies (four from each sample were identified to be C. jejuni. Table 9.4 shows the counts obtained from the enumeration study. The highest cfu of C. jejuni/g of faeces was present in sample No. 1 (the mother) whose faeces contained small amounts of blood. The lowest cfu of C. jejuni/g of faeces was obtained from the youngest child in the family (sample No. 4).

A total of 60 different BRENDA types were identified from the 327 C. jejuni isolates examined. Fifty nine (59) types were identified from the 311 isolates of C. jejuni received from NHI and other Laboratories. All the 15 isolates of C. jejuni from the family outbreak had identical DNA patterns. This pattern was identical to that of one of the 311 isolates from the NHI (see Table 9.5). The C. jejuni type strain had a different DNA pattern from all other isolates. (See Table 9.5).

Nineteen (19) different BRENDA types were identified from the 25 isolates of C. coli.

Tables 9.5 and 9.6 show the number of isolates of C. jejuni and C. coli which were of the same BRENDA type. Plates 9.1 and 9.2 illustrate representative BRENDA types of both C. jejuni and C. coli.

As can be seen from Table 9.5 and Plate 9.1, some BRENDA types have similar or almost identical patterns. Sometimes these minor differences are difficult to see in the printed photographs, but can be seen in the original negatives.

TABLE 9.4 : NUMBER OF C. JEJUNI/G OF FAECES FROM THE FAMILY OUTBREAK

(No patients had signs of disease at time of culture)

Faeces No.	Age of Patient (years)	Estimated Days Post Infection	Log_{10} Numbers
1	32	7	$10^{8.11}$
2	8	21	$10^{6.43}$
3	4	?	$10^{6.25}$
4	3	?	$10^{5.90}$

TABLE 9.5 : DISTRIBUTION OF DIFFERENT BRENDA TYPES IN ISOLATES OF C. JEJUNI FROM HUMANS

BRENDA Type No.	Comments		No. of Isolates
$H1_J$			70
2	almost identical	similar	9
3	almost identical	similar	13
4	almost identical	similar	3
5	almost identical	similar	8
6	almost identical		6
7	almost identical		1
8	almost identical	similar	5
9	almost identical	similar	28
10	almost identical	similar	2
11	almost identical	similar	2
12	NCTC 11368		1
13			8
14			2
15			2
16			19
17	almost identical		3
18	almost identical		3
$H19_J$			2
19	Sub Totals		187

(To be cont.)

Table 9.5 (cont)

BRENDA Type No.	Comments	No. of isolates
$H20_J$		2
21		12
22		8
23		3
24		3
25		1
26		5
27		2
28	almost identical	7
29	almost identical	4
30	almost identical	7
31	almost identical	7
32	almost identical	3
	Similar	
33		3
34		2
35		8
36		1
37	almost identical	5
$H38_J$	almost identical	7
37	Sub Totals	90

(To be cont.)

Table 9.5 (cont)

BRENDA Type No.	Comments	No. of isolates
$H39_J$		3
40		6
41		2
42		2
43		2
44		4
45	almost identical	2
46		1
47		2
48	Includes the 15 isolates from the outbreak in a family of four	16
$H49_J$ - $H60_J$	Each isolate of a different BRENDA type	12
60	TOTAL	327

TABLE 9.6 : DISTRIBUTION OF DIFFERENT BRENDA TYPES IN ISOLATES OF C. COLI FROM HUMANS

BRENDA Type No.		No. of isolates
$H1_C$		2[a]
2		2[b]
3		2[c]
4		2
5		2
6		2
$H7_C$ - $H19_C$		13[d]
19	Total	25

a Brothers living in the same house

b Same patient sampled 5 weeks apart

c Same patient sampled 6 weeks apart

d Each isolate of a different BRENDA type

PLATE 9.1 : EXAMPLES OF DNA 'FINGERPRINTS' PRODUCED BY VARIOUS ISOLATES OF C. JEJUNI (J) RECOVERED FROM HUMANS (H)

Lanes 1, 2 and 3 are isolates of BRENDA type $H2_J$

Lane 4 is an isolate of BRENDA type $H4_J$

Lanes 5 and 6 are isolates of BRENDA type $H5_J$

Lanes 7 and 8 are isolates of BRENDA type $H1_J$

$H4_J$ and $H5_J$ are almost identical BRENDA types.
$H2_J$, $H4_J$ and $H5_J$ are similar BRENDA types.
BRENDA type $H1_J$ is different from the other types.

PLATE 9.2 : EXAMPLES OF DNA 'FINGERPRINTS' PRODUCED BY VARIOUS ISOLATES OF C. COLI (C) RECOVERED FROM HUMANS (H)

Lane 1 : Bacteriophage C1857 S7.

Lane 2 : BRENDA type $H7_C$

Lane 3 : BRENDA type $H8_C$

Lane 4 : BRENDA type $H3_C$

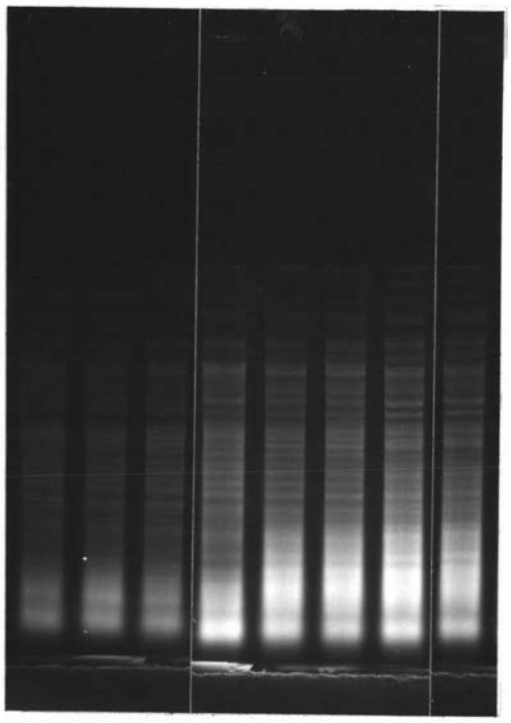
Lane 5 : BRENDA type $H10_C$

Lane 6 : BRENDA type $H11_C$

Lane 7 : BRENDA type $H12_C$

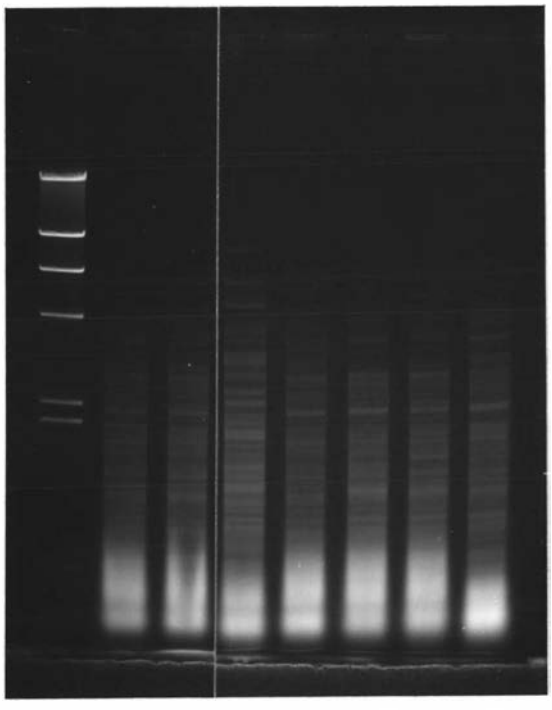
Lane 8 : BRENDA type $H1_C$

PLATE 9.1



1 2 3 4 5 6 7 8

PLATE 9.2



1 2 3 4 5 6 7 8

Patients from the same households were affected by strains with identical BRENDA patterns (see Table 9.7). All the paired isolates (four in the case of a group of Vietnamese patients), except isolates Nos 952 and 968, produced identical DNA patterns to each other. At the time the BRENDA analyses were being carried out, the identity of the individual patients from whom the samples had been taken, were not known. Plate 9.3 shows the DNA patterns of the isolates recovered from patients from the same households (see also Table 9.7).

Development of Resistance to Nalidixic Acid by C. jejuni

Seven cloned colonies of C. jejuni of the 362 examined, (see Table 9.2) were found to develop a resistance to 30 µg nalidixic acid discs on subculture. These colonies on several subsequent subcultures remained resistant to nalidixic acid, but retained their ability to hydrolysis hippurate; a characteristic feature identical to the original non resistant colonies. These resistant colonies retained a DNA pattern identical to the original non resistant colonies (see Plate 9.4).

Age Distribution of Infected Persons

Table 9.8 and Figure 9.1 indicate the age distribution of infection of the 139 people from whom Campylobacter isolates were received and whose ages were known.

All age groups were affected, but the highest rates of infection were in the 1 to 4-year-old group (38.1%), followed by those between 15 to 24 years (18%) and those between 25 to 34 years (14.4%). The lowest rates were in the less than 1-year-old group (2.9%), the 55 to 64-year-old (2.9%) and those more than 65 years old (2.2%).

Monthly Notified Cases of Campylobacter and Salmonella Enteritis in New Zealand

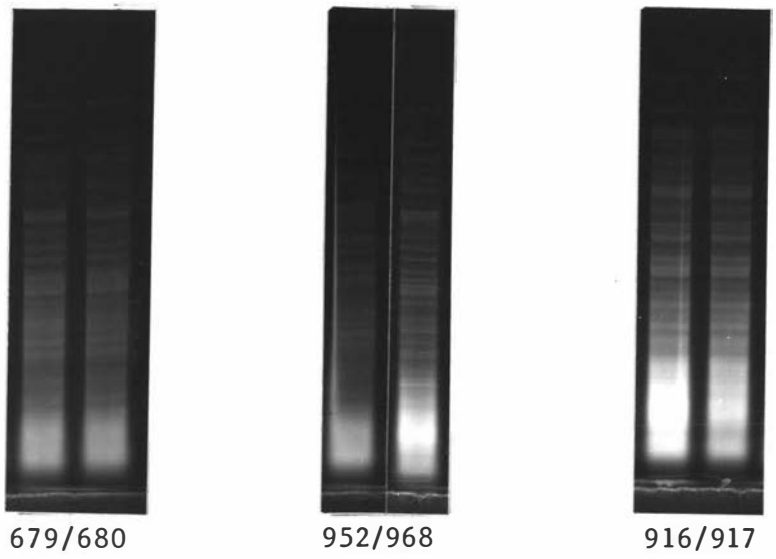
Figure 9.2 shows the monthly distribution from 1.7.80 to 31.6.84.

TABLE 9.7 : GROUPS OF IDENTICAL BRENDA TYPES OF C. JEJUNI AND C. COLI AND ASSOCIATION BETWEEN PATIENTS

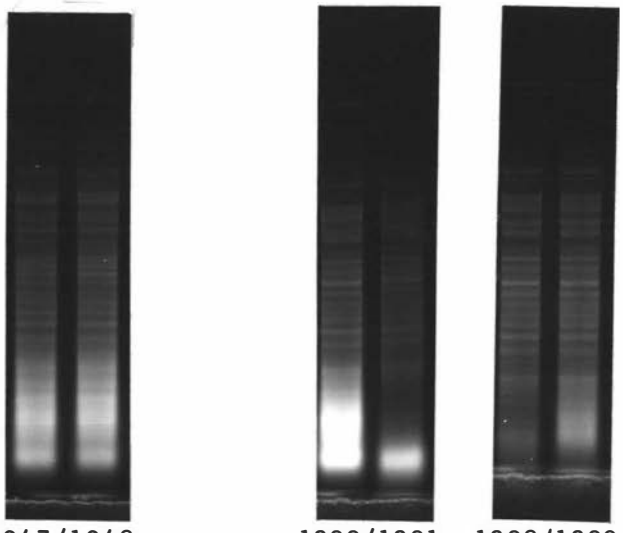
C. jejuni		C. coli	
Isolates in groups	Association between Patients	Isolates in groups	Association between Patients
679) 680)	Brothers in the same house	611) 612)	Brothers in the same house
952) 968)	Same person samples, 3 days apart	771) 789)	Same person samples, 6 weeks apart
916) 917)	Daughter Mother	100) 425)	Same person samples, 5 weeks apart
1247) 1248)	Sisters in the same house		
1300) 1301) 1308) 1309)	Two Vietnamese families in the same house		

PLATE 9.3 : DNA 'FINGERPRINTS' OF ISOLATES C. JEJUNI (J) AND C. COLI (C) RECOVERED FROM PATIENTS FROM THE SAME HOUSEHOLDS

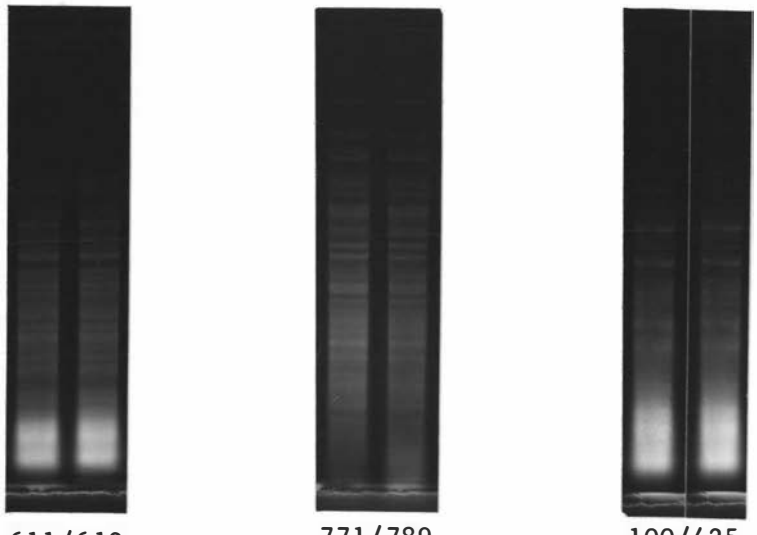
(For explanation see Table 9.7)



679/680 952/968 916/917

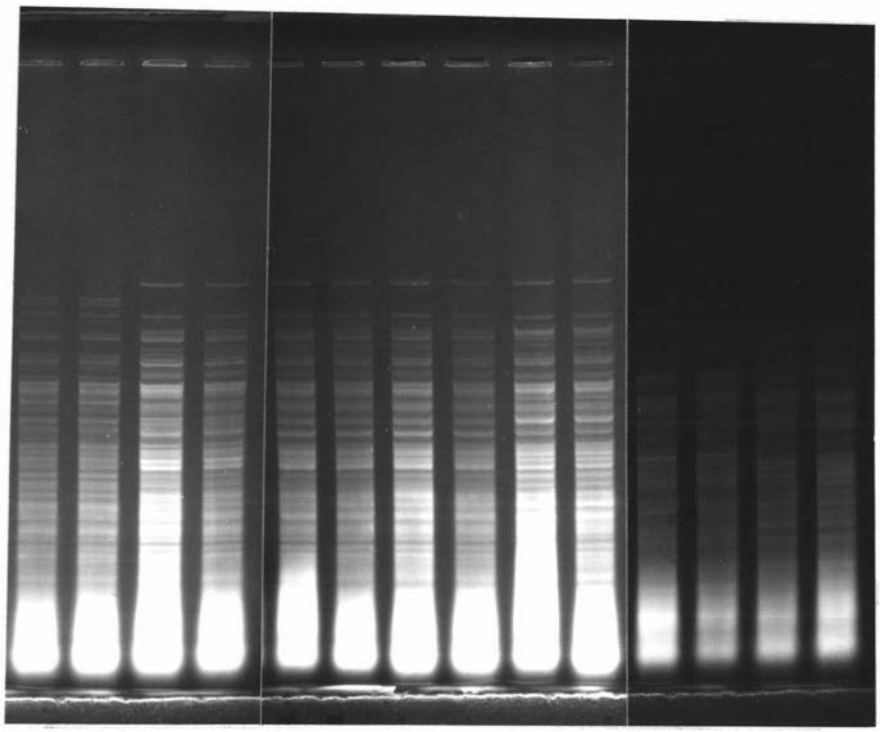


1247/1248 1300/1301 1308/1309



611/612 771/789 100/425

PLATE 9.4 : DNA 'FINGERPRINTS' OF ISOLATES OF C. JEJUNI NON RESISTANT TO NALIDIXIC ACID, AND THEIR RESISTANT SUBCULTURES



Lanes without N represent isolates of C. jejuni non resistant to nalidixic acid and lanes with N, their resistant subcultures.

TABLE 9.8 : AGE DISTRIBUTION OF CAMPYLOBACTER INFECTIONS IN HUMANS (BASED ON THE CAMPYLOBACTER ISOLATES RECEIVED BETWEEN 1.5.82 AND 28.2.83

Age	Male	Female	No. %
1	2	2	4 (2.9)
1-4	39	14	53 (38.1)
5-14	11	5	16 (11.5)
15-24	15	10	25 (18.0)
25-34	8	12	20 (14.4)
35-44	1	7	8 (5.8)
45-54	2	4	6 (4.3)
55-64	2	2	4 (2.9)
65	3	-	3 (2.2)
Total	83 (80%)	55 (40%)	139 (100%)

FIGURE 9.1 : AGE DISTRIBUTION OF CAMPYLOBACTER INFECTIONS IN HUMANS (CALCULATED FROM 139 ISOLATES RECEIVED WITH APPROPRIATE INFORMATION BETWEEN MAY 1982 AND FEBRUARY 1984)

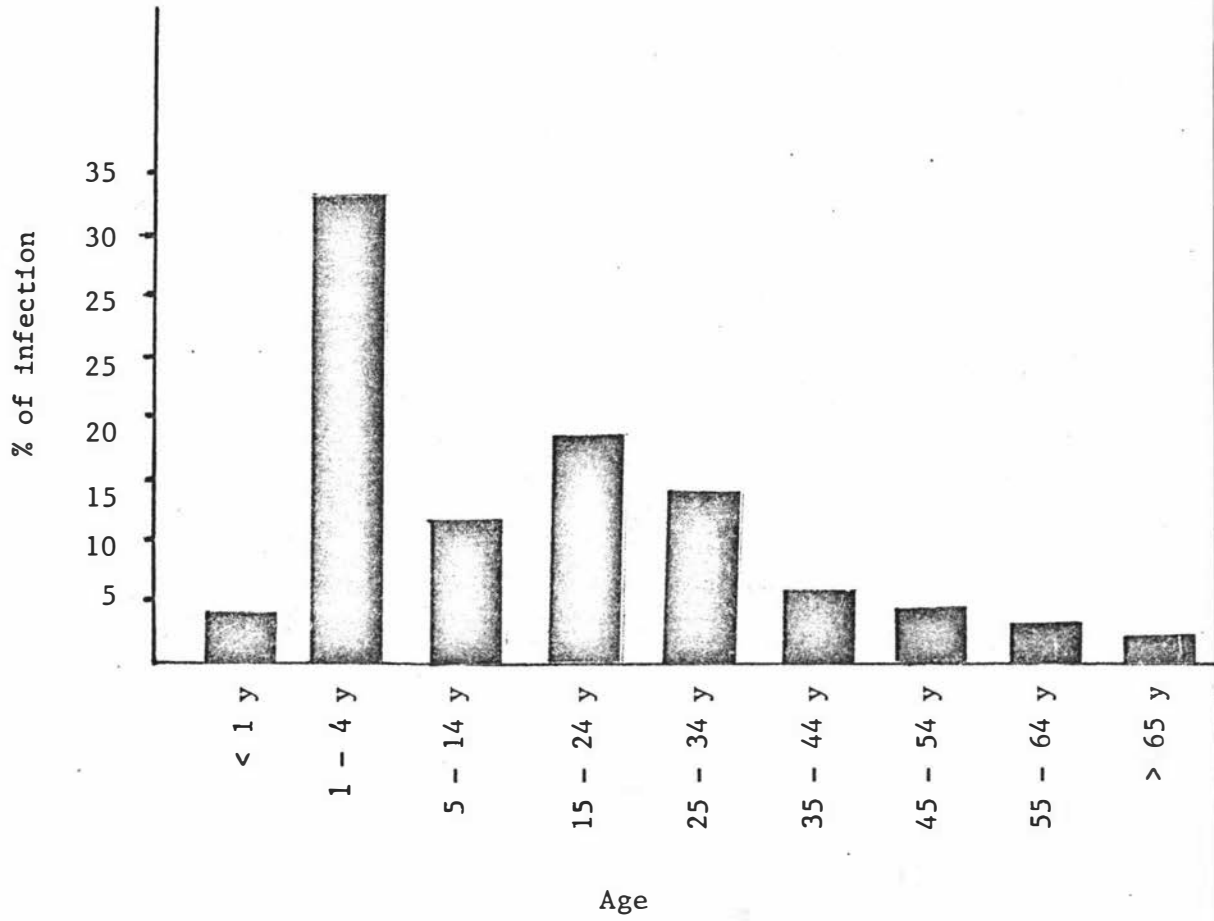
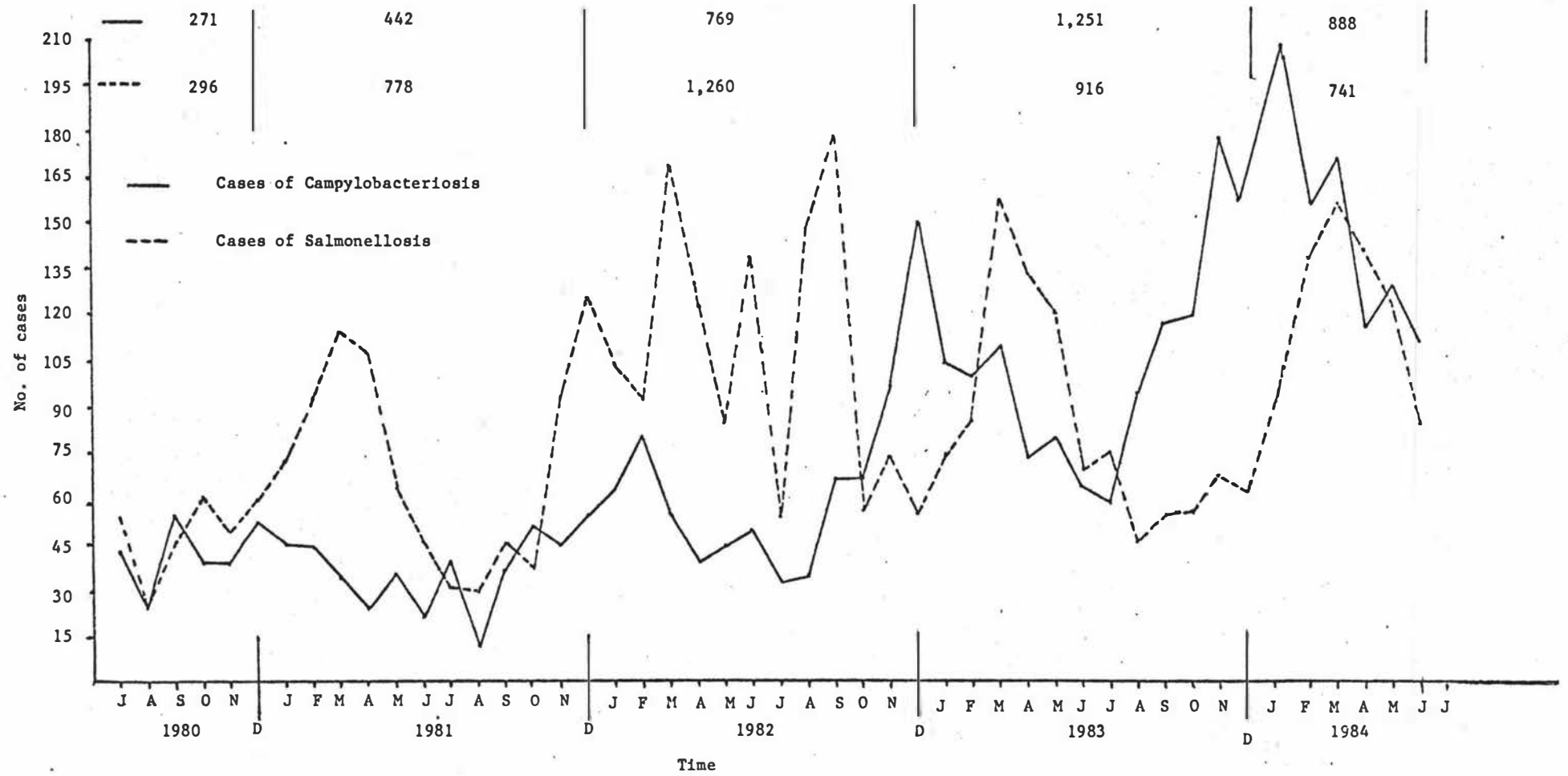


FIGURE 9.2 : MONTHLY DISTRIBUTION OF THE NOTIFIED CASES OF CAMPYLOBACTER AND SALMONELLA INFECTION IN NEW ZEALAND FROM 1.7.80 TO 31.6.84

(Data were analysed from the monthly bulletin issued by the Department of Health)



The isolation rate of Campylobacter appeared to stabilise in the last six months of 1980 (271 cases) and in 1981 (442 cases). Thereafter there was a steady increase, and in 1982 the number of notified cases of Campylobacter were almost double that of the previous year.

In 1983 the number of notifications of Campylobacter infections rose to 1251 cases, and for the first time was greater than the 916 notified cases of salmonellosis. This trend has continued into the first six months of 1984 (888 cases versus 741 cases).

The highest number of Campylobacter infections occurs in the summer months starting to rise in October and reaching its highest level in December to January. The incidence of notifications in the summer months is two to three times greater than in the winter months. The highest rate of notifications of salmonellosis appears to occur three to four months later than that of Campylobacteriosis, and occurs in the autumn.

Campylobacter Rates/100,000 N.Z. Population

Table 9.9 shows annual notifications of campylobacteriosis from the 18 Health Districts in New Zealand, for the last full year (1.7.82 to 31.6.83).

The mean rate was 31/100,000. The highest rate of 80.4/100,000 was reported from the Christchurch Health district and the lowest of 1.6/100,000 came from the Rotorua Health district.

DISCUSSION

The low proportion of C. coli (6.4%) compared with C. jejuni, in cases of human Campylobacteriosis is in agreement with the findings from other countries (see Chapter II). The low isolation rates of C. coli from humans could be related to two factors. First, its limited distribution in animals, with the exception of pigs, compared with C. jejuni. This would be of importance if infected animals are a major source of human infection. Secondly, the differences in infectivity of these two species of Campylobacter demonstrated experimentally in chickens and rats (Chapter VIII) may reflect the infectivity of these organisms for humans.

TABLE 9.9 : CAMPYLOBACTER RATES/100,000 POPULATION FROM 1.7.82 TO 31.6.83

Health District	Population	No. of Cases	Rate/ 100,000
Whangarei	107,600	23	21.4
Takapuna	270,300	67	24.8
Auckland	289,100	47	16.3
South Auckland	246,400	54	21.9
Hamilton	274,000	134	48.9
Rotorua	190,000	67	35.3
Gisborne	62,700	1	1.6
Napier	125,400	41	32.7
New Plymouth	99,700	56	52.2
Wanganui	87,000	6	6.9
Palmerston North	147,000	14	9.5
Hutt	190,000	11	5.8
Wellington	193,000	103	53.4
Nelson/ Greymouth	131,000	28	21.4
Christchurch	346,000	278	80.4
Timaru	109.000	9	8.3
Dunedin	157,000	24	15.3
Invercargill	116,000	8	6.9
Totals	3,144,700	971	30.9

There is no satisfactory explanation for the high proportion of C. coli isolates received from the Hamilton Laboratory (HML) compared with the other laboratories. In Chapter II, it was reported that several workers considered that the consumption of pig meat was associated with human C. coli infections. The high proportion (15%) of C. coli isolated from human cases of campylobacteriosis in Belgium was related to the high consumption of pork in that country (Skirrow and Benjamin 1980b). However, there is no evidence to suggest that pork is an especially popular food in the Hamilton area or that this is an area with a particularly large pig population.

It was interesting that among the Campylobacter isolates received, one was C. fetus subsp. fetus. This species is known to cause systemic campylobacteriosis, particularly in debilitated patients (see Chapter II). This isolate was from a blood culture of a hospitalised patient diagnosed as suffering from a subacute bacterial endocarditis. In Australia, Harvey and Greenwood (1983) associated the isolation of this species from a patient with gastroenteritis and in Switzerland, Heinzer (1983) reported that during a 3 year period isolated C. jejuni from 214 patients with enteritis and C. fetus subsp. fetus from 12 patients. However, Lauwers et al (1978) isolated C. fetus subsp. fetus from only three cases of 22,000 faecal samples examined, and infections were not associated with diarrhoea (Butzler and Skirrow 1979). It would therefore appear that C. fetus subsp. fetus is seldom associated with enteric disease of humans.

It has been suggested that only low numbers of Campylobacter are excreted in the faeces of infected patients after the cessation of diarrhoea (Robinson 1981). Therefore, as the organisms only survive in the environment for a short period, people who no longer suffer from diarrhoea should not constitute a danger for others, and should be able to return to their work, including the handling and preparation of food (Norkrans and Svedhem 1982, Skirrow 1982). However, the numbers of cfu of C. jejuni/g of faeces found in patients without diarrhoea (10^6 to 10^8) in the present investigation, are extremely high and were presumably even higher (around 10^7 to 10^9) as there was a delay of approximately 48 h between the collection and

examination of faecal samples. Any relaxation of standards of personal hygiene by such asymptomatic patients might constitute a risk for others.

The identical DNA patterns of the isolates recovered from the cases in contact with each other, indicates the potential value of the BRENDA technique in investigating the epidemiology of the intestinal thermophilic Campylobacter and in attempts to identify point sources of infection.

Previous workers had suggested that less than 1% of untreated patients still excrete intestinal thermophilic Campylobacter, by the end of the ninth week post infection (see Chapter II). The two patients from whom C. coli of the same BRENDA type was recovered, five and six weeks apart, were presumably suffering from such a transitory chronic infection. These cases are the first which have been documented with such convincing evidence of a persistent infection for up to six weeks. These findings together with the other results presented, provide further evidence that both C. coli and C. jejuni maintain stable BRENDA patterns during the course of an infection.

The dissimilar BRENDA types of C. jejuni recovered from the same person sampled three days apart provides evidence that humans can be affected with more than one strain of the same species at the same time.

Interpretation of the analysis of the ages of people infected, need to be made with caution. It is possible that with such small numbers of isolates studied, many confounding variables could have affected the prevalence observed in the different age groups. However it appears that although enteric Campylobacter infections affect all age groups of humans in New Zealand, a bimodal pattern occurs and the age groups 1 to 4 years and 15 to 34 are most susceptible. Similar results have been reported by Young (1982) in England. In Young's investigation of 11,850 cases, it was found that incidence of Campylobacter infection in patients was highest in 0 to 4-year-olds (19%) and in the 15 to 24-year-olds (22%), and 25 to 34-year-olds (20.2%). The incidence in those more than 44 years

old was 7.2% or less. Blaser (1983) reported that in the U.S.A. the most commonly affected groups were also infants and young adults 15 to 29 years old.

It has been observed in many countries that the peak of Campylobacter isolations occur during the summer months. This has been reported in England and Wales (Skirrow 1982 and Young 1982), Belgium (Butzler and Skirrow 1979), Sweden (Kaijser and Svedhem 1982, Walter and Forsgren 1982), Germany (Kist 1982), and United State (Blaser et al 1982). The reasons for this seasonal prevalence has been discussed in Chapter II. An additional factor predisposing to this increased incidence in the summer, could be related with the breeding season and increased activity of flies during this period. Most of the flies captured in a piggery and a poultry flock where the animals were infected with C. jejuni/ C. coli were also found to be carrying these species of Campylobacter (Rosef and Kapperud 1983). No attempts were made by these authors to demonstrate whether the flies were actively infected or were merely mechanical carriers. In view of the apparently low infective dose of C. jejuni for humans, the mechanical carriage of the organism by flies could be an important mode of transmission.

Marshall and Clements (1984) reported that the rates of isolation of thermophilic Campylobacter during 1982 and 1983, from people living in rural areas in the Christchurch Health District were significantly higher ($P < 0.001$ and $P > 0.005$) than from urban areas. Among the hypotheses proposed by them to account for this phenomenon, was that more flies and more primary sources of infection exist in rural areas compared with urban areas.

The marked differences between the notifications from different Health Districts (80/100,000 - 1.6/100,000) probably indicates that in many Health Districts, the true rates are underestimated. Nevertheless intestinal thermophilic Campylobacter appeared to be the most common agent associated with enteric infections of humans in New Zealand.

CONCLUSIONS

1. Campylobacter infections are the most common of the notified human enteric diseases in New Zealand with an annual incidence rate of 31/100,000.

2. Approximately 94% of human cases of campylobacteriosis are due to C.jejuni and 6% to C. coli.

3. Some strains of C. jejuni isolated from humans developed a resistance to nalidixic acid. This observation is important in relation to previous reports of the isolation of C. laridis (NARTC) from man.

4. All ages can be affected by campylobacteriosis, but children aged one to four years and young adults (15 - 35 years old) appear to be most frequently affected.

5. Patients who no longer have clinical signs of infection, may shed up to 10^8 cfu of C. jejuni/g of faeces.

6. Based on BRENDA analysis of isolates of Campylobacter, it would appear that there are more than 80 different strains of C. jejuni and C. coli which can infect humans.

7. Some patients may be infected concurrently with more than one strain of C. jejuni.

CHAPTER X

BRENDA TYPING OF INTESTINAL THERMOPHILIC CAMPYLOBACTER RECOVERED FROM ANIMALS AND POSSIBLE RELATIONSHIPS WITH ISOLATES FROM HUMANS

INTRODUCTION

Until recently the precise relationship between the intestinal thermophilic Campylobacter of animals and humans has been confused. The major reason for this situation was the lack of a technique sufficiently sensitive to differentiate between different strains of the same species of Campylobacter.

The biotyping scheme of Skirrow and Benjamin (1980b) has received international recognition and provides a method for differentiation between C. jejuni, C. coli and C. laridis. It also allows for the subdivision of C. jejuni into biotypes I and II. Although the system provides the basis for further development of more detailed taxonomic criteria, it is insufficient to differentiate critical strain differences. Another biotyping system (Weaver et al 1982) has divided the thermophilic Campylobacter into eight biotypes. Biotypes 1 to 4 represent C. jejuni biotype I and II of Skirrow and Benjamin (1980b), and biotypes 5 to 8 represent C. coli.

Blaser (1980) in a review of the intestinal thermophilic Campylobacter, discussed the inadequacies of these methods of classification in relation to epidemiological investigations and stated "that the situation was like attempting to solve the epidemiology of Salmonellae infections by not applying the existing serological typing system." The first International Workshop on Campylobacter (1981) stressed the urgent need for the development of typing schemes. Skirrow (1982) also pointed out that "it is unlikely that there will be any major advances in the field of Campylobacter epidemiology until a comprehensive and practical strain identification scheme is developed."

In some countries, including England (Abbott et al 1980, Sweden (Kosumen et al 1980), Belgium (Lauwers et al 1981), Israel (Rogol et al 1982), Japan (Itoh et al 1982) and Canada (Penner and Hennessy 1980 and Lior et al 1982), serological methods were attempted to resolve the problem of strain differentiation of C. jejuni and C. coli.

The two Canadian and one Belgian serotyping schemes have gained the widest acceptance. That of Lior et al (1982) was developed as a slide agglutination test of heat-labile antigens which can differentiate 53 serogroups and serotypes, but is still incapable of typing approximately 7% of isolates examined from both humans and animals (Lior et al 1983). The serotyping system of Penner and Hennessy (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 1983b). Certain difficulties with the Penner system have been encountered and it has been suggested that plasmid typing may be necessary as an additional means of discrimination (Bradbury et al 1983). Both Canadian systems are dependent on the hippurate hydrolysis test (Skirrow and Benjamin 1980b) to differentiate serotypes as being either C. jejuni or C. coli. The Belgian scheme (Lauwers et al 1981) is based on O-serotyping by passive haemagglutination.

Bacterial restriction endonuclease DNA analysis (BRENDA) has already been shown to be a most useful technique for the identification 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 prowazekii (Regnery et al 1983), Neisseria meningitidis (Bjorvatn et al 1984) and Moraxella bovis (Marshall et al 1985).

Recently, Penner et al (1983c) successfully used chromosomal restriction endonuclease analysis to investigate a laboratory acquired case of C. jejuni enteritis, in order to confirm their initial conclusions based on serological methods. Bradbury et al (1984) used restriction endonuclease DNA analysis as an adjunct to serological typing (Penner's typing scheme), in an investigation of

the source of a suspected milk borne outbreak of C. jejuni infection, carried out by the Public Health Laboratory Service (England). The serological technique indicated that isolates recovered from both milk and patients were dissimilar in spite of some antigenic relationship between the isolate recovered from milk, the isolates from the patients, isolates from cattle on the farm and serotype 50 of Penner. Subsequent restriction endonuclease DNA analysis showed that all the human isolates from the outbreak, a few of the cattle isolates and serotype No. 50 had identical DNA patterns, but were dissimilar to the isolate of C. jejuni from the milk.

The main objective of the work in this chapter was to explore the use of BRENDA analyses for typing intestinal thermophilic Campylobacter and, if possible, to use the technique to investigate the epidemiology of thermophilic Campylobacter infections in animals and humans. Secondary objectives include the study of the phenomenon on the development of resistance to nalidixic acid by certain isolates of C. jejuni and C. coli and a study of the relationship between C. coli, C. laridis and C. jejuni cultured in the presence of TMAO and NaCl. These later studies were included because it was felt that the taxonomic relationship between C. coli and C. laridis was unclear.

MATERIALS AND METHODS

BRENDA Analysis of Animal Isolates

A total of three hundred and ninety two (392) isolates of thermophilic Campylobacter (159 C. jejuni, 190 C. coli and 43 C. laridis) were subjected to BRENDA analysis. Table 10.1 shows the source and the numbers of all the isolates examined.

The methodology of the BRENDA technique was described in Chapter III.

BRENDA Analysis of Isolates of C. jejuni and C. coli Resistant and Non Resistant to Nalidixic Acid

Fourteen(14 C. jejuni and three C. coli) isolates, which on subculture developed colonies resistant to 30 µg nalidixic acid (see

TABLE 10.1 : BRENDA ANALYSIS: SOURCE AND NUMBER OF ISOLATES FROM ANIMALS EXAMINED

Source of Isolation			Farm No	Flock No	Rate of infection or contamination of samples	No. of isolates analysed by BRENDA		
						C. jejuni	C. coli	C. laridis
POULTRY	Farm Survey	Broilers	5	5	20/20	10	-	-
			8	8	20/20	10	-	-
			9	9	17/20		17	-
			12	12a	10[a]/10	5	2	-
			"	12b	10[b]/10	5	1	-
			19	19a	10/10	5	-	-
			"	19b	10/10	5	-	-
		Young Breeding Stock	23	23a	16/20	5	-	-
			"	23b	14/20	5	-	-
		Layers	25b	25b	7/10	5	-	-
			26	26	9/10	27[c]	-	-
	Super-market Survey	Processed Chicken Wings		Batch 1 (10 packets)	10/10	10[d]	-	-
				Batch 2 (10 packets)	10/10	-	10[d]	-
	Received from NHI	Campylobacter isolates from live birds				6	-	-
				Sub Total		98	30	

To be cont.

[a] = 8 samples were C. jejuni and 2 C. coli
[b] = 9 samples were C. jejuni and 1 C. coli

[c] = 3 colonies examined per sample
[d] = 2 colonies examined per packet

Table 10.1 (cont)

Source of isolation			Rate of infection or contamination of samples	No. of isolates analysed by BRENDA C. jejuni	C. coli	C. laridis
PIGS	Piggery No.1	Piglets	23/64	-	23	-
		Weaners and Growers	27/30	-	24	-
		Sows	14/15	-	14	-
		Sub Total			61	
	Piggery No. 2 - 20	Growers and sows	122/138	-	86[a]	-
		TOTAL			147	

[a] = includes 30 isolates from nine pigs

Source of isolation		Rate of infection or contamination of samples	No. of isolates analysed by BRENDA C. jejuni	C. coli	C. laridis
RATS	Abattoir rubbish tip	18/30	18	-	-
GULLS	P. North rubbish tip, Foxton Beach	82(95)[b]/140	34(39)[b]	11(11)[b]	43(45)[b]
DUCKS	P.N. pond	4/13	2	2	-
	Massey pond	3/8	3	-	-
	Wanganui pond	2/10	2	-	-
CALF	Received (NHI)		1	-	-
HORSE	Received (NHI)		1	-	-
	TOTAL		43	13	43
	OVERALL TOTAL		159	190	43

( )[b] = Number of isolates recovered (13 birds had dual infections)

Chapters V, VII and IX), were subjected to BRENDA analysis using both resistant and non resistant cultures. One of the isolates of C. coli was from a gull with a concurrent infection with C. laridis, which was also subjected to BRENDA typing. Table 10.2 shows the origin of these isolates of C. jejuni and C. coli studied by BRENDA analysis, which were either resistant or non-resistant to nalidixic acid.

Comparative Study to Assess the Growth of C. jejuni, C. coli and C. laridis Isolated from Various Sources, in the Presence of TMAO and Sodium Chloride

The growth of twenty seven isolates of intestinal thermophilic Campylobacter including C. coli, C. laridis and C. jejuni were studied:

1. in the presence of trimethylamine N-oxide hydrochloride (TMAO) supplement in a semisolid medium (Benjamin et al 1983);

2. on agar plates supplemented with 1.5% sodium chloride (Benjamin et al 1983).

Of the 27 isolates studied, 22 were of gull origin. These included two primary isolates of C. jejuni and two of C. coli, together with their subcultures which developed a resistance to nalidixic acid, and the isolate of C. laridis referred to above. Two isolates originated from a pig (an isolate of C. coli and its nalidixic acid resistant subculture). The remaining three isolates were type strains (C. coli NCTC 11366 isolated from pig faeces, C. jejuni NCTC 11351 of bovine origin and 11368 isolated from human faeces). Table 10.3 outlines the origin of all the isolates studied.

The media used and the tests employed, have been described in Chapter III (see non-obligatory test).

Use of a Second Endonuclease Enzyme for BRENDA Analysis

Twelve intestinal thermophilic Campylobacter subjected to BRENDA analysis using Hind III and found to belong in five different

TABLE 10.2 : BRENDA ANALYSIS : SOURCE AND NUMBER OF DEVELOPING RESISTANT TO NALIDIXIC ACID ON SUBCULTURE

Source of isolation	C. Jejuni		C. coli		C. laridis
	Non resistant	Resistant	Non resistant	Resistant	Resistant
Pig	-	-	1	1	-
Poultry	2	2	-	-	-
Gulls	3	3	2*	2*	1
Rats	2	2	-	-	-
Humans	7	7	-	-	-
TOTAL	14	14	3	3	1

* One strain recovered concurrently with strain of C. laridis

TABLE 10.3 : GROWTH OF ORGANISMS IN PRESENCE OF TMAO AND NACL : SOURCE AND NUMBER OF ISOLATES EXAMINED

Source	No. of *C. laridis*[b]	Source	No. of *C. coli*[b]	Source	No. of *C. jejuni*[b]	Total
	1		1		1	
	2		2		2	
	3		3		3	
Gull	4[a]	Gull	4[a´]		4	
	5		4_1	Gull	5	
	6		5		5_1	
	7		5_1		6	
			6		6_1	
		Pig		Bovine NCTC 11351		
			6_1	Human NCTC 11168		
		Pig NCTC 11366				
Sub Total	7		10		10	27

4_1, 5_1, 6_1 = Resistant to nalidixic acid; derived from subcultures of the non resistant 4, 5, 6 isolates respectively.

[a] = Concurrently recovered with *C. coli* a´ from a faecal sample from a gull.

[b] = All were of faecal origin.

BRENDA types (each type included at least two isolates), were subjected to further BRENDA analyses using a second endonuclease enzyme (XhoI).

BRENDA Typing and Serotyping of a Limited Number of Intestinal Thermophilic Campylobacter of Animal and Human Origin

Thirty eight isolates of both C. jejuni and C. coli typed by BRENDA were also serotyped by Professor Penner[a] who was unaware of the BRENDA results at the time he carried out the tests. Table 10.4 shows the source and number of the two species of Campylobacter examined.

RESULTS

BRENDA Analysis of Animal Isolates

Table 10.5 presents the results of this investigation.

Chicken isolates: A total of seventeen (17) different BRENDA types of C. jejuni and three different BRENDA types of C. coli were recorded from the 98 isolates of C. jejuni and 30 of C. coli examined. These isolates originated from both the farm and supermarket surveys and the isolates from NHI. The farm isolates of C. jejuni consisted of 11 BRENDA types while those examined from the supermarket survey of only two, one of which was identical to a BRENDA type from all the birds from one flock. Two of the five packets of processed chicken wings examined were found to be contaminated with two different BRENDA types, and the remaining three packets with only one, (two C. jejuni colonies were examined per packet). The remaining five BRENDA types of C. jejuni were from six of the isolates received from the NHI. (The sixth isolate was identical to one type isolated during the course of the farm survey.)

Of the three BRENDA types of C. coli, two were from isolates of farm birds and one from processed chicken wings.

As can be seen from Table 10.5, some flocks were either exclusively infected with the same BRENDA type, or by up to three different types. These circumstances also occurred in different flocks on the same farm. However, not more than one BRENDA

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a Soluble heat-stable antigens extracted from each isolate (Penner and Hennessy 1980) were sent to Canada for serotyping.

TABLE 10.4 : ISOLATES SEROTYPED BY PROFESSOR PENNER : SOURCE AND TYPE

Species	Source	Number
C. jejuni	Chickens (alive)	6
"	Processed chicken wings	2
"	Rats	6
"	Gulls	2
"	Humans	9
C. coli	Chickens (alive)	3
"	Processed chicken wings)	2
"	Pigs	6
C. laridis	Gulls	2
TOTAL		38

TABLE 10.5 : BRENDA TYPES IDENTIFIED FROM ANIMAL ISOLATES

Source	Flock No	BRENDA types of C. jejuni (J)	BRENDA types of C. coli (C)	BRENDA types of C. laridis (L)
POULTRY	5	$P4_J(10)$		
	8	$P3_J(10)$		
	9		$P1_C(17)$	
	12a	$P3_J(3)$, $P5_J(2)$	$P2_C(2)$	
	12b	$P3_J(3)$, $P9_J(1)$, $P11_J(1)$	$P2_C(1)$	
	19a	$P13_J(5)$		
	19b	$P13_J(5)$		
	23a	$P1_J(4)$, $P6_J(1)$		
	23b	$P1_J(3)$, $P6_J(2)$		
	25b	$P2_J(3)$, $P3_J(1)$, $P7_J(1)$		
	26	$P8_J(9)$, $P13_J(12)$, $P14_J(6)$		
	Processed chickens batch 1	$P4_J(6)$, $P8_J(4)$		
	batch 2		$P3_C(10)$	
	Campylobacter isolates from live birds (NHI)	$P1_J(1)$, $P12_J(1)$, $P10_J(1)$ $P15_J(1)$, $P16_J(1)$, $P17_J(1)$		
	TOTAL	17 types	3 types	

P = poultry
( ) = Number of isolates

(To be cont)

Table 10.5 (cont)

Source		Group of Pigs	BRENDA types of C. coli (C)
PIGS (p)	Piggery No. 1	Piglets and sows	$p14^*_C$ (37)
		Weaners and growers	
		Weaners and growers	$p14^*_C$ (24)
	Piggery No. 2 - 20	Growers and sows	$p48^*_C$ (86)
	Total		p76*

* Identical BRENDA types either in each group of pigs examined or between them are not included in the types reported

Source		BRENDA types of C. Jejuni (J)			BRENDA types of C. coli (C)	BRENDA types of C. laridis (L)
RATS (R)	Abattoir rubbish tip	$R1_J$(15), $R3_J$(1),	$R2_J$(1) $R4_J$(1)	4 types		
GULLS (G)	P.North rubbish tip Foxton Beach	$G1_J$-$G22_J$		22 types[a]	$G1_C$-$G6_C$ 6 types[a]	$G1_L$-$G27_L$ 27 types[a]
DUCKS (D)	P.North pond Massey pond Wanganui pond	$D1_J$(1), $D2_J$(2), $D4_J$(2)	$D2_J$(1) $D3_J$(1)	4 types	$D1_C$(1), $D2_C$(1) (2 types)	
CALF (Ca)		$Ca1_J$(1)		1 type		
HORSE (Ho)		$Ho1_J$(1)		1 type		

a = Table 10.9 for full explanation

$P6_J = R3_J = H17_J$ $R2_J = Ho1_J = H16_J$

type of C. coli was recovered from any one flock or from the batches of processed chicken wings.

Of the eight flocks (Nos 5, 8, 19a, 19b, 23a, 23b, 25b and 26) infected only with C. jejuni, isolates from four (Nos 5, 8, 19a and 19b) were of a single BRENDA type. Flocks No. 5 and 8 were infected with different types while both 19a and 19b with an identical BRENDA type which was also found in another flock. The isolates from the remaining four flocks (Nos 23a, 23b, 25b and 26) and the processed chicken wings, were of up to three different BRENDA types. Isolates from flock No. 9 infected only with C. coli and batch No. 2 of processed chickens consisted of only one BRENDA type each.

Of the 17 BRENDA types of C. jejuni recovered from live and processed poultry, 11 were identical to BRENDA types of human isolates and another four were very similar to human isolates. The remaining two BRENDA types were quite different from any of the human types. One of the BRENDA types which was identical with a human BRENDA type was also identical to an isolate from a rat (see further results).

One of the three BRENDA types of C. coli recovered from poultry was found to be identical to an isolate of C. coli from a human patient (see Chapter IX).

Table 10.6 shows all the BRENDA types of C. jejuni and C. coli isolated from chickens and indicates those isolates which have identical and similar BRENDA patterns to isolates of C. jejuni and C. coli from humans. Examples are illustrated by Plate 10.1.

Pig isolates: Table 10.5 shows the results of the BRENDA analysis of 147 isolates of C. coli from 126 pigs. A total of 76 different BRENDA types of C. coli were identified, 28 of which were recovered from the G.I. tract of animals from piggery No. 1. The remaining 48 types were from the 86 isolates from animals from the other 19 piggeries.

Fourteen different BRENDA types were recovered from both the

TABLE 10.6 : ISOLATES OF C. JEJUNI AND C. COLI FROM POULTRY WITH IDENTICAL OR SIMILAR BRENDA PATTERNS TO ISOLATES FROM HUMANS

CAMPYLOBACTER JEJUNI (J)				
BRENDA Type: Poultry (n=98)	Number of isolates	BRENDA Type: Human (n=316) Type	No. identical	No similar
$P1_J$	8	$H14_J$	2	-
$P2_J$	3	$H28_J$	7	-
"		$H29_J$	-	4
"		$H30_J$	-	7
"		$H31_J$	-	7
$P3_J$	17	$H1_J$	70	-
$P4_J$	16	$H2_J$	9	-
"		$H3_J$	-	13
$P13_J$	22	$H4_J$	3	-
"		$H5_J$	-	8
$P5_J$	2	$H35_J$	8	-
$P6^*_J$	3	$H17_J$	3	-
"		$H18_J$	-	3
$P7_J$	1	$H58_J$	1	-
$P8_J$	13	Not found in humans		
$P9_J$	1	$H45_J$	2	-
"		$H46_{1J}$	-	1
$P10_J$	1	$H40_J$	6	-
$P11_J$	1	$H25_J$	-	1

(To be cont)

* Also identical with BRENDA type $R3_J$

Table 10.6 (cont)

CAMPYLOBACTER JEJUNI (J)

BRENDA Type: Poultry (n=98)	Number of isolates	BRENDA Type: Humans (n=316) Type	No. identical	No. similar
$P12_J$	1	$H20_J$	-	2
$P14_J$	6	$H15_J$	-	2
$P15_J$	1	$H41_J$	-	2
$P16_J$	1	Not found in humans		
$P17_J$	1	$H43_J$	2	-
TOTAL 11 types	84	11 types	113(35.8%)	-
" 4 types		11 types	-	50(15.8%)

CAMPYLOBACTER COLI (C)

BRENDA Type: Poultry (n=30)	Number of isolates	BRENDA Type: Humans (n=25) Type	No. identical	No. similar
$P1_C$	17	$H3_C$	2[a] (8.6%)	-

[a] = same person sampled six weeks apart (see Chapter IX)

PLATE 10.1 : IDENTICAL DNA 'FINGERPRINTS' OF ISOLATES OF C. JEJUNI AND C. COLI FROM POULTRY AND HUMANS

P = Poultry BRENDA type

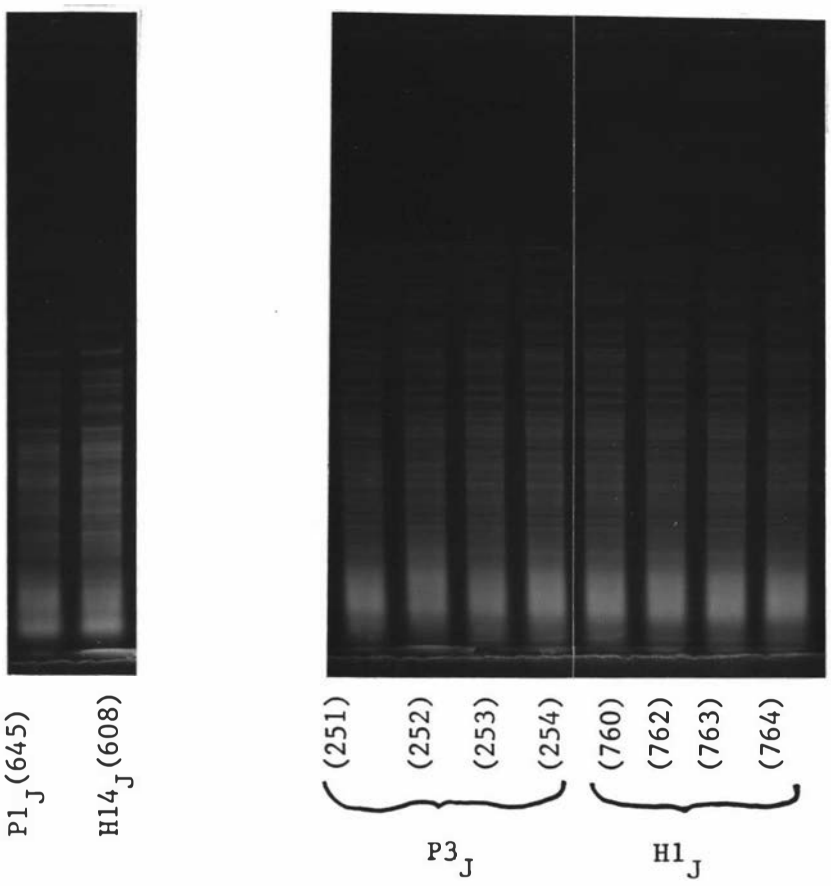
H = Human BRENDA type

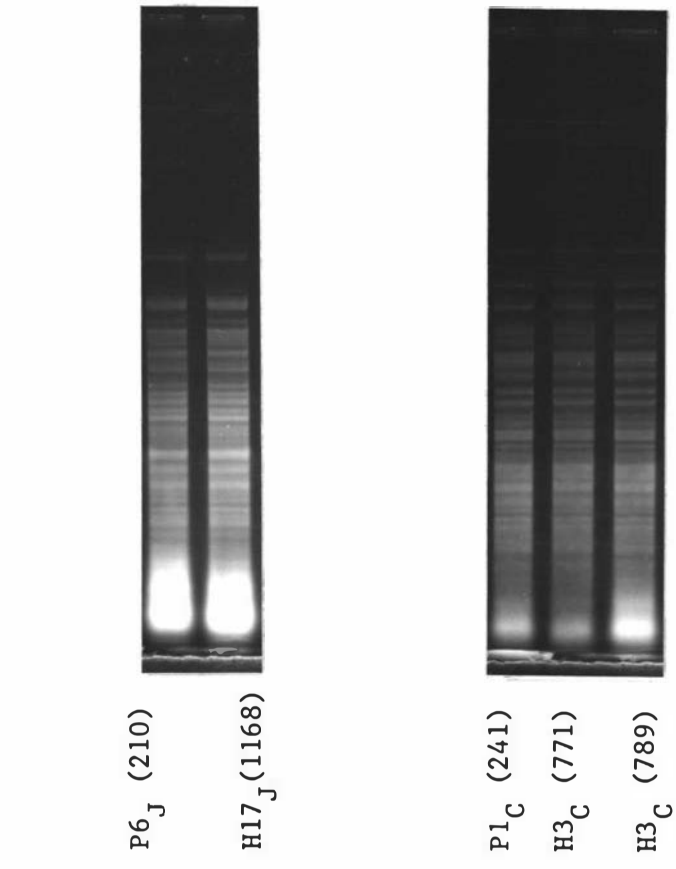
J = C. jejuni

C = C. coli

Isolate ref. no. in parenthesis.

PLATE 10.1


P1J(645)
H14J(608)
(251)
(252)
(253)
(254)
(760)
(762)
(763)
(764)
P3J
H1J


P6J (210)
H17J(1168)
P1C (241)
H3C (771)
H3C (789)

14 sows and their 23 piglets which were examined from piggery No. 1. In six cases the DNA pattern of the isolates from the sows and their own piglets were identical (Plate 10.2 illustrates such examples). The remaining 14 of the 28 BRENDA types, were found in the older growing pigs in this piggery. Some pigs were infected with up to three different BRENDA types of C. coli. Only one of these isolates from pigs had an identical BRENDA pattern to an isolate from a human (see Plate 10.3).

Rat isolates: Four BRENDA types were recovered from the 18 isolates of C. jejuni from rats. Fifteen of these isolates were of the same BRENDA type and the remaining three isolates belonged to three distinct BRENDA types (see Table 10.5). Two of these later BRENDA types were identical with two human types and each was also ~~similar~~ identical to isolates from chickens and a horse. Table 10.7 and Plate 10.4 show the four BRENDA types recovered from rats and in comparison with isolates from humans, chickens and the horse.

Duck isolates: Four different BRENDA types were detected in the seven isolates of C. jejuni from ducks and two isolates of C. coli were of different BRENDA types. Both isolates of C. jejuni and both of C. coli, recovered from the faeces of ducks from the banks of the pond at Palmerston North, were of different BRENDA types. The three isolates from the banks of the Massey University pond consisted of two BRENDA types. One of these types (two isolates) was identical with an isolate recovered from the banks of the pond at Palmerston North. Both the isolates from the Wanganui pond were identical. None of the BRENDA types recovered from ducks were similar to those found in any other animals, bird or human. These findings are shown in Table 10.5 and illustrated in Plate 10.5.

Gull isolates: Twenty two BRENDA types of C. jejuni were identified from 34 isolates from gulls. The 11 C. coli isolates consisted of six BRENDA types while the 43 C. laridis isolates were of 27 BRENDA types. However, two of the BRENDA types of C. coli from six isolates, were found to be identical to two BRENDA types of C. laridis from three isolates (see Plate 10.6). One of these three isolates of C. laridis was recovered concurrently with C. coli from the same faecal sample and the subculture of this C. coli which developed a resistance to nalidixic acid. (See results on the development of resistance by C. jejuni and C. coli.)

PLATE 10.2 : DNA 'FINGERPRINTS' OF ISOLATES OF C. COLI RECOVERED FROM SOWS AND THEIR SUCKLING PIGLETS

Lane 1: Bacteriophage C1857 S7.

Lane 2: Isolate from a sow and lanes 3*, 4 and 5, her piglets.

Lane 6: Isolate from another sow and,

Lanes 7, 8 and 9: her piglets.

* Not identical to her mother (2).

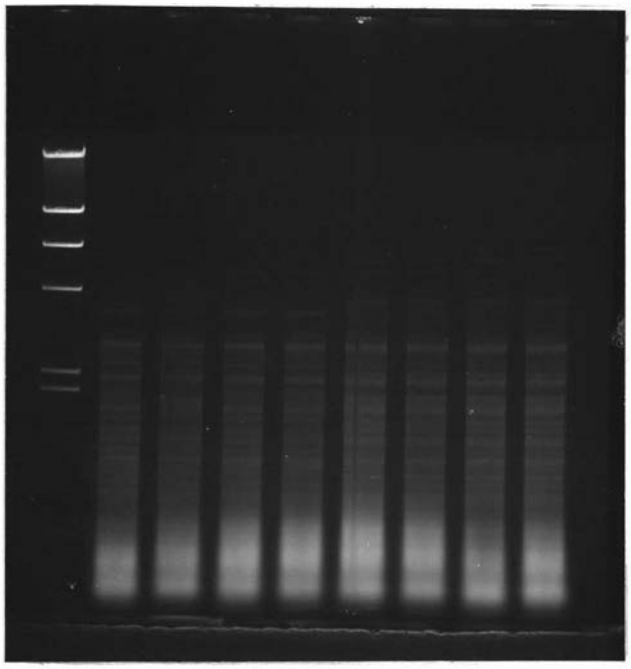
PLATE 10.3 : IDENTICAL DNA 'FINGERPRINTS' OF A C. COLI (C) ISOLATE FROM A PIG (p) AND A HUMAN (H)

$p4_C$: BRENDA type recovered from a pig

$H9_C$: BRENDA type recovered from a human

( ) Ref. No. of isolates

PLATE 10.2



1 2 3 4 5 6 7 8 9

PLATE 10.3

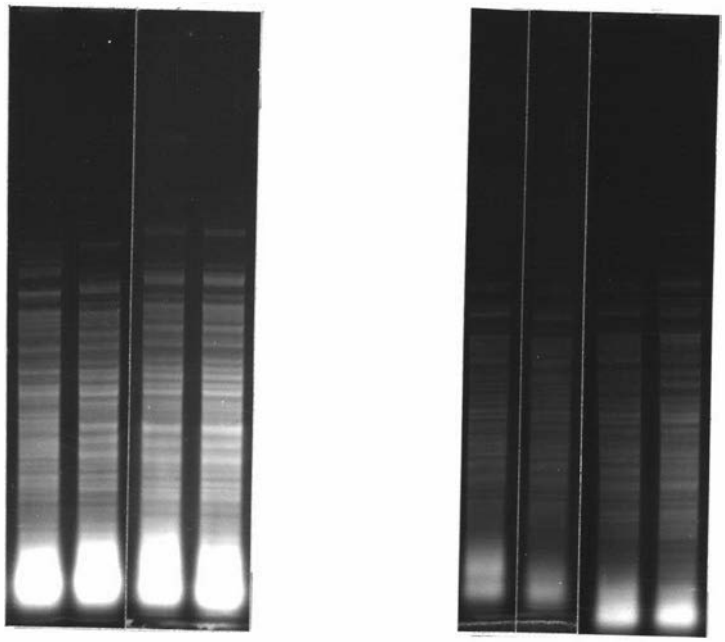
$P4_C$(315) $H9_C$(692)

TABLE 10.7 : ISOLATES OF C. JEJUNI FROM RATS WITH IDENTICAL OR SIMILAR BRENDA PATTERNS TO ISOLATES FROM OTHER ANIMALS AND HUMANS

Rat BRENDA type	No. of isolates from rats (n=18)	Relation with isolates from other animals and humans	No. of isolates identical	No. of isolates similar
$R1_J$	15	-	-	-
$R2_J$	1	Horse	1 (1)	-
"		Human	19 (316)	-
R3	1	Chicken	3 (98)	-
"		Human	3 (316)	-
"		Human	-	3 (316)
R4	1	-	-	-

( ) = number of isolates examined

PLATE 10.4 : COMPARISON OF DNA 'FINGERPRINTS' OF FOUR C. JEJUNI (J) BRENDA TYPES RECOVERED FROM RATS (R) WITH BRENDA TYPES FROM HUMANS (H), POULTRY (P) AND A HORSE (Ho)

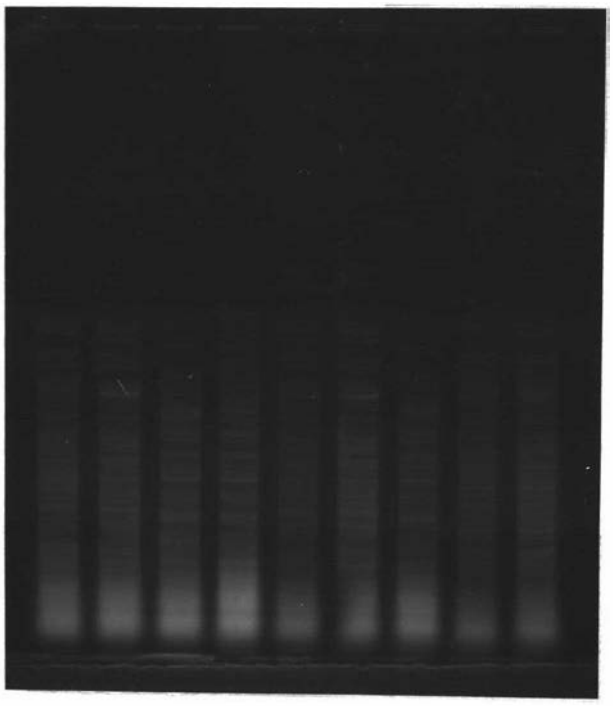


$R1_J$ $R3_J$ $P6_J$ $H17_J$ $H16_J$ $Ho1_J$ $R2_J$ $R4_J$

$R3_J$, $P6_J$, $H17_J$ } Identical

$R2_J$, $Ho1_J$, $H16_J$ } Identical

PLATE 10.5 : DNA 'FINGERPRINTS' OF ISOLATES OF C. JEJUNI (J) AND C. COLI (C) RECOVERED FROM DUCKS (D)



1 2 3 4 5 6 7 8 9

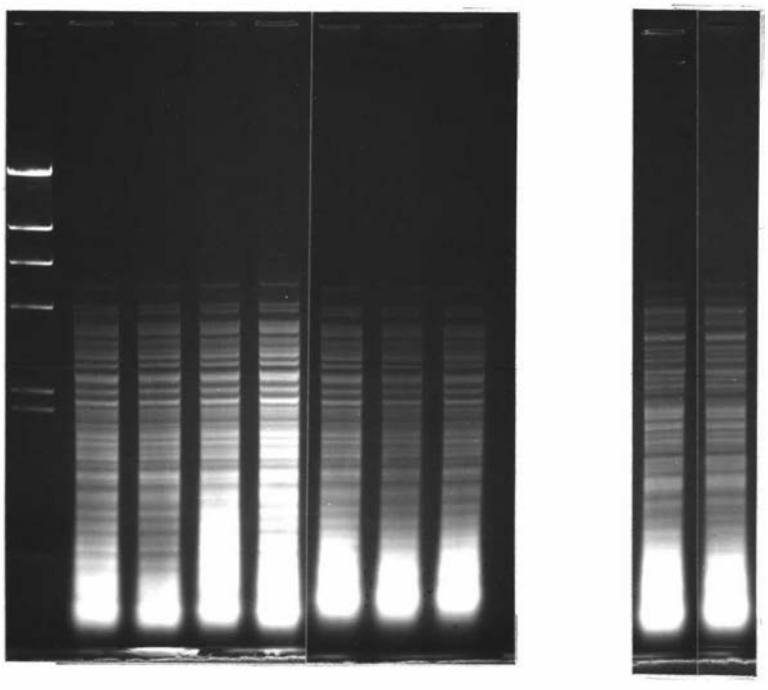
Lanes 1, 2 and 3, 4 are isolates of *C. jejuni* and *C. coli* ($D1_J$, $D2_J$ and $D1_C$, $D2_C$) from the pond at Palmerston North.

Lanes 5, 6, 7 are isolates of *C. jejuni* ($D2_J$, $D2_J$, $D3_J$) from the pond at Massey.

Lanes 8, 9 are isolates of *C. jejuni* ($D4_J$, $D4_J$) from the pond at Wanganui.

PLATE 10.6 : IDENTICAL DNA 'FINGERPRINTS' OF 'C. COLI' (C) AND C. LARIDIS (L) RECOVERED FROM GULLS (G)

(see also Table 10.8)



1 2 3 4 5 6 7 8 9

Lanes 1 - 6 and 8 are isolates of 'C. coli' BRENDA types $G5_C$ and $G4_C$.

Lanes 7 and 9 are isolates of C. laridis BRENDA types $G16_L$ and G12.

Lanes 5 and 6 are the 'C. coli' and its nalidixic acid resistant variant, and lane 7, the C. laridis recovered concurrently with the 'C. coli' (5).

Isolates 1 - 7 and 8 - 9 have identical DNA 'fingerprints'.

Table 10.8 shows the different BRENDA types of C. jejuni, C. coli and C. laridis identified from the isolates recovered from gulls. Plate 10.7 illustrates some of these BRENDA types. Seven BRENDA types (five C. jejuni, one C. laridis and one 'C. coli' were common to faecal samples from gulls from both the rubbish tip of Palmerston North and Foxton Beach. None of the BRENDA types found in gulls were similar to any human or other animal isolates.

Calf and horse isolate: The isolates of C. jejuni from a calf and a horse were different BRENDA types (see Table 10.5). Both of these types were identical to types found in humans. Plate 10.8 shows the pattern of the isolates from the calf and a human. The identical pattern of the horse and a human isolate has already been described (see Plate 10.4). The horse isolate was also identical with one of the two BRENDA types recovered from rats which were identical to human types (see the results from rats and Plate 10.4).

Isolates of C. jejuni and C. coli from Humans Identical or Similar to BRENDA Types recovered from Animals

Table 10.9 summarises those BRENDA types from animals and humans which appeared to be related to each other (the data are a composite of that from Tables 9.4 and 9.5, 10.5 and 10.6, and Plates 10.1, 10.3 and 10.10.)

Fifteen of the BRENDA types of C. jejuni from poultry were either identical (11) or very similar (4) to those isolated from humans. Thus 61% of the 316 isolates of C. jejuni from humans are similar to those which occur in animals.

Ten types of Campylobacter jejuni isolated previously only from chickens accounted for almost half (49.7%) of the 316 human isolates examined (34.8% were identical and 14.9% were similar to the chicken BRENDA types). The identical BRENDA type found in both chickens and a rat was similar to the six (1.9%) human isolates (three were identical and three very similar). The identical BRENDA type isolated from a rat and a horse was similar to 19 (6.0%) of the human isolates, while the calf BRENDA type was similar to 12 (3.8%) human isolates (2.5% identical and 1.3% similar). Two C. coli BRENDA types recovered from animals (one from 17 chickens and the other from a pig) were identical to two of the 23 isolates of C. coli from humans (see Table 10.9).

TABLE 10.8 : BRENDA TYPES OF THERMOPHILIC CAMPYLOBACTER FROM GULLS

C. jejuni BRENDA Types	No. of isolates	*C. laridis* BRENDA Types	No. of isolates	'*C. coli*' BRENDA Types	No. of isolates
Gl_J	1 Rt	Gl_L	1 Rt	Gl_C	2 Rt
2	1 Fb	2	2 Rt	2	1 Fb
3	2 Rt,Fb[c]	3	1 Rt	3	1 Fb
4	1 Rt	4	1 Rt	4[a]	1 Fb
5	3 Fb	5	1 Rt	5[a]	5 (1Rt,4Fb)
6	2 Fb	6	1 Fb	6	1 Rt
7	2 Fb	7	5 Fb		
8	1 Fb	8	1 Fb		
9	2 Rt,Fb	9	1 Fb		
10	1 Fb	10	8 (3Rt,5Fb)		
11	2 Rt,Fb	11	4 Fb		
12	1 Fb	12[a]	2 Fb		
13	1 Fb	13	1 Fb		
14	2 Fb	14	1 Fb		
15	1 Fb	15	1 Fb		
16	4 (3Rt,1Fb)	16[a]	1 Fb		
17	1 Rt	17	1 Fb		
18	1 Rt	18	1 Fb		
19	2 Rt,Fb	19	1 Fb		
20	1 Rt	20	1 Fb		
21	1 Fb	21	1 Fb		
22	1 Fb	22	1 Rt		
		23	1 Rt		
		24	1 Rt		
		25	1 Fb		
		26	1 Rt		
		27	1 Fb		
22	34	27	43	6	11 TOTALS

a = '*C. coli*' $G4_C$ and $G5_C$ were identical to $G12_L$ and $G16_L$ respectively.

Rt = Palmerston North Rubbish tip

Fb = Manawatu estuary at Foxton Beach.

C = Seven BRENDA types (5 *C. jejuni*, 1 *C. laridis* and 1 '*C. coli*' types) were common to faecal samples from gulls from both the Rt and the Fb.

PLATE 10.7 : SOME DNA 'FINGERPRINTS' FROM VARIOUS ISOLATES OF C. JEJUNI (J), C. LARIDIS (L), AND 'C. COLI' (C) RECOVERED FROM GULLS (G)

(see also Table 10.8)

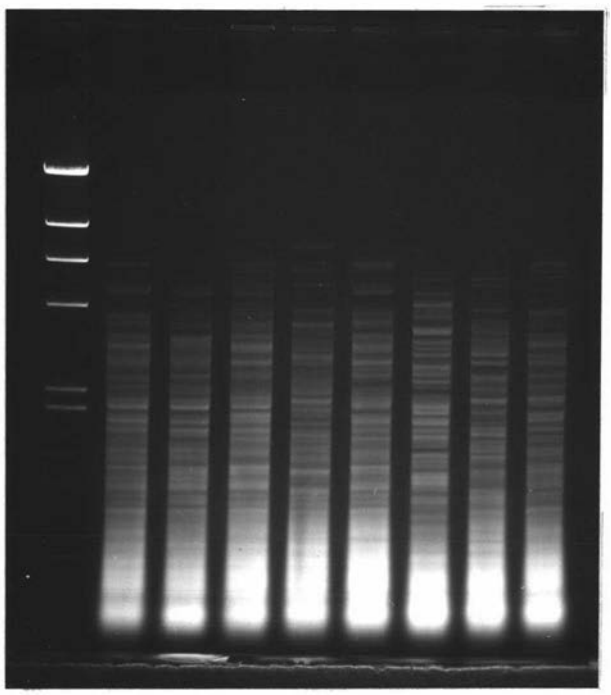
Lane 1 is the bacteriophage C1857 S7

Lanes 3, 4, 6, 9 are C. jejuni ($G17_J$, $G5_J$, $G18_J$, $G19_J$)

Lanes 2, 5, 8 are C. laridis ($G24_L$, $G26_L$, $G10_L$)

Lane 7 is a 'C. coli' ($G4_C$) which was shown to be C. laridis (see Plate 10.6, Lane 8).

PLATE 10.8 : IDENTICAL DNA 'FINGERPRINTS' OF C. JEJUNI (J) FROM THE CALF (Ca) AND A HUMAN (H)



1 2 3 4 5 6 7 8 9

$Cal_J/H16_J$

TABLE 10.9 : ISOLATE OF C. JEJUNI AND C. COLI FROM ALL ANIMALS EXAMINED WITH IDENTICAL OR SIMILAR BRENDA PATTERNS TO ISOLATES FROM HUMANS

CAMPYLOBACTER JEJUNI

Source of animal isolates	No. of animal BRENDA types	Nature of Relationship	No. of human isolates (n=316)	% of human isolates with similar patterns	
Chickens only	10 (17)	identical	110	34.8	49.7
	4 (17)	similar	47	14.9	
Chicken + rat	1 (17)	identical	3	0.95	
	1 (4)	similar	3	0.95	
Rat + Horse	1 (4)	identical	19	6.0	
	1 (1)				
Calf only	1 (1)	identical	8	2.5	
		similar	4	1.3	
Total	17 (23)		19.4	61.4	

CAMPYLOBACTER COLI

Origin of animal isolates	No. of animal BRENDA types	Nature of Relationship	No. of human isolates (n=23)*	% of human isolates with similar patterns
Chicken	1 (3)	identical	1	4.3
Pig	1 (76)	identical	1	4.3

( ) = Total number of BRENDA types identified in the species of animal

* = Multiple isolates from the same patient were not included

Development of Resistance to Nalidixic Acid by C. jejuni and C. coli and BRENDA Analysis of the Original Non Resistant Isolates and their Resistant Cloned Colonies

The non-resistant isolates of C. jejuni and C. coli and their resistant colonies produced identical DNA patterns to each other. The DNA patterns of C. coli and C. laridis recovered from the same faecal sample were identical. Plate 10.9 shows examples of the patterns of the DNA fragments of the isolates studied. The patterns of the isolates of C. jejuni from humans, which developed resistance to nalidixic acid, have been described in Chapter IX and are shown in Plate 9.4.

Study to Assess the Growth of C. jejuni and C. coli in the Presence of TMAO, NaCl

Table 10.10 shows the result of this study. Campylobacter coli and C. laridis from gulls, including both the C. coli and the C. laridis recovered from the same sample, as well as the cloned colony of this isolated C. coli, had identical BRENDA patterns. They all grew anaerobically in the presence of TMAO, and microaerophilically in the presence of 1.5% NaCl. However, the type strain of C. coli (NCTC 11366) and the C. coli isolated from a pig and its cloned colony which was resistant to nalidixic acid, were unable to grow in the presence of TMAO or 1.5% NaCl. Similarly, all the C. jejuni isolates from gulls, including those cloned colonies that were resistant to nalidixic acid, and the two type strains of C. jejuni (NCTC 11351 and 11168 originally isolated from bovine and human faeces respectively) failed to grow in the presence of these substances.

Use of a Second Endonuclease Enzyme

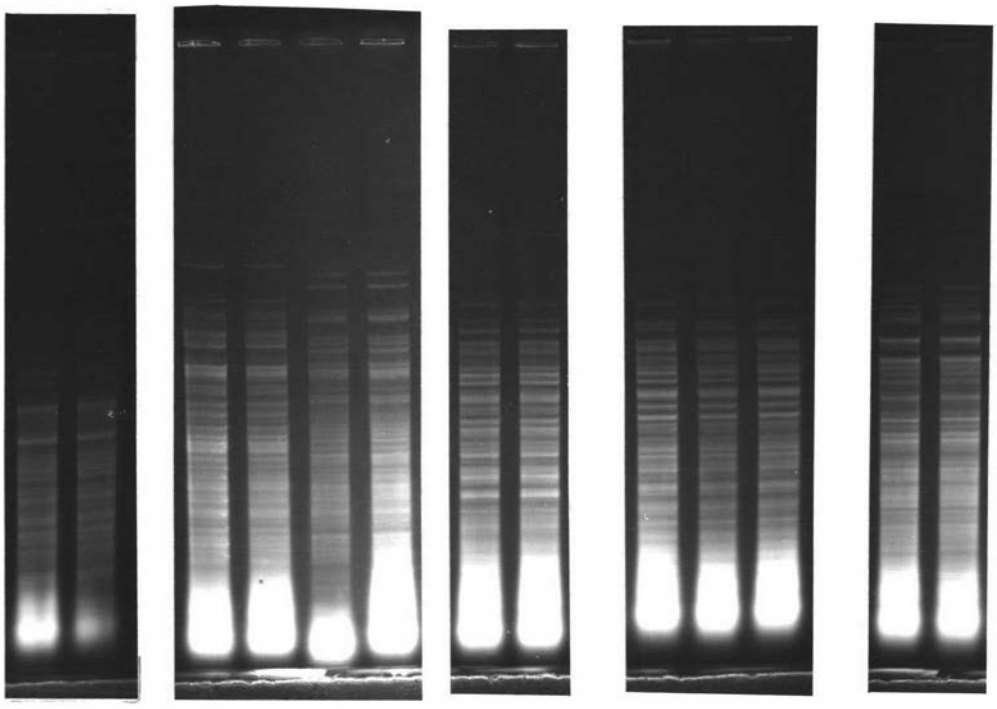
Plate 10.10 shows the results obtained when both endonuclease enzymes were used. Isolates which were found to produce identical DNA patterns with the first enzyme (Hind III), also had identical patterns when digested by the second enzyme.

BRENDA Typing and Serotyping of Intestinal Thermophilic Campylobacter Recovered from both Animals and Humans

From the results shown in Table 10.11, the correlation between BRENDA and serological typing can be seen. Based on the limited results, it appears that there is overall, a reasonably good correlation between the two techniques. The major exceptions were five different BRENDA types (three C. jejuni and two C. coli) which were classified as being serologically the same (serotype 2 and 48 respectively) and five isolates which were untypeable by the serological system.

PLATE 10.9 : SOME EXAMPLES OF DNA 'FINGERPRINTS' OF VARIOUS ISOLATES OF C. JEJUNI AND C. COLI AND THEIR SUBCULTURES WHICH WERE RESISTANT TO NALIDIXIC ACID

(see also Table 10.2)



1/1N 2/2N 3/3N 4/4N 5/5N L 6/6N

Lanes without N are the original non resistant isolates.

Lanes with N are their resistant subcultures.

Lane 1: *C. coli* from a pig

Lanes 2, 3: *C. jejuni* from chickens

Lanes 4, 5: '*C. coli*' from gulls

Lane L: *C. laridis* concurrently recovered with 5 from the same faecal sample from a gull.

Lane 6: *C. jejuni* from a rat.

TABLE 10.10 : GROWTH OF C. LARIDIS, C. COLI AND C. JEJUNI IN THE PRESENCE OF TMAO AND NACL

Source[b]	Ref. No. of isolate	Growth at 42°C	Catalase	Oxidase	30 µg nalidixic disc	30 µg cephalothin disc	Hippurate hydrolysis test	TMAO (0.1%) anaerobic	NaCl 1.5%
	C. laridis								
	1	+	+	+	R	R	-	+	+
	2	+	+	+	R	R	-	+	+
	3	+	+	+	R	R	-	+	+
Gull	4[a]	+	+	+	R	R	-	+	+
	5	+	+	+	R	R	-	+	+
	6	+	+	+	R	R	-	+	+
	7	+	+	+	R	R	-	+	+
Sub total	7								
	C. coli								
	1	+	+	+	S	R	-	+	+
	2	+	+	+	S	R	-	+	+
	3_a	+	+	+	S	R	-	+	+
Gull	4	+	+	+	S	R	-	+	+
	4_1	+	+	+	R	R	-	+	+
	5	+	+	+	S	R	-	+	+
	5_1	+	+	+	R	R	-	+	+
	6	+	+	+	S	R	-	-	-
Pig	6_1	+	+	+	R	R	-	-	-
Pig NCTC 11366		+	+	+	S	R	-	-	-
Sub total	10								

(To be cont)

Table 10.10 (cont)

Source[b]	Ref. No. of isolate	Growth at 42°C	Catalase	Oxidase	30 g nalidixic disc	30 g cephalothin disc	Hippurate hydrolysis test	TMAO (1% anaerobic)	NaCl 1.5%
	C. jejuni								
	1	+	+	+	S	R	+	-	-
	2	+	+	+	S	R	+	-	-
	3	+	+	+	S	R	+	-	-
Gulls	4	+	+	+	S	R	+	-	-
	5	+	+	+	S	R	+	-	-
	5_1	+	+	+	R	R	+	-	-
	6	+	+	+	S	R	+	-	-
	6_1	+	+	+	R	R	+	-	-
Bovine	NCTC 11351	+	+	+	S	R	+	-	-
Human	NCTC 11168	+	+	+	S	R	+	-	-
Sub total	10								
TOTAL	27								

4_1, 5_1, 6_1 = Resistant to nalidixic acid derived from subcultures of the non resistant 4, 5, 6 isolates respectively.

a = Concurrently recovered with '*C. coli*'[a] from a faecal sample from a gull.

b = All except NCTC 11366 originated from faecal samples.

PLATE 10.10 : DNA 'FINGERPRINTS' OBTAINED FROM VARIOUS ISOLATES OF C. JEJUNI AND C. COLI SUBJECTED TO RESTRICTION ENDONUCLEASE DIGESTION WITH TWO ENZYMES (Hind III and XhoI)

Lanes 1 - 6: C. coli from pigs

Lanes 7 - 8: C. coli from humans

Lanes 9 - 12: C. jejuni from humans

Note that isolates identical by Hind III digestion are also identical by XhoI digestion.

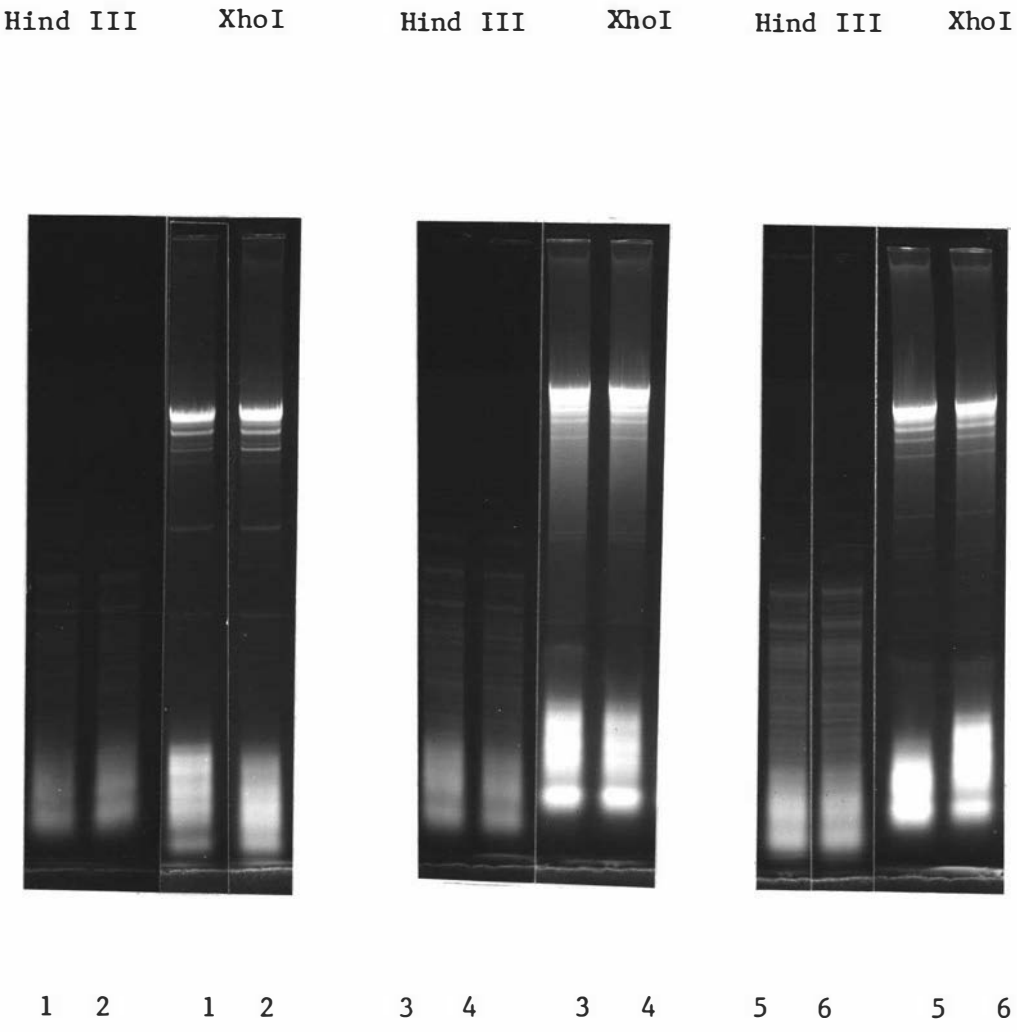

Hind III
XhoI
Hind III
XhoI
Hind III
XhoI
1 2 1 2
3 4 3 4
5 6 5 6
Hind III
XhoI
Hind III
XhoI
7 8 7 8
9 10 11 12
9 10 11 12

TABLE 10.11 : RELATIONSHIP BETWEEN BRENDA AND SEROTYPING OF INTESTINAL THERMOPHILIC CAMPYLOBACTER RECOVERED FROM ANIMALS AND HUMANS (n=38)

Source	Ref. No. of isolate	BRENDA type		PENNER Serotype
CAMPYLOBACTER JEJUNI (J)				
Chickens (Flock No.8)	231	$P4_J$	Identical BRENDA types (IBT)	0:1
	232			0:1
Humans	600	$H2_J$		0:1
	602			0:1
Chickens (Flock No.23a)	210	$P6_J$	IBT	0:19
Chickens (Flock No.23b)	212			0:19
Rat	R15	$R3_J$		0:19
Rats	R4	$R1_J$		0:12
	R7			0:12
	R9			0:12
	R11			0:12
	R18			0:12
Humans	604	$H8_J$	Three dissimilar BRENDA types	0:2
	606			0:2
Chickens (Flock No.	251	$P3_J$	IBT	0:2
	253			0:2
Humans	743	$H1_J$		0:2
	745			0:2
Human	942	$H28_J$		0:2
	942N*			0:2
Gulls	888	$G16_J$		0:27
	886			0:27
Processed chicken wings (Batch No. 1)	1078	$P4_J$	IBT	NT (not
	1079			NT typeable)
Human	623	$H2_J$		NT
Total	25			

* 942N = Isolate 942 developed a resistance to nalidixic acid

(To be cont.)

Table 10.11 (cont)

Source	Ref. No. of isolate	BRENDA type		PENNER Serotype
CAMPYLOBACTER COLI (C)				
Processed chicken wings (Batch No. 2)	1080	$P3_C$		0:5
	1083	$P3_C$		0:5
Chickens (Flock No.12a)	261	$P2_C$		0:5:31
	267	$P2_C$		0:5:31
Chicken (Flock No.12b)	273	$P2_C$		0:5:31
Piggery No. 1 Sow No. 3	307	$p2_C$	Three dissimilar BRENDA types	0:48
Piglets of Sow No. 3	308	$p2_C$		0:48
	309	$p2_C$		0:48
	310	$p2_C$		0:48
Pigs in the same piggery	348	$p39_C$		0:48
	349	$p52_C$		0:48
Total	11			
CAMPYLOBACTER LARIDIS (L)				
Gulls	824	$G19_L$		NT
	887	$G19_L$		NT
	2			
Overall total	38			

DISCUSSION

Lauwers *et al* (1981) examined 300 isolates of *C. jejuni* from humans and subdivided them into 50 serotypes. The system was unable to type approximately 40% of the isolates. The serotyping system of Lior *et al* (1983) and Penner *et al* (1983b) recognised 53 and 59 serotypes respectively. Approximately 7% and 3% are untypeable by the two systems respectively, but up to 20% of the typeable isolates react with more than one antiserum (Penner *et al* 1983b). In the future, it is likely that even more serotypes will be recognised. The identification of so many BRENDA types among the intestinal thermophilic *Campylobacter* is not therefore surprising. One of the advantages of BRENDA analysis is that the DNA 'fingerprint' of all isolates can be described and therefore it is reasonable to be expected that the total number of types will be more than the number created by a serotyping system. Also, it is unlikely that any serotyping system, or other routinely used system of subspecies identification, will be as sensitive as BRENDA typing.

Karmali *et al* (1983) stated that serological typing of thermophilic *Campylobacter* does not necessarily indicate a definite identity among isolates of the same serotype, and suggested that additional differential markers may be required. This has been substantiated by the limited comparison of isolates by both BRENDA and serological techniques. Of the limited number of isolates examined, a few which were found to belong to the same serogroup (see Penner's serogroup 2), were shown to consist of three different BRENDA types. The existence of these three different BRENDA types makes epidemiological sense as they were recovered from three different ecological niches.

The general agreement between the Penner typing system and BRENDA typing is of particular interest. It demonstrates the usefulness of both methods in epidemiological investigations. However, it must be stressed that this comparative study was only of a preliminary nature and a further 100 selected isolates which have been analysed by the BRENDA technique have been sent to Professor Penner for serotyping. (These results have not yet been received.)

The stability of the BRENDA patterns produced by isolates C. jejuni and C. coli is of significance. It is believed that evidence of a definite stability has been demonstrated from the results of the in vivo experiments (see Chapter VIII). In these experiments, the challenging BRENDA types either were given to the same species of animals from which they were originally isolated, or to different species. In all cases the infecting organisms retained their DNA patterns of identity. Further evidence of the stability of the DNA 'fingerprint' is provided by the recovery of the same BRENDA type from related human cases (see Chapter IX), the recovery of similar BRENDA types of C. jejuni and C. coli recovered from different chickens from the same flock and the recovery of C. coli of the same DNA pattern from both sows and their piglets. Moreover, in an in vitro experiment reported elsewhere, (Kakoyiannis et al 1984), four strains of C. coli of different BRENDA types retained their specific restriction endonuclease DNA patterns after 23 passages on agar over a period of 45 days.

A number of C. jejuni and C. coli have been found to carry plasmids (Austen and Trust 1980). Non chromosomal DNA (plasmids) may contribute to part of the BRENDA pattern of some thermophilic Campylobacter obtained by the BRENDA technique. However, the plasmid components of C. jejuni and C. coli have been shown to be stable, and it has been suggested that these fragments of DNA could be used to identify strains of C. jejuni and C. coli carrying plasmids (Bradbury et al 1983). If this hypothesis is correct, the presence of plasmids will not affect the accuracy of the BRENDA technique for identifying specific strains of Campylobacter. The BRENDA technique has the advantage over plasmid typing alone, in differentiating between two isolates carrying the same plasmid but with different chromosomal DNA. When attempting to demonstrate the presence of a plasmid, electrophoresis can be carried out on the extracted DNA without subjecting it to restriction enzyme digestion. Although this is not a sensitive method for the detection of plasmids of large molecular weight, by comparing electrophoretic patterns, before and after restriction enzyme analysis, the presence of plasmids can be detected.

The use of restriction endonuclease enzyme Hind III produced sufficient fragments from the genome to provide a satisfactory 'fingerprint'. The number of bands of digested DNA produced by Hind III ranged from approximately 30 to 60. The usefulness of this enzyme has also been shown in limited studies of isolates of C. jejuni by Penner et al (1983c)and Bradbury et al (1984). The present work indicates that the use of a second enzyme (Hsu I) provides no extra taxonomic data. Organisms which have different DNA patterns, presumably have a different arrangement of base pairs within the DNA of their chromosome and therefore cannot be considered as having been derived from the same parent stock.

Some discussion on the in vitro development of resistance to nalidixic acid by C. jejuni and C. coli is to be found in Chapter VII. It has already been pointed out that the development of nalidixic acid resistance by C. jejuni, has been recorded previously (Rosef and Yndestad (1982), but not by C. coli. It has also been mentioned that strains of C. jejuni which are nalidixic acid resistant retain their ability to hydrolyse hippurates which allows them to be distinguished from C. laridis. Hippurate hydrolysis cannot be used to differentiate between C. coli and C. laridis, because both fail to hydrolyse hippurate. It was unfortunate that attempts to carry out tests for H_2S production in an iron-metabisulphite pyruvate medium (FBP medium) as recommended by Skirrow and Benjamin (1980b) were unsuccessful. This test has been suggested as a further criterion for the differentiation between C. coli and C. laridis (C. laridis produces H_2S while C. coli does not). It appears however, that other workers have had difficulties with this test (Skirrow and Gill, personal communications).

When an organism develops nalidixic acid resistant after being subcultured in the laboratory, the development of these resistant isolates are easily identified. The 20 isolates, which developed this resistance and were studied in the work reported in this chapter, were confirmed by BRENDA analysis as retaining the same pattern in-vitro.

Development of resistance to nalidixic acid by C. coli other than strains of 'C. coli' from gulls, and C. jejuni may possibly

occur *in vivo*, either in patients treated with nalidixic acid or in wild animals and birds. Such isolates resistant to nalidixic acid could be incorrectly classified as *C. laridis*.

It is believed that these studies indicate that the organisms recovered from gulls and classified according to conventional criteria as *C. coli* and *C. laridis*, may be a single species. The reasons for this hypothesis are as follows:

1. Both organisms, including the nalidixic resistant strains of '*C. coli*', grew in the presence of TMAO. This is a characteristic considered unique for *C. laridis* (Benjamin *et al* 1983).

2. Both were capable of growing in media containing 1.5% NaCl, but *C. coli* is not considered to have this ability (Benjamin *et al* 1983).

3. '*C. coli*', including the nalidixic acid resistant strains, isolated from gulls, had the same DNA patterns as *C. laridis* isolated from gulls.

4. Neither 'species' of organisms isolated from gulls would infect rats or chickens, yet *C. coli* from other sources caused infection.

It would appear that resistance to nalidixic acid is not an absolute criterion for differentiation between *C. coli* and *C. laridis*, but it is unfortunate that the H_2S production test of Skirrow and Benjamin (1980b) could not be performed by the author. However, the weight of the other evidence presented, strongly indicates that the organisms isolated from gulls and initially classified as '*C. coli*' and *C. laridis*, were phenotypic variants of the same species, and were probably both *C. laridis*.

The taxonomic and somewhat academic question which remains, is whether *C. laridis* is a separate species or a subspecies of *C. coli*. In this respect, it is interesting to note that Benjamin *et al* (1983) reported that in DNA hybridization studies, *C. coli* and *C. laridis* appeared to be more closely related to each other than to other *Campylobacter*.

The limited number of BRENDA types of C. coli (3) obtained from chickens in general and the limited number (3) strains of C. jejuni found in any one flock, is in contrast to the much greater number recorded from pigs, ducks and gulls. A similar situation was recorded by Munroe et al (1983) in relation to the serotypes of both C. coli and C. jejuni in pigs and poultry. It is believed these results are an indication of the more closed nature of poultry flocks, compared with piggeries and flocks of wild birds, which would limit the introduction of different types of C. coli and C. jejuni to a poultry flock.

The limited number of BRENDA types of C. coli in poultry, compared with C. jejuni is probably a reflection of the lower infectivity of C. coli for poultry.

The similarities of the BRENDA patterns of C. jejuni and C. coli isolated from both chickens and humans are in keeping with the serological findings of others (Lior et al 1981, 1982, Banffer 1983; Konsumen et al 1982, Munroe et al 1983). Although a direct link between infected chickens and human infection cannot be proved, the similarities in BRENDA patterns of isolates from both, and the high levels of contamination by Campylobacter on processed carcases (see Chapter VI) supports the view that chickens are an important source of infection for humans.

The finding that two of the four BRENDA types seen in rats were identical with isolates from humans, chickens and a horse, is of interest. Although this finding cannot be taken as direct evidence that the human isolates were of a rat origin, it indicates the possibility that rats may be a source of infection for other animals and humans. Experimentally (see Chapter VIII) a rat isolate was able to infect chickens. The BRENDA type of those isolates of C. jejuni which was recovered most frequently from rats was, when serotyped by Penner, found to belong to the same type as isolates from humans which were recovered from a waterborne outbreak in Norway and with many isolates from humans from Mexico. This serotype had not been reported previously from any other animal sources (Penner 1984, pers. communication).

Cattle isolates have been shown by chromosomal restriction endonuclease analysis (Bradbury et al 1984) and by serotyping (Lior et al 1981, 1982, Lauwers 1982, Munroe et al 1983) to share similarities with isolates from humans. In this respect, it is interesting to note that the only isolate of C. jejuni from cattle which was examined by BRENDA analysis was identical to an isolate from a human. These findings suggest that cattle may also be an important source of infection for humans.

Although many C. coli BRENDA types were seen in pigs, ~~none of~~ only one of them ~~were similar~~ was identical to isolates of C. coli from humans. This apparent dissimilarity between C. coli from pigs and those of human origin has also been recorded by Munroe et al (1983) using the serological typing system of Penner (Penner and Hennessy 1980). These findings are in contrast with those of Lior et al (1981, 1982) who reported that serotypes of C. coli from pigs commonly occur in humans.

Hudson and Roberts (1982) observed that the contamination rate of pig carcases by Campylobacter is considerably reduced after chilling the carcases for 24 hours at $0^{o}C$. Campylobacter could not be isolated from more than 2% and 26% of the wet and dry areas of the carcases respectively when the initial rate of carcase contamination was approximately 60%. The number of Campylobacter recovered were generally less than $1/cm^{2}$. This decrease of carcase contamination is in contrast to that which has been reported in this thesis in relation to processed chicken carcases (see Chapter VI). It might explain why no human isolates of C. coli had the same BRENDA pattern as those isolated from pigs and why Munroe et al (1983) showed no association between serotypes recovered from humans and pigs.

CONCLUSIONS

1. Bacterial restriction endonuclease DNA analysis (BRENDA) is a technique of considerable value in epidemiological studies of infections associated with intestinal thermophilic Campylobacter.

2. The endonuclease enzyme, Hind III, appears to be capable of producing specific DNA 'fingerprints' of intestinal thermophilic Campylobacter.

3. One hundred and ninety four (61%) of 316 isolates of C. jejuni from humans had similar BRENDA patterns to isolates of C. jejuni from animals.

4. Poultry appears to be a major source of infection of C. jejuni for humans. (Fifteen of the 17 BRENDA types from humans with similar patterns to those from animals, were from poultry, the remaining two were individual isolates from a calf and a horse.)

5. Rats can be infected with strains of C. jejuni which can infect both poultry and humans.

6. Only one isolate of C. coli from a pig and one strain of C. coli from a flock of poultry were of a similar BRENDA pattern to isolates from humans.

7. No isolates of Campylobacter from wild birds were similar to isolates from humans.

8. Individual pigs can be concurrently infected with up to three different BRENDA types of the same species of Campylobacter.

9. Up to 28 different BRENDA types of C. coli have been identified in pigs from one herd, but no more than three BRENDA types of C. jejuni from chickens in the same flock.

10. The majority of preweaning piglets are infected with the same strain of C. coli as their dams.

11. Some strains of C. jejuni and C. coli can develop an in-vitro resistance to nalidixic acid. This is an important finding in relation to the conventional taxonomic criteria for differentiating C. laridis from other intestinal thermophilic Campylobacter.

12. Isolates of so called '*C. coli*' from gulls are probably phenotypic variants of *C. laridis* which may be either resistant or non resistant to nalidixic acid.

13. At present, the most reliable test for differentiating *C. coli* from *C. laridis* is by determining whether or not, the organisms will grow under anaerobic conditions in the presence of TMAO.

CHAPTER XI

GENERAL DISCUSSION

Although, before this work was carried out, it was known that intestinal thermophilic Campylobacter survive better at lower than ambient temperatures, there was a lack of information on the duration of survival of these organisms under field conditions and in faecal samples being transported to a laboratory. Although many studies, based on the culture of faeces, have been carried out on the prevalence of intestinal thermophilic Campylobacter in various species of animals, few workers appeared to have taken account of possible inaccuracies associated with the death of organisms during the transport of specimens to the laboratory.

From the work reported in the first part of this thesis, it would appear that the sensitivity of prevalence studies, based on the culture of rectal or cloacal contents, will not be affected if specimens are collected on dry swabs, transported on ice and cultured within six hours of collection. However, if longer periods between collection and culture are necessary, a transport medium, such as FBP broth, is required. In most long term epidemiological studies of bacterial infections, it is necessary to preserve many isolates for more detailed investigation at a later date. It is therefore important, that it has been shown that cultures of intestinal thermophilic Campylobacter can be preserved for long periods in FBP broth at -70^{o}C.

When attempting to investigate possible inter-species associations of infection, particularly between animals and man, it is important that some form of sub-species identification of infectious agents is developed. Obvious examples include the phage typing of Salmonellae and Staphylococci and the serotyping of Leptospira. Without such sub-species differentiation, it is often impossible to gain accurate epidemiological information of a particular disease.

It was thought that the technique of restriction endonuclease

DNA analysis (BRENDA) might provide such a tool to help understand the natural history and the epidemiology of intestinal thermophilic Campylobacter infections. The technique was chosen because the Department of Veterinary Pathology and Public Health at Massey University had played a leading role in the development of this technique in relation to other organisms (Marshall et al 1981, Robinson et al 1982). Thus the facilities and expertise to carry out the technique, were readily available to the author.

The BRENDA technique can be used either on its own, or in conjunction with serological typing systems for the study of intestinal thermophilic Campylobacter. The BRENDA technique has the advantage that the DNA patterns of all isolates can be defined, whereas with serological systems, similar but not necessarily identical organisms are grouped together, and the number of subgroups depends on the accuracy of antigen detection. An additional advantage of the BRENDA technique, is that a photograph of the DNA 'fingerprint' of an organism can be sent anywhere in the world for comparison with the results obtained by other workers investigating similar problems providing they are using the same restriction endonuclease enzyme. A bank of reference 'fingerprints' for intestinal thermophilic Campylobacter has already been started and subsequent isolates of these organisms can be compared to BRENDA types already identified and new types added as they are discovered.

The present work confirms that of others, which indicate that intestinal thermophilic Campylobacter are very widely distributed in nature in the G.I. tract of animals, and with the exception of pigs, C. jejuni is the predominate species recovered from mammals including humans. In pigs, C. coli is the predominant isolate, and C. laridis is a species specific for seabirds.

There appears to be some analogies which can be made between Salmonellae and the intestinal thermophilic Campylobacter, particularly in relation to birds. The obvious host specificity of C. laridis for gulls and other seabirds, is perhaps analogous with the host specificity of S. gallinarum and S. pullorum for poultry. On the other hand, C. jejuni, like S. typhi murium appears to lack any particular host specificity.

Intestinal thermophilic Campylobacter can survive in natural waters for a few days or a few weeks, depending on the temperature. Whenever other workers have isolated Campylobacter from water, the organisms have been recovered together with Escherichia coli type I, indicating concurrent recent faecal contamination. However, untreated surface water can never be considered free of Campylobacter because of the large number of free-living animals and birds potentially capable of continually contaminating such waters.

In developed countries, as opposed to developing countries, there is no apparent reservoir of Campylobacter in the human population. Animals and animal products have been implicated as the most likely sources of infection for humans. Common serotypes and BRENDA types of Campylobacter have been found in both animals and humans. Thus it would appear that in developed countries, the weight of evidence is in favour of human campylobacteriosis to be considered a zoonosis.

The work presented in this thesis, demonstrates for the first time that the infectivity of C. jejuni for chickens and rats, is considered greater than C. coli. Apart from pigs, which appear to be particularly adapted to infection with C. coli, it is possible that C. jejuni is more infective than C. coli for other species of animal including man. This hypothesis tends to be supported by the greater prevalence of C. jejuni, compared with C. coli, in most of the species of animal investigated.

Another important finding from this work is that, in the species of animal studied, Campylobacter infections are self-limiting and in the case of poultry, birds which have eliminated a primary infection are resistant to reinfection. It is suggested that this elimination and resistance to infection is likely to be associated with an immunological response, possibly mediated by the production of Ig.A in the intestine. This is a topic which should be investigated further as it could lead to the development of appropriate methods of control. However, the multiplicity of different BRENDA types involved in infection in the same species of animal may cause problems. In this respect, it is interesting to

note that Karmali et al (1981b) reported that over a period of a few months, a female patient has been infected on two separate occasions by two different strains of C. jejuni.

The finding that organisms which would have been previously described as 'C. coli' from gulls were in fact C. laridis, is an important observation, and provides further evidence of the inadequacy of using resistance to nalidixic acid, as a taxonomic criterion. The only reliable criterion for differentiating between C. coli and C. laridis are their ability to grow anaerobically in the presence of TMAO.

As a result of this new information on the identification of C. laridis, previous reports of the isolation of this species from humans and other animals, with the exception of seabirds, must be treated with caution. Further work may well show that C. laridis is limited to birds adapted to a marine environment. It appears that C. coli, a species closely related to C. laridis, also has a restricted range of animal reservoirs, and at present the pig appears to be the major source of infection. Conversely, C. jejuni appears to be adapted to a much wider range of animal hosts, and is the species of greatest public health significance.

One of the most important considerations arising from the concept that human campylobacteriosis is a zoonoses, is the action which can be taken to reduce the incidence of human disease.

Apparently, intestinal thermophilic Campylobacter are so widely prevalent in wildlife, that it appears at first sight, to be an impossibility to break the chain of infection between wild and domestic animals. A further problem is that infection in the domestic animal seldom results in clinical disease. Thus, there is no economic incentive for farmers to tackle the problem. From the public health point of view, most of the pressure for a control programme should be directed towards the poultry, and possibly the pig industry, in order to reduce the rate of contamination. However, it is believed that action could be taken to reduce the prevalence of infection in poultry.

As opposed to salmonellosis, infection by Campylobacter is not transmitted vertically. Thus, by standard, and relatively simple, methods of hygiene at the hatchery and during transport, newborn chicks could be protected from infection. At the farm, as was indicated from the survey of poultry flocks, relatively simple barrier techniques should be capable of maintaining freedom from infection. This view is also supported from the results of the experimental infections of chickens, where a simple partition between cages prevented cross infection. Special attention would have to be paid to vermin control and entry to the building by insects, rodents and wild birds. Maintenance of supplies of normal potable water should eliminate waterborne infections. Unlike salmonellosis, food should not normally consitute a good milieu for the survival of Campylobacter, especially if it is stored in a clean environment for a few days before use.

The Campylobacter status of flocks could easily be checked by taking and culturing a pooled faecal sample four to five days before they are to be slaughtered and processed. If flocks are found to be infected, the farmer would have to thoroughly fumigate and disinfect the building before introducing a new batch of chickens.

From a public health point of view, it would be better to process infected birds at the end of the week, to prevent cross contamination of carcases from uninfected flocks. However, this could create an economic problem for the farmer. Other procedures within the poultry processing plant conducive to cross-contamination, also apply to other infectious agents, and are beyond the scope of the present discussion.

In New Zealand, pigs appeared to be an insignificant source of C. coli infections to humans. This may be associated with a relatively small pig industry, the limited consumption of pig meat, and the theoretical low levels of contamination to be expected on pig carcases subsequent to chilling. However, in some countries with well developed pig industries and a high consumption of pork, C. coli may be of greater public health significance. However, it would be almost an impossible task, compared with poultry, to

produce pigs free of intestinal thermophilic Campylobacter. The major reason being, that piggeries have a continuous population of animals of different ages and unless strict SPF techniques were employed, sows would continue to be a primary source of infection for all other animals. However, there are better opportunities at the abattoir, in relation to conditions of carcase chilling and humidity control compared with poultry processing plants, to reduce the degree and rate of carcase contamination.

It is difficult to envisage any method for controlling infection in cattle and sheep populations as it is likely that there are reservoirs of infection of C. jejuni in a wide variety of species of wildlife. Strains of C. jejuni isolated from gulls during the course of this work, were not of similar BRENDA types to those examined from other animals. However, on this evidence alone, gulls cannot be dismissed as a potential reservoir of infection. Contamination of milk should be controlled by pasteurisation and unpasteurised milk must be always considered as a possible source of infection.

In view of the high infectivity of C. jejuni for man, the public needs to be educated about the potential risks of contracting campylobacteriosis from handling animals, including young dogs and cats with diarrhoea. Processed chicken carcases must be considered one of the major potential sources of infection, and cross contamination in the kitchen from such products to other prepared foods must be avoided. Any information given to the public should emphasise that adequate cooking and standards of good hygiene in the kitchen and elsewhere, are the ultimate measures in the defence against infection by intestinal thermophilic Campylobacter. The poultry industry should not have to take the blame for a biological problem not of their making.

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APPENDIX I

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

The components of this medium consist of:

a) Columbia blood agar base
b) Defibrinated sheep blood
c) Skirrow's antibiotic supplement
d) Campylobacter growth supplement (FBP supplement)

a) Columbia blood agar base

This is a standard Difco product.

b) Defibrinated sheep blood

Blood is only collected from healthy sheep which have not been subjected to any form of prior medication. Blood is collected from a jugular vein by a needle connected by a rubber tube to a 500 ml flask at an initial negative pressure of 400 nm of mercury, and containing 100 - 150 glass beads. The flask is kept agitated during the time of blood collection.

The defibrinated blood is dispensed into 100 ml bottles which are kept at 2 - 3°C. Samples more than three weeks old are discarded.

c) Skirrow's antibiotic supplement

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

d) Campylobacter growth supplement (FBP supplement)

Four g of each of ferous sulphate$^{\beta}$, sodium pyruvate$^{\gamma}$, sodium metabisulfite$^{\delta}$ are dissolved in 16 ml of distilled water (250 mg/ml). The solution is passed through 0.45 mm millipore filter$^{\varepsilon}$ and concentrated in a 20 ml sterile universal bottle, and the supplement is kept at 2 - 3°C until used. One ml (250 mg) of the FBP supplement is added to 500 ml of the medium.

To rehydrate the Columbia blood agar base, 22 g is suspended in 500 ml of distilled water and heated to boiling, until completely dissolved. Autoclave for 15 minutes at 121°C. Allow the base to cool at 43 - 45°C and add 7% sterile defibrinated sheep blood (component b), a vial of the antibiotic selective supplement (component c) dissolved in 2 ml sterile distilled water and 1 ml of the FBP growth supplement (see component d). After thorough mixing, 12 - 15 ml of the medium is poured into sterile plastic petri dishes. The plates are allowed to cool for a few hours and stored at 2 - 3°C for up to two weeks.

For routine isolation of Campylobacter the plates require to be reasonably moist. For enumeration studies, the plates require to be dried, by prior incubation at 37°C for 30 minutes to prevent spreading of colonies.

β BDH Chemicals Ltd., Poole, England.

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

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

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

APPENDIX II

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

The modified Campy-BAP selective medium is 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] is dissolved in 10 ml distilled water (7.5 mg/ml). The solution is passed through 0.45^{o} millipore filter and kept in a universal bottle at 2 - $3^{o}C$, and 1 ml is added to 500 ml of medium.

Fifteen (15) mg of amphotericin b[b] is first dissolved in 1 - 2 ml ethylalcohol and distilled water is 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 is added to 500 ml of medium. Both antibiotics are added to the medium after it has cooled to 43 - $45^{o}C$ (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 AN IRON METABISULFITE PYRUVATE MEDIUM (FBP MEDIUM)

The components of the FBP medium are:

a) Nutrient broth

b) Agar

c) FBP supplement

Rehydrate 12.5 g nutrient broth No. 2 (CM 67, Oxoid) and 0.6 g of Technical Agar No. 3 (L13, Oxoid) in 500 ml of distilled water and heat to boiling, to dissolve the agar. Autoclave for 15 minutes at 121°C. Allow to cool at 45 - 50°C and add 1 ml (250 mg) of sterile FBP supplement (see Appendix I) and mix well. Dispense the FBP medium in aliquots of 10 ml in sterile 20 ml universal bottles and allow to cool and store at 2 - 3°C. The medium is slightly semisolid because of the 0.12% agar which was added to the nutrient broth. When the medium is needed for testing for H_2S production, it needs to be kept for half an hour at an ambient temperature before inoculation.

APPENDIX IV

PREPARATION OF THE MEDIUM FOR THE TEST OF ANAEROBIC GROWTH IN THE PRESENCE OF TMAO

The components of this medium are:

a) Nutrient broth
b) Yeast extract
c) Agar
d) Trimethylamine N-oxide hydrochloride (TMAO) supplement

Rehydrate 23 g of nutrient broth No. 2 (CM 67, Oxoid), 1 g yeast extract (L21, Oxoid) and 2 g New Zealand agar in 1000 ml of distilled water (this is termed Yeast Nutrient Broth YNB). Heat to boiling until the components have dissolved and autoclave for 15 minutes at 121°C. Allow to cool at 45° - 50° and add 2 ml of TMAO supplement (see details below). After thorough mixing, the 60 - 70 ml of the medium is dispensed into 100 ml glass bottles, which are stored at 2 - 3°C.

Trimethylamine N-oxide hydrochloride supplement: Five g of trimethylamine N-oxide hydrochloride (TMAO)* are dissolved in 10 ml of distilled water. The solution is passed through a 0.45 millipore filter and collected in a universal bottle. Two ml of the TMAO supplement are added to one litre of the medium to obtain a final concentration of TMAO of 0.1%.

* = BDH Chemicals Ltd., Poole, England.

APPENDIX V

PREPARATION OF YEAST EXTRACT NUTRIENT AGAR (YNA) MEDIUM

The components of the medium are:

a) Nutrient agar

b) Yeast extract

Rehydrate 11.5 g of nutrient agar (CM 271, Oxoid) and 0.5 g yeast extract (L21, Oxoid) in 500 ml of distilled water and heat to boiling to dissolve. Autoclave for 15 minutes at 121^{o}C and allow to cool at 45 - 50^{o}C. Dispense the medium into sterile plastic petri dishes which are left to cool for a few hours at an ambient temperature before being stored at 2 - 3^{o}C.

APPENDIX VI

PREPARATION OF THE FBP BROTH

The components of this broth are:

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

Rehydrate 12.5 g of nutrient broth No. 2 (CM 67, Oxoid) and 0.6 g Bacto-agar (Difco) in 500 ml of distilled water. Heat to boiling until dissolved. Add 15% (v/v) glycerol and autoclave for 15 minutes at 121^{o}C. Cool at 45 - 50^{o}C and add 1 ml (250 mg) of the FBP supplement (see Appendix I). Dispense into sterile universal bottles and store at 2 - 3^{o}C.

APPENDIX VII

REAGENTS USED FOR BRENDA TECHNIQUE

TEB = Tris-Ethylenediaminetetraacetic acid disodium salt-Buffer

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

100 mM Ethylenediaminetetraacetic 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 $MgCl_2$

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 ethidium bromide[d].

Protease[e] solution

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

Sodium percholate

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. Calbiochem. Behring Co., San Diego, California 92112, U.S.A.