THE SIGNIFICANCE OF WILD BIRDS (IN PARTICULAR, *LARUS* SPP.) IN THE EPIDEMIOLOGY OF CAMPYLOBACTER INFECTIONS IN SCOTLAND

A thesis submitted to the Faculty of Science,
University of Glasgow, for the degree of
Master of Science

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May 1986

ACKNOWLEDGEMENTS

I would like to thank Colin Fricker, who introduced me to this project. I am sincerely grateful for all of his help in the the production of antisera and serotyping.

Many thanks go to R.W.A. Girdwood, who gave me moral support during this project and good advice in the preparation of this manuscript. Without his help, this project could not have taken place at Stobhill Hospital. I appreciate the assistance given to me by the staff at the Bacteriology Laboratory in Stobhill Hospital, who kept the cultures alive in my occasional absence.

Kenny Ensor was involved in several areas of this project. His expertise in the field (and refuse tips) catching gulls was greatly appreciated, as was his collecting of water samples in all types of miserable Scottish weather. Without his assistance in my learning of the intricacies of Wordstar and the Digiplotter, this thesis would have taken twice as long to prepare!

I am grateful to Professor S. Phillips for the facilities made available to me in the Department of Zoology, University of Glasgow.

I wish to thank the samplers at the Strathclyde Water Board for collecting water samples, and in particular, K. Benton, for her interest and support.

A special thanks must be given to P. Monaghan, who was instrumental in the catching and sampling of the gulls. I deeply appreciate the invaluable advice and criticism she gave me throughout the writing of this thesis.

This work was supported by the Scottish Home and Health Department and the University of Glasgow.

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SUMMARY

A total of 650 human Campylobacter spp. isolates were obtained from 4 localities in Scotland. Approximately 3% of these were designated to be C. coli; the remaining isolates were C. jejuni. With a panel of 29 antisera prepared in rabbits to Penner reference strains, 492 (75.7%) were able to be assigned a serotype using passive haemagglutination for the detection of heat stable antigens. The four most common serotypes were Penner 2, 4, 1 and 31, accounting for 38.3% of the isolates examined, and 50.6% of those which were typable.

Cloacal swabs from herring gulls were cultured for Campylobacter spp. throughout the year. Gulls were sampled from refuse tips in the Glasgow area as well as Aberdeen. Unfledged chicks from the breeding colonies at Inchmarnock and Flanders Moss were also sampled. The breeding colony on Horse Island, off the west coast of Scotland, provided Campylobacter spp. isolates from lesser black backed gulls and herring gulls. There was no consistent age or sex effect in the carriage of Campylobacter spp., C. laridis or C. jejuni/coli.

Geographical location was a factor in the number of gulls that cultured positive for Campylobacter spp., with the refuse tip birds in the Glasgow area having a higher proportion of C. jejuni/coli than the Aberdeen tip birds and the gulls caught in Thurso and Bowmore (from previous data). A seasonal effect was exhibited in the refuse tip birds from the Glasgow area, with C. jejuni/coli responsible for the majority of Campylobacter spp. isolated from the gulls in the November-December period. In January-February, there was a large increase in the proportion of gulls carrying Campylobacter spp., mainly due to an increase in the number of gulls with C. laridis.

Feeding ecology plays an important role in the carriage of Campylobacter spp. in gulls. Tip birds had a higher proportion of

both C. jejuni/coli and C. laridis when compared with the birds from a breeding colony. The proportion of unfledged chicks with Campylobacter spp. was less than that of the adult gulls, although the difference in the proportion of C.jejuni/coli in both populations was not statistically significant. Twenty-nine percent of the C. jejuni/coli strains isolated from the gulls were able to be serotyped. The percentage of C. jejuni/coli that serotyped in the unfledged chicks was significantly higher than that of the fledged gulls. Serotypes obtained from the chicks were similar to those most commonly found in humans.

There was an increased amount of Campylobacter spp. found in reservoirs used by gulls as nocturnal roosting sites. The levels of Campylobacter spp. were shown to increase with the increase in the number of nocturnally roosting gulls. C. laridis was isolated from bodies of water during periods of medium (August-September) and high (November-December) gull usage, but was not found in reservoirs not utilised by gulls as nocturnal roosts. These latter reservoirs had numbers of Campylobacter spp. that followed a different monthly pattern from the utilised reservoirs.

The role of gulls in the epidemiology of human Campylobacter infections in Scotland is discussed.

CHAPTER 1. INTRODUCTION

Aims of the Study

The aim of this study was to investigate the relationship between the intestinal carriage of *Campylobacter* spp. in gulls and humans and to determine whether the organisms isolated from gulls were biologically the same as those commonly associated with human intestinal infection. To date, the use of biotyping schemes has given a limited amount of information concerning the relationship between the *Campylobacter* spp. found in man and those found in birds. With the use of serotyping techniques in this study, the identification of these human and avian isolates is taken one step further in order to identify in more detail the epidemiological relationship, if any, between the two. The project has involved the study of three species of catalase positive thermophilic *Campylobacter* spp., *C. jejuni*, *C. coli* and *C. laridis*, from gulls and humans in Scotland, and the subsequent serotyping of *C. jejuni* and *C. coli*. Water in storage reservoirs utilised by gulls as roosting sites was also cultured for *Campylobacter* spp. and compared with water not utilised by gulls to establish whether the role of avian carriage of *Campylobacter* spp. and its effect on the environment could be documented.

CHAPTER 2. THE GENUS CAMPYLOBACTER

In this chapter, a general description of organisms belonging to the genus Campylobacter provided. The history, taxonomy, and characteristics of the thermophilic catalase-positive Campylobacter spp. are given and their relationship with man and animals discussed. Some of the various methods currently used to elucidate the epidemiology of Campylobacter spp., such as serotyping, are also described.

2.1. GENERAL DESCRIPTION OF CAMPYLOBACTER SPECIES

Campylobacter spp. are small, non-sporing Gram negative bacteria with curved, s-shaped or spiral morphology. In order to observe this morphology, the use of a strong stain such as carbol fuchsin or crystal violet is essential, as Campylobacter spp. do not take up stain readily (Goosens et al., 1984). The cells may vary from 0.5 to 0.8 μm in length and 0.2 to 0.5 μm in width. A polar flagellum at one or both ends of the cell gives it a rapid, darting, corkscrew-like motility. Almost all members of the genus are oxygen sensitive; they require either an anaerobic or microaerophilic atmosphere for growth, depending on the species, although an aerotolerant group has been described (Neill et al., 1979; Logan et al., 1982). Another characteristic feature of some of the species is the formation of coccoid forms after a few days of culture, especially on solid media (Goosens et al., 1984).

Campylobacter spp. are oxidase positive and reduce nitrates. Some species are catalase positive. They use amino acids and tricarboxylic acid cycle intermediates as their principal source of energy. They will neither ferment nor oxidise carbohydrates. In

conventional biochemical media, Campylobacter spp. are relatively inert, and classification using such methods is insufficiently discriminative (Karmali and Skirrow, 1984).

The various species and subspecies of the genus Campylobacter can be readily separated into two groups by the catalase test (Bryner and Frank, 1955). Only C. jejuni, C. coli and C. laridis, which are the Campylobacter spp. commonly found in man and birds, are considered in this study. These "thermophilic" catalase positive Campylobacter spp. distinguish themselves by their ability to grow at 42 to 43°C. The key characteristics of the three species are listed in Table 2.1.

2.2. HISTORY OF CAMPYLOBACTER SPECIES

The known history of the genus Campylobacter begins at the turn of the century, although the name "campylobacter", Greek for "curved rod", was not used for the bacterium until 1963, when it was renamed by Sebald and Veron from the original Vibrio. The historical background of our knowledge of Campylobacter spp. is summarised below; a more detailed account of the genus Campylobacter can be found in Karmali and Skirrow (1984).

For many years, Campylobacter spp. were known strictly for their role in veterinary diseases. In 1909, two veterinary surgeons, McFadyean and Stockman, isolated an unknown vibrion shaped bacterium from aborted foetuses in epizootic abortion in ewes. These workers further associated a similar organism with infectious abortions in sheep in 1913, but were prevented from carrying out further studies because of the start of World War I (McFadyean and Stockman, 1909, 1913). When Theobald Smith isolated a bacterium from infectious abortions in bovines in the U.S. in 1919, he assumed it was the same bacterium described by McFadyean, and proposed Vibrio fetus as a name

Table 2.1

Differential Characteristics
of Some Catalase Positive Campylobacters

	C. jejuni	C. coli	C. laridis
Morphology :			
Wavelength of spiral	short	short	short
Rapid coccal formation	+	*	+
Flagellar arrangement	A	A	A
Swarming on moist agar	+	d	d
Growth at :			
25°C.	-	-	-
30.5°C.	-	d	+
43°C.	+	+	+
45.5°C.	d	d	+
Hippurate hydrolysis	+	-	-
Susceptibility to :			
Naladixic acid (30 µg. disc)	S	S	R
Cephalothin (30 µg. disc)	R	R	R
Metronidazole (5 µg. disc)	d	d	R
Tolerance to :			
TTC[1] 1.0 g/L	-	+	-
TTC 0.4 g/L	*	+	d
NaCl 1.5% (agar)	-	-	+
Glycine 1.0%	+	+	+
H_2S Production in :			
Nut.broth with lead acetate strip	+	+	+
Iron/metabisulfite medium	-	-	+
Anaerobic growth in the presence of :			
Fumarate (2 g/L)	-	-	d
TMAO[2] (1 g/L)	-	-	+
Mol. % G+C ± std. dev.	31.6 ± 0.6	32.9 ± 0.8	31.7 ± 0.7

- negative, 0-15% strains positive
d 16-84% strains positive (or sensitive)
* 85-100% strains weak positive
+ 85-100% strains positive
A predom. amphitricate
R resistant
S sensitive

[1] 2,3,5-triphenyltetrazolium chloride
[2] trimethylamine N-oxide hydrochloride

Based on: Benjamin et al., 1983.

(Smith, 1919).

In the early 1930's, Jones et al. (1931) discovered that winter dysentery in cattle was caused by a "vibrio", and named it Vibrio jejuni. A few years later, Doyle (1944) found the causative agent in swine dysentery to be similar to V. jejuni and named it V. coli. Organisms similar to V. jejuni were later found in man after a milkborne outbreak of acute diarrhoea by Levy (1946). The bacterium was seen in the blood of the infected patients and in 20% of faecal smears but could not be isolated on solid media and positively identified (Levy, 1946).

Vinzent et al. (1947) isolated what was considered to be V. fetus from the blood of three pregnant women admitted to hospital for fever of unknown origin. After an illness of approximately four weeks, two of the three patients aborted, and their placentae revealed large necrotic, inflammatory lesions. A similar vibrio was also described by King (1957), but it differed antigenically, biochemically and in its high optimum growth temperature of 42°C. from the vibrio described by Vinzent. King labelled this organism "related vibrio". Due to the inadequacy of culture techniques, and the fact that such organisms could be isolated only from bacteraemic patients, infections remained largely unrecognised for many years, with only 12 cases being reported in the 15 years following King's original description (Wheeler and Borchers, 1961; Middelcamp and Wolf, 1961; Darrell et al., 1967; White, 1967). King's work with the organism stressed the need to develop a selective medium to isolate the vibrio from faeces, as she believed the infection was more common than the reported numbers indicated.

It was in the late 1950's that two distinct types of V. fetus were characterised. Florent (1959) showed that there were two varieties of V. fetus; V. fetus var. intestinalis and V. fetus var.

venerealis. V. fetus var. intestinalis was responsible for sporadic abortions in cattle and originated in the intestine, while V. fetus var. venerealis was transmitted venereally, causing bovine infertility.

It was not until 1972 that Butzler and colleagues reported the first culturally confirmed human case of acute enteritis attributed to Campylobacter species (Dekeyser et al., 1972). The technique used to isolate the organism involved passing the faeces through a 0.65 μ Millipore filter, which held back most of the coliforms but let the vibrios pass through. In Belgium, Butzler et al. (1973) isolated C. jejuni from 5% of children with diarrhoea and 1.3% of asymptomatic children. Five years later in Britain, Skirrow confirmed the isolation of Campylobacter spp. from 7.1% of patients with diarrhoea (Skirrow, 1977).

C. fetus subsp. fetus (formerly called V. fetus var. intestinalis), C. jejuni and to a lesser extent, C. coli and C. laridis are the only Campylobacter spp. known to be pathogenic to man. C. fetus subsp. fetus is a comparatively rare cause of human disease, usually attacking the debilitated or immunocompromised patient. It can easily be distinguished in the laboratory from the thermophilic catalase positive Campylobacter spp. by its ability to grow only at temperatures between 25 and 37^{0}C. Reports of campylobacter enteritis have increased yearly and C. jejuni is now thought to be the commonest cause of human bacterial enteritis (Butzler and Skirrow, 1979).

2.3. TAXONOMY AND NOMENCLATURE OF CAMPYLOBACTER SPECIES

In the 1970's, the genus Campylobacter was classified with the genus Spirillum in the family Spirillaceae (Krieg and Smibert, 1974).

Recently, the genus Spirillum has been divided into three genera: Spirillum, Aquaspirillum and Oceanospirillum. Two additional genera have also been added to the family, Azospirillum and Bdellovibrio (Krieg, 1984). Krieg (1984) admits the difficulty in finding a useful definition for the family Spirillaceae that could include all six genera. He states that it is best "to consider them merely as forming a loose assemblage of taxa that exhibit some morphological and/or physiological similarities." Nonetheless, of the six genera included in this family, the genus Campylobacter is the only genus whose habitat includes humans or mammals, and in which carbon dioxide is required for growth.

Prior to the publication of the Approved Lists of Bacterial Names (Skerman et al., 1980), there were 2 classifications of the thermophilic Campylobacter spp.: Veron and Chatelain (1973) and Smibert (1974). Smibert grouped the thermophilic Campylobacter spp. first described by King as "related vibrios" into a single subspecies he called C. fetus subsp. jejuni. Veron and Chatelain used the names C. jejuni and C. coli for the two catalase positive Campylobacter spp., basing the names on the original Vibrio jejuni found in calves by Jones et al. (1931) and Vibrio coli found in swine by Doyle (1944). The Approved List has chosen the Veron and Chatelain (1973) nomenclature as the officially recognised one (Skerman et al., 1980).

At the time the Approved List was published, it was still not certain whether C. jejuni and C. coli were different species or biotypes of the same species. Harvey (1980) introduced the use of the hippurate test to distinguish between strains of C. fetus subsp. jejuni and C. fetus subsp. intestinalis (see 3.2.1.). Skirrow and Benjamin (1980a) took Harvey's work a step further and attributed hippurate positivity to C. jejuni and hippurate negativity to C. coli. With the use of DNA hybridisation, it has now been proved that C.

jejuni and C. coli represent two distinct species (Owen and Leaper, 1981; Belland and Trust, 1982). It is therefore apparent that studies carried out prior to this time on what was reported to be C. jejuni could possibly include C. coli.

2.4. CAMPYLOBACTER JEJUNI AND CAMPYLOBACTER COLI

C. jejuni accounts for the majority of Campylobacter spp. isolated from man (Skirrow and Benjamin, 1980; Karmali et al., 1983). It has also been isolated from a wide variety of animal species, including dogs, cats, cattle, sheep, pigs, poultry, wild birds, monkeys and even some rare species found in zoos (Skirrow and Benjamin, 1980b; Luechtefeld and Wang, 1982). C. jejuni is also a cause of sporadic abortion in sheep (Smibert, 1978).

C. coli is most commonly associated with pigs. However, it has also been isolated from poultry, cattle and domestic animals (Skirrow and Benjamin, 1982; Walder et al., 1983). It is responsible for between 3% and 5% of all human cases of campylobacter gastroenteritis (Skirrow and Benjamin, 1982; Karmali et al., 1983).

Colonies of C. jejuni on solid moist media have a greater tendency to spread than C. coli. C. coli tends to grow faster and is not prone to such a rapid coccal transformation in the presence of oxygen as C. jejuni (see Table 2.1.). The optimum temperature for growth for both C. jejuni and C. coli is 43°C., although both will grow at 37°C. (see Table 2.1.). At 30.5°C., 78% of C. coli strains will grow and 90-100% of C. jejuni strains will not (Karmali and Skirrow, 1984). Hippurate hydrolysis is a distinguishing feature which separates C. jejuni from the rest of the catalase-positive Campylobacter species (see 3.2.1.).

The use of different characteristics to distinguish between the thermophilic Campylobacter spp. has evolved over the past few years in order to elucidate the epidemiology of the different species in man and animals. Several schemes have been proposed to subdivide C.jejuni and C. coli into various subgroups and biotypes (see 2.6. and 3.2.).

2.5. CAMPYLOBACTER LARIDIS

In 1980, when Skirrow and Benjamin (1980b) set out to classify 1220 Campylobacter spp. strains from various human and animal sources, they found a group of 42 isolates that did not conform to any of the previously described Campylobacter species. These strains had been isolated from 19% of locally caught gulls and only occasionally from other animals and man. They were similar in some ways to the C. jejuni/C. coli group but resistant to naladixic acid. They also exhibited salt tolerance in comparison with C. jejuni and C. coli, sensitivity to 2,3,5 - triphenyltetrazolium chloride (TTC), had a wide range of growth temperatures and readily formed coccoid bodies. Since this initial discovery, similar naladixic acid resistant thermophilic campylobacters (NARTC) have been isolated from water, a dog, a cow, a duck, a healthy Rhesus monkey and a few humans (Benjamin et al., 1983).

After the careful analysis of 10 strains of NARTC by extensive physiological and biochemical tests including DNA base composition and DNA-DNA hybridizations, Benjamin et al. (1983) proposed that the new strain be given the name C. laridis (Laros- sea bird, Greek) The characteristics differentiating it from C. jejuni and/or C. coli are its growth at 30.5°C., negative hippurate hydrolysis, resistance to naladixic acid (30 µg disc), cephalothin (30 µg disc), and metronidazole (5 µg disc), tolerance to 2,3,5 - triphenyltetrazolium

chloride (TTC), tolerance to 1.5% NaCl, its pronounced tendency to undergo coccoid transformation upon exposure to air, H_2S production in an iron/metabisulfite medium and anaerobic growth in the presence of trimethylamine N-oxide hydrochloride (TMAO) (see Table 2.1.). It is interesting to note that the ability of C. laridis to grow anaerobically in the presence of TMAO is a unique feature of this species. It has been suggested that since TMAO is commonly found in marine fish as a product of urinary excretion, the ability of C. laridis to use the oxygen in TMAO could facilitate its growth in the gut of seabirds, whose diet consists mainly of marine fish (R.W.A. Park quoted in Benjamin et al., 1983).

The majority of NARTC isolates has come from birds. Kapperud and Rosef (1983) recorded 20% of the Campylobacter species recovered from Larus gulls were NARTC. They also found a high isolation rate of NARTC from puffins (Fratercula arctica) with all 39 samples yielding NARTC strains. NARTC accounted for 35% of the Campylobacter spp. found in wading birds (Fricker and Metcalfe, 1984).

Human illness associated with C. laridis is rare. C. laridis has been isolated from the faeces of 4 children, 2 with mild recurrent diarrhoea and 2 who were symptomless (Karmali and Skirrow, 1984). Six clinical isolates were sent to the Center for Disease Control in Atlanta in 1982 and 1983 (Tauxe et al., 1985). The illness associated with C. laridis in these cases involved enteritis in four, severe cramping abdominal pain in one and terminal bacteraemia in an immunocompromised host in one. None of these patients was found to have any specific contact with seabirds.

2.6. SEROTYPING OF CAMPYLOBACTER SPECIES

Serotyping as a method for subspecific classification of Campylobacter spp. has proven to be an important step in understanding the epidemiology of the organism. It is only through a more detailed analysis of the organism that we are able to compare the Campylobacter spp. found in man and animals, thus enabling us to complete another piece in the epidemiological puzzle. Several methods of serotyping have been established and will be discussed briefly.

It is natural for the basis of the serotyping techniques for Campylobacter spp. to evolve from techniques currently used successfully to characterise other Gram negative species. The Kauffmann-White scheme (Kauffman, 1966) for the typing of Salmonellae is often used as a model when developing serotyping schemes for Gram negative bacteria. Antigens used for the speciation and subspeciation of Gram negative bacteria are most often associated with the material found outside the capsule, flagellum, and the lipopolysaccharide (LPS) of the outer membrane. This scheme is based on the antigens of each of the three structural components and are known as K, H and O antigens, respectively. These three types of antigens are distinguished by their different tolerance to heat treatment. The O antigen is heat tolerant, or thermostable. It remains unaltered after heating at 100°C. or 120°C. for two hours. The H antigen comes from the flagellum, which is protein and therefore thermolabile. The presence of the K antigen may complicate the easy speciation by these two antigens, as it may be either polysaccharide or protein, being species dependent. It is the heat stable and heat labile antigens that form the basis of the serotyping schemes for Campylobacter species.

The research into serotyping is still in its early stages. It

will probably take many more years before the structure of the LPS can be characterised properly and a reliable scheme developed to serotype the seemingly heterogeneous C. jejuni and C. coli. Several methods have been used successfully in different parts of the world to study many different Campylobacter spp. isolates.

2.6.1. Slide Agglutination of Heat Labile Antigens

Lior et al. (1982) have developed a serotyping scheme for the heat labile antigens of C. jejuni and C. coli using slide agglutination of live bacteria with rabbit antisera that has been absorbed with homologous heated and heterologous unheated cross reactive antigens. All the antisera are absorbed with the homologous heated bacterial suspensions to ensure that only agglutinins against the heat labile factors were present. Cross reactive antibodies to heat labile factors were removed similarly. The slide agglutinations are performed on glass slides using antisera diluted 1:5 with phosphate buffered saline (PBS). A loopful of bacteria is emulsified in a drop of antiserum and observed after 45-60 seconds for agglutination. Due to the stickiness of some of the Campylobacter spp. isolates, the use of a nuclease solution containing 0.1% DNase is necessary to emulsify the organism. Autoagglutinability is determined by mixing a small loopful of the organism with a drop of PBS (Lior et al., 1982).

Since the original publication on this method, Lior has increased the serotyping scheme from the identification of 21 serogroups to 36 serogroups (Lior, 1984b). Of 1504 C. jejuni and C.coli isolates Lior investigated, 1163 from human and 341 from nonhuman sources, 85% (1273 cultures) were typable in a single serum or various pairs of sera. Eleven percent (174 strains) were found to be untypable and 4% (58 strains) were autoagglutinable in saline.

Wenman et al. (1985) have recently analysed the outer membrane proteins of C. jejuni and other Campylobacter spp. using immunoblotting, a technique that allows the identification of specific polypeptide antigens within a complex protein mixture. The most highly conserved outer membrane protein antigen was the flagellum, with a molecular weight of 62,000. The flagellar protein appears to be an essential determinant of the heat labile antigen typing scheme; an aflagellar mutant could not be successfully typed using the heat labile serotyping system, although its parental isolate was able to be typed.

Wenman et al. (1985) also illustrated that the flagellar protein is the dominant immunogen recognised during C. jejuni infections in humans by reacting the sera of convalescent patients with their homologous C. jejuni isolates using immunoblot analysis. This technique also illustrated a unique outer membrane protein antigenic profile recognised by rabbit antisera for each Campylobacter species.

2.6.2. The Passive Haemagglutination Test For Heat Stable Antigens

The passive haemagglutination test (PHA) for serotyping C. jejuni and C. coli strains was developed by Penner and Hennessy (1980) and Lauwers (1981). It uses the ability of the thermostable antigen to modify mammalian erythrocytes and make them agglutinable in antisera containing antibodies directed against these particular antigens as the basis for serotyping Campylobacter spp. isolates. The Penner and Hennessy method was chosen to serotype isolates collected in this study. For a detailed description of the technique, see 3.3.

Penner et al. (1982) typed a total of 609 isolates from human, animal and environmental sources by the PHA method, using 36 antisera. The majority of the isolates typed with only one antiserum but a few (2.6%) reacted to two or more antisera. Ninety percent of the

isolates were able to be assigned a Penner serotype. Also tested in Penner's study was a "blind" trial of isolates from outbreaks mixed with unrelated isolates. The PHA system was able to show that the epidemiologically linked isolates could be separated from the other isolates by their serotypes.

The Lauwers method is similar to the Penner and Hennessy method, differing slightly in the use of 5 subcutaneous inoculations with Freund's complete adjuvant followed by intravenous injection for the production of antisera in rabbits and the use of human Rh negative erythrocytes for sensitisation (Lauwers, 1981).

Lauwers (1984) also confirmed the idea of a two species classification scheme for C. jejuni and C. coli. After serotyping a large number of isolates, he found that C. jejuni isolates generally reacted with the antisera prepared against C. jejuni strains, and C. coli strains reacted against C. coli antisera. Only 4 of the 222 isolates that were hippurate negative reacted with C. jejuni antisera. However, more studies need to be carried out on these isolates before they can be definitively classified as C. coli rather than hippurate negative C. jejuni. Lauwers results indicate that each species possesses its own set of specificities for the thermostable antigen. Lauwers also used his system to investigate the serotypes of isolates from known outbreaks and correlated a single serotype with a single outbreak (Lauwers, 1984).

There are several advantages in using the PHA method to serotype Campylobacter spp. isolates. The antigen extract is easily prepared, and can be used in many additional immunological techniques that may further enlighten the epidemiological and serological picture: immunodiffusion, immunoelectrophoresis and polyacrylamide gel electrophoresis. The use of microtitre trays allows for automation to be used for the titrations and also lessens the quantity of antisera

needed to perform the test. There is no need for the laborious absorbing of antisera used in the Lior method, as cross reactions are rare.

At the time Penner and Hennessy (1980) and Lauwers (1981) adapted the passive haemagglutination test for serotyping C. jejuni and C. coli isolates, the composition of the extracted thermostable material was unknown. Penner hypothesised that it was the O antigen that was being recognised, particularly since the extracted material possessed characteristics similar to the O antigen: stability at 100°C., the capability of adsorbing to erythrocytes to make them agglutinable in specific antisera, and the ability to be extracted both by heating saline suspensions and by treatment with EDTA (Penner and Hennessy, 1980).

Buck et al. (1984) have subsequently performed a variety of biochemical and immunological tests on the antigenic material to discover its composition. The results show a complex mixture of components, including cell wall lipopolysaccharides, the major outer membrane component, flagellin, and possibly some cytoplasmic components.

Mills et al. (1985) have further studied the LPS found in C. jejuni and confirmed the thermostable antigen scheme is based on the LPS (O) antigens. The extracted and purified LPS from 8 strains of C. jejuni was used to sensitise sheep erythrocytes and proved to be more sensitive than the routine boiled suspension, resulting in higher titres in the PHA test. With the use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining, Mills et al. showed differences in C. jejuni serotypes corresponded to differences in the molecular weights of the fast migrating LPS, with strains of the same serotype having the same rate of migration.

2.6.3. Coagglutination and Other Methods

Further progress in the serotyping of Campylobacter spp. has been described by Wong et al. (1985) and involves the typing of both heat stable and heat labile antigens of C. jejuni and C. coli using coagglutination. This enables both typing schemes to be utilised simultaneously to characterise the strains. Wong et al. (1985) prepared the two different types of antisera according to the original methods of Penner and Hennessy (1980) and Lior (1982). These were then used to sensitise a 1% suspension of protein A positive Staph. aureus (Cowan strain). The 35 Penner antisera were pooled in groups of 4 or 5 to facilitate the testing of each isolate with all of the available antisera. The isolate was then tested with the individual antisera within the reactive pool. This was also done with the Lior antisera. To confirm their findings, Wong et al. (1985) serotyped the isolates using the passive haemagglutination test and slide agglutination.

The coagglutination test has several advantages. It was shown that by the use of Dnase in the antigen suspension, rough strains could easily be typed by coagglutination as the specific agglutination occurred rapidly before autoagglutination could take place. There were fewer cross reactivities occurring among the different heat stable antigens compared with the PHA test, with the homologous typing reagents always giving a stronger, faster reaction (Wong et al., 1985). Coagglutination is much less time consuming than the PHA method which involves extraction, sensitisation and washing steps. The use of monospecific antisera for the detection of the heat labile antigen is only necessary for the final confirmation of the serotype, as unabsorbed pooled antisera is used in the presumptive part of the test.

There have been many reports of serotyping C. jejuni and C. coli isolates. Abbott et al. (1980) prepared antisera from strains from patients with C. jejuni and C. coli infections and used these to test for both heat labile and heat stable antigens using tube agglutination with heated and formolised suspensions. He was able to distinguish epidemic strains from non epidemic strains using these antisera. Kosunen et al. (1982) performed direct slide and tube agglutinations as well as coagglutination and latex agglutination to demonstrate the heat labile and heat stable antigens. Direct immunofluorescence has proved to be a useful way to serotype isolates (Hebert et al., 1983). This method has an advantage in that it can be used to test environmental and clinical specimens for Campylobacter species directly. However, not all laboratories have the expensive fluorescence equipment needed to carry out these procedures.

CHAPTER 3. MATERIALS AND METHODS

3.1 GROWTH OF CAMPYLOBACTER SPECIES

3.1.1. Culture Media

There have been several selective media developed to isolate Campylobacter spp., among them Skirrow's (Butzler et al., 1973), Butzler's (Lauwers et al., 1978), Blaser's (Blaser et al., 1978), and Preston's (Bolton and Robertson, 1982). Skirrow's medium contains vancomycin, polymixin B and trimethoprim (TMP) and uses Blood Agar Base No. 2 (Oxoid, CM 271) and 5% lysed horse blood. Butzler's medium is more selective for the isolation of Campylobacter spp. as it contains cefazolin and novobiocin instead of TMP to suppress Proteus species. The addition of actidione (cycloheximide) inhibits the presence of yeast. It contains bacitracin and colistin and uses 5% sheep blood (or other animal blood), with a basal medium of thioglycollate medium USP. Blaser's medium is similar to Skirrow's with the addition of cephalothin and amphotericin. Preston's is also similar to Skirrow's medium but with rifampicin replacing vancomycin as a more economic way of inhibiting the swarming Bacillus species. Cycloheximide is also added to inhibit fungi and yeasts.

In a comparative study (Bolton et al., 1983) made between the aforementioned media using human and animal faecal specimens as well as environmental specimens, it was shown that Preston's medium gave the highest isolation rate. It was also shown that the Preston's enrichment broth gave a higher isolation rate than direct plating onto Preston's medium. In another trial, Bolton and Robertson (1982) showed the superiority of Preston's medium over Skirrow's medium when dealing with faecal specimens from human, cattle, pigs, and gulls.

Not only were less organisms other than Campylobacter spp. such as normal faecal flora, found growing on the Preston's medium, but the amount of growth of Campylobacter spp. was greater than that found on Skirrow's medium. The use of Preston's enrichment broth produced more positive Campylobacter spp. isolations than direct plating when culturing both animal and avian specimens (Bolton and Robertson, 1982). This is an advantage when dealing with samples containing very small numbers of Campylobacter species (Fricker et al., 1983). Therefore, the selective media used in this study for isolating small numbers of Campylobacter spp. from water and avian sources was Preston's enrichment broth and Preston's solid medium (see Appendix 1).

The only modification made to the Preston's medium and enrichment broth was the omission of the antifungal agent, amphotericin, for the human and water samples. As all the human isolates received were pure Campylobacter species, and not mixed with faecal flora, it was deemed an unnecessary addition. It was also found to be unnecessary in the water samples. However, in the case of the gull samples, the antifungal agent was necessary, as fungi would grow over the entire plate if not inhibited by amphotericin. The advantage of the Preston's plate when dealing with human isolates was the ease with which individual colonies could be picked for further study. Blood agar plates, even when streaked for isolation, produced wet spreading colonies that ran together, making individual colonies impossible to distinguish.

FBP supplement (see Appendix 1) was added to the Preston's plates, enrichment broths and Columbia blood agar plates. This combination of ferrous sulphate, sodium metabisulphite and sodium pyruvate destroys the toxic hydrogen peroxide and superoxide anions and therefore increases aerotolerance and enhances the growth of

Campylobacter species (George et al., 1978).

3.1.2. Cultural Characteristics

Since Campylobacter spp. are sensitive to oxygen, a microaerophilic environment is essential for growth and viability. The atmosphere for incubation should contain approximately 5% oxygen and 10% carbon dioxide (Goosens et al., 1984). The easiest way to obtain this environment, and the method used in this study, involves the use of the Gaspak® jar (BBL; without a catalyst) and a gas generating envelope. The gas generation is activated with the addition of 10 mls. water, with the lid subsequently finger tightened to seal the jar. It should be noted that once the jar is opened, an additional hour is needed for the atmosphere to resume the microaerophilic condition should a new envelope be activated (Goosens et al., 1984). All Preston's plates and blood agar plates were placed in Gaspak® jars and left to incubate for 48 hours at 42° C. before opening.

3.1.3. Human Isolates

Human Campylobacter spp. isolates were supplied on a cotton wool swab immersed in Amies Charcoal Medium (Medical Wire and Equipment) from Aberdeen City Hospital, Monklands District General Hospital (MDGH), Falkirk Royal Infirmary, and Dumfries Royal Infirmary. Fresh isolates were also obtained from Stobhill Hospital, Glasgow when available. Upon arrival, the isolates were swabbed on a Preston plate and inoculated into an enrichment broth (see Appendix 1 and 3.1.1.). The surface of a Preston plate was streaked for isolation and incubated in a Gaspak® jar (see 3.1.2.) for 48 hours at 42°C. The broth was incubated at 42°C. for the same length of time, after which it was stored at 4°C. The broth culture was retained as a reserve

isolate.

A single colony randomly selected from the Preston plate was placed in an enrichment broth and incubated for 48 hours at 42°C. The broth was then plated for confluent growth onto 4 blood plates containing FBP supplement (see Appendix 1) and incubated as above, with a nalidixic acid (NA) disc (30 µg) placed on one of the plates (see 3.2.2.).

After 48 hours incubation, the NA sensitivity was recorded (see 3.2.2.), a hippurate test performed (see 3.2.1.), and the purity checked by a Gram stain. A 10 µl loopful of the organism was placed in each of two FBP glycerol vials to be stored at -70°C.(see Appendix 1). The bacterial growth from the remaining three plates was used to make the antigen for serotyping (see 3.3.2.).

3.1.4. Gull Samples

The gull samples were treated similarly to the human isolates. A cloacal swab was taken from each gull (see 5.1.2.) and placed immediately into a Preston's enrichment broth. After 48 hours' incubation at 42°C., 10 ul of broth were plated for separate colonies onto half of a freshly made Preston's plate. The plates were then placed in a Gaspak® jar (see 3.1.2.) and incubated for 48 hours at 42°C.

After incubation, a single colony with the characteristic growth and Gram film appearances of *Campylobacter* spp. was inoculated into a Preston's broth and incubated as before. The broth was then swabbed for confluent growth onto three blood agar plates. A NA disc (30 µg.) was placed on one of the plates. After incubation at 42°C. for 48 hours, the sensitivity was recorded (see 3.2.2.) and a hippurate hydrolysis test was also performed (see 3.2.1.). A carbol fuchsin stain was carried out on the organism from one of the remaining two

blood agar plates to confirm the typical Campylobacter spp. morphology. If on the basis of the above tests it was suggested that the organisms were C. jejuni or C. coli (see 3.2) a 10 µl loopful of these organisms was placed in an FBP glycerol broth (see Appendix 1) and frozen at -70°C. Two blood agar plates were swabbed to obtain confluent growth using the broth originally inoculated with a single colony. After a 48 hour incubation period in a Gaspak® jar at 42°C., the confluent growth on the two plates was used to make the antigen for the passive haemagglutination test (see 3.3.2.).

3.1.5. Water Samples

One litre dipped samples of raw water were collected monthly (see 6.2.). All samples were processed within 5 hours of collection. The following two methods were used in the study to isolate Campylobacter spp. from water.

A) The Filter Method

The filter method was strictly a qualitative method. Five hundred millilitres of raw water were coarsely filtered using a Whatman filter No. 113, to remove particulate matter. Two hundred millilitres of the filtered water were then passed through a 0.45 micron filter (Millipore) using a sterilised membrane filtration apparatus consisting of a base supporting a porous disc (Gallencamp) on which the sterile filter is placed. After filtration, the filter was aseptically placed in 25 mls. of Preston's enrichment broth (see Appendix 1) and incubated at 42°C. It was then subcultured after both 24 and 48 hours onto Preston's plates which were then incubated microaerophilically at 42°C. for 48 hours before examination. All suspect colonies were processed exactly as described in 3.1.4. above.

B) The Most Probable Number (MPN) Method

The MPN method (Bolton et al., 1982) was the quantitative method used to enumerate the Campylobacter spp. found in the water samples. Using the 500 mls. of remaining unfiltered water, one ml. was placed in each of 10 bijoux containing 5 mls. of Preston's enrichment broth. The lids on the bijoux were loosened and the broths placed in the Gaspak® jar at 42°C. The broths were subcultured to Preston's plates after both 24 and 48 hours incubation and incubated as before (see 3.1.2.).

After performing a confirmatory Gram stain on any growth found on the Preston plate, a single colony was taken to a Preston's enrichment broth, incubated for 48 hours at 42°C. and processed in the same manner as the isolates from the gulls (see 3.1.4.).

3.2. BIOTYPING

The increasing numbers of cases of campylobacter enteritis being reported, together with the increasing interest in the epidemiology of the organism, has resulted in the development of several schemes for biotyping. The hippurate hydrolysis test, resistance to naladixic acid and a rapid H_2S test in an iron containing medium have been used to distinguish between C. jejuni, C. coli and naladixic acid resistant thermophilic campylobacters (Skirrow and Benjamin, 1980a). The addition of the DNA hydrolysis test to the hippurate hydrolysis test and rapid H_2S test has enabled C. jejuni isolates to be separated into 4 biotypes, and C. coli and C. laridis each into 2 biotypes (Lior, 1984a). A combination of hippurate hydrolysis, DNA hydrolysis and growth on Charcoal Yeast Extract agar has also been proposed as a biotyping system for C. jejuni, resulting in 8 biotypes. Using these criteria, however, it has proved difficult to identify C. coli (Hebert

et al., 1982). The importance of identifying strains at the species level before subdividing them into biotypes was stressed by Roop et al. (1984a). They point out that the differentiation of C. jejuni and C. coli by means of hippurate hydrolysis, growth in MM (minimal media) and H_2S production in a triple sugar iron slant can be used in addition to alkaline phosphatase activity and DNase activity to place C. jejuni isolates into one of four "biovars" and C. coli into the same number of biovars.

To speciate the Campylobacter spp. found in this study, the two simplest tests were used: hippurate hydrolysis and naladixic acid (NA) susceptibility. These two tests allowed the thermophilic Campylobacter spp. to be divided into C. jejuni (hippurate positive, NA sensitive), C. coli (hippurate negative, NA sensitive), and C. laridis (hippurate negative, NA resistant).

3.2.1. The Hippurate Hydrolysis Test

The rapid hippurate hydrolysis test was originally described for the differentiation of Streptococcus agalactiae (group B) from Streptococcus pyogenes (group A) (Hwang and Ederer, 1975). Glycine, one of the end products of the hydrolysis of hippurate was detected using the Hwang and Ederer method and thin layer chromatography in parallel by Harvey (1980). The test was performed on isolates of C. fetus subsp. intestinalis and C. fetus subsp. jejuni and proved to be instrumental in differentiating between the two. Harvey also found two strains of what he thought were C. fetus subsp. jejuni which differed in their inability to hydrolyse hippurate. He hypothesised that these two strains might represent the first detectable biotype within the group.

The rapid hippurate hydrolysis test was performed on each isolate in this study (Hwang and Ederer, 1975). A 1% solution of hippuric

acid was freshly made and aliquoted in 0.4 ml amounts into glass tubes. A 10 µl loopful of the organism was then emulsified into the solution to obtain a heavy suspension. The tubes were placed in a waterbath at 37°C. for two hours. A 3.5% ninhydrin solution was freshly made in equal parts of butanol and acetone. After the two hour incubation period, 0.2 ml of the ninhydrin reagent was added to each tube. After 10 minutes incubation in the 37°C waterbath, the results were recorded. A positive reaction was noted when the colour of the suspension was dark blue-violet, which indicated the presence of glycine resulting from the hydrolysis of hippurate. A colourless or light purple colour was considered negative.

3.2.2. Nalidixic Acid Susceptibility

After 48 hours incubation in a Preston enrichment broth, the organism was swabbed onto a blood agar plate for confluent growth. A 30 µg disc of Nalidixic Acid (Mast Labs) was placed on the plate, which was then incubated at 42°C in a microaerophilic environment for 48 hours. An inhibition zone of 6 mm or more from the edge of the disc was recorded as sensitive (Skirrow and Benjamin, 1982).

3.3. SEROTYPING

Serotyping in this study was performed by passive haemagglutination as described by Penner and Hennessy (1980).

3.3.1. Production of Antisera

All Penner strains were obtained from Dr. J. L. Penner, Toronto, Canada, via Dr. D. M. Jones, Public Health Laboratories, Manchester, England, and the the University of Goteborg, Sweden, Department of

Clinical Bacteriology Culture Collection.

Confluent growth of two blood agar plates of the Penner strain was washed twice in sterile 0.85% saline and resuspended in sterile 0.85% saline to an optical density of .375 at 625 nm. New Zealand white rabbits of approximately 3 kilograms weight were inoculated intravenously 5 times over a two week period at doses of 1,2,2,4 and 4 mls, with the last of the 2 ml. and the last of the 4 ml. doses being double the concentration. One week after the last dose, cardiac puncture was performed. The blood was allowed to clot at 4°C overnight before being spun down and separated. The sera were aliquoted in 0.5 ml amounts and stored at -70°C. Approximately 60 mls of antisera was produced from each rabbit.

The antisera were produced in batches of 3 or 4, therefore a stock of several were collected before beginning the serotyping of the isolates. The human isolates were the first to be serotyped using the antisera to the following Penner strains: 20,18,24,27,11,16,8,15, 1,4,6,19,2,9, and 10. When the next group was produced, antisera to Penner 7,31,37,55,and 44, the gull and water isolates were typed with all 20 available antisera. Aliquots, stored throughout at -70°C., of the isolates that did not type with the aforementioned antisera were typed with the antisera produced at a later date. The last group to be added was the antisera to Penner strains 30,42,48,5,45,3,13,23, and 35.

The decision on which antisera to use for this study was based on the collaborative study of human, animal and environmental isolates made by Jones *et al.* (1984).

3.3.2. Preparation of Antigen

Extracts from the Penner type strains, and extracts from the human, gull and water *Campylobacter* isolates were all prepared in the

same way. Cells from three blood plates yielding confluent growth were washed twice in 20 mls. sterile 0.85% saline. A heavy suspension of organisms was obtained by resuspending the washed cell pellet in 2 mls. of sterile saline. The suspension was placed in a waterbath at 100°C. for one hour. After cooling to room temperature, the suspension was spun for 15 minutes at 3000g. The supernatants from the Penner strains were stored at 4°C. and used for determining the homologous titre in the haemagglutination test (see 3.3.3.). The extracts from the isolates were frozen at -70°C.

3.3.3. Obtaining An Homologous Titre

To obtain the homologous titres for the Penner type strains the following procedure was carried out. Each Penner antigen extract was diluted 1:5 with phosphate buffered saline (PBS-3.5 g Na_2HPO_4, 6.8 g NaCL per litre adjusted to pH 7.0 with concentrated HCl) in 75 by 12 mm. soda glass test tubes (Samco). An equal volume of a 1% suspension of sheep red blood cells which were washed twice in PBS was then added. The tubes were placed in a waterbath at 37°C. for one hour. After centrifugation (approximately 300g. for 5 minutes), the cells were washed with PBS three times and finally resuspended to give a 0.5% erythrocyte suspension.

The antisera were diluted 1:40 with PBS and doubly diluted in 25 ul amounts in an 8 by 12 U-welled microtitre tray (NUNC). Twenty five microlitres of the appropriate sensitized erythrocytes were added to each well, gently shaken, and incubated for one hour at 37°C. The tray was then incubated at 4°C. overnight.

The homologous titre of each antiserum was determined by the highest dilution of antiserum that showed agglutination. Wells with an intact red cell pellet were read as negative and wells with a

diffuse pattern of red blood cells were read as positive. The homologous titre was then used in determining the dilutions of each antiserum used in the haemagglutination test with the extracts from the human, gull, and water isolates (see 3.3.4. and Table 3.1).

In order to ensure consistent results, a monthly homologous test of all antisera was performed. The antisera were purposely aliquoted into small amounts of 0.5 ml. to reduce the possibility of contamination and loss of titre. At no time during the experiment did the homologous titres vary more than one dilution.

The antisera produced were also tested heterogeneously, i.e. every Penner antigen was tested against every Penner antiserum. This was ensure the specificity of each antiserum (see Table 3.1).

3.3.4. Serotyping of Isolates

Serotyping of the isolates was divided into two parts. The first part involved testing the antigen extracts with pools of the antisera. The second part involved the testing of the antigen extract with the individual constituents within the pool or pools which yielded a positive reaction.

To make the pools of antisera, the antisera were divided into groups of four having similar titres ranging from 160 to 5120 (Table 3.2.). Each antiserum was diluted to a concentration four doubling dilutions below the required titre obtained with the homologous antigen. For instance, an antiserum with a titre of 2560 was diluted 1:160, then equal amounts of three other diluted antisera in the group were combined, thus increasing the dilution fourfold, to make it a 1:640 dilution. The final result was a pool of antiserum to four Penner serotypes, each at a concentration two doubling dilutions below the homologous titre. Twenty five microlitres of each pool was placed in the microtitre tray and doubly diluted once, using PBS as

Table 3.1. Homologous and Heterologous Titres of *C. jejuni* and *C. coli* antisera (unabsorbed)

\- Titre < 40
* *C. coli* strain

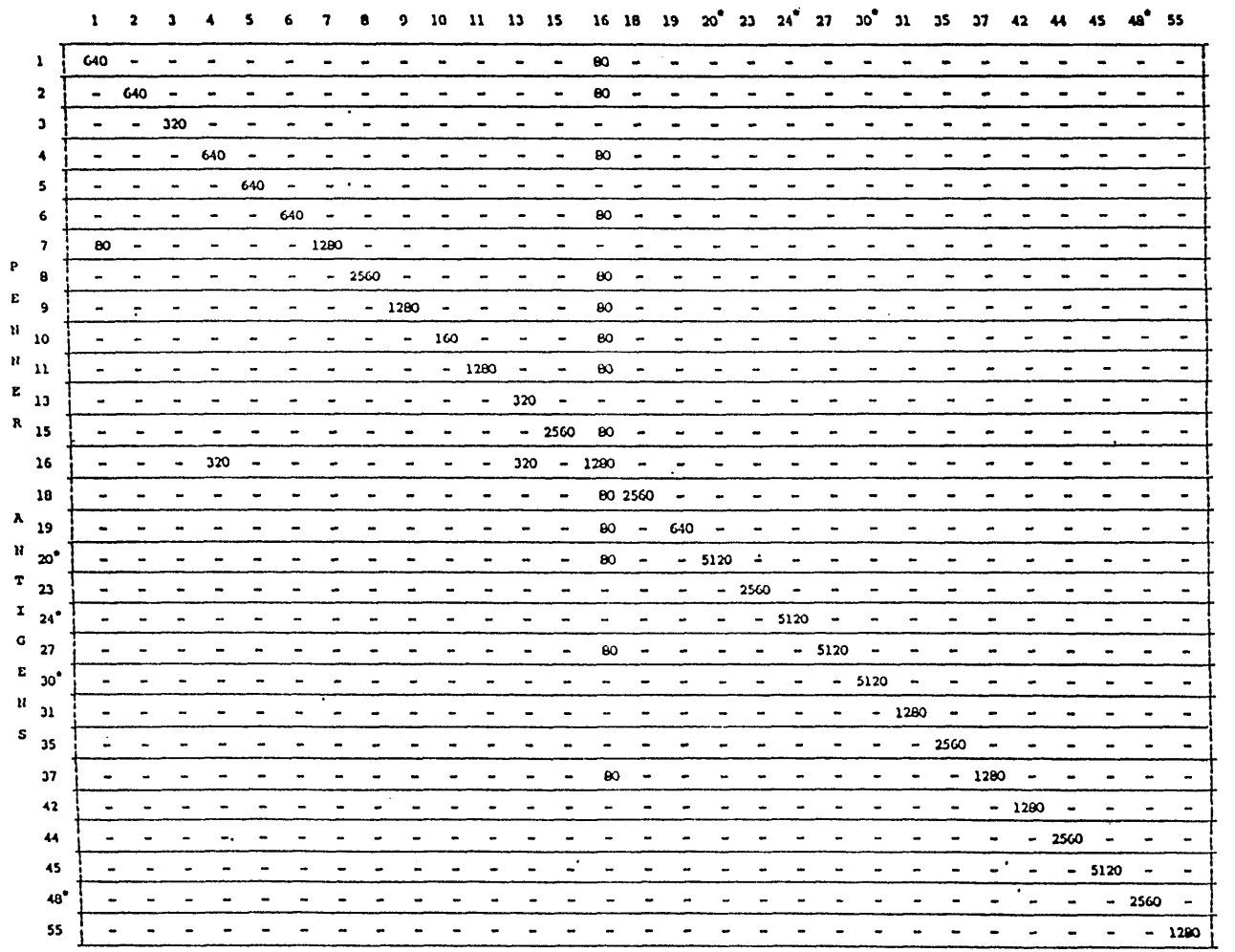

PENNER ANTISERA
1 2 3 4 5 6 7 8 9 10 11 13 15 16 18 19 20° 23 24° 27 30° 31 35 37 42 44 45 48° 55
PENNER ANTIGENS
1 640 80
2 640 80
3 320
4 640 80
5 640
6 640 80
7 80 1280
8 2560 80
9 1280 80
10 160 80
11 1280 80
13 320
15 2560 80
16 320 1280
18 80 2560
19 80 640
20° 80 5120
23 2560
24° 5120
27 80 5120
30° 5120
31 1280
35 2560
37 80 1280
42 1280
44 2560
45 5120
48° 2560
55 1280

Table 3.2.

Penner Antisera Groups

	Penner Antisera	Titre
Group 1	20	5120
	18	2560
	24	5120
	27	5120
Group 2	11	1280
	16	1280
	8	2560
	15	2560
Group 3	1	640
	4	640
	6	640
	19	640
Group 4	2	640
	9	1280
	10	160
	44	2560
Group 5	31	1280
	37	1280
	55	1280
	7	1280
Group 6	30	5120
	42	1280
	48	2560
	5	640
Group 7	3	320
	45	5120
	13	320
	23	2560
	35	2560

the diluent at all times. Each antiserum was therefore at a concentration one and two dilutions below the homologous titre. Twenty five microlitres of the sensitised washed cells were added to the antisera, shaken and incubated at 37°C. for one hour.

After the hour, the wells were observed for agglutination. The sensitised red blood cells were then reacted with the individual antiserum within the pool in which there was agglutination in one or both of the wells.

For the final titration, the antiserum was doubly diluted so that the cells would be titrated against the dilution equivalent to the homologous titre as well as two dilutions below. It was incubated, as before, for one hour at 37°C. and then placed at 4°C. overnight. The Penner serotype was then obtained by observing agglutination in one or more of the wells with the individual Penner antiserum.

3.4. STATISTICAL ANALYSES

All statistical methods used for analysing data in this study were according to Siegel (1956). Yates correction was used in all 2x2 contingency tables.

CHAPTER 4. CAMPYLOBACTER INFECTIONS IN HUMANS

4.1. INTRODUCTION

Campylobacter enteritis is now the most commonly reported form of acute bacterial diarrhoea in Britain (Skirrow, 1982). The continuing increase in reported cases can be attributed to the increase in the number of laboratories that now routinely test for Campylobacter spp. coupled with the development in the selective media used to culture faeces successfully for Campylobacter species. General practitioners can now be more confident that sending faeces for culturing may result in a positive result and are therefore submitting more faecal samples for culturing. The numbers of cases reported are still far less than the actual amount of campylobacter enteritis found in the population.

Another way of examining the impact of Campylobacter spp. is by looking at the number of working days lost due to diarrhoeal illness. Skirrow (1982) estimates that allowing for the proportion of patients unemployed and assuming an average incapacity of three days, approximately 20,000 working days per year are lost, using the numbers from 200 public health and hospital laboratories in England and Wales reported to the Communicable Disease Surveillance Centre (CDSC) in London. This underestimated figure can be compared to a more realistic figure based on a study carried out by Kendall and Tanner (1982) when they estimated one in five patients who consulted general practitioners because of enteritis were infected with Campylobacter spp., giving a projected rate of 1.1% of the population per year. This estimate gives a figure of about 600,000 cases per year for the United Kingdom, which is about 40 times the figure derived from laboratory reports (Skirrow, 1982). While this figure may be an overestimate, nonetheless the amount of discomfort suffered due to

Campylobacter spp. is of a significant enough proportion to warrant extensive studies into its epidemiology.

This chapter provides a background on the epidemiology of Campylobacter spp. in humans. The results of this study concerning Campylobacter spp. isolated from humans in Scotland are presented and discussed.

4.1.1. Human Enteritis

Campylobacter enteritis can present itself in a variety of ways, from the very mild and even asymptomatic cases found in close contacts of symptomatic cases to abdominal pain and cramping severe enough to make the patient consult a doctor or even become hospitalised. It is through these reported cases that we learn the typical picture of gastroenteritis associated with Campylobacter species.

The dose of Campylobacter spp. required to infect a person and cause symptoms of the illness has not been fully researched. A volunteer became mildly ill after he drank milk containing 500 organisms of a strain of C. jejuni isolated from humans after a milk-associated outbreak (Robinson, 1981). This indicates that the infective dose may be relatively small.

The incubation period can range from 1.5 to 7 days, with 3 to 5 days being the average (Butzler and Skirrow, 1979). Half of the patients suffer a febrile, prodromal period followed by nausea, headache, dizziness, abdominal pain, backache and profuse diarrhoea. Fresh blood may appear in the stool after 2 or 3 days (Mandal et al., 1984). The illness usually lasts no longer than a week, but 25% of patients will subsequently have a recurrence of the symptoms, especially the abdominal pain (Mandal et al., 1984). After several days of illness, the patient is weak and exhausted. Once the symptoms

begin to subside, a common mistake is to resume the eating of solids. This will often bring a recurrence of the symptoms and a possible relapse (Butzler and Skirrow, 1979). Although the uncomfortable symptoms may only last for a week or two, the patient may feel unwell for much longer.

Symptomatic patients shed 10^6-10^9 organisms per gram of stool (Mandal et al., 1984). If patients are untreated, their stools can remain positive for 2-5 weeks and occasionally even longer (Butzler and Skirrow, 1979). The infection may be localised in the upper gastrointestinal tract, but recent evidence shows tissue injury may include not only the jejunum and ileum, but also the colon (Mandal et al., 1984).

The use of antibiotics in the treatment of campylobacter enteritis is often unneccessary, as the patient may naturally recover within several days. In cases requiring antimicrobial treatment, erythromycin has been most successful (Mandal et al., 1984). This eradicates the organism from the faeces but if treatment is not started before 4 days after the onset of symptoms, does not alter the natural course of the uncomplicated enteritis (Anders et al., 1981). Erythromycin resistance has been reported in Sweden (8% - Walder and Forsgren, 1978) and in Canada (1% - Karmali et al., 1980). Alternative antimicrobial agents include tetracyline, doxycycline, and clindamycin. Almost all strains of C. jejuni are resistant to penicillin G, cephalosporin, colistin and trimethoprim (Mandal et al., 1984).

Rarely, abdominal pain can become so severe that the patient is admitted to hospital because of what is thought to be acute appendicitis (Skirrow, 1977), cholecystitis (Mertins and De Smet, 1979), or peritonitis. Upon laparotomy, acute inflammation of the ileum, jejunum, and associated mesenteric lymph nodes has sometimes

led the physician to mistake the campylobacteriosis for typhoid fever (Butzler and Skirrow, 1979).

A case of meningitis due to C. jejuni has been reported (Norrby et al., 1980), as well as urinary tract infections (Davis and Penfold, 1979), Reiter's syndrome (Matti Saari and Kauranen, 1980) and Guillain Barré syndrome (Rhodes and Tattersfield, 1982). The reporting of C. jejuni bacteraemia has become more frequent as more laboratories become capable of isolating the organism from blood samples.

4.1.2. Animal Reservoirs

Campylobacter jejuni and Campylobacter coli have been established as commensals in the intestinal tracts of a variety of animal species, including cows, chickens, pigs and sheep (Manser and Danziel, 1985). Since these animals may come in daily contact with man indirectly through meat consumption, it is important to establish procedures to elucidate any epidemiological links between humans and animals. Biotyping and serotyping have helped to reveal the possible relationship between infections in man and those in animals.

A) Poultry

Banffer (1985) reported that C. jejuni and C. coli were isolated from 96% of healthy chickens tested, with fresh faecal samples of chickens from the local slaughterhouse positive (86%) more often than livers bought from the local poulterers shop (56%). Using passive haemagglutination based on thermostable antigens developed by Lauwers (1981) and the Skirrow and Benjamin biotyping scheme (1980a), Banffer illustrated a resemblance between the pattern of bio- and serotypes from patients with the bio- and serotypes found in chickens. Five of the six most common bio- and serotypes found in humans were also common to 48% of the Campylobacter spp. positive chickens. The

correlation was not found when human and pig Campylobacter spp. isolates were compared (Banffer, 1985).

The process of slaughtering chickens allows the intestinal flora to grossly contaminate the surface of the carcass. Washing does not eliminate these organisms (Mehle et al., 1982). The presence of Campylobacter spp. in chickens does not apparently affect their weight at the time of slaughter or increase mortality (Cruikshank et al., 1982).

The three most common Penner serotypes found in humans (1/44, 2, and 4) accounted for just over 50% of the human isolates serotyped and were also common to 32% of the chicken isolates serotyped by Jones et al. (1984). Munroe et al. (1983) found 96% of the chicken isolates serotyped belonged to the 11 C. jejuni serotypes most frequently encountered in human cases of enteritis.

Several outbreaks of human campylobacter enteritis have been attributed to the consumption of raw or undercooked chicken (Rosenfeld et al., 1985; Mouton et al., 1982; Kist, 1982). Severin (1982) found that the method of cooking chicken made a difference in the number of poultry associated cases found. Shorter cooking times, used for fondue or barbeque, were more likely to have a positive correlation with the campylobacter cases than the longer cooking times used in baking, roasting or boiling.

The fact that the majority of uncooked poultry has been found to have such a high contamination rate with Campylobacter spp. makes it a potential vehicle of infection in homes as well as larger industrial sized kitchens. The problem of cross contamination should not be ignored, as it has been demonstrated that a person may have been infected from eating a piece of lettuce served with contaminated chicken livers (Mouton et al., 1982).

B) Cattle

C. jejuni is a normal commensal of cows, with stool positivity peaking in the summer months and declining in the winter months (Robinson, 1982). Sampling of cattle carcasses and offal at abattoirs has shown that the percentage positive rate may vary from 0-32 (Hudson and Roberts, 1982; Bolton et al., 1982; Bolton et al. 1985). Although widespread contamination of the carcasses does take place at the abattoir, samples taken at the wholesale and retail outlets have shown less contamination with Campylobacter species (Bolton et al., 1985; Turnbull and Rose, 1982).

Although contaminated cattle carcasses do not appear to be a major factor in the epidemiology of human campylobacter enteritis, milk has been implicated as a vehicle for numerous outbreaks (Robinson et al., 1979). Faecal contamination or possibly mastitis may lead to the introduction of C. jejuni into milk. Several outbreaks have implicated raw milk as the suspected vehicle and have shown identical serotypes found in the infected humans and from the faeces of cows who have yielded the suspect milk (Blaser et al., 1982; Vogt et al., 1984), but recovery of Campylobacter spp. from the suspected milk has proved difficult. Campylobacter spp. experimentally inoculated into milk has survived for up to 3 weeks when kept at 4^{o}C. (Blaser et al., 1980b). The lack of positive results when culturing milk suspected to be the source of outbreaks could be due to the organism's sensitivity to increased concentrations of lactic acid found in spoilt milk (Waterman and Park, 1982).

Pasteurisation has been found to be an effective method for eradicating Campylobacter spp. from milk (Doyle and Roman, 1981). The consumption of unpasteurised milk has been the most common cause of milk-borne campylobacter enteritis in humans (Blaser et al., 1984). A failure in the pasteurisation process might have been the cause of an

extensive milk-borne outbreak in Luton affecting 2500 school children (Jones and Willis, 1982).

C) Swine

Swine are known to carry mainly C. coli, and occasionally C. jejuni as intestinal commensals (Banffer, 1985; Manser and Danziel, 1985; Munroe et al., 1983). Munroe et al. (1983) found that the serotypes of the C. coli isolates from pigs were not commonly found among human isolates. Banffer (1985) also showed negative correlation between human and pig serotypes using the Lauwer serotyping scheme. Although pig carcasses may be contaminated with Campylobacter spp. during the slaughtering process, the level of contamination diminishes upon storage so that at the retail level, contamination is relatively low. This could possibly be due to the oxygen sensitivity of Campylobacter spp., which would prevent it from growing on cold storage at the abattoirs and butcher's shops (Bolton et al., 1982; Banffer, 1985).

In a study by Manser and Danziel (1985), an interesting correlation was found between the isolation rate of C. coli in healthy pigs and pigs with diarrhoea. Seventy seven percent of the pigs with diarrhoea had C. coli in their faeces compared to 47% found in healthy pigs. This difference in the isolation rate between sick and healthy animals was not found in cattle or sheep in the same study. Since C. coli accounts for only 3-5% of human cases of campylobacter enteritis in Britain (Skirrow and Benjamin, 1982), pigs are not seen as a significant reservoir of Campylobacter species. This figure, however, could be related to the amount of pork and pork products eaten in this country. In some European countries, where pork products make up a larger part of the diet, the percentage of C. coli isolated from human faeces is greater than in Britain (Skirrow, 1982).

D) Sheep

C. jejuni is found as an intestinal commensal in sheep without apparant morbidity (Blaser et al., 1984). It is also responsible for sporadic abortion in sheep (Smibert, 1978). A study of 100 strains of Campylobacter spp. of ovine origin by Jones et al. (1984) showed 95% to be C. jejuni; 62% of these 100 isolates serotyped as either Penner 1/44, Penner 2, or Penner 4, the most common serotypes found in man. Faecal samples from 281 sheep yielded 61 Campylobacter spp. isolates, with 82% of them being C. jejuni (Manzer and Dalziel, 1985). A survey of abattoirs found 88 out of 126 (77%) of sheep positive for Campylobacter spp. when swabbings of carcasses were cultured (Bolton et al., 1982). However, in the same study, when swabbings were taken at a wholesale butcher and 20 butcher's shops, no Campylobacter spp. were isolated.

E) Dogs and Cats

There have been several differing results in the isolation of Campylobacter spp. from dogs. The parameters needed to be taken into consideration when looking at the figures include the age of the dog (adult vs. puppy), whether the dog is healthy or has diarrhoea, and also the habitat of the dog, whether it is a household pet, resides in a kennel, or is a working dog. Bruce and Fleming (1983) surveyed 100 dogs and found 16% of the 18 faecal sample from dogs less than 6 months old to be positive for Campylobacter species. Seven of the 82 (9%) dogs over 6 months old were found to be positive. Although the study found no significant variation in the recovery rate of Campylobacter spp. between mongrel or pedigree, dog or bitch, symptomatic or asymptomatic, the finding of Campylobacter spp. in 2 of the 4 working dogs was of interest. Unfortunately, this sample size is too small to make any significant judgement, but considering the

environment of the working dog, which includes contact with other domestic animals that are known to carry Campylobacter spp. as intestinal commensals, this is probably not an unexpected result.

Blaser et al. (1980c) showed 5 out of 9 puppies (less than 3 months old) from households of patients positive for Campylobacter spp. excreted C. fetus subsp. jejuni. Unfortunately, the isolates from both the puppies and their symptomatic owners were not able to be compared by serotyping. Blaser et al. found kennel dogs had a higher percentage positive rate than household animals, with puppies in kennels having a higher rate (35%) compared to adult dogs (27%).

Blaser et al. (1980c) also tested kennel cats and found 6% of the kittens under 3 months of age to excrete Campylobacter spp., but found no positives in adult cats. Although there have been documented cases substantiated by serotyping of human campylobacter infections acquired from close contact with kittens and puppies (Blaser et al., 1982; Skirrow et al., 1980; Svedham and Norkrams, 1980), dogs and cats should not be considered a major reservoir of Campylobacter species.

4.1.3. Seasonality

Reports sent to the Communicable Disease Surveillance Centre in London from Public Health Laboratories and hospitals in England, Wales and Ireland have consistently shown the same pattern of isolation, with seasonal peaks occurring in the summer and early autumn (Young, 1982). The Communicable Diseases (Scotland) Unit has also recorded this seasonal trend (Anon., 1986). Similar seasonal patterns have been seen in Belgium, Canada, the Netherlands and the U.S. (Skirrow, 1982).

4.1.4. Age and Sex Specificities

In developed countries, the data on age- and sex-specific

incidences of Campylobacter spp. are usually based on the number of isolations obtained from patients with diarrhoea, and are therefore affected by age-specific differences in the infection-to-illness ratio and by differences in the age-specific rates for which the faecal samples were obtained from ill persons (Blaser et al., 1984). Keeping this in mind, Blaser et al (1984) postulate a bimodel distribution of Campylobacter spp. by age with the highest incidence in infants and persons 20-29 years of age. In developing countries, the incidence in children seems to be greater. Forty percent of children in South Africa and Bangladesh aged nine months to two years excreted C. jejuni (Bokkenhauser et al., 1979; Blaser et al., 1980a). It is difficult, however, to assess the true isolation rate from children in these countries, especially when healthy children are also found to be excreters. In Gambia, this asymptomatic carriage was found only in children less than 5 years old (Billingham, 1981). The incidence in developed countries of healthy people with Campylobacter spp. is approximately 1-2% per year (Blaser et al., 1984). The prevalence of C. jejuni in developing countries can be much greater than in industrialised countries, however more studies need to be carried out before these differences can be quantified.

Sex specificities do not seem to be of significant importance in Campylobacter spp. infections. The CDSC report for 1977-80 showed a male:female ratio of 1.16:1.0 (Young, 1982). These data do not include information concerning the proportion of males and females who submit faeces for culturing for Campylobacter species. Blaser et al. (1983) took this into account when producing the isolation rates for males and females in a study of eight hospitals in the U.S. and found no difference in the rates. Interestingly, a sex difference was seen on the same study when the seasonality was examined, with males having

a higher isolation rate in the peak months of June-September (9.3%) as compared to the rate for the remaining months (4.5%). Females showed a peak isolation rate of 7% for June-September and 5.7% for the remainder of the year.

4.2. RESULTS

Human campylobacter isolates were received from 5/7/84 to 29/6/85 from the following hospitals: Stobhill General Hospital, Glasgow; Monklands District General Hospital (MDGH); Falkirk District Royal Hospital (FDRI); Dumfries Royal Infirmary and Aberdeen City Hospital.

The total number of isolates received and confirmed as Campylobacter spp. was 650. Figure 4.1 shows the total number of isolates received and confirmed as Campylobacter spp. in four-weekly periods during the study and the total numbers of Campylobacter spp. reported to the Communicable Diseases (Scotland) Unit for the same period (S. Moffatt, personal communication).

The total number of strains typable with the Penner and Hennessy passive haemagglutination test (Penner and Hennessy, 1980) using 29 antisera was 492 (75.7%). Of 610 isolates tested for hippurate hydrolysis (see 3.2.1.), 29 isolates were hippurate negative, and therefore believed to be C. coli (Skirrow and Benjamin, 1980a). Of these 29 hippurate negative isolates, 9 typed with the C. jejuni antisera, thus leaving a total of 20 isolates thought to be C. coli. This makes C. coli accountable for approximately 3.3% of the Campylobacter spp. isolates submitted.

The overall distribution of the Penner serotypes found is shown in Table 4.1. The four most common serotypes were Penner 2, 4, 1 and 31, and accounted for 38.3% of the isolates examined and 50.6% of those which were typable.

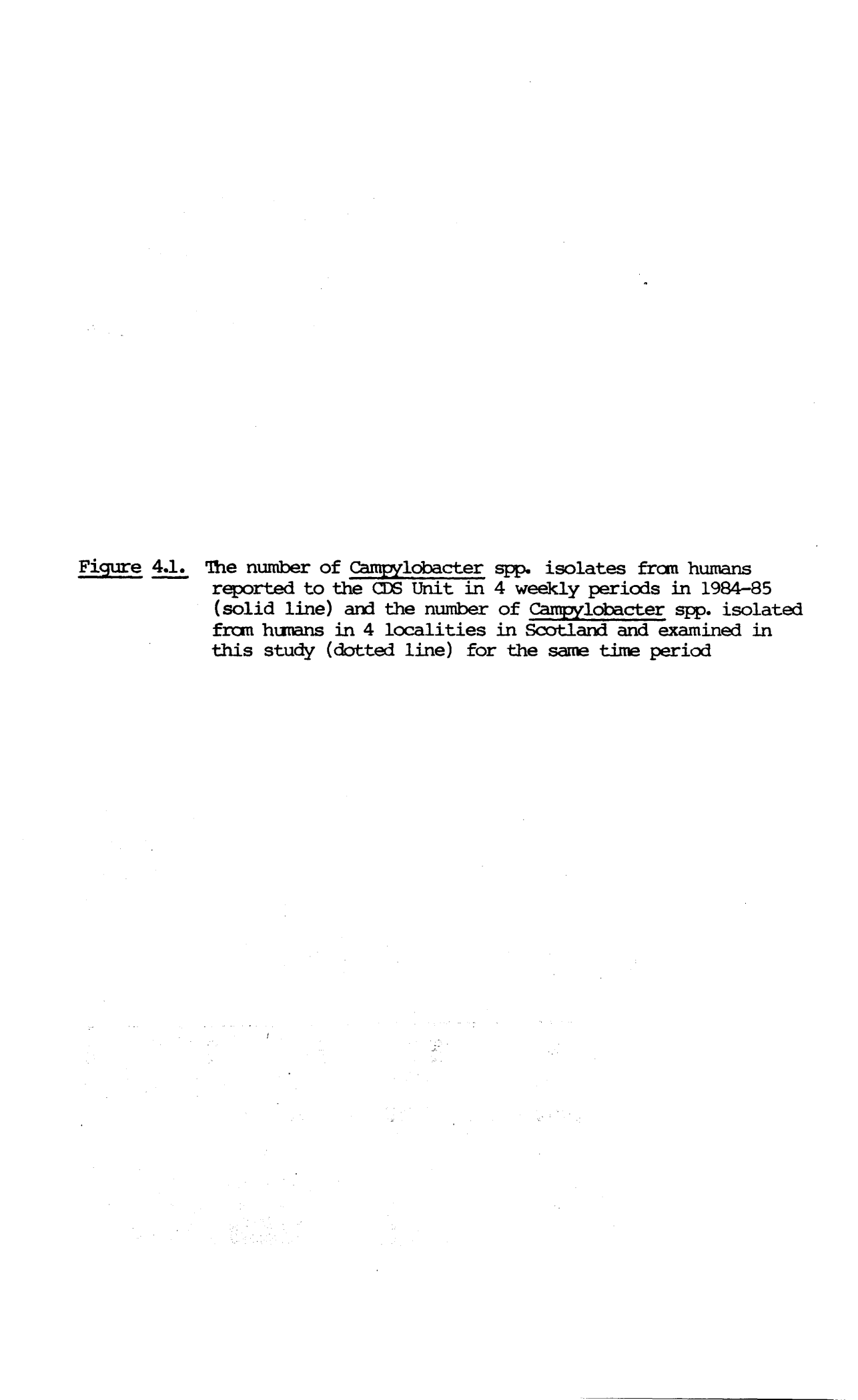


Figure 4.1. The number of Campylobacter spp. isolates from humans reported to the CDS Unit in 4 weekly periods in 1984-85 (solid line) and the number of Campylobacter spp. isolated from humans in 4 localities in Scotland and examined in this study (dotted line) for the same time period

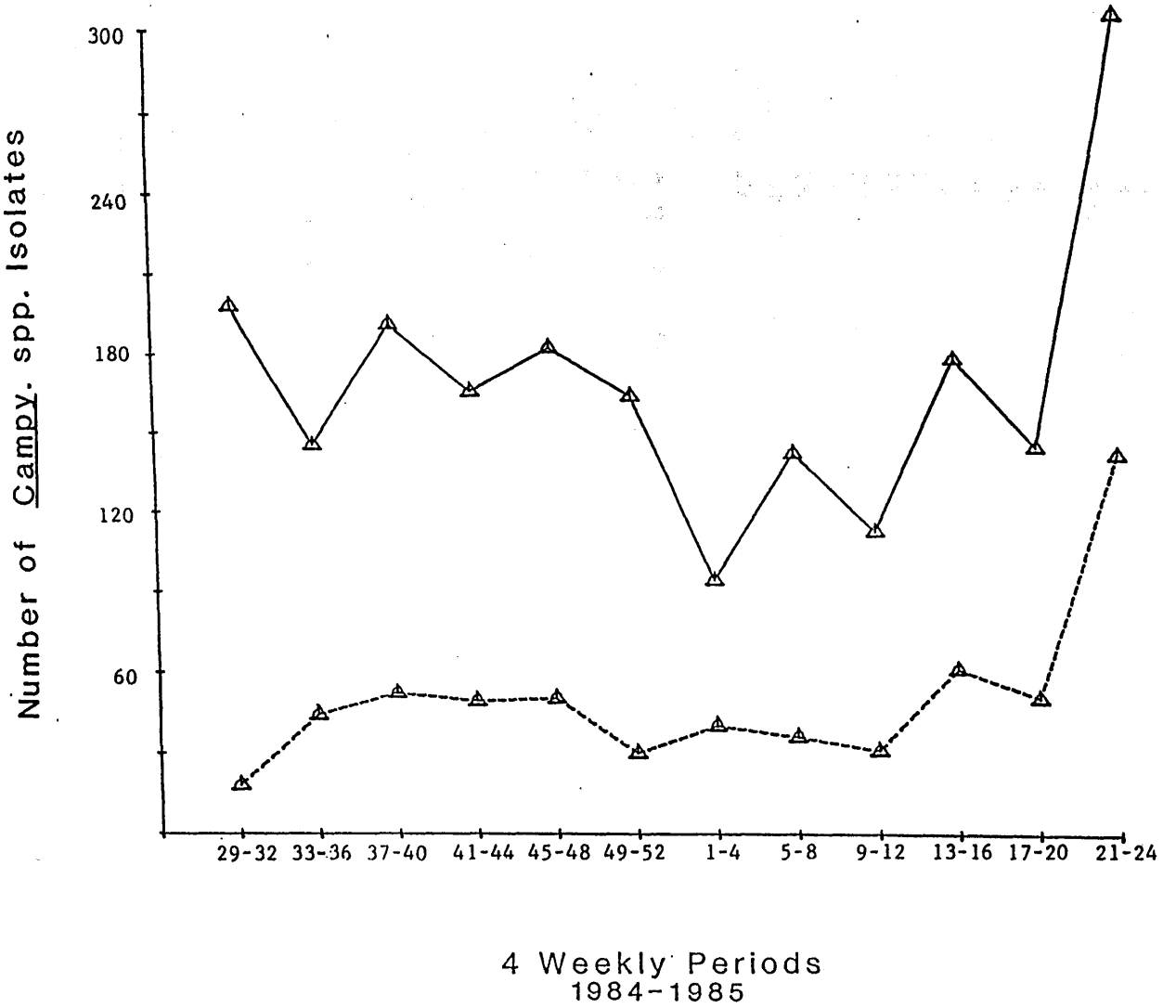

Number of Campy. spp. Isolates
300
240
180
120
60
29-32
33-36
37-40
41-44
45-48
49-52
1-4
5-8
9-12
13-16
17-20
21-24
4 Weekly Periods
1984-1985

Table 4.1.

Overall Frequency Distribution of Penner Serotypes of 650 Human Campylobacter Isolates Received From Various Sources in Scotland
(see text for details)

Serotype	Number	%	Serotype	Number	%
1	43	6.6	20	1	0.2
2	95	14.6	23	18	2.8
3	11	1.7	24	8	1.2
4+	78	12.0	27	5	0.8
5	15	2.3	30	6	0.9
6	21	3.2	31	33	5.2
7	—	—	35	1	0.2
8	15	2.3	37	8	1.2
9	14	2.2	42	5	0.8
10	14	2.2	44	6	0.9
11	20	3.1	45	1	0.2
13	27	4.1	48	—	—
15	4	0.6	55	6	0.9
16	12	1.8	5/31	3	0.4
18	10	1.5	NT*	158	24.3
19	12	1.8			

+ includes 4/16

*NT=not typable

Table 4.2.

Human Campylobacter Isolates Received From Five Hospitals

	Total Rec'd	Total Confirmed as Campylobacter spp.	Total Typed (%)
Aberdeen City Hospital	281	258	200 (78)
Monklands District General Hospital	167	152	123 (81)
Dumfries Royal Infirmary	149	138	106 (77)
Stobhill General Hospital	59	54	36 (67)
Falkirk District Royal Infirmary	43	40	27 (67)
Total Numbers	699	642	492 (77)

Isolates from the five hospitals were examined in more detail (see Table 4.2.). There was no significant difference in the proportion of typable strains found from the individual hospitals (X^2= 6.54, d.f.= 4, p> 0.05).

The distribution of the serotypes within the four localities is presented in Table 4.3. It must be noted that "Greater Glasgow" in this table refers to the combined totals for MDGH and Stobhill General Hospital. Penner 2 and 4 were the two most common serotypes found in the 4 localities. The third most common serotype was Penner 1, except in Dumfries, where Penner 31 and 23 were jointly the third most common serotype. The number of different serotypes found increased with the number of isolates typed. The four most common serotypes from each locality accounted for between 31% and 46% of the total number of isolates received from each locality.

The degree of similarity in the relative ranking of the Penner serotypes in each of three localities, Aberdeen, Greater Glasgow, and Dumfries, was examined using the Kendall coefficient of concordance (Siegel, 1956). The rankings were significantly related (X^2= 65.63, d.f.= 27, p < 0.001). Falkirk DRI was not used for this analysis due to the small sample size.

The comparative occurrence of serotypes with a frequency of greater than or equal to 5.0%, (P1,P2,P4,P6,P13,P23,P31) in one or more of the 3 localities, Aberdeen, Greater Glasgow, and Dumfries, was examined. There was no statistically significant difference between the proportions of each of these seven serotypes found in each of the three localities (X^2= 20.71, d.f.=12, p > 0.05). In Table 4.4., the number of isolates of each serotype is expressed as a percentage of the sum total of the seven serotypes from each locality.

Table 4.3.

Percentage Distribution of Penner Serotypes From Four Different Localities In Scotland

Penner Serotype	Aberdeen	Dumfries	Greater Glasgow	Falkirk
1	9.0	1.5	6.8	10.0
2	14.0	19.5	14.1	7.5
3	2.3	0.7	1.9	—
4*	12.0	15.9	8.8	17.5
5	2.3	2.9	2.4	—
6	1.9	4.3	4.8	—
7	—	—	—	—
8	3.1	2.9	1.5	—
9	1.6	0.7	3.9	2.5
10	1.2	2.2	3.9	—
11	4.6	2.2	2.4	—
13	5.0	4.3	2.9	2.5
15	0.8	0.7	0.5	—
16	1.9	1.5	2.4	—
18	1.6	—	2.4	2.5
19	1.6	1.5	2.4	2.5
20	0.4	—	—	—
23	3.1	5.1	1.9	—
24	0.4	1.5	0.5	7.5
27	1.6	—	0.5	—
30	0.8	—	1.5	2.5
31	3.9	5.1	6.8	5.0
35	—	0.7	—	—
37	1.6	—	1.9	—
42	1.2	0.7	0.5	—
44	1.2	0.7	0.5	2.5
45	—	—	0.5	—
48	—	—	—	—
55	0.4	1.5	1.0	2.5
5/31	—	0.7	0.5	2.5
Not Typable	22.5	23.2	22.8	32.5
Total Numbers	258	138	206	40

* includes 4/16

Table 4.4.

Percentages Of The Seven Penner Serotypes With A Frequency Of 5% Or Greater In One Or More Of The Localities

	Penner Serotypes							Total Number
	1	2	4	13	6	31	23	
Aberdeen	18	28	25	11	4	8	6	127
Greater Glasgow	15	30	19	6	11	15	4	95
Dumfries	3	35	28	8	8	9	9	77

4.3 DISCUSSION

The number of isolates received throughout the year from the various hospitals in Scotland corresponded to the established seasonal trends found in the U.K. (Young, 1982; Jones et al., 1984) .The peak rate of isolation occurs in the summer months and early autumn, with almost twice as many cases in the third quarter of the year as in the first quarter. The frequency of C. coli found in this study was 3.3%. This is in agreement with the findings of Skirrow and Benjamin (1982) who state the frequency in Britain to be between 3 and 5%.

The predominance of serotypes 2, 4 and 1 shown in this study has also been documented by others. Karmali et al. (1983) serotyped 285 campylobacter isolates from children in Toronto over a three year period and found Penner serotypes 2, 4, 3 and 1 to predominate. The most common three Penner serotypes derived from human sources by Jones et al. (1984) in Britain were also Penner 4, 2 and 1. Penner et al. (1983) illustrated the same predominance of these three serotypes in a Canadian study of 1586 isolates. Although statistically no significant difference was found between the proportion of the top seven serotypes from the three locales examined in this study, Penner

1 represented only 3% of the top seven serotypes in Dumfries, compared with 18% and 15% for Aberdeen and Greater Glasgow, respectively (see Table 4.4). The reason for this cannot be ascertained from this study.

The degree of association between the rankings of the predominant serotypes in these three localities was significant. This shows the similarity in the rankings, which is not surprising, considering the relatively small area covered by these three localities.

Although in the documented studies throughout the world a few strains seem to be predominant, the number and variety of serotypes is ever increasing. The serotyping scheme serves a useful purpose in separating strains within the C. jejuni and C. coli group. For epidemiological purposes, finer distinction such as may be provided by phage typing, plasmid analysis, biotying and antimicrobial susceptibility tests will be necessary.

CHAPTER 5. CAMPYLOBACTER SPECIES IN GULLS

5.1. INTRODUCTION

There has been a dramatic increase in the numbers of herring gulls in Britain during this century (Chabryzk and Coulson, 1976). Accompanying this increase has been the spread into inland and urban areas. Gulls have come into much closer contact with man than ever before- from feeding on his refuse at tips to nesting on urban dwellings (Monaghan and Coulson, 1977) and roosting on water storage reservoirs (Shedden, 1983). This close contact with humans allows gulls to acquire pathogens from humans and possibly disseminate them by faecal contamination to other areas of the environment (Monaghan, 1983). In addition, in Scotland, two-thirds of all sewage is discharged untreated into the sea (Reilly *et al.*, 1981). This contamination will more than likely enter the diet of gulls, whether indirectly through the contamination of the fish and debris eaten by gulls or directly by the ingestion of the sewage.

Since the main aim of this project was to assess the role of *Larus* gulls in the epidemiology of *Campylobacter* spp., it was clearly necessary to have a realistic picture of the proportion of gulls carrying the organism and how this relates to the biology of the birds. Variations in the proportion of gulls carrying *Campylobacter* spp., *C. jejuni/coli* and *C. laridis* were examined in relation to age, sex, season, geographical location and the feeding ecology of the gulls. The nature of the isolates of *C. jejuni* and *C. coli* from gulls was examined in more detail through the use of serotyping; this enabled the comparisons of the types of *C. jejuni* and *C. coli* found in the gulls with the types of *C. jejuni* and *C. coli* found in the human population in Scotland and in water bodies in the Glasgow area.

5.1.1. General Occurrence of Campylobacter Species in Birds

The lifestyle of wild birds such as gulls makes them ideal candidates for the dissemination of bacteria and possibly disease. Several bird species are known to be capable of harbouring as well as transmitting bacteria which are pathogenic to man and domesticated livestock (Williams et al., 1977; Reilly et al., 1981). Their comparatively high internal body temperature of 42°C. makes them perfect incubators for the growth of thermophilic Campylobacter species.

In trying to discover more about the epidemiology of Campylobacter spp. found in livestock, Smibert (1969) cultured the intestinal contents of sparrows (Passer domesticus), starlings (Sturnus vulgaris), pigeons (Columba livia), blackbirds (Turdus merula merula), chickens and turkeys and was able to isolate Campylobacter spp. from several of each of these avian species which are commonly found around livestock in barns and fields in the United States. In a study of 445 migratory waterfowl in northern Colorado in the U.S., 35% yielded C.fetus subsp. jejuni (Luechtefeld et al., 1980). The frequency of isolation varied among different species, with shovelers (Spatula clypeata) having a significantly higher isolation rate (66%) and green winged teal (Anas carolinensis) having a significantly lower isolation rate (16%) than the remaining five species of waterfowl tested. It was postulated that the different feeding habits of the various species could be the cause of the difference in isolation rates. Further studies have shown that when different species of waterfowl are maintained on different diets, they harbour different total numbers of intestinal bacteria (Hussong et al., 1979). This difference in the populations of intestinal bacteria was also noted in waterfowl of the same species maintained on different diets (Hussong,

et al, 1979).

Fenlon et al. (1982) looked for thermophilic Campylobacter spp. in the droppings of birds noted for roosting in large numbers on grazed pastures in Britain, another potential vehicle for infection of livestock and man. Herring gulls were 55% positive for Campylobacter spp., urban pigeons 41% positive, rooks (Corvus frugilegus) 40% positive, and wild geese 33% positive.

In a sample of 311 wading birds (Charadrii) from the Firth of Clyde and Firth of Forth in Scotland, 71.4% were found to be carrying Campylobacter species (Fricker and Metcalfe, 1984). The feeding habits of these birds are such that the estuarine invertebrates they ingest may be contaminated with human waste in the form of sewage. During high tide, they are likely to move inland to fields and pasturelands for food, where their faecal deposits, if ingested, could possibly result in the infection of livestock.

5.1.2. Description of Study Areas and Methods Used

Figure 5.1. shows the areas where gulls were caught. Appendix 2 contains a list of the sites where samples were obtained. The gulls were aged using plumage characteristics (Grant, 1981). The fledged birds were divided into 3 age classes: first years, intermediates (2-4 yrs.) and adults (5 years or older). The fledged birds were sexed by their body size, using the head and bill length (Coulson et al., 1983). Cloacal swab samples were taken from all of the birds, with the swab immediately submerged in a Preston's enrichment broth for the culturing of Campylobacter species (see 3.1.4.).

There were four types of sites at which herring gulls were sampled for this study:

1) The majority of gulls tested for Campylobacter spp. in this study were caught at refuse tips using a cannon net. The 90' by 45'

Figure 5.1. Map of the sites in Scotland where gulls were caught and cloacally sampled for *Campylobacter* species

1) Thurso (tip)

2) Aberdeen (tip)

3) Moodiesburn (tip)

4) Bishopbriggs (tip)

5) Horse Island (breeding colony)

6) Bowmore (tip)

7) Inchmarnock (breeding colony)

8) Firth of Clyde (boat)

9) Flanders Moss (breeding colony)


1
2
3
4
5
6
7
8
9
40 km.

net was set up near an area of freshly dumped refuse. Once the gulls settled on the area to feed, the cannon net was fired over the group of feeding gulls, catching them under the net. Gulls feeding at the refuse tips at Moodiesburn and Bishopbriggs outside Glasgow were sampled from November 1984 to August 1985 during this study, for a total of 6 catches. One catch was made at a refuse tip in Aberdeen in June 1985. In addition to the Moodiesburn and Bishopbriggs catches, data obtained in previous work from gull catches in Bishopbriggs, Bowmore, on the island of Islay, and Thurso, on the northern coast of Scotland from September 1982 to December 1982 were analysed. The culturing of these samples for Campylobacter spp. was performed by C. Fricker (Microbiology Dept., University of Reading). These samples were not available for serotyping. Plumage details and standard body measurements were taken by P. Monaghan (Zoology Dept., Univ. of Glasgow) for all the birds sampled for the isolation of Campylobacter species.

2) Eighty-four unfledged chicks were sampled at a coastal breeding colony on the island of Inchmarnock and 40 chicks were sampled at an inland colony at Flanders Moss (Fig. 5.1.) in June 1985; both these sites hold large numbers of herring gulls and the closely related lesser black backed gulls (Larus fuscus). At the Inchmarnock colony, the difference in the type of nesting site chosen by the two species of gull made it comparatively easy to distinguish between the young of the two species; differences in the plumage pattern of older chicks also aided the distinction. Only herring gull chicks were sampled from this site. Flanders Moss is inhabited predominantly by lesser black backed gulls, containing thousands of breeding pairs of this species, compared to only hundreds of breeding pairs of the herring gull (P. Monaghan, personal communication). For analysis, all

samples from Flanders Moss were considered to be from lesser black backed gulls.

3) An annual cull of herring and lesser black backed gulls is carried out in late May by the Royal Society for the Protection of Birds on Horse Island, a breeding site off Ardrossan on the west coast of Scotland (Fig. 5.1.), as part of a management strategy. A large number of freshly dead birds were sampled at that time in 1985, although the majority of these were lesser black backed gulls.

4) A small number (19) of herring gulls feeding at a prawn trawler (boat birds) in the Firth of Clyde were netted and cloacal swabs cultured for Campylobacter species. These birds were treated in the same manner as the tip caught birds.

The methods used for the isolation of Campylobacter spp. from the gulls samples may be found in 3.1.4.

In order to discover if age, sex, and time of year affects the carriage rate of Campylobacter spp., the refuse tip birds were examined together; the chicks, "boat birds" and culled birds were considered separately, in order to examine the effects of differences in their feeding ecology. The 1982 data were used for the examination of geographical differences in Campylobacter spp. carriage.

5.2. RESULTS

In order to examine the various factors affecting the avian carriage rate of Campylobacter spp. in the 1984-85 data for Moodiesburn tip, the data for the catches in November and December were combined, as were the data for the January and February catches. This was necessary to give a sufficient sample size for analysis and enabled seasonal effects to be examined. This therefore gave 3 sets of data for the Moodiesburn tip--birds caught in November and

December, January and February, and April. These three time periods can be related to the annual cycle of the birds, with November-December and January-February representing a nonbreeding time and the April catch representing the beginning of the breeding season. Data for the individual catches can be found in Appendices 2 and 3.

5.2.1. Age and Sex Effects In Fledged Birds Caught At Refuse Tips

The proportion of herring gulls with and without *Campylobacter* spp. was compared in relation to the age of the birds. There was no significant difference in carriage rates in relation to age in any of the five 1982 tip catches. This was also found to be true when the ratio of gulls with respect to *C. laridis* carriage and *C. jejuni/coli* carriage were considered separately.

The three sets of data from the Moodiesburn catches were examined separately for an age effect in the same manner as the 1982 data. There was no significant difference between the age classes concerning the numbers positive for *Campylobacter* spp., *C. laridis* or *C. jejuni/coli* for the Jan.-Feb., April or in the June samples from Aberdeen. There was no significant difference in the *C. laridis* carriage between the different classes in the Nov.-Dec. catch. This catch did show a significant difference between the age classes for the ratio of gulls with *Campylobacter* spp. to those without (X^2=13.58, d.f.=2, $p<0.005$), which was largely due to the ratio of gulls with *C. jejuni/coli* to those without (X^2=24.50, d.f.=2, $p<0.001$). However, as this result was obtained with one set of data out of 8 considered in total, it was clearly not the typical pattern; as it was based on a sample of only 7 first years, the difference may have been due to small sample size. Thus, considering all of the data analysed, no consistent age effect was apparent in the positivity rate for *Campylobacter* species, both *C. laridis* and *C. jejuni/coli*; the age

classes were therefore combined in subsequent analyses.

Data on the sex of the sampled birds was available for the 1984/1985 Moodiesburn catches, the June Aberdeen catch and the August Bishopbriggs catch. The five sets of data for 1984-85 were examined separately to compare the proportion of males and females with Campylobacter spp., with C. jejuni/coli, and with C. laridis (Table 5.1. and 5.2.). The results showed that there were no significant differences between the two sexes in any of these samples, therefore males and females were combined in subsequent analyses.

5.2.2. Effect of Geographical Location

The possibility of geographical variation in Campylobacter spp. carriage in herring gulls was examined using the 1982 data, as these covered the widest range of locations sampled at the same time of year. The data from the two catches at Bishopbriggs were combined, as were the two catches at Bowmore. These two sets of data together with those obtained from Thurso provided information for 3 locations sampled at the same time of year (Sept.-Nov., 1982). There was a significant difference in the proportion of Campylobacter spp. isolated at these sites (Table 5.3.). There was a similarity between the percentage of Campylobacter spp. found in the Bowmore and Thurso results, and the difference was largely due to the high percentage of Campylobacter spp. found in the Bishopbriggs catch (Table 5.3). When these data were examined further in relation to the species of Campylobacter isolated, there was no significant difference in the proportion of C. laridis isolated from the 3 different locations (Table 5.4.), but a highly significant difference was found in the proportion of C. jejuni/coli isolated (Table 5.4.). A higher proportion of the Bishopbriggs birds carried C. jejuni/coli, as compared to both the Thurso and Bowmore data.

Table 5.1.

Comparison of the Proportion of C. laridis in Male and Female Herring Gulls Able to be Sexed at Refuse Tips in 1984-85

		No. With C. laridis	No. Without C. laridis
Nov.-Dec. (Moodies.)			
	Male	14 (18%)	63 (82%)
	Female	4 (17%)	20 (83%)
	X^2=0.02, d.f.=1, NS.		
Jan.-Feb. (Moodies.)			
	Male	34 (56%)	27 (44%)
	Female	24 (68%)	11 (32%)
	X^2=1.04, d.f.=1, NS.		
April (Moodies.)			
	Male	15 (65%)	8 (35%)
	Female	3 (60%)	2 (40%)
	X^2=.003, d.f.=1, NS.		
June (Aber.)			
	Male	31 (49%)	32 (51%)
	Female	14 (48%)	15 (52%)
	X^2=0.02, d.f.=1, NS.		
August (B'riggs)			
	Male	13 (52%)	12 (48%)
	Female	12 (35%)	22 (65%)
	X^2=1.03, d.f.=1, NS.		

Table 5.2.

Comparison of the Proportion of C. jejuni/coli in Male and Female Herring Gulls Able to be Sexed at Tips in 1984-85

		No. With C. jejuni/coli	No. Without C. jejuni/coli
Nov.-Dec. (Moodies.)			
	Males	36 (47%)	41 (53%)
	Females	6 (25%)	18 (75%)
	X^2=2.72, d.f.=1, NS.		
Jan.-Feb. (Moodies.)			
	Males	12 (20%)	49 (80%)
	Females	6 (17%)	29 (83%)
	X^2=0.001, d.f.=1, NS.		
April (Moodies.)			
	Males	7 (30%)	16 (70%)
	Females	2 (40%)	3 (60%)
	X^2=0.01, d.f.=1, NS.		
June (Aber.)			
	Males	11 (17%)	52 (83%)
	Females	6 (21%)	23 (23%)
	X^2=0.01, d.f.=1, NS.		
August (B'riggs)			
	Males	10 (40%)	15 (60%)
	Females	16 (47%)	18 (53%)
	X^2= 0.07, d.f.=1, NS.		

Table 5.3.

Comparison of the Proportion of Tip Birds With Campylobacter Species For Three Different Locations Caught at the Same Time of Year in 1982

	No. With *Campylobacter* spp.	No. Without *Campylobacter* spp.
Bishopbriggs	131 (66%)	69 (34%)
Bowmore	97 (53%)	86 (47%)
Thurso	98 (54%)	83 (46%)

$X^2=7.58$, d.f.=2, $p<0.025$

Table 5.4.

Comparison of the Proportion of Tip Birds With C.laridis and With C. jejuni/coli In Three Different Locations Caught at the Same Time of Year in 1982

	No. With *C. laridis*	No. Without *C. laridis*	No. With *C. jej./coli*	No. Without *C. jej./coli*
Bishopbriggs	58 (29%)	142 (71%)	73 (37%)	127 (63%)
Bowmore	61 (33%)	122 (67%)	36 (20%)	147 (80%)
Thurso	48 (27%)	133 (73%)	50 (28%)	131 (72%)

$X^2=2.08$, d.f.=1, NS.

$X^2=13.41$, d.f.=2, $p<0.005$

Table 5.5.

Comparison of the Proportion of Tip Caught Birds With Campylobacter Species From Two Locations At the Same Time of Year in 1985

	No. With *Campylobacter* spp.	No. Without *Campylobacter* spp.
Aberdeen	65 (67%)	32 (33%)
Bishopbriggs	43 (86%)	7 (14%)

$X^2=4.23$, d.f.=1, $p< 0.05$

The only location effect during the same time of year that could be examined in 1984-85 was between Aberdeen (June) and Bishopbriggs (August). There was a significant difference in the ratio of gulls with *Campylobacter* spp. to those without, with Bishopbriggs having a much higher proportion with *Campylobacter* species. The 1982 data also exhibited a higher proportion of *Campylobacter* spp. carriage in the gulls from Bishopbriggs (Table 5.5.and Table 5.3). There was no significant difference between the two locations in the proportion of gulls with and without *C. laridis* (Table 5.6.). There was a significant difference between the proportion of *C. jejuni/coli* in the two locations, with Bishopbriggs having over twice the proportion of birds with *C. jejuni/coli* as Aberdeen (Table 5.6.).

Table 5.6.

Comparison of the Proportion of Tip Caught Birds With C. laridis and With C. jejuni/coli From Two Locations At the Same Time of Year in 1985

	No. With *C. laridis*	No. Without *C. laridis*	No. With *C. jej./coli*	No. Without *C. jej./coli*
Aberdeen	47 (48%)	50 (52%)	18 (19%)	79 (81%)
Bishopbriggs	21 (42%)	29 (58%)	22 (44%)	28 (56%)

$X^2=0.32$, d.f.=1, NS. $X^2=12.30$, d.f.=1, $p<0.001$

5.2.3. Seasonal Effect

The results from the catches in 1984-85 were used to examine seasonal variation in Campylobacter carriage by herring gulls feeding at the same refuse tip, since these data spanned the widest time period.

The difference in the proportion of herring gulls with Campylobacter spp. in the three time periods was highly significant (see Table 5.7.). There is a 20% increase in the numbers of gulls with Campylobacter spp. in the January-February period (Table 5.7.). The proportion of gulls with and without C. jejuni/coli, and with and without C. laridis in the three time periods was also highly significant (Table 5.8.). This illustrates a seasonal effect in carriage rates, with C. jejuni/coli responsible for the majority of the Campylobacter spp. isolated from gulls in the November-December period. In the January-February period, this picture is reversed, with a large increase in the proportion of gulls with C. laridis, and a decrease in the number of gulls with C. jejuni/coli (See Figure 5.2.).

Table 5.7.

Comparison of the Proportion of Herring Gulls Feeding At the Same Refuse Tip Positive For Campylobacter Species For Three Time Periods

	No. With Campylobacter spp.	No. Without Campylobacter spp.
Nov./Dec.	60 (58%)	43 (42%)
Jan./Feb.	78 (79%)	21 (21%)
April	30 (97%)	1 (3%)

$X^2=21.40$, d.f.=2, $p< 0.001$

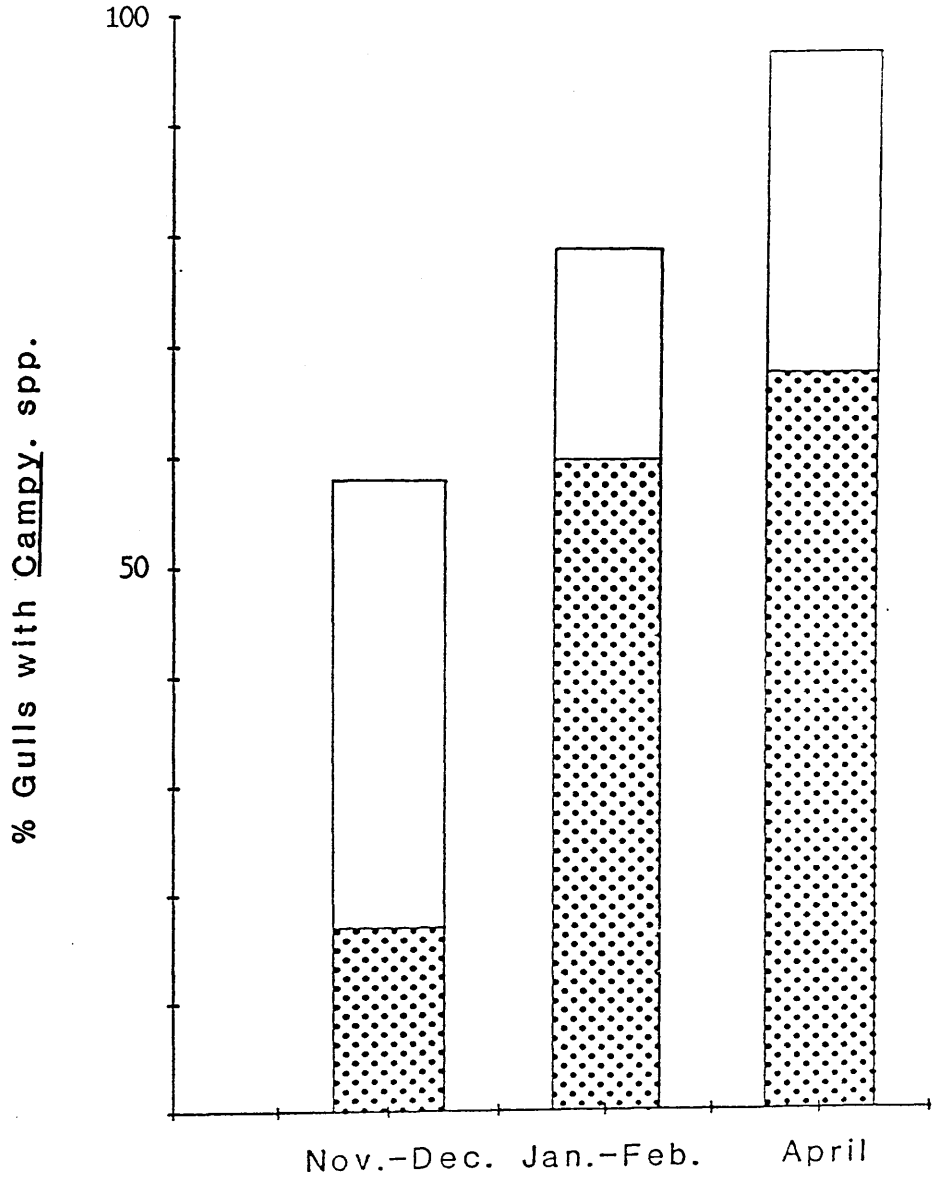


Figure 5.2. The percentage of herring gulls, caught at Moodiesburn tip in three time periods, which carried Campylobacter species.
The data has been broken down into those which carried C. laridis (shaded portion), and C. jejuni/coli (unshaded portion).

Table 5.8.

Comparison of the Proportion of Herring Gulls Feeding at the Same Refuse Tip Positive With C. laridis and C. jejuni/coli For Three Time Periods

	No. With C. laridis	No. Without C. laridis	No. With C. jej./coli	No. Without C. jej./coli
Nov./Dec.	18 (17%)	85 (83%)	42 (41%)	61 (59%)
Jan./Feb.	59 (60%)	40 (40%)	19 (19%)	80 (81%)
April	21 (68%)	10 (37%)	9 (29%)	22 (71%)

X^2=46.43, d.f.=2, $p<0.001$ X^2=11.21, d.f.=2, $p<0.005$

5.2.4. Effect of Differences in Feeding Ecology On Campylobacter Carriage in Gulls

A) Breeding Gulls and Tip Feeding Gulls

To examine the effect that the type of feeding site has on the carriage of Campylobacter spp., the data from the birds culled at the breeding colony at Horse Island were analysed and compared with the tip caught birds. It is likely that a proportion of the gulls caught at the breeding colony would not feed at inland refuse tips, but along the coast; they therefore have, on average, a more marine diet than the refuse tip birds. The majority of the birds obtained at this site were lesser black backed gulls, although some herring gulls were also sampled. These two species are very closely related and similar in their ecology and behaviour. Indeed, they occasionally interbreed (Harris, 1970). The proportion of gulls with Campylobacter spp. was compared between the two species of gull, as well as the proportion with C. laridis and the proportion with C. jejuni/ coli. There were no significant differences between carriage rates of the Campylobacter spp. isolated from the two species of gull, and so the data for the two species were combined.

Ideally, it would have been preferable to compare Campylobacter spp. carriage in the breeding colony birds with those caught at a refuse tip in the same general locality at the same time of year. However, these data were not available. The breeding colony birds were therefore compared with a tip catch at Aberdeen at the same time of year. There was a highly significant difference in the proportion of gulls positive for Campylobacter spp. between the two types of sampling sites (Table 5.9.). In a comparison of the numbers of birds with or without C. laridis in the two sites, a highly significant difference was found (Table 5.10.). There was no significant difference in the proportion of birds with C. jejuni/coli between the two sites (Table 5.10.).

Table 5.9.

Comparison of Campylobacter Carriage Between Breeding Colony Birds and Those Caught at a Refuse Tip At the Same Time of Year (May-June) in Aberdeen, and Those Caught at Refuse Tips in the Same Geographical Area in April and August

	No. With Campylobacter spp.	No. Without Campylobacter spp.
Breeding Colony	112 (43%)	148 (57%)
Refuse Tip (Aber.)	65 (67%)	32 (33%)

$X^2=15.24$, d.f.=1, $p<0.001$

Refuse Tip (Mood. and B'riggs.)	73 (90%)	8 (10%)

$X^2=53.20$, d.f.=1, $p<0.001$
(as compared with breeding colony above)

Since there may be an effect of geographical location in the above analyses, a further comparison of the breeding colony birds with the tip feeding birds in the same geographical area, at as similar a time of year as possible, was made. The Glasgow area catches at the beginning and end of the 1985 breeding season were used (Moodiesburn in April and Bishopbriggs in August-see Appendices 2 and 3.). This gave results similar to the Aberdeen comparison, with the tip birds again showing even higher levels of Campylobacter spp. carriage, and in this case, with significantly higher levels of both C. laridis and C. jejuni/coli (Tables 5.9. and 5.10.).

Table 5.10.

Comparison Of Both C. jejuni/coli and C. laridis Carriage Between Breeding Colony Birds and Those Caught at a Refuse Tip At the Same Time Of Year (May-June) in Aberdeen, and With Those Caught at Refuse Tips in the Same Geographical Area in April and August

	No. With C. laridis	No. Without C.laridis	No. With C.jej./coli	No. Without C.jej./coli
Breeding Colony	75 (29%)	185 (71%)	37 (14%)	223 (86%)
Refuse Tip (Aber.)	47 (48%)	50 (52%)	18 (19%)	79 (81%)
X^2=11.22, d.f.=1, p<0.001			X^2=0.71, d.f.=1, NS.	
Refuse Tip (Mood. and B'riggs)	42 (52%)	39 (48%)	31 (38%)	50 (62%)
X^2=13.50, d.f.=1,p<0.001 (as compared with breeding colony above)			X^2=20.90, d.f.=1, p<0.001	

B) "Boat Birds" and Tip Feeding Gulls

A small number of herring gulls (19) feeding from a prawn trawler in the Firth of Clyde were sampled in December, 1985 to examine a possible difference in Campylobacter spp. carriage due to feeding ecology. For the comparison, a tip catch from Moodiesburn taken at

the same time of year in December 1984 was used. There was no significant difference found in the number of gulls with and without Campylobacter spp. between the two feeding sites (Table 5.11.). There was also no significant difference in the proportion of birds with C. laridis and the proportion of birds with C. jejuni/coli between the two sites (Table 5.12).

Table 5.11.

Comparison of Campylobacter Carriage in Birds Sampled at Two Different Feeding Sites In the Same Area at the the Same Time of Year

	No. With Campylobacter spp.	No. Without Campylobacter spp.
Boat Birds	8 (42%)	11 (58%)
Tip Birds	30 (61%)	19 (39%)

X^2= 1.32, d.f.=1, NS.

Table 5.12.

Comparison of Gulls With C. laridis and C. jejuni/coli From Two Different Feeding Sites in The Same Area at the Same Time of Year

	No. With C. laridis	No. Without C. laridis	No. With C. jej./coli	No. Without C. jej./coli
Boat Birds	2 (11%)	17 (89%)	6 (31%)	13 (69%)
Tip Birds	8 (16%)	41 (84%)	22 (45%)	27 (55%)

X^2=0.50, d.f.=1, NS. X^2=0.53, d.f.=1, NS.

5.2.5. Comparison of Campylobacter Carriage in Unfledged Chicks And Fledged Gulls

The gull chicks sampled at the Inchmarnock breeding colony in the Firth of Clyde were compared with adult gulls sampled in the same time

period in the same area at the nearby breeding colony on Horse Island (see Figure 5.1.). When the proportion of birds with and without Campylobacter spp. were compared, there was a significant difference between the chicks and the adult gulls (Table 5.13.). There was no significant difference between the two samples in the proportion of C. jejuni/coli isolated (Table 5.14.). However, there was a highly significant difference in the proportion of gulls with C. laridis (Table 5.14.). The chicks had a lower percentage of C. laridis than the adult birds.

Table 5.13.

Comparison of Campylobacter Carriage In Unfledged Chicks and Adult Gulls From the Firth of Clyde For The Same Time Period (May-June)

	No. With Campylobacter spp.	No. Without Campylobacter spp.
Unfledged Chicks	25 (30%)	59 (70%)
Adult Gulls	112 (43%)	148 (57%)

$X^2=4.15$, d.f.=1, $p<0.05$

Table 5.14.

Comparison of C. laridis and C. jejuni/coli Carriage Between Unfledged Chicks and Adult Gulls From the Firth of Clyde in the Same Time Period (May-June)

	No. With C. laridis	No. Without C. laridis	No. With C. jej./coli	No. Without C. jej./coli
Unfledged Chicks	10 (12%)	74 (88%)	15 (18%)	69 (82%)
Adult Gulls	75 (29%)	185 (71%)	37 (14%)	223 (86%)
	$X^2=8.9$, d.f.=1, $p<0.005$		$X^2=0.40$, d.f.=1, NS.	

5.2.6. Serotyping

Table 5.15. lists the dates and sites of the catches with the serotypes found in the herring gulls examined in this study. Twenty one different serotypes were represented.

Appendix 4 shows the number of C. jejuni/coli isolates that could be serotyped for each age class of herring gull examined for each location. There was no significant difference between the age classes in the proportion of C. jejuni/coli isolates that typed for any of the locations. Thus, all of the data for the age classes were combined for each tip catch. In order to see if there was any seasonal effect in the percentage of C. jejuni/coli isolates which could be typed, the combined November-December tip catch and the combined January-February tip catch were compared; no significant difference was found.

In a comparison of the proportion of C. jejuni/coli isolates which were found to be typable between the herring gull chicks at Inchmarnock and breeding adults from Horse Island during the same time period, there was a highly significant difference (Table 5.16), with the chicks having considerably more typable strains.

Table 5.15.

Serotypes of C. jejuni and C. coli Isolated From Herring Gulls

Date	Site	Serotype ()=number of isolates
29/11/84	Moodiesburn	P30(2)
		P18(2)
		P 3
		P 4
		P37
		P42
13/12/84	Moodiesburn	P30(2)
		P15
		P16
		P42
21/2/85	Moodiesburn	P31
		P44
17/4/85	Moodiesburn	P24(2)
		P44/7
24/5/85	Horse Island*	P24
24/5/85	Horse Island*	P55(3)
		P 3(2)
		P1/7
		P 8
		P31
		P45
24/6/85	Aberdeen	P 1
		P 8
		P24
13/8/85	Bishopbriggs	P42(2)
		P 2
		P3/13
11/12/85	Boat-Ayr	P42

Serotypes of Chicks From Flanders Moss and Inchmarnock

27/6/85	Flanders Moss*	P 4(2)
		P 6(2)
		P 2
		P1/7
4/7/85	Inchmarnock	P 2(5)
		P 4(2)
		P1/7
		P 8
		P10

* lesser black backed gulls

Table 5.16.

Comparison of the Proportion of C. jejuni/coli Isolates That Typed Between Adult Gulls and Unfledged Chicks from the Firth of Clyde During The Same Time Period (May-June)

	No. Isolates Serotyped	No. Isolates Did Not Serotype
Unfledged Chicks	10 (66%)	5 (34%)
Fledged Gulls	10 (27%)	27 (73%)

$X^2=5.5$, d.f.=1, $p<0.005$

5.3. DISCUSSION

This study investigated the carriage of Campylobacter spp. in Larus gulls and its relation to the age, sex, time of year, geographical location and feeding ecology of the birds. It was established that the age and sex of fledged gulls was not a consistently contributing factor in the number of gulls that carried Campylobacter species.

The geographical location, the seasonal variation and the feeding ecology are all very closely related. It was shown that the tip samples from Bowmore and Thurso had very similar results in the proportion of C. jejuni/coli and C. laridis, with lower proportions of the Campylobacter spp. at these sites than the tip birds from Bishopbriggs. While these sites are at opposite ends of Scotland geographically, these sites are coastal and are in areas of low human population density. The gulls in such areas rely on fishing ports and marine life in general for part of their diet, along with the refuse tips. The gull samples from Bishopbriggs showed a higher proportion of Campylobacter spp. and in particular, a higher proportion of C. jejuni/coli than the Thurso and Bowmore samples and the 1985 Aberdeen

samples. The Bishopbriggs tip is in the Strathclyde region of Scotland, one of the regions highest in human population density. It is possible that the gulls would be more likely to acquire the Campylobacter spp. most common in humans, C. jejuni and C. coli, in an area with a higher population density, where there is more of a chance of ingesting material contaminated with human waste. According to Monaghan et al. (1985), gulls in the Clyde area appear to be more dependent on refuse tips for food than the traditional food obtained from fishing ports and fishing fleets, as these food sources have been drastically reduced in the Clyde area.

It is difficult to explain the reason for the increase in the percentage of gulls with Campylobacter spp. in the months of January and February, and in particular, the reversal in the proportion of C. jejuni/coli and C. laridis as compared to the November-December data. Not enough information is known about the aetiology of C. laridis in gulls to explain this phenomenon. It has been shown that in the Clyde area, more birds are present at tips in the winter months than in the summer months, with more than three quarters of the wintering birds feeding at refuse tips (Monaghan et al., 1985). The large increase in birds feeding in the same areas and roosting nearby could facilitate cross contamination within the gull colonies, thus possibly increasing the number of gulls with Campylobacter species. The small sample size of the April catch should be considered when observing the high percentage of gulls with Campylobacter species for that time period.

The difference in the proportion of gulls with Campylobacter spp. between the tip birds and the breeding colony birds was the larger proportion of tip birds with C. laridis. A possible explanation for this could be the cross contamination of the tip birds with C. laridis due to the cleaning of plumage in puddles and small bodies of water

near the tips, which could easily become contaminated with the faeces of the birds using them. It is interesting that the proportion of C. jejuni/coli is not higher in the breeding colony on Horse Island than in the tip birds, as is the case for Salmonella spp. carriage (Girdwood, et al., 1985). The breeding colony has a wider range of foraging specialists, who are known to frequent the sewage outfalls and sludge boats off the coast of Ayr (N. Metcalfe, personal communication), and would be more likely to acquire the human strains.

The birds caught around the fishing boat in the Firth of Clyde did not differ from the tip birds at the same time of year in the carriage of Campylobacter species. The "boat birds" would also probably feed on the sewage outfalls near the Ayr coast, which would be a likely source of C. jejuni and C. coli. The number of birds sampled on the boat was very small, and more data would need to be collected before a true pattern of Campylobacter spp. of birds feeding in this habitat could be established.

It was not surprising that the percentage of unfledged chicks with Campylobacter spp. was lower than that of fledged gulls, as they have not had the increased time or the opportunity to acquire it. The difference between the two was in the proportion of birds with C. laridis, which was lower in the chicks. However, the diet of the unfledged chicks is dependent upon that of the parents. While up to 50% of the chick's diet in some areas may be provided from refuse tips, according to a survey in the Netherlands made by Spaans (1971), refuse tends to be fed to older chicks. This could be a possible way of acquiring the human strains, C. jejuni and C. coli. Young chicks are typically fed on more traditional foods, such as earthworms and those of a more marine origin (Spaans, 1971). In the serotyping results, a larger proportion of the C. jejuni/coli strains from the chicks were typable, compared to the tip birds. It could be that the

digestive tract of the young birds is colonised first by C. jejuni and C. coli before later acquiring C. laridis , known to be a commensal in the intestinal tracts of gulls.

There seems to be a link between gulls feeding at refuse tips and the presence of C. laridis in the gulls. There was an increase in the number of gulls with C. laridis in the severest winter months (Jan.and Feb.), when tip usage by the birds is highest (Shedden, 1983). The birds caught at tips had a higher percentage of C. laridis than the breeding colony birds, which feed on a wider range of food types. The fact that the chicks were low in C. laridis would also support this theory, as adults are more likely to feed the very young the more traditional foods, rather than refuse (Spaans, 1971). However, further study would be needed to establish the connection between refuse feeding and the preponderance of C. laridis in adult gulls.

Most of the serotyped strains from the chicks were of the two most common serotypes found in humans, Penner 2 and 4. The Inchmarnock colony is very near sewage outfalls and sewage sludge dumping grounds, where contaminated material could be obtained by the gulls to feed their chicks. The Flanders Moss colony is an inland one, with the birds possibly using a more natural diet for their chicks. Further study would be needed to investigate the feeding habits of this breeding colony in order to find out the source of these strains.

It is difficult to give an overall figure of Campylobacter spp. carriage in the gulls as it has just been illustrated that several factors are involved. Carriage rates of between 30% and 97% were found in this study, depending on the time of year, geographical location, feeding ecology and sample size. The small percentage (29%) of gull Campylobacter spp. isolates that serotyped with the panel of

29 Penner antisera in this study indicates that gulls are not a major reservoir for the strains that are, at this time, pathogenic in man.

CHAPTER 6. CAMPYLOBACTER SPECIES IN WATER

6.1. INTRODUCTION

6.1.1. Gulls and the Bacterial Contamination of Water

Since the turn of the century, it has been known that storing raw water improved its bacteriological quality (Houston, 1909). Studies by Holden (1970) showed that when at least 10 days retention occurred, between 75% and 99% of the excremental organisms were eliminated. These studies did not investigate the effect of additional bacterial pollution occurring during storage. In particular, faecal contamination introduced by roosting gulls was not considered.

Since around 1930, the numbers of herring gulls have been doubling approximately every 6 years (Chabryzk and Coulson, 1976). This has been accompanied by large numbers of gulls flying inland to roost on storage reservoirs, which provide gulls with large, safe and convenient locations near feeding sites to spend the night. The use of reservoirs as nocturnal roosting sites by gulls was documented in both Great Britain (Metropolitan Water Board, 1928) and the United States (Houser, 1931) over 50 years ago. Since that time, several studies have been carried out to establish the role of gulls in the bacterial contamination of water supplies in Britain. For example, Fennell *et al.* (1974) illustrated the correlation between the increase in gulls during the winter and the degree of deterioration in water quality found for that same period in a reservoir in England. Benton *et al.* (1983) studied a storage reservoir in Scotland that showed a marked deterioration in bacterial quality during the winter months. A significant correlation was found between the number of gulls roosting on the reservoir and the number of *E. coli* isolated from the water.

The use of bioacoustics to deter gulls from roosting on this reservoir resulted in a significant decrease in the level of bacterial contamination.

A short background to the Campylobacter spp. found in water and the documented outbreaks of human campylobacter enteritis attributed to water is provided. This chapter is principally concerned with the study of reservoirs and lochs in the Glasgow area utilised and not utilised by gulls as roosting sites and the connection between the numbers of roosting gulls and the level of Campylobacter spp. found in these bodies of water.

6.1.2. Isolation of Campylobacter Species From Water

Campylobacter spp. have been isolated from fresh water as well as salt water (Knill et al., 1982). Bolton et al. (1982) used the most probable number method to enumerate Campylobacter spp. in raw water samples and found counts ranging from 10 to 239 Campylobacter spp. per 100 mls. for 11 of 49 coastal and estuary water samples, and from 10 to 36 Campylobacter spp. per 100 mls. for 7 of 44 river samples in Britain. C. jejuni, C. coli, and NARTC were all represented in the isolates obtained from these samples, with some samples containing more than one species. Ribeiro and Price (1984) investigated the optimum conditions needed to isolate thermophilic Campylobacter spp. from river water using Preston's enrichment broth. The overall highest rate for isolation was obtained using enrichment broth incubated for 48 hours in a microaeric (70% nitrogen and 10% carbon dioxide) environment. Representatives of the three thermophilic Campylobacter spp., C. jejuni, C. coli, and NARTC were isolated using this procedure, with some of the samples yielding more than one species.

6.1.3. Waterborne Outbreaks of Campylobacter Enteritis

The first recorded outbreak of human campylobacter enteritis which was attributed to water affected approximately 3000 people (19% of the population) in Bennington, Vermont in June, 1978 (Vogt et al., 1982). Although Campylobacter spp. could not be isolated from the water in question, it was suggested that a heavy rainfall prior to the outbreak could have been responsible for increase runoff from the watershed. This could have caused the contamination of the water system via the main unfiltered water source, which was chlorinated. The lack of filtration combined with the increased waterflow could have made adequate levels of chlorine difficult to maintain (Vogt et al., 1982). C. jejuni was cultured from 15 of 42 rectal swabs obtained from symptomatic persons. Further investigation revealed at least two different serotypes, Penner 13/16 and Penner 36/23, when four of the isolates were serotyped, indicating a possible multi-source contamination.

Mentzing (1981) reported a Swedish outbreak of campylobacter enteritis affecting approximately 2000 people. C. jejuni was isolated from 221 of the 263 stools cultured. After ruling out food and milk as possible sources and examining the extent of the illness in the surrounding areas, it was suggested that the infection was spread via a mains water supply after surface water was introduced into the unchlorinated water supply system. Bacteriological examination of the suspected water failed to grow Campylobacter spp., but the investigations subsequent to the outbreak pointed to water as the likely infective vehicle (Mentzing, 1981).

Rural populations that depend on surface water from open reservoirs for drinking are especially at risk of acquiring water-borne campylobacter enteritis. A campylobacter outbreak affecting 700

persons in British Columbia in July, 1980 was thought to have been caused by the ingestion of contaminated water from the town water supply, which was gravity fed from a reservoir, or pumped directly from a creek (Anon, 1981).

Palmer et al (1983) reported a waterborne outbreak of campylobacter enteritis in which samples taken from the suspect water supply proved to be positive for C. jejuni. The outbreak affected 257 pupils and staff at a boarding school over an eight week period. Upon examination of the water supply system, unchlorinated bore hole water was found to be stored in a cast iron open top tank in the clock tower of the main school building. This water fed into the hot and cold water systems of the main buildings and was only supplemented by mains water in times of heavy demands. It was this holding tank that yielded the Campylobacter jejuni isolates. Evidence of roosting birds was found in the clock tower, and it was postulated that birds could have been responsible for the outbreak by faecal contamination (Palmer et al., 1983). Ten isolates from infected pupils and staff were available for serotyping; 6 were the same serotype as the two isolates recovered from a water storage tank.

6.2. DESCRIPTION OF STUDY AREAS AND METHODS USED

Table 6.1. lists the lochs and reservoirs examined during this study. Water samples were taken from Strathclyde Park, Fannyside Loch, Hogganfield Loch, Johnston Loch and Gadloch by K. Ensor, a technician at the University of Glasgow Department of Zoology. Samples from Barcraigs Reservoir, Cochno Reservoir and Balgray Reservoir were obtained via Strathclyde Water Department. Figure 6.1. shows the location of these lochs and reservoirs. Also shown are the

locations of refuse tips, which are in close proximity to almost all of these bodies of water, and are commonly used as feeding sites for gulls especially during the winter months of November to February (Shedden, 1983). All sites were sampled from June 1985 to January 1986 on a monthly basis. All samples were of raw, untreated water. Strathclyde Park, Fannyside Loch, Hogganfield Loch, Johnston Loch, and Gadloch were also sampled in March and April, 1985.

Table 6.1.

Lochs and Reservoirs in Scotland Examined For Campylobacter Species

Site	Water Supply Status	Surface Area (km^2)	Alt. m.	Water Treatment
Strathclyde Park	none	0.89	30	No
Barcraigs	Service (feeds Muirdykes)	0.61	150	Yes
Hogganfield	none	0.19	60	No
Balgray	Service (feeds Gorbals)	0.43	100	Yes
Fannyside	none	0.19	170	No
Cochno	Storage	0.27	260	Yes
Gadloch	none	0.19	46	No
Johnston	none	0.19	60	No

Figure 6.1. Map of the lochs and reservoirs (circles) tested for Campylobacter spp. in this study and nearby refuse tips (squares)

1) Cochno Loch

2) Gadloch

3) Hogganfield Loch

4) Johnston Loch

5) Fannyside Loch

6) Strathclyde Park Pond

7) Balgray Reservoir

8) Barcraigs Reservoir

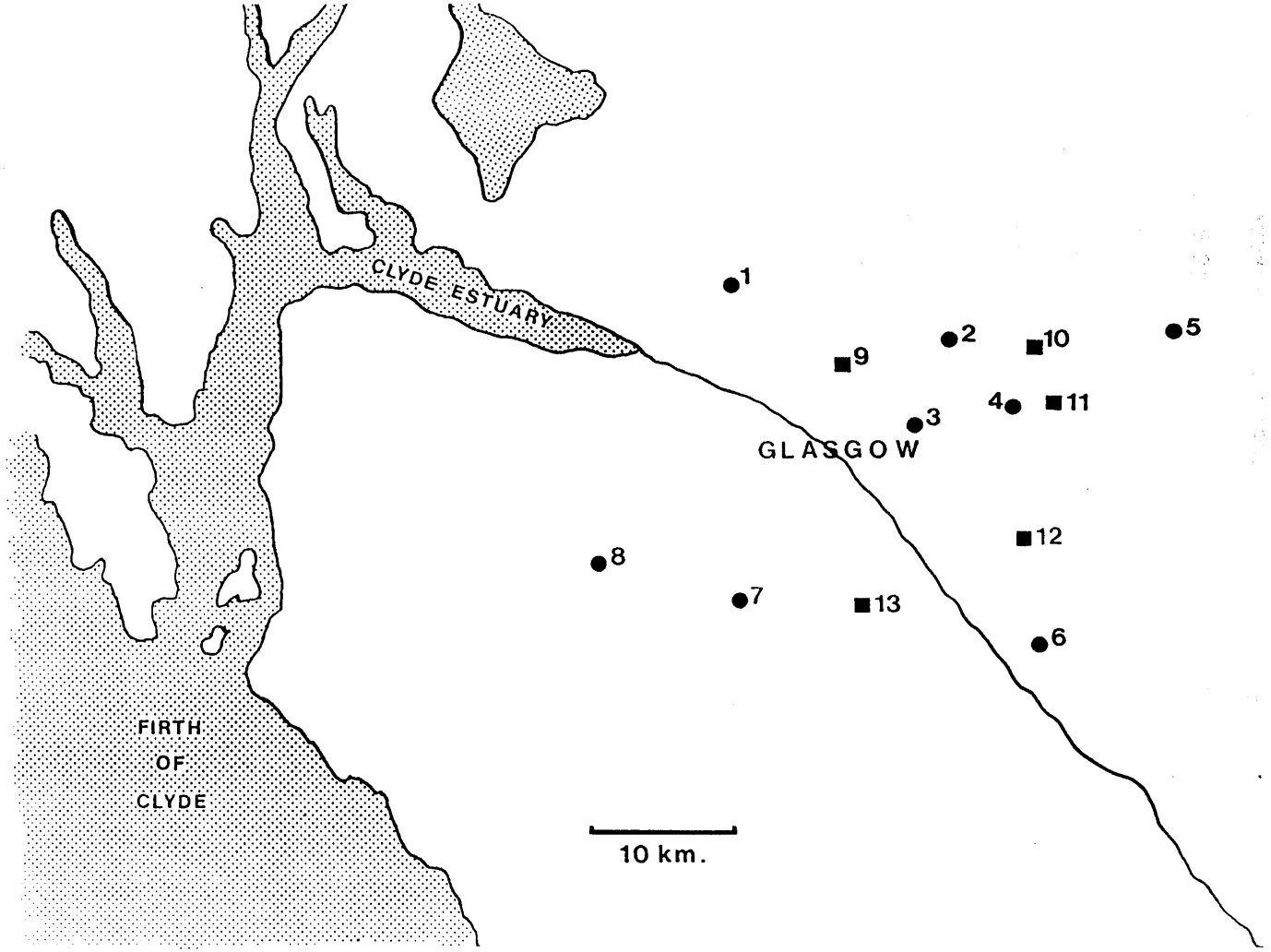
9) Bishopbriggs Tip

10) Moodiesburn Tip

11) Gartcosh Tip

12) Bellshill Tip

13) Cathkin Tip


CLYDE ESTUARY
GLASGOW
FIRTH
OF
CLYDE
1
2
3
4
5
6
7
8
9
10
11
12
13
10 km.

The MPN method (Bolton et al., 1982) was used to estimate the numbers of Campylobacter spp. organisms isolated from the water samples (see 3.1.5.B.). The most probable number of Campylobacter spp. for each water sample was derived using the table of negative broths and the corresponding number of Campylobacter spp. per 100 mls. of water (Table 6.2). In addition, all water samples were cultured for Campylobacter spp. using filtration (see 3.1.5.A.). With some samples, Campylobacter spp. were obtained by filtration, but like organisms were not detected by the MPN method. Such filter results are included in the appendices when appropriate, but filtration results were not considered when examining the data quantitatively, as only the MPN method gave quantitative data.

Table 6.2.

Determination of Most Probable Number of Campylobacter Spp. Using the Number of Negative Broths (out of 10)

	MPN	
No. of Neg. Broths	Organisms/100 ml.	95% conf. limits
0	>230	118->600
1	230	81-600
2	160	59-368
3	120	43-270
4	92	30-211
5	69	21-168
6	51	12-134
7	36	7-106
8	22	3-81
9	10	.025-59
10	0	<0-37

From Bolton et al., 1982.

The data concerning the pattern of usage of these reservoirs by gulls as roosting sites were based on counts and observations made by Shedden (1983) and personal observations made by P. Monaghan. Strathclyde Park, Fannyside, Barcraigs, and Balgray reservoirs were established as sites utilised by nocturnally roosting gulls. Three of the lochs in Shedden's study, Cochno, Johnston and Gadloch, were found not to be utilised by gulls as overnight roosts. These three lochs were used as "negative" controls, to examine the difference in the pattern of bacterial contamination between reservoirs used by gulls and reservoirs not used by gulls for overnight roosting. The pattern of usage by herring gulls roosting overnight at these reservoirs is such that numbers are very low in the summer, corresponding to a shift of birds to the breeding colonies; numbers roosting at reservoirs rise in the autumn and peak in the winter months. Numbers begin to decline again in February, with the departure of the birds to the breeding areas. Accordingly, it is possible to split the reservoir sampling periods into periods of low (June-July), medium (Aug.-Sept.) and high (Oct.-Jan.) gull usage.

In order to examine the effect of the numbers of gulls roosting on the reservoirs, it was necessary to consider months when the intestinal carriage rate of Campylobacter spp. was relatively constant. It was previously shown that the relative percentage of gulls excreting Campylobacter spp. increased greatly in January and February (see 5.2.3.). Data for the month of January were therefore omitted from this analysis in order to avoid the inclusion of an additional confounding variable.

6.3. RESULTS

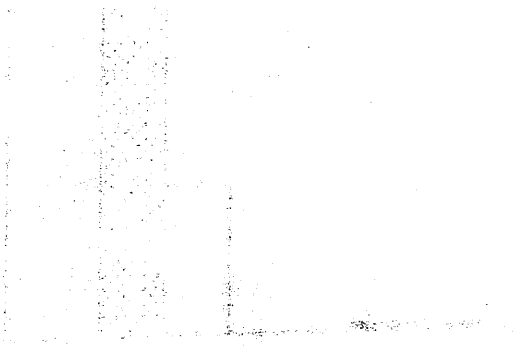
6.3.1. Comparison of relative numbers of Campylobacter spp. in reservoirs utilised and not utilised by roosting gulls

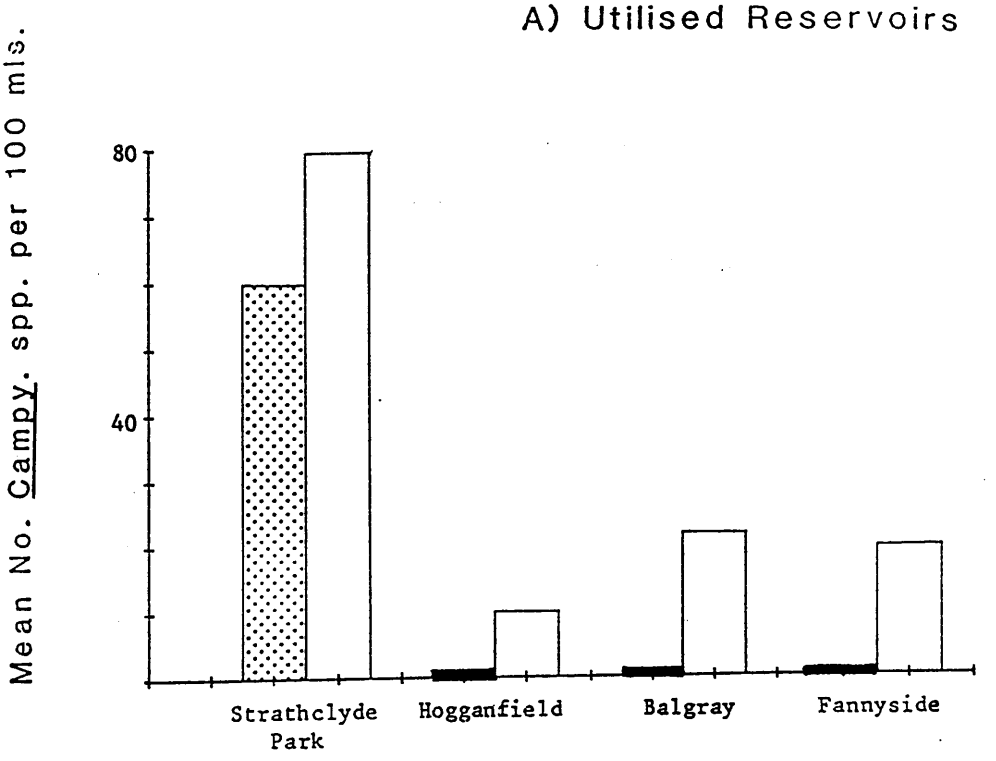
Appendix 5 lists the loch and reservoirs samples cultured for Campylobacter spp. during the months of March, April, June and July and the corresponding number of negative MPN broths (out of 10). Appendix 6 shows the number of negative broths (out of 10) for the reservoirs utilised and not utilised by gulls as roosting sites for the months of August to January. The amount of Campylobacter spp. isolated from the reservoirs in the March to July period was consistently low, with only an occasional isolation using filtration (see Appendix 5). The only exception to this was Gadloch and Johnston Lochs in the months of July, and Hogganfield Loch in the month of March.

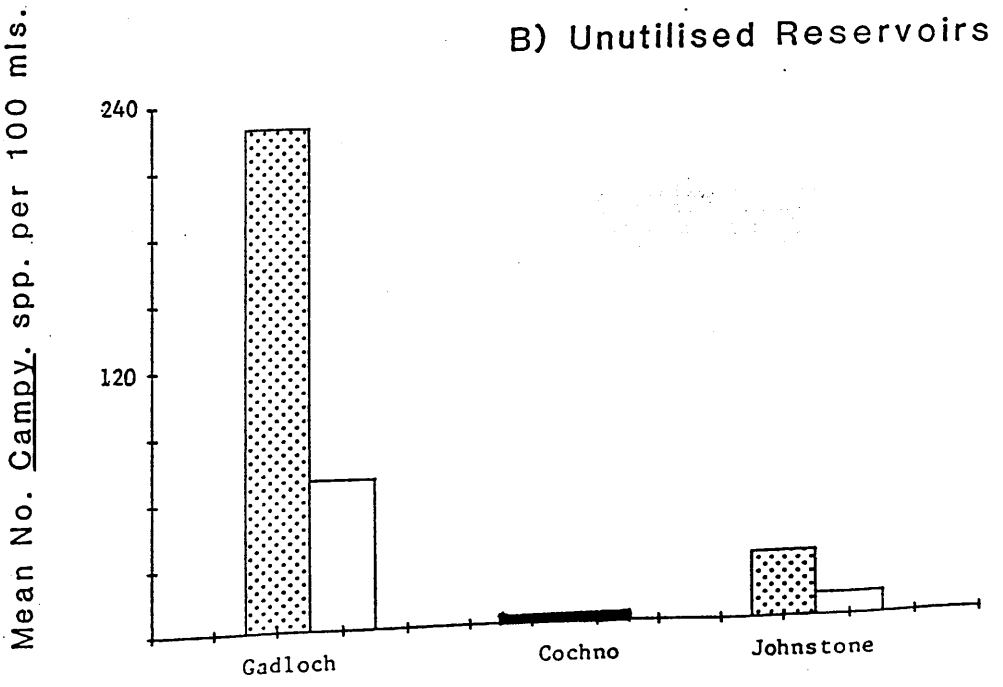
The mean number of herring gulls was determined for Strathclyde Park, Hogganfield, Fannyside, Balgray and Barcraigs for the high usage period (Oct.-Dec.) and the medium usage period (Aug.-Sept.) using the data collected by Shedden (1983). The mean number of negative broths obtained for each of these periods for each of the reservoirs was also calculated and the most probable number of Campylobacter spp. per 100 mls. of water was extrapolated from the mean negative broth figures using a graph of $-\log_e x/10$ (where x= the number of negative broths) plotted against the number of Campylobacter spp. in 100 mls. of water (Bolton et al., 1982). The same calculations for the mean most probable number of Campylobacter spp. per 100 mls. were made for the non utilised reservoirs, Gadloch, Cochno and Johnston (see Appendix 7). Figure 6.2. shows the mean number of Campylobacter spp. per 100 mls. in the medium usage (Aug.-Sept.) and high usage (Oct.-Dec.) periods for reservoirs utilised and not utilised by gulls as roosting

Figure 6.2. The mean number of Campylobacter spp. per 100 mls. of water in the medium (Aug.-Sept., shaded) and high (Oct.-Dec., not shaded) usage periods for reservoirs A) Utilised and B) Not Utilised by gulls as nocturnal roosting sites

The solid line on the X axis is used to indicate no detectable Campylobacter species.




A) Utilised Reservoirs
Mean No. Campy. spp. per 100 mls.
80
40
Strathclyde Park
Hoggansfield
Balgray
Fannyside


B) Unutilised Reservoirs
Mean No. Campy. spp. per 100 mls.
240
120
Gadloch
Cochno
Johnstone

sites. There was a significant difference between the ratio of positive to negative broths found in the used reservoirs for the period of high usage compared to the period of medium usage (see Table 6.3.). There was no statistically significant difference between the ratio of positive to negative broths found in the unused reservoirs for the period of high usage compared to the period of medium usage (Table 6.4).

Table 6.3.

Comparison of the Ratio of Positive to Negative MPN Broths in Utilised Reservoirs During the Medium and High Usage Periods

	No. Pos. Broths	No. Neg. Broths
Medium Usage	11 (11%)	89 (89%)
High Usage	37 (25%)	113 (75%)

$X^2=6.37$, d.f.=1, $p<0.02$

Table 6.4.

Comparison of Ratio of Positive to Negative MPN Broths in Reservoirs Not Utilised By Gulls During Aug.-Sept. and Oct.-Dec.

	No. Pos. Broths	No. Neg. Broths
Aug.-Sept.	23 (38%)	37 (62%)
Oct.-Dec.	20 (22%)	70 (78%)

$X^2=3.82$, d.f.=1, NS.

In order to examine the relationship between the numbers of roosting gulls and the levels of Campylobacter spp. in the reservoirs in more detail, the reservoirs for which there were sufficiently detailed gull counts (Shedden, 1983) were investigated: Strathclyde Park, Fannyside, Balgray, and Hogganfield. The levels of usage by roosting herring gulls on these reservoirs for the months of August to December were ranked from the month with the least number of gulls (1) to the highest number of gulls (5). The corresponding Campylobacter spp. results for each of these reservoirs for the five months were also ranked from the least amount of Campylobacter spp. (1) to the highest amount of Campylobacter spp. (5) (see Appendix 8). The relationship between these rankings was examined (see Figure 6.3.) and the Spearman Rank Correlation Coefficient was used to assess the degree of correlation between the relative numbers of gulls and the relative numbers of Campylobacter spp. found. There was a significant correlation (r_s=0.60, n=20, $p< 0.01$).

6.3.2. Comparison of C. jejuni/coli and C. laridis in utilised and unutilised reservoirs

Appendix 9 lists the number of broths positive with C. jejuni and/or C. coli compared with the number of broths positive with C. laridis using the MPN method for the lochs and reservoirs utilised and not utilised by gulls as roosting sites for Aug.-Dec. 1985. For utilised reservoirs, there was a significant difference between the high and medium usage periods in the ratio of broths positive with C. laridis and C. jejuni/coli (Table 6.5). This contrasts sharply with the results in the unused reservoirs, where no broths were found to be positive with C. laridis in either the medium or high usage period (Table 6.6.- No X^2 value because expected < 5). The number of broths positive with C. jejuni/coli in the unutilised reservoirs actually

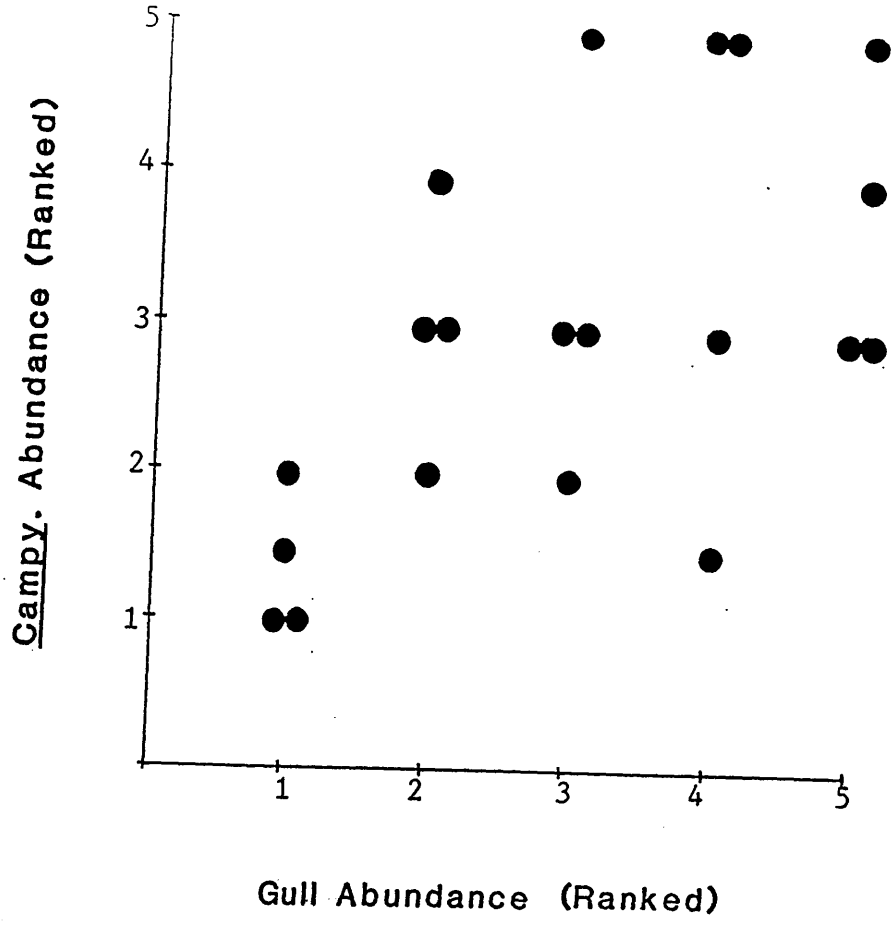


Figure 6.3. The relationship between the relative number of gulls roosting on the reservoirs during the period Aug.-Dec. and the relative numbers of Campylobacter spp. per 100 mls. present in those reservoirs (see text for details). The relationship is statistically significant (r_s=0.60, n=20, p<0.01).

fell in numbers during the period when gull usage is considered high in utilised reservoirs. In addition, the ratio of broths positive with C. jejuni/coli to the broths positive with C. laridis isolated in the period of high usage (Oct.-Dec.) was highly significant between the used and unused reservoirs (Table 6.7.). There was no C. laridis isolated from either the used or unused reservoirs during the Aug.-Sept. period using the MPN method (Table 6.7.-No X^2 value because expected < 5). However, C. laridis was isolated by filtration in several of the utilised reservoirs during this period (see Appendix 9).

Table 6.5

Comparison of MPN Broths Positive With C. jejuni/C. coli and C. laridis During Periods of Medium and High Usage In Reservoirs Utilised By Gulls As Roosting Sites

	C. jejuni/coli	C. laridis
Med. Usage	11 (100%)	0 (0%)
High Usage	18 (49%)	19 (51%)

X^2=7.33, d.f.=1, p<0.01

Table 6.6.

Comparison of MPN broths Positive With C. jejuni/coli and C. laridis During Periods of Aug.-Sept. and Oct.-Dec. In Reservoirs Not Used By Gulls As Roosting Sites

	C. jejuni/coli	C. laridis
Aug.-Sept.	23	0
Oct.-Dec.	20	0

Table 6.7.

Comparison of MPN Broths Positive With C. jejuni/C. coli and C. laridis Isolated In The Period Of Medium (Aug.-Sept.) and High Usage (Oct.-Dec.) For Reservoirs Used and Not Used By Gulls As Roosting Sites

	Aug.-Sept.		Oct.-Dec.	
	C. jej./coli	C. laridis	C. jej./coli	C. laridis
Used Reservoirs	10	0	18 (49%)	19 (51%)
Unused Reservoirs	23	0	20 (100%)	0 (0%)

$X^2=15.41$, d.f.=1, $p<0.001$

6.3.3. Serotyping the C. jejuni/coli Strains Isolated From Water Sources

Every C. jejuni/coli isolate from a positive MPN broth was serotyped using the PHA method (see 3.3.4.). Fifteen different serotypes were isolated from the reservoirs studied (Table 6.8.). Multiple serotypes were isolated from a single monthly sample from Hogganfield, Strathclyde Park and Gadloch. It must be noted that the likelihood of finding more than one serotype increased with the amount of Campylobacter spp. per 100 mls. found, as each positive MPN broth had a single colony taken for identification and serotyping.

Gadloch had the widest range of serotypes and was the most consistently positive for Campylobacter species of all the reservoirs and lochs examined. The serotypes isolated from all of the bodies of water examined were found in the general population of the Greater Glasgow area with the exception of Penner 1/7 and Penner 48, which were not found at all in the Scotland survey of human isolates, and Penner 20, a C. coli serotype, found only in the Aberdeen area survey.

A more detailed discussion of the serotypes isolated in the reservoirs compared to those found in avian and human population in Scotland may be found in Chapter 7.

Table 6.8.

Penner Serotypes Isolated From Lochs and Reservoirs

Body of Water	Month	Penner Serotype
Barcraigs	Oct.	P13
Hogganfield	Mar.	(P8)
	Jan.	P20
		P55
Strath. Park	July	(P8)
	Aug.	P 8
		P37
		P48
	Sept.	P 2
	Oct.	P16
	Jan.	P 8
		P18
Fannyside	Oct.	(P9)
	Dec.	P37
Balgray	Nov.	(P8)
Cochno	Nov.	P48
Johnston	July	P 2
	Aug.	P 4
	Sept.	P 4
	Dec.	P 2
Gadloch	July	P 2
		P37
	Aug.	(P27)
		P 2
	Sept.	P 2
		P 8
		(P4)
	Oct.	P 9
	Dec.	P42
	Jan.	P 5
		P 9
		P1/7
		P18
		P42

() refers to the serotype isolated from the filter only

6.4. DISCUSSION

The utilised reservoirs showed a pattern of gull usage relating to the lifestyle of the birds: small numbers of gulls were observed roosting in the summer months during their breeding season, with the number of gulls increasing in the autumn (Aug.-Sept.) and peaking in the winter months (Oct.-Jan.). The increase in numbers of gulls at the utilised sites during the winter period should correlate with a higher rate of positive MPN broths from these reservoirs if the hypothesis that gulls contribute significantly to the Campylobacter spp. contamination of the reservoir is true. This was found to be true in this study, with a significant difference found between the ratio of positive to negative broths found in these two periods. It was shown that in all of the utilised reservoirs, the pattern remained the same--little or no Campylobacter spp. isolated in the medium usage period (Aug.-Sept.) and an increase in the winter months (Oct.-Jan.).

The lochs not utilised by gulls showed a completely different pattern of bacterial contamination in regard to the amount and species of Campylobacter isolated. Gadloch remained consistently positive throughout the study, with a wide range of serotypes isolated from it, indicating a possible contamination due to sewage, or runoff from nearby fields. It showed a much larger increase during the months of August and September compared to the months of October to December, in contrast to the pattern shown by the reservoirs utilised by gulls. Cochno was consistently negative for Campylobacter spp., which also ties in with the fact that it was not used by gulls for overnight roosting. Johnston had a pattern similar to Gadloch, with increased numbers of Campylobacter spp. isolated in the medium usage months for gulls, although not to the same high degree of positivity as Gadloch. The absence of C. laridis in the Aug.-Dec. period in the unutilised

reservoirs also suggests that gulls are not contributing to its bacterial content.

Almost no Campylobacter spp. were detected by the MPN method during the months of March, April, June and July. The only exception to this was Hogganfield Loch (March), a utilised loch, and Gadloch and Johnston (July), two of the unutilised lochs. This lack of Campylobacter spp. could be due to the decrease in numbers of gulls visiting and using the reservoirs for roosting during the breeding season. The absence of Campylobacter spp. in the unutilised reservoirs suggests that other factors are involved. It could also be due to the increase in the temperature of the water. Studies carried out on the effect of temperature on the survival of Campylobacter spp. in water by Blaser et al. (1980b) showed that C. jejuni died within days if kept in water at 25°C., but survived for 1-4 weeks in water kept at 4°C. The combination of possible small numbers of Campylobacter spp. during this season combined with the effect of temperature reducing the numbers viable at the time of study could be responsible for the absence of Campylobacter spp. found during this period.

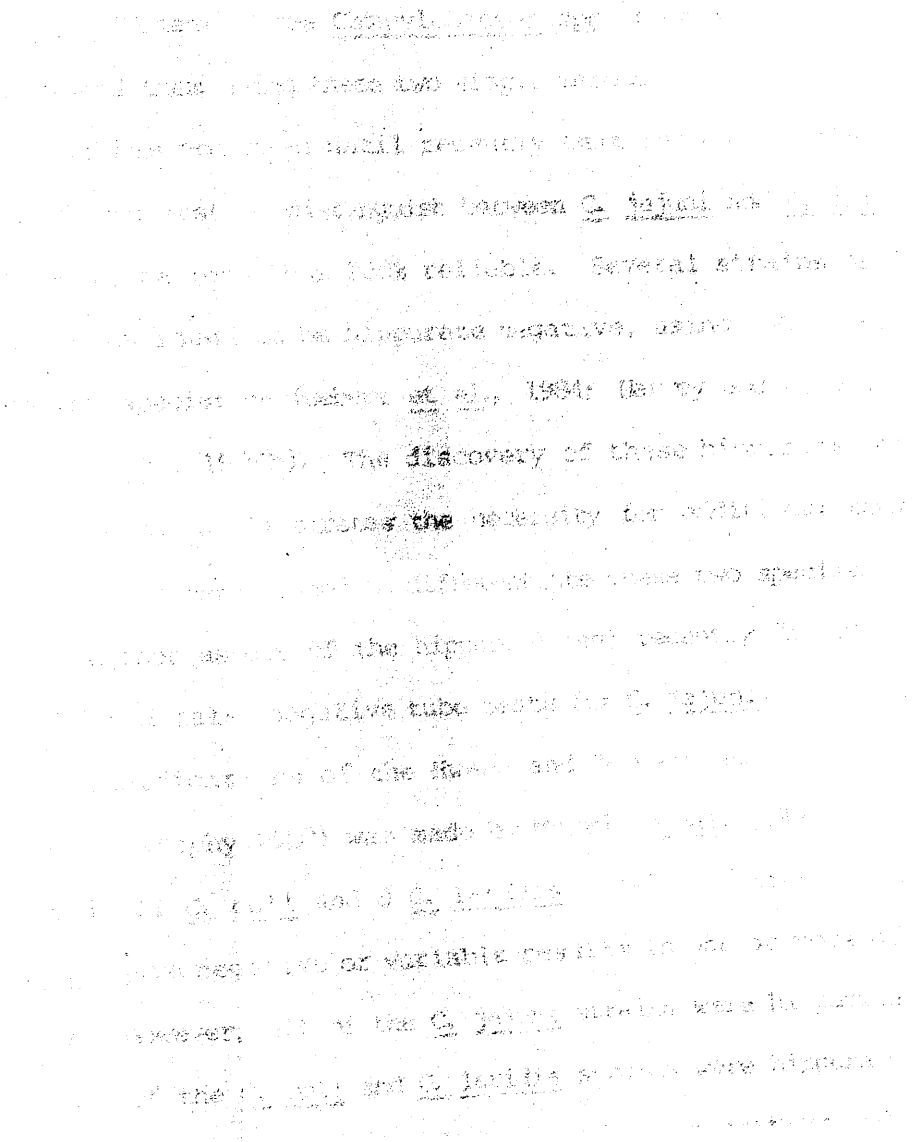
The difference in the Campylobacter spp. found during the two periods studied in the utilised reservoirs also indicated avian contamination. C. laridis is found mainly in wild birds, (especially gulls) and therefore would be expected to be isolated in larger numbers in the high usage period in the utilised reservoirs. The difference in the ratio of C. jejuni/coli to C. laridis in the high usage period between the used and unused reservoirs was highly significant, suggesting that the gulls were contributing to the bacterial pollution of the utilised reservoirs. The ratio of C. jejuni/coli to C. laridis isolated from the utilised reservoirs in the

medium usage period compared to the high usage period was also highly significant. The absence of quantifiable C. laridis during the medium usage period of the utilised reservoirs suggests that the numbers of gulls needed to be above a threshold number for a given water body before the organisms they excreted were isolated by the MPN method. C. laridis was isolated from the filter during the medium usage period in several of the reservoirs where only C. jejuni or C. coli was found in the MPN broths (see Appendix 9).

There are many factors that may contribute to the population of Campylobacter spp. found in rivers, ponds or reservoirs. The environment surrounding the water source could affect the isolation rate, especially if domestic animals graze nearby, making faecal contamination by livestock a possibility. The various types of animal species inhabiting the area could not only affect the numbers found but also the species of Campylobacter isolated, as in the case of avian contribution of C. laridis. The possibility of sewage contamination must also be considered. Knill et al. (1982) correlated the isolation of Campylobacter spp. with the presence of faecal coliforms, supporting the theory that the presence of Campylobacter spp. in water is probably due to faecal contamination.

The isolation of Campylobacter spp. in water was investigated to find out if a correlation could be made with the numbers of gulls roosting on the body of water at night and the level of Campylobacter species. The evidence presented here suggests that nocturnally roosting gulls can be a contributory factor in the bacterial contamination of water. Increased numbers of Campylobacter spp. were present in high usage periods by gulls and corresponding increased numbers of C. laridis, the Campylobacter sp. predominant in gulls, were present during these high usage periods compared with bodies of water not utilised by gulls for roosting. The contribution to the

faecal contamination of reservoirs by gulls, especially during the winter months when gull numbers are at their peak, may have serious implications, as extra treatment may be necessary if the water is to be safe for human use.



CHAPTER 7. GENERAL DISCUSSION

At first glance, the thermophilic Campylobacter spp. seem easily distinguishable by the use of a few simple tests. The detection of hippurate hydrolysis was the original test that distinguished C. jejuni and C. coli (Harvey, 1980). The nalidixic acid resistance of a Campylobacter sp. isolated from gulls by Skirrow and Benjamin (1980b) (later to be designated as C. laridis) introduced a method to differentiate C. jejuni and C. coli from C. laridis. This project isolated these three Campylobacter spp. from various sources and speciated them using these two simple methods.

It has not been until recently that the use of the hippurate hydrolysis test to distinguish between C. jejuni and C. coli has been shown to be less than 100% reliable. Several strains of C. jejuni have been found to be hippurate negative, using DNA relatedness to confirm speciation (Hebert et al., 1984; Harvey and Greenwood, 1983; Roop et al., 1984b). The discovery of these hippurate negative C. jejuni strains illustrates the necessity for additional confirmatory tests to be established to differentiate these two species.

Another aspect of the hippurate test recently discovered is the finding of false negative tube tests for C. jejuni. A comparison of four modifications of the Hwang and Ederer test with gas liquid chromatography (GLC) was made by Morris et al. (1985), using 22 C. jejuni, 11 C. coli and 8 C. laridis strains. Three strains of C. jejuni gave negative or variable results in one or more of the tube tests. However, all of the C. jejuni strains were hippurate positive and all of the C. coli and C. laridis strains were hippurate negative by the GLC method, proving it to be a more sensitive and reliable method for detecting hippurate hydrolysis.

The method used for the hippurate hydrolysis test in this study

was, on occasion, very difficult to interpret. The final colour of the suspension in the tube often appeared to be negative, as it was not the deep, blue-black colour found in the positive tubes. Upon serotyping, some of the isolates believed to be C. coli due to "negative" hippurate tests would serotype with a C. jejuni strain. This did not occur often with the human or gull strains, but occurred frequently with the water isolates. This was not considered crucial in the analysis of the water isolates, as the major differences in Campylobacter spp. that were examined in the water were the amount of C. laridis as compared to the numbers of C. jejuni and C. coli in relation to the numbers of gulls roosting on the water body. The use of more sophisticated methods, such as GLC, could have assisted in this difficulty, but was not within the scope of this project. Naladixic acid resistance is one of the tests used to distinguish C. laridis from C. jejuni and C. coli. Although this characteristic was one of the first to indicate a new species of Campylobacter, it is not to be used solely as a method of speciation. Resistance to this drug has recently been found in a few strains of C. jejuni (Benjamin and Leaper, 1983). One of eight strains of C. laridis has been found to be susceptible to nalidixic acid (Morris et al., 1985).

The Penner and Hennessy (1980) typing scheme for C. jejuni and C. coli has proved to be a straight forward method for the serological characterisation of these two Campylobacter species. The antisera produced in rabbits was relatively easy to accomplish. Only two strains proved difficult to produce, Penner 21 and Penner 3. Penner 21 was never produced, although repeated attempts were made with various isolates of this reference strain. Penner 3 never elicited an antibody response in rabbits, despite several attempts with numerous stock strains. The antisera was finally supplied by C. Fricker, but

the titre was unfortunately reduced during transport.

The Penner 16 antiserum gave several heterologous reactions. It reacted unilaterally (nonreciprocally) against Penner 1,2,6,8,9,10,11,15,18,19,20,27 and 37 antigens (see Table 3.1.). The strength of the cross reactions was fairly weak, at a 1:80 dilution of the antiserum. Since the homologous titre was 1280, cross reactions at a 1:80 dilution during the haemagglutination testing were unlikely, as the lowest dilution of P16 antiserum used was 1:320.

The only Penner antiserum to give a reciprocal cross reaction with P16 antigen was Penner 4 (Table 3.1.). Penner 4 antiserum gave a titre of 320 with the P16 antigen, and Penner 4 antigen gave a titre of 80 with the Penner 16 antiserum. This reciprocity was also found by Penner and Hennessy (1980). Human isolates were found during this study that reacted with both antisera, although in most cases, one of the two presented a much stronger reaction.

Penner 13 antiserum reacted unilaterally with the Penner 16 antigen, with a titre equal to its homologous titre. This cross reactivity was not observed in the isolates tested because the two antisera were not used simultaneously to test the isolates.

Penner 1 antiserum reacted unilaterally with the Penner 7 antigen, giving a titre of 80. This cross reactivity was also found in some of the water and avian isolates. The isolates usually reacted with equal strength against both P1 and P7 antisera.

Such unilateral reactions found in PHA can be explained on a molecular basis, according to Penner et al. (1982). The antigenic determinant (or hapten) that elicits an antibody response is covalently linked on some strains. However, on other strains, this same antigenic determinant may be more loosely attached, so that it is non-immunogenic. The antibodies elicited by the strain with the covalently bound hapten react with another strain with the similar

hapten that is loosely bound, therefore causing a one way, or unilateral reaction in the PHA test.

In comparing the serotypes isolated from water, avian and human sources, it would be logical to assume that if gulls are acquiring C. jejuni/coli strains from human waste, the serotypes of strains found in humans would be found in the gulls. A variety of serotypes were isolated from the gulls, including a few multiple reacting strains not seen in the human population in the study. All of the strains isolated from the gulls in the Strathclyde region were present in the human population in that area.

There was not a predominance of the most common human strains in gulls, except in the case of the unfledged chicks at Flanders Moss and Inchmarnock, but the sample size was quite small, as only 27 C. jejuni/coli strains were isolated. The difference between the chicks and adults in the carriage of Campylobacter spp. could relate to the dietary differences which are known to occur, but vary geographically, or the differences in the gut physiology. Chicks, unlike adults, because of a lower pH in the stomach, are capable of digesting fine bones (Spaans, 1971). However, this would require further investigation into the effect of pH on the survival of Campylobacter spp. found in gulls.

Among the gulls, the 1/7 serotype was found only in the three breeding colonies, Inchmarnock, Flanders Moss and Horse Island. It was also isolated from Gadloch in January, but was not found in the human population in Scotland.

The serotypes isolated from the reservoirs used by gulls were also isolated from the gulls. There were no predominant serotypes in the reservoir isolates, but it is interesting to note that the unutilised reservoirs had the largest proportion of Penner 2 and 4 of all the bodies of water examined. These serotypes, which are among

the most common found in humans, were found mainly in the months when Campylobacter spp. isolations in humans are at their peak, in the summer and early autumn. This indicates possible sewage contamination into these bodies of water, although more detailed analysis of these samples would be necessary to confirm this.

Information on the use of the reservoirs by gulls as overnight roosts has shown that gulls can contribute to the salmonella contamination of water (Benton et al., 1983). This study has shown that the amount of water contamination with Campylobacter spp. relates to the numbers of gulls using these bodies of water as nocturnal roosting sites. Fenlon et al. (1982) have quantified the number of organisms per gram of faeces for herring gulls and great black backed gulls in Scotland as being between 1.8×10^2 and 4.9×10^6. Obviously, the size of the body of water and the number of gulls utilising the site are important factors, but as the gulls spend approximately 8-16 hours roosting on the water (depending on the time of the year), their contribution to the bacterial content of the water is considerable.

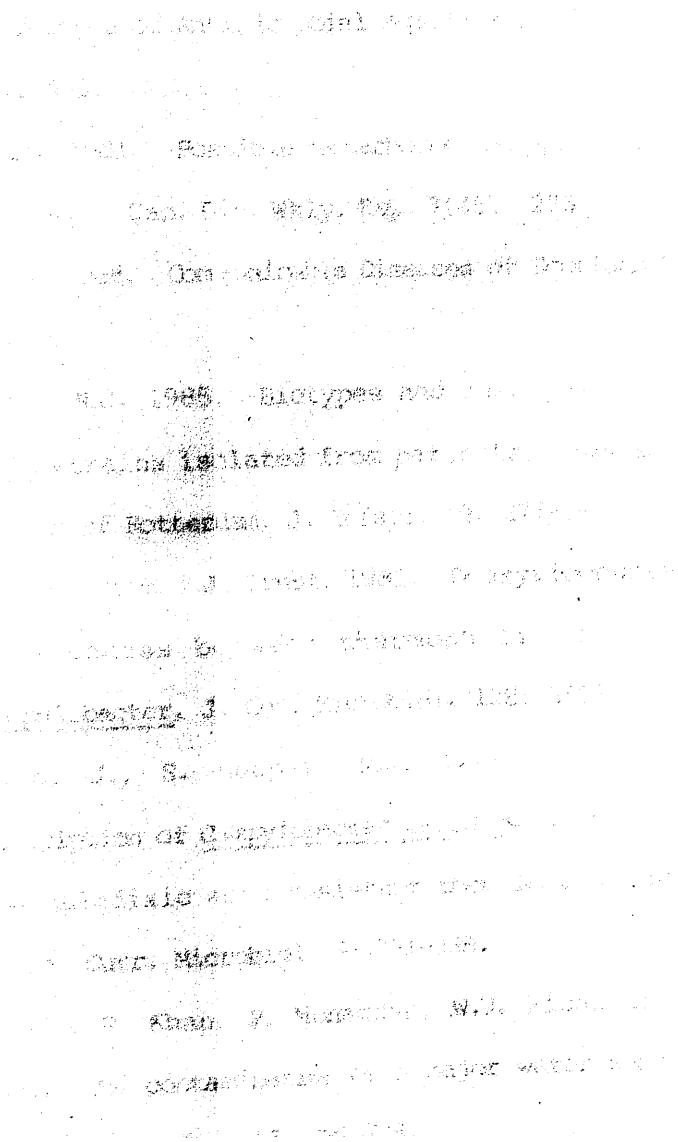
All of the reservoirs used for potable water supply in this study are treated by the Strathclyde Water Board to remove bacterial contamination. This treatment should be sufficient for the destruction of Campylobacter spp. in the raw water. However, if the numbers of gulls increase dramatically, the methods currently in use may not be adequate. This was found to be the case with Salmonella spp. in the Mugdock and Craigmaddie Reservoirs (Benton, et al., 1983). The judicious use of bioacoustics to scare the gulls from roosting on the water has proved successful, thus restoring the quality of the stored water.

The feeding ecology of the birds plays an important role in the species of Campylobacter they carry. Their diet in the Strathclyde

region consists mainly of refuse from tips and sewage outfalls, making them likely reservoirs for the Campylobacter spp. strains found in man. From this study, only a small percentage (29%) of the C. jejuni/coli strains isolated from gulls were able to be serotyped by the panel of 29 Penner antisera that typed 75% of the human strains. This indicates that the proportion of human strains in the gulls is relatively low. It must be pointed out, however, that as only one colony was used from each gull specimen for further study, the fact that C. laridis was isolated from a gull does not exclude the possibility of the colonisation of additional biotypes in the gut of the gull. Overall, C. laridis was the most common Campylobacter spp. in gulls. More research would be needed on the feeding ecology of these birds before all of the various differences in carriage rates can be fully explained. More than likely, gulls are an indicator of the extent of contamination in the environment, recycling the organisms in the environment in a manner dependent upon their feeding ecology.

The PHA method for the serotyping of C. jejuni and C. coli has been effective in elucidating the epidemiology of Campylobacter species. It has been useful in clinical studies to identify links between the sources of outbreaks and those exhibiting clinical symptoms (Blaser et al., 1982). In this study, it has been used to illustrate similiarities in the types of C. jejuni and C. coli isolated from human, avian and water sources. As a practical method, passive haemagglutination is time consuming and too cumbersome to be incorporated into a routine bacteriology laboratory. This type of test would ideally be suited to a reference laboratory, which could tailor the procedure to handle large numbers of samples. The coagglutination procedure, as described by Wong et al. (1985) and

Fricker et al. (1986) is based on the saline extractable, heat stable antigens used in the Penner scheme, but is less time consuming and easier to manipulate. This type of procedure would clearly extend the serotyping of Campylobacter spp. to a wider range of laboratories and thus increase the knowledge of the epidemiology of Campylobacter species.



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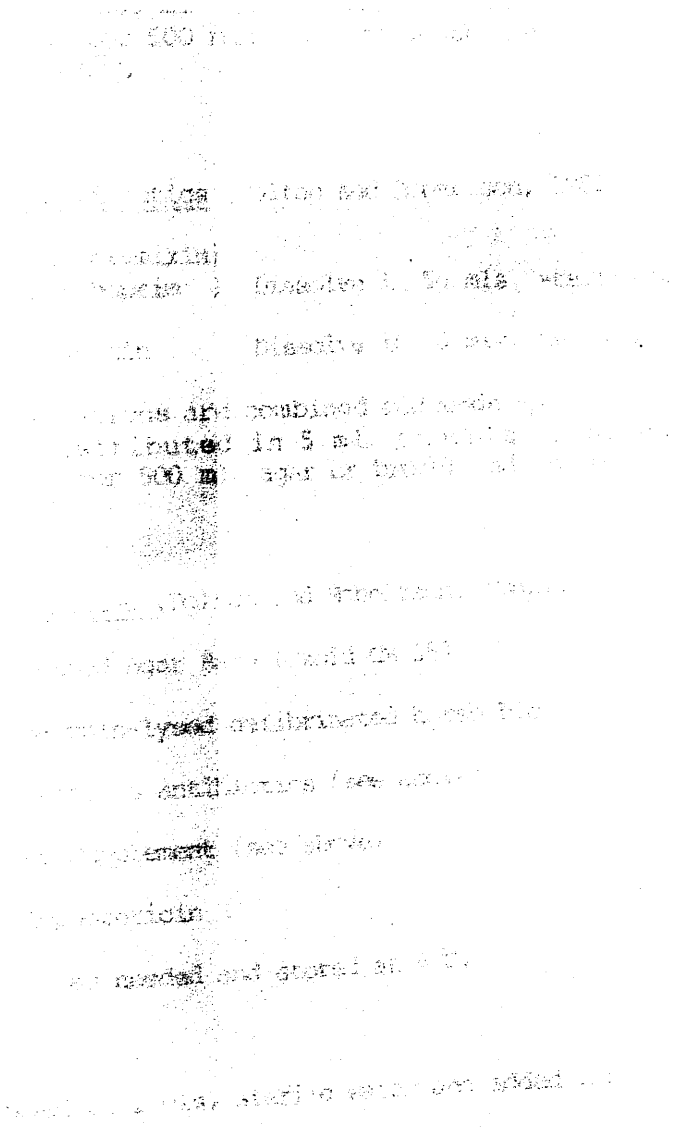

APPENDIX 1.

Description Of Media Used In This Study For The Isolation Of Campylobacter Species

FBP Supplement (George et al., 1978).

18.75 g. sodium metabisulphite

18.75 g. sodium pyruvate

18.75 g. ferrous sulphate

Dissolved in 300 ml. sterile water. Distributed in two ml. amounts (sufficient for 500 mls. agar or broth) and stored in screw capped bijoux at -20°C.

Preston's Antibiotics (Bolton and Robertson, 1982).

100,000 IU. polymixin)
0.2 g. trimethoprim) Dissolve in 50 mls. sterile water.

0.2 g. rifampicin Dissolve in 50 mls. methanol.

The two solutions are combined and made up to 200 mls. with sterile water. Distributed in 5 ml. amounts in screw capped bijoux (sufficient for 500 ml. agar or broth) and stored at -20°C.

Preston's Plates (Bolton and Robertson, 1982).

500 mls. Blood Agar Base (Oxoid CM 55)

25 mls. saponin-lysed defibrinated horse blood (Gibco)

5 mls. Preston's antibiotics (see above)

2 mls. FBP Supplement (see above)

1.0 mg. Amphotericin B*

Made fresh as needed and stored at 4°C.

* Dissolved in 2 mls. sterile water and added for gull specimens only.

Preston Enrichment Broth (Bolton and Robertson, 1982).

500 mls. Nutrient Broth No. 2 (Oxoid CM 67)

2 mls. FBP supplement

5 mls. Preston's antibiotics

1.0 mg. Amphotericin B*

Screw capped bijoux filled to the top and stored at 4°C.

FBP Storage Broth (Goosens et al., 1984).

2.5 g. Nutrient Broth No. 2 (Oxoid CM 67)

0.12 g. Bacteriological Agar No. 1 (Oxoid)

15 mls. glycerol

0.4 mls. FBP supplement (see above)

The two powders are dissolved in 85 mls. distilled water and the glycerol added. Sterilize at 121°C. for 15 minutes and cool. The FBP supplement is added, and the broth aliquoted in one ml. amounts into sterile Eppendorfs (Alpha Laboratories) and stored at -20°C.

Blood Agar Plates

500 mls. Columbia Agar Base (Lab m)

25 mls. defibrinated horse blood

2 mls. FBP supplement

Made fresh as needed and stored at 4°C.

* Dissolved in 2 mls. sterile water and used for gull specimens only.

APPENDIX 2

The Numbers of First Year and Intermediate Aged Herring Gulls Positive With Campylobacter Species In Scotland

		FIRST YEARS			INTERMEDIATES		
DATE	LOCATION	C.LAR.	C.JEJ./COLI	NO. EXAM.	C.LAR.	C.JEJ./COLI	NO. EXAM.
28/09/84	B'riggs	13	15	39	9	9	27
20/10/82	B'riggs	4	6	15	1	8	13
04/10/82	Bowmore	1	1	5	9	2	17
18/11/82	Bowmore	0	1	1	4	3	15
12/10/82	Thurso	7	1	23	24	30	91
29/11/84	Moodies.	0	5	5	2	7	12
13/12/84	Moodies.	0	2	2	0	7	7
24/01/85	Moodies.	1	0	1	1	0	0
21/02/85	Moodies.	4	3	8	6	2	8
17/04/85	Moodies.	2	2	4	12	4	16
24/05/85	Horse I.	0	0	0	0	2	2
	Horse I.[1]	1	0	1	0	0	0
25/06/85	Aberdeen	0	0	0	16	4	25
27/06/85	Flanders Moss[1,2]	2	12	40	0	0	0
04/07/85	Inchmar.[2]	10	15	84	0	0	0
13/08/85	B'riggs.	12	12	28	3	2	7
11/12/85	Boat-Ayr	0	3	4	1	0	2

[1] Lesser Black Backs

[2] unfledged birds

B'riggs =Bishopbriggs
Moodies.=Moodiesburn
Horse I.=Horse Island
Inchmar.=Inchmarnock

APPENDIX 3

The Numbers of Adult Herring Gulls With Campylobacter Species and The Total Number Of Gulls Examined In Each Catch

DATE	LOCATION	C.LARIDIS	C.JEJ./COLI	NO. EXAM.	TOTAL NO.EXAM.
28/09/82	Bishopbriggs	16	24	61	127
20/10/82	Bishopbriggs	15	11	45	73
04/10/82	Bowmore	26	18	85	107
18/11/82	Bowmore	21	11	60	76
12/11/82	Thurso	17	19	67	181
29/11/84	Moodiesburn	8	8	37	54
13/12/84	Moodiesburn	8	13	40	49
24/01/85	Moodiesburn	29	9	53	53
21/02/85	Moodiesburn	18	5	28	44
17/04/85	Moodiesburn	7	3	11	31
24/05/85	Horse Island	9	2	31	33
	Horse Island[1]	65	33	226	227
25/06/85	Aberdeen	31	14	72	97
13/08/85	Bishopbriggs	6	8	15	50
11.12.85	Boat-Ayr	1	3	13	19
		277	181	891	

[1] Lesser Black Backs

761 Camp = 62%
422 Lari 55%
339 Jej/Col 45%

APPENDIX 4

The Number of C. jejuni/coli Serotypes Isolated from Herring Gulls Which Were Able to be Serotyped Divided Into Age Classes

()=Total Number of C. jej./coli examined

Date	Location	1st Year	Intermed.	Adult
29/11/84	Moodiesburn	4 (5)	3 (7)	2 (8)
13/12/84	Moodiesburn	1 (2)	3 (7)	1 (13)
24/01/85	Moodiesburn	0 (0)	0 (0)	0 (9)
21/02/85	Moodiesburn	0 (3)	0 (2)	2 (5)
17/04/85	Moodiesburn	0 (2)	2 (4)	1 (3)
24/05/85	Horse Island	0 (0)	1 (2)	0 (2)
	Horse Island[1]	0 (0)	0 (0)	9 (33)
25/06/85	Aberdeen	0 (0)	2 (4)	1 (14)
13/08/85	Bishopbriggs	3 (12)	0 (2)	1 (8)
11/12/85	Boat-Ayr	1 (3)	0 (0)	0 (3)

Number of C. jejuni/coli Isolates From Unfledged Chicks Which Were Able to be Serotyped

27/06/85	Inchmarnock	10 (15)
04/07/85	Flanders Moss[1]	6 (12)

[1] Lesser Black Backed Gulls

APPENDIX 5

The Number of Negative MPN Broths (out of ten) From Reservoirs Utilised By Gulls As Overnight Roosting Sites For the Period March to July

Month	Barcraigs	Fannyside	Balgray	Strathclyde Park	Hogganfield
March	nd.	10	nd.	10	8
April	nd.	10*	nd.	10*	10*
June	10*	10	10	10	10
July	10	10	10	10*	10

Number of Negative MPN Broths (out of ten) In Reservoirs Not Used By Gulls For the Period March to July

Month	Gadloch	Cochno	Johnston
March	10	nd.	10
April	10	nd.	10
June	10	10	10*
July	6	10	9

* Indicates isolation of *Campylobacter* spp. by filtration only.

nd.=not done

See p. 76 for MPN values

APPENDIX 6

The Number Of Negative MPN Broths (out of ten) In Reservoirs Utilised By Gulls During The Periods Of Medium (Aug.-Sept.) And High (Oct.-Jan.) Usage

Month	Barcraigs	Fannyside	Balgray	Strathclyde Park	Hogganfield
Aug.	10	10	10	2	10
Sept.	9	10	9	9	10
Oct.	9	9	9	1	10
Nov.	8	6	6	9	10
Dec.	8	6	9	3	6
Jan.	6	5	9	3	0

The Number Of Negative MPN Broths (out of ten) In Reservoirs Not Utilised By Gulls During The Period Aug.-Jan.

Month	Gadloch	Cochno	Johnston
Aug.	1	10	7
Sept.	1	10	8
Oct.	2	10	8
Nov.	10	9	9
Dec.	3	10	9
Jan.	3	10	6

APPENDIX 7

The Mean Number of Herring Gulls and the Mean Number of Campylobacter spp. for Periods of Medium (Aug.-Sept.) and High (Oct.-Dec.) Usage In Reservoirs Utilised and Not Utilised By Gulls As Roosting Sites

	Aug.-Sept.		Oct.-Dec.	
Reservoir	Mean No. Herring gulls	Mean No. Campy. spp. per 100 mls.	Mean No. Herring gulls	Mean No. Campy. spp. per 100 mls.
Strath. Park	512	60	1762	80
Hogganfield	89	0	389	10
Balgray	80	0	641	22
Fannyside	147	0	117	20
Gadloch	—	230	—	69
Cochno	—	0	—	0
Johnston	—	30	—	10

APPENDIX 8

Ranking of Numbers of Herring Gulls and Numbers of Campy. spp. per 100 mls. During the Months of Medium (Aug.-Sept.) and High (Oct.-Dec.) Usage For Reservoirs Utilised As Gull Roosts

Month	Number of H. gulls	Rank	Number of Campy. spp. per 100 mls.	Rank
Strath. Park				
Aug.	628	2	160	4
Sept.	395	1	10	1.5
Oct.	822	3	230	5
Nov.	1964	4	10	1.5
Dec.	2500	5	120	3
Fannyside				
Aug.	82	2	0	2
Sept.	130	3	0	2
Oct.	200	5	10	4
Nov.	0	1	0	2
Dec.	150	4	51	5
Balgray				
Aug.	0	1	F*	1
Sept.	161	3	10	3
Oct.	150	2	10	3
Nov.	800	4	51	5
Dec.	972	5	10	3
Hogganfield				
Aug.	5	1	0	1
Sept.	172	2	F	3
Oct.	174	3	F	3
Nov.	397	4	F	3
Dec.	596	5	51	5

*F refers to Campylobacter spp. isolated from the filter only. The Campylobacter spp. in the filter was taken to rank above the situation where no Campylobacter spp. were isolated by the MPN method.

APPENDIX 9

Number of MPN Broths (Out of Ten) Positive With C. jejuni[1] and C. laridis For Reservoirs and Lochs Utilised by Gulls As Roosting Sites

Month	Strath. Park	Balgray	Fannyside	Hogganfield	Barcraigs
	C. jejuni[1]/C. laridis				
Aug.	8/0	*/0	0/0	0/0	*/0
Sept.	1/0[2]	1/0	0/0	0/*	1/0[2]
Oct.	2/7	1/0[2]	1/0	*/0	1/0
Nov.	1/0[2]	1/3	0/0	*/0	0/2
Dec.	3/4	0/1	4/0[2]	2/2	2/0[2]

Number of MPN Broths Positive (Out Of Ten) For C. jejuni[1] and C. laridis For Lochs Not Utilised By Gulls As Roosting Sites

Month	Cochno	Gadloch	Johnston
	C. jejuni[1]/C. laridis		
Aug.	0/0	9/0	3/0
Sept.	0/0	9/0	2/0
Oct.	0/0	8/0	2/0
Nov.	1/0	0/0	1/0
Dec.	*/0	7/0	1/0

[1] includes C. coli

[2] Indicates the isolation of C. laridis using filtration, when the isolates from the MPN method were C. jejuni or C. coli.

* This refers to the isolation of C. jejuni/coli or C. laridis obtained only from the filter, when the MPN method showed no positive broths. The finding of Campylobacter spp. in the sample via the filter was a positive result that was not able to be enumerated by the MPN method, which gives the finding of 10 negative broths as being the equivalent of 0-37 organisms per 100 mls. The positive filter in this case confirmed the presence of Campylobacter spp. in very small numbers that were not picked up by the quantitative method.