THE EPIDEMIOLOGY OF *YERSINIA* INFECTIONS IN GOAT FLOCKS

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY AT MASSEY UNIVERSITY

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ABSTRACT

Due to the increasing frequency of yersiniosis in goats, it was considered necessary to study the behaviour of *Yersinia* species in goat flocks. The aim of this study, carried out in several phases among goat farms in the Manawatu, was to identify factors involved in the epidemiology of *Yersinia* infections, which might eventually lead to the formulation of effective control measures.

The first phase of the study was the screening of goat flocks for the presence or absence of *Yersinia* species infections. This phase was considered necessary, since prior to the study the prevalence of such infections among apparently healthy goats were unknown. The results of this phase showed that 18 of the 30 farms screened (60%) were positive for the infection.

Concurrent with the screening phase, a postal survey was undertaken involving the farmers of the thirty goat farms. This survey gave an indication of the production and health management practices implemented on goat farms in the region, and how these related to the presence of *Yersinia* species infections in the goat flocks. The results of the survey (97% response rate) showed that farmers generally managed goats in the same manner as sheep, and that the farmers' knowledge of the presence of gastrointestinal bacterial infections such as yersiniosis was almost nonexistent. Stress-related management practices which might be associated with the presence of *Yersinia* species infections were also identified in this phase of the study.

The farms found positive during screening were included in the next phase of the study, the prevalence survey. This phase involved the sampling of three age groups from each flock: kids (less than one year old), hoggets (one to two years old), and adults (two to five years old). The results of this phase showed that the mean level of *Yersinia* prevalence of all the combined age groups from the 18 farms was 15%. In kids, the prevalence was 24.7%, in hoggets 11.8%, and in adults 9.6%. The predominant *Yersinia* species recovered from kids was *Y. enterocolitica* biotype 5, comprising 94.5% of all the isolates. Among hoggets, *Y. enterocolitica* biotype 5 and the environmental strains (*Y. frederiksenii*, *Y. kristensenii* and *Y. intermedia*) were about equal in prevalence, while among the adults, the environmental strains predominated, comprising 92.7% of all the isolates in that group.

The prevalence survey also revealed that infection levels among the different goat flocks were extremely variable, and since sampling was conducted only once, the results were obviously only minimum estimates of flock infection levels.

In order to explain the inherent drawbacks associated with a single sampling event, it was decided to carry out repeated samplings on the same group of animals over time, particularly as *Yersinia* species infections had been reported in the past to be commonest during the colder months of the year. Thus a cohort study was implemented, where selected groups of goats stratified into three age groups (kids, hoggets and adults) were subjected to repeated monthly samplings for at least 12 months.

Several key points were brought to light by the cohort study. It was shown that the incidence of potentially pathogenic *Yersinia* species (*Y. pseudotuberculosis* and *Y. enterocolitica* biotypes 2, 3 and 5) appeared to have a distinct seasonal variation, a characteristic which was absent in the incidence of the majority of the environmental strains (*Y. enterocolitica* biotype 1A, *Y. frederiksenii*, *Y. kristensenii*, *Y. intermedia* and *Y. rohdei*). Of the climatic factors studied, low daily minimum temperatures were particularly influential on the incidence of the potentially pathogenic strains, while increased monthly precipitation levels were highly influential on the incidence of the environmental strains. Age was also an important factor in the incidence of the infections, with the younger age groups showing a higher incidence of the potentially pathogenic strains and the older age groups showing a higher incidence of the environmental strains. The ability of the animals to develop apparent immunity against subsequent reinfection by the potentially pathogenic *Yersinia* species was another finding of the cohort study.

The numerous strains of *Yersinia* isolated throughout the study exhibited heterogeneity in their reactions to biochemical testing, even among strains within the same species. An attempt was therefore made to classify these strains using numerical taxonomy. This procedure indicated that the pathogenic and environmental *Yersinia* strains were quite different, as shown by a number of distinct clusters.

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CHAPTER 1

GENERAL INTRODUCTION

Prior to this study, published records of yersiniosis among goats worldwide were limited to isolated reports of clinical cases and outbreaks in goat flocks, with the probable exception of a study made by Krogstad (1974) in Norway, where a 12-month serological and bacteriological survey on *Yersinia enterocolitica* infection in a goat flock was undertaken. Apart from this study, no other literature has been found on formally organised surveys on the carriage of *Yersinia* species by healthy goats. In New Zealand, the situation was the same, where reports of yersiniosis in goats came only from isolated outbreaks of the disease (McSporran, 1983; Orr, 1987), or were just parts of larger surveys of diseases of livestock and goats (Hodges *et. al.*, 1984a; Buddle *et. al.*, 1988).

This present study was therefore an attempt to establish the epidemiology of *Yersinia* species infections within goat flocks, with the specific aims of determining if *Yersinia* infections are affected by seasonal changes and by the age of the animals under consideration.

The study was undertaken in several phases. The first phase was the screening of goat farms in the Manawatu for the presence or absence of *Yersinia* species infection. This phase was considered necessary for determining the percentage of flocks infected and deciding on the number of farms to be included in the next phase. Concurrent with the screening survey, a questionnaire-interview was conducted of the farmers concerned to obtain general knowledge of the production and health management practices implemented on these farms, and how they relate to the health problems on a particular farm. The second phase of the study involved intensive sampling of the farms found positive in the first phase, with the idea of establishing statistically sound figures for the prevalence of *Yersinia* species infections. Since this prevalence survey, as expected, provided only minimum estimates of the prevalence of infection, the third phase, a cohort study, was conducted with the specific aim of following up three farms to evaluate the monthly levels of *Yersinia* infections for at least a year. In this phase of the study, the degreee of underestimation of prevalence which resulted from the second phase was assessed, and a better understanding of the dynamics of the infections was obtained.

It was anticipated that as a result of this study, a better understanding of *Yersinia* species infection could be achieved, and the formulation of better control methods would be possible. The identification of the specific *Yersinia* biotypes and serotypes from goats could then be compared with the isolates coming from other animal species, including humans, worldwide. Of particular interest would be the isolation of human biotypes and serotypes from goats, as this would mean that goats may serve as reservoirs of the infection for human beings.

CHAPTER 2

REVIEW OF LITERATURE

Introduction

The term yersiniosis originally referred only to infections of animals and man due to either *Yersinia pseudotuberculosis* or *Yersinia enterocolitica*. Recent research, however, has resulted in the identification of new species of *Yersinia*, necessitating the wider use of the term to include infections by these additional species. The use of the term "pseudotuberculosis" has sometimes led to confusion because it has been used in veterinary pathology to describe not only the lesions due to *Y. pseudotuberculosis* but also to those caused in sheep and goats by *Corynebacterium pseudotuberculosis* (Mair, 1975). With the acceptance of the new generic name *Yersinia* for *Pasteurella pestis*, *Pasteurella pseudotuberculosis* and *Yersinia enterocolitica* (Mollaret and Thal, 1974), the modern practice is to avoid the term pseudotuberculosis entirely and to refer instead to "yersiniosis" to refer to infections caused by *Y. pseudotuberculosis*, *Y. enterocolitica*, and the new *Yersinia* species.

Members of the Genus *Yersinia*

The following *Yersinia* species have been recovered from a variety of animal and inanimate sources and therefore will be considered in this review: *Yersinia pseudotuberculosis*, *Yersinia enterocolitica*, *Yersinia frederiksenii*, *Yersinia kristensenii*, *Yersinia intermedia*, *Yersinia aldovae*, *Yersinia rohdei*, *Yersinia mollaretii*, and *Yersinia bercovieri*. Although it causes disease in man and rodents, *Yersinia pestis* is absent from New Zealand (Blackmore and Humble, 1987) and therefore will not be discussed. Likewise, *Yersinia ruckeri* (Ewing *et. al.*, 1978), one of the causes of redmouth disease in rainbow trout and other fishes, has never been reported to infect man and other animals and therefore will not be considered.

Yersinia pseudotuberculosis

Y. pseudotuberculosis was first isolated in 1853 by Malassez and Vignal after they produced what appeared to be tuberculous lesions in guinea pigs inoculated with pus from a child who had died of tuberculous meningitis (Mair, 1975). Typical biochemical reactions of *Y. pseudotuberculosis* are given by Kapperud and Bergan (1984), Brenner *et. al.* (1976), and Bisset (1981).

Yersinia pseudotuberculosis was originally grouped into serotypes I to VI, with serotypes I, II, IV, and V divided into subtypes A and B (Thal, 1978). The addition of subtype IIC and serotypes VII and VIII has been proposed by Tsubokura *et. al.* (1984b).

Yersinia enterocolitica

Y. enterocolitica was first described by Schleifstein and Coleman in 1939 when they isolated a pathogen from a human infection which had some cultural, biochemical, and pathogenic features in common with *Y. pseudotuberculosis* (Obwolo, 1976). Following this isolation, little attention was given to this organism until the 1960's when several outbreaks in chinchillas occurred in Europe, the United States, and Mexico, which were later identified as due to *Y. enterocolitica* (Hurvell, 1981). *Yersinia enterocolitica sensu stricto* has been characterised by Bercovier *et. al.* (1980a).

The latest biotyping scheme for *Y. enterocolitica* has been proposed by Wauters *et. al.* (1987). These authors grouped *Y. enterocolitica* strains into 6 biotypes (1 to 6) with biotype 1 divided into subtypes A and B. Also, several antigenic O-factors have been described for this organism (Wauters, 1981; Aleksic and Bockemuhl, 1984).

Yersinia frederiksenii

Enterobacterial strains that are rhamnose-positive and were formerly called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like are now included in a new *Yersinia* species, *Yersinia frederiksenii* (Ursing *et. al.*, 1980).

Yersinia kristensenii

Enterobacterial strains which were also previously called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like but do not have the ability to ferment sucrose are now included in a new *Yersinia* species, *Yersinia kristensenii* (Bercovier *et. al.*, 1980).

Yersinia intermedia

Another group of *Yersinia* strains formerly called *Yersinia enterocolitica* or *Yersinia enterocolitica*-like but able to ferment rhamnose, melibiose, and raffinose are now included in a new species, *Yersinia intermedia*. Eight biotypes of this species have been identified (Brenner *et. al.*, 1980). An additional biotype (biotype 9) has been proposed by Agbonlahor (1986).

Yersinia aldovae

Formerly called Group X2 *Yersinia enterocolitica*, isolates which are capable of producing acid from L-rhamnose, but do not ferment sorbose, cellobiose, melibiose, or raffinose, and rarely ferment sucrose (5% in 48 hours, 10% in 7 days), are now included in a new species, *Yersinia aldovae* (Bercovier *et. al.*, 1984).

Yersinia rohdei

Yersinia rohdei can be distinguished from other *Yersinia* species by its positive reactions in tests for citrate and sucrose fermentation, and negative reactions in tests for indole, acetoin (Voges-Proskauer), and rhamnose (Aleksic *et. al.*, 1987).

Yersinia mollaretii

Formerly called Biogroup 3A *Yersinia enterocolitica*, this species is characterised by its negative Voges-Proskauer reaction and positive reactions in tests for pyrazinamidase, acid production from mucate, proline peptidase, and acid production from D-xylose. It also ferments L-sorbose but not L-fucose (Wauters *et. al.*, 1988).

Yersinia bercovieri

Formerly called Biogroup 3B *Yersinia enterocolitica*, this species has the same biochemical reactions as *Y. mollaretii*, except for its inability to ferment L-sorbose and the ability to ferment L-fucose (Wauters *et. al.*, 1988).

Yersiniosis in Man and Animals: The Global Situation

The role of *Yersinia pseudotuberculosis* as a pathogen in humans and animals is long-established (Thal, 1978), but interest in *Yersinia enterocolitica* as a human pathogen has increased enormously in the last twenty years (De Groote *et. al.*, 1982). *Y. enterocolitica* is a much-heralded microorganism in various parts of the world, particularly in European countries (Bottone, 1977), and infections by this organism are probably common but unrecognised in many other countries (Morris and Feeley, 1976). It is unclear whether the rapid increase in the incidence of *Y. enterocolitica* infections is due to an increased awareness and improved isolation procedures, or represents a genuine increase in the occurrence of the infection in man and animals. Of these two explanations, Morris and Feeley (1976) speculated that the increase in reported isolations is more likely to be a result of greater awareness about this organism and about its potential role in human and animal disease.

The frequency of isolations from man and animals is greatly influenced by the activities of a few laboratory workers and researchers who actively look for the organism in routine enteric specimens. Routine laboratory procedures often do not result in the isolation of *Yersinia* species, mainly because they are difficult to identify using ordinary media and methods.

Human Infections

Yersinia pseudotuberculosis infections. Yersiniosis in man caused by *Y. pseudotuberculosis* usually occurs sporadically, in contrast to infections in various species of birds and animals, where the disease can reach epidemic proportions, usually accompanied by high mortalities (Stovell, 1980). Cases of yersiniosis due to *Y. pseudotuberculosis* have been reported from all continents, with most cases coming from the northern hemisphere, especially Europe (Obwolo, 1976). While the prevalence in Europe is generally high, the disease is almost unknown in the Iberian peninsula, Italy, and Greece (Stovell, 1980). Also, human infection is

almost unknown in the African continent, India, and Southeast Asia (Mair, 1975). Full awareness of human disease due to this microorganism only dates back to the 1950's, when a series of cases described as abscess-forming reticulocytic lymphadenitis were described, occurring mostly in children operated on for supposed appendicitis (Knapp, 1958).

According to Thal (1978), the most common *Y. pseudotuberculosis* serotype causing human disease in Europe has been serotype I. The first descriptions of pseudoappendicitis cases, mostly caused by serotype I, were described in Germany (Knapp, 1958). Reports from Great Britain in the 1960's also showed serotypes IA and IB to be the most common serotypes involved in human infections (Mair *et. al.*, 1960; and Randall and Mair, 1962), although Randall and Mair (1962) were not able to isolate *Y. pseudotuberculosis* from the cases of pseudoappendicitis and mesenteric adenitis encountered in their study. Subsequent reports by Mair (1965), Macaulay *et. al.* (1967), and El-Maraghi and Mair (1979) confirmed these same serotypes as the most commonly involved in human infections in Great Britain.

Serotypes IA and IB have also been the predominant serotypes involved in human *Y. pseudotuberculosis* infections in North America (Hubbert *et. al.*, 1971; Yamashiro *et. al.*, 1971; Paff *et. al.*, 1976; Toma, 1986).

Although serotype I has been the most prevalent *Y. pseudotuberculosis* strain involved in human infections, serotypes II and III have also been recorded (Thal, 1978). Serotype II has been reported by El-Maraghi and Mair (1979) in Britain. Serotype IIC, a new subdivision of this serotype, was isolated from a human case in Japan (Tsubokura *et. al.*, 1984b). Hubbert *et. al.* (1971) reported a case of mesenteric lymphadenitis involving serotype III, and Paff *et. al.* (1976) reported a fatal case of septicaemia involving the same serotype. Serotype III has also been isolated in Finland by Tertti *et. al.* (1984), and found to be implicated (through serology only) in a case of ileitis in Great Britain by Tobin *et. al.* (1988).

Serotypes IV and V have rarely been encountered in human disease (Thal, 1978; and Stovell, 1980). Almost all human cases involving serotype IV (particularly serotype IVB) have come from Japan (Tsubokura *et. al.*, 1970; Tsubokura *et. al.*, 1982; Fukushima *et. al.*, 1988; Tsubokura *et. al.*, 1989). One report of serotype IV involvement in human disease was described by Attwood *et. al.* (1987) in Ireland, but the role of *Y. pseudotuberculosis* in that report was only determined serologically.

The first isolation of *Y. pseudotuberculosis* serotype V from a human case (inconclusively designated as serotype I-V) was made by Yamashiro and his co-workers in 1971. This was followed by a report from Bradley and Skinner (1974) concerning a case of *Y. pseudotuberculosis* serotype V septicaemia concurrent with sickle-cell anaemia. Since the subdivision of serotype V into serotypes VA and VB (Tsubokura *et. al.*, 1971), most isolates of these serotypes have come from Japan (Tsubokura *et. al.*, 1982; Tsubokura *et. al.*, 1984a; Tsubokura *et. al.*, 1989; Nakano *et. al.*, 1989).

Serotype VI has never been isolated from humans; all isolations have been from animals in Japan (Tsubokura *et. al.*, 1971; Tsubokura *et. al.*, 1982; Tsubokura *et. al.*, 1989).

The new *Y. pseudotuberculosis* serotypes (VII and VIII) as proposed by Tsubokura *et. al.* (1984b) have been encountered only from rats and dogs in Japan.

Yersinia enterocolitica infections. The highest incidence of *Y. enterocolitica* infections in humans has been observed in Scandinavia (Nilehn, 1969a; Ahvonen, 1972; WHO Scientific Working Group, 1980; Christensen, 1987), Belgium (Vandepitte and Wauters, 1979; De Groote *et. al.*,1982; Tauxe *et. al.*, 1987), Canada (Toma and Lafleur, 1974; Marks *et. al.*, 1980), and Japan (Fukushima *et. al.*, 1985). Infections are less often reported in the United States, the United Kingdom, and France, in spite of extensive surveys (De Groote *et. al.*, 1982).

Investigations in recent years have indicated that diseases due to *Y. enterocolitica* are usually associated with specific biotypes, serotypes, and phage types. In addition, differences exist in the geographical locations of these types, even between countries with contiguous borders.

The importance of *Y. enterocolitica* as a human pathogen in Scandinavia was first determined by Winblad *et. al.* in 1966. Later research by workers in these countries resulted in the establishment of *Y. enterocolitica* serotype O:3 as the most common *Y. enterocolitica* strain involved in human infections, followed by serotype O:9 (Nilehn, 1969a; Ahvonen, 1972; Christensen, 1987).

The majority of human *Y. enterocolitica* isolates from Belgium belong to biotype 4 serotype O:3. *Y. enterocolitica* biotype 2 serotype O:9 make up the next largest group of strains isolated. During the period 1963-1975, 89.2% of all the *Y. enterocolitica* strains isolated in that country belonged to serotype O:3, with O:9 making up just 8.6% of the total (Vandepitte and Wauters, 1979). A follow-up study on the incidence of *Y. enterocolitica* isolations three years later

revealed that serotype O:3 remained the most predominant, accounting for 84% of all the isolations (De Groote *et. al.*, 1982). The same study also revealed that there was an increasing proportion of serotype O:9 isolates and of serotypes other than O:3 and O:9.

In other European countries, such as the Federal Republic of Germany, Hungary, the Netherlands, France and Italy, *Y. enterocolitica* serotypes O:3 and O:9 were also found to be the most prominent serotypes isolated from human cases of yersiniosis (Feller *et. al.*, 1983; WHO Scientific Working Group, 1980; Lecomte *et. al.*, 1989; Mingrone *et. al.*, 1987; Chiesa *et. al.*, 1987).

A different situation regarding *Y. enterocolitica* infections has been reported from Great Britain. Results from two surveys on the carriage rate of *Yersinia* spp. by human patients showed that most of the *Y. enterocolitica* strains isolated belonged to serotypes O:5,27 and O:6,30 (Lewis and Chattopadhyay, 1986; Greenwood and Hooper, 1987). The results of these surveys are of considerable interest because serotypes O:3 and O:9 were never isolated in these studies mentioned, even though Britain is only separated from mainland Europe by the English Channel. These surveys showed that serotype O:3 is relatively rare in the United Kingdom.

South African isolates of *Y. enterocolitica* usually belong to biotype 4, serotype O:3, phage type 9a (Rabson *et. al.*, 1975; Robins-Browne *et. al.*, 1981).

In Japan, most of the early isolates of *Y. enterocolitica* were biotype 4 serotype O:3, with a minority made up of biotype 2 serotype O:9 (Zen-Yoji and Maruyama, 1972; Zen-Yoji *et. al.*, 1973; Asakawa *et. al.*, 1973;). Recently, however, other serotypes of *Y. enterocolitica* have been involved in human yersiniosis. Fukushima *et. al.* (1985) reported the isolation of *Y. enterocolitica* biotype 3B serotype O:3 from clinical cases of yersiniosis in Shimane prefecture. The same serotype was likewise observed by Shiozawa *et. al.* (1987) in Shizuoka prefecture. Serotype O:5,27 was also isolated from clinical cases, as reported by Maruyama (1987) and Fukushima *et. al.* (1987). Most of the Japanese isolates of *Y. enterocolitica* biotype 4 serotype O:3 belong to phage type 8 (Fukushima *et. al.*, 1984c).

Y. enterocolitica isolates from the North American continent show an interesting distribution. Early reports from Canada revealed strains of *Y. enterocolitica* that were quite distinct from the *Y. enterocolitica* strains isolated in the United States. Toma and Lafleur (1974) summarised the characteristics of *Y. enterocolitica* isolates from the different Canadian provinces and found that the most predominant *Y. enterocolitica* strain present was biotype 4 serotype O:3

phage type 9b, the so-called "Canadian strain". These authors pointed out that this strain is different from the serotype O:3 found in Europe and Japan (phage type 8), and in South Africa (either phage type 8 or 9a). Mention was also made by these authors that this "Canadian strain" was practically the only serotype present in Quebec and was the predominant serotype in Ontario. The other *Y. enterocolitica* serotypes mentioned in this study as being significant in human yersiniosis in Canada were serotypes O:5,27, O:8, and O:4,32, but these were isolated from relatively few cases.

Subsequent surveys on *Y. enterocolitica* isolates in Canada revealed similar results. Caprioli *et. al.* (1978) found serotype O:3 to be the most common serotype, followed by serotypes O:6,30 and O:5,27. One isolate of *Y. enterocolitica* serotype O:8 was encountered by these authors. In cases of *Y. enterocolitica* gastroenteritis, biotype 4 serotype O:3 was found to be the most commonly isolated serotype (Marks *et. al.*, 1980; Thompson and Gravel, 1986). The other significant *Y. enterocolitica* serotypes recovered from these patients were O:6,30, O:5,27, and O:7,8 (Pai *et. al.*, 1979). Simmonds *et. al.* (1987) reported that in the cases of gastroenteritis encountered in their study the most common *Y. enterocolitica* strains isolated were non-serotypable, with serotypes O:6,30, O:5, and O:7,8 occurring in considerable numbers.

The significance of these findings is that *Y. enterocolitica* serotype O:9, which is very common in Europe and Japan, has not been encountered in the above-mentioned surveys. *Y. enterocolitica* serotype O:8, a very common serotype in the United States, appears to be rare in Canada as well. Isolated reports of outbreaks and clinical cases in Canada have been caused by other serotypes of *Y. enterocolitica*, such as serotype O:5 (Ratnam *et. al.*, 1982), biotype 3 serotype O:21 (Karmali *et. al.*, 1982), and biotype 1 serotype O:21 (Martin *et. al.*, 1982).

Prior to 1978, most of the *Y. enterocolitica* strains isolated from cases of yersiniosis in the United States belonged to serotype O:8 (Bottone, 1983). This serotype has been involved in an inter-familial outbreak of yersiniosis in North Carolina in 1972 (Gutman *et. al.*, 1973), and in a 1976 epidemic among children in New York, in which the source of infection was identified as contaminated chocolate milk (Black *et. al.*, 1978). It has also been isolated from human patients in outbreaks of *Yersinia* enteritis (Shayegani *et. al.*, 1983; Tacket *et. al.*, 1985).

More recently, however, other serotypes have assumed greater importance as a cause of human yersiniosis in the United States. Shayegani *et. al.* (1981) summarised the characteristics of *Yersinia* species isolated from human, animal, and environmental sources in New York. During the course of the study (1976-

1980), it was determined that *Y. enterocolitica* serotype O:8 was the most predominant serotype found during the early part of the study, but in the later years isolations of other serotypes became more frequent. *Y. enterocolitica* biotype 4 serotype O:3 phage type 9b ("Canadian strain") was first isolated by the authors in 1977, and was isolated with increasing frequency towards the end of the study period. The other *Y. enterocolitica* serotypes isolated by the authors from human sources were O:5, O:6,31, and O:7,8. According to Bottone (1981), another *Y. enterocolitica* serotype that has become strongly associated with human disease in the United States is serotype O:5,27.

Recently, *Y. enterocolitica* biotype 4 serotype O:3 phage type 9b ("Canadian strain"), which was formerly isolated only from Canada, has been shown to be the single most common serotype causing human infection in New York City (Bottone, 1983; Bottone *et. al.*, 1987), superseding the importance of serotype O:8 and other *Y. enterocolitica* serotypes.

In the United States, other *Y. enterocolitica* serotypes previously considered to be rare or unknown in human disease have gained prominence as human pathogens. *Y. enterocolitica* biotype 2 serotype O:20 was isolated by Wilson *et. al.* (1976) from an infant with adenopathy. Serotypes O:2,3 and O:5 have been shown to cause mesenteric adenitis and septicaemia (Chester *et. al.*, 1981). Serotypes O:13 and O:18 have been involved in cases of pharyngitis (Tacket *et. al.*, 1983), and in a large, multistate *Y. enterocolitica* epidemic characterised by diarrhoea, abdominal pain, and fever (Tacket *et. al.*, 1984). Toma *et. al.* (1984) characterised a new *Y. enterocolitica* serotype (O:13a, 13b) considered pathogenic for humans.

Human yersiniosis caused by other *Yersinia* species. There have been rare instances where *Yersinia* species other than *Y. pseudotuberculosis* and *Y. enterocolitica* have been implicated in human disease. Lewis and Chattopadhyay (1986) were able to isolate *Y. frederiksenii* and *Y. intermedia* from stool samples of diarrhoeic patients. Both *Y. frederiksenii* and *Y. intermedia* have also been isolated by Greenwood and Hooper (1987). Simmonds *et. al.* (1987) were able to isolate considerable numbers of *Y. frederiksenii* from human cases of yersiniosis characterised by gastroenteritis. Van Noyen *et. al.* (1987a) were also able to isolate other *Yersinia* species such as *Y. frederiksenii*, *Y. intermedia*, and *Y. kristensenii* from stool samples of patients with gastroenteritis.

Agbonlahor (1986) isolated *Y. intermedia*-like bacteria from patients in Nigeria and proposed the classification of these as *Y. intermedia* biotype 9. This biotype has also been isolated by Punsalang *et. al.* (1987) from one patient out

of six who had *Y. intermedia* isolated from their stool samples. However, *Y. intermedia* is considered to have a limited pathogenic potential for man and their isolation was thought to be without clinical significance in the patients as they were only recovered through cold enrichment (Punsalang *et. al.*, 1987).

Yersiniosis in Animals

Yersinia organisms are isolated from a wide variety of domestic and wild animals, where their presence may or may not be associated with clinical disease. Infections in animals, as in man, are characterised by the association of different biotypes, serotypes, and phage types of *Yersinia* with certain animal species. Geographical location may also play a role in the host specificities of these organisms.

Yersinia pseudotuberculosis infections. The following is a listing of *Y. pseudotuberculosis* infections in domestic and wild animals, from both diseased and healthy sources.

1. Cattle - *Y. pseudotuberculosis* has been involved in different pathologic conditions of cattle. The first fully authenticated isolation of *Y. pseudotuberculosis* from a pathological case in cattle was reported in Great Britain by Mair and Harbourne in 1963, where the authors were able to isolate *Y. pseudotuberculosis* serotype IA from an aborted foetus.

In Canada, Langford (1969), reported the isolation of *Y. pseudotuberculosis* from an aborted foetus submitted to his laboratory, but he did not specify which serotype was involved. A lung sample from a steer examined by this author within the same year revealed the same organisms. Toma (1986), in summarising *Y. pseudotuberculosis* infections in Canada from 1962 to 1985, reported 15 cases of *Y. pseudotuberculosis* infections in cattle, mostly involving serotype III. One case of calf pneumonia in the United States also yielded *Y. pseudotuberculosis* serotype III (Hubbert, 1972).

Hodges *et. al.* (1984a) reported that among the 56 isolates of *Y. pseudotuberculosis* obtained from diseased cattle in the North Island of New Zealand, 51 (91%) belonged to serotype III, with the remainder belonging to serotype I (7%) and serotype II (2%).

Recent reports of *Y. pseudotuberculosis* infection of cattle in Australia have also involved serotype III. Callinan *et. al.* (1988) reported outbreaks of enterocolitis in several cattle herds in coastal river valleys in New South Wales, and Slee *et. al.* (1988) also reported cases of enteritis among weaned calves in Victoria, with serotype III being the most predominant isolate. Cases of abortion in cattle caused by *Y. pseudotuberculosis* serotype III have recently been reported by Jerrett and Slee (1989).

While it has been shown to cause disease, *Y. pseudotuberculosis* can also be isolated from healthy cattle. Fukushima *et. al.* (1983b) described the isolation of *Y. pseudotuberculosis* serotype IIB from bovine faeces, an event the authors described as the first isolation of *Y. pseudotuberculosis* from cattle in Japan, as well as the first isolation of serotype IIB from cattle in the world.

Hodges and Carman (1985) carried out a survey to determine the faecal carriage of *Y. pseudotuberculosis* in healthy cattle in the North Island of New Zealand. The results of their survey showed a prevalence of 26.3% from all the cattle sampled. 93.2% of the isolates were identified by the authors as serotype III. Bullians (1987) was able to recover one isolate of *Y. pseudotuberculosis* from a survey of cull cows at an abattoir in New Zealand.

2. Sheep - *Y. pseudotuberculosis* has been implicated in systemic diseases of sheep in Australia as early as 1932 by Pullar, and 1937 by Beveridge. The 1937 report described a systemic infection among sheep characterised primarily by enteritis and pneumonia, with some involvement of the liver. A systemic *Y. pseudotuberculosis* infection in a ewe characterised by the presence of multiple abscesses in the liver and the intestinal tract has been reported by Hubbert (1972) in the United States.

Abortion seems to be the most common manifestation of *Y. pseudotuberculosis* infection in sheep. Watson and Hunter (1960) reported the isolation of *Y. pseudotuberculosis* from an aborted foetus in Great Britain, and this organism has been reported to cause sheep abortion in New Zealand (Hartley and Kater, 1964). Mair (1965) was also able to report the recovery of *Y. pseudotuberculosis* serotype III from nine aborted foetuses in Great Britain. More recent reports include cases of sheep abortion in New Zealand and the isolation in the

United States of *Y. pseudotuberculosis* serotype III from a flock of sheep with multiple abortions (Karbe and Erickson, 1984).

Another manifestation of *Y. pseudotuberculosis* infection in sheep is epididymo-orchitis, as reported by Jamieson and Soltys (1947). In this report, the *Y. pseudotuberculosis* isolate was identified as serotype IB.

Other surveys on the prevalence of *Y. pseudotuberculosis* in sheep have shown that serotype III is a common serotype in sheep infections (Hodges *et. al.*, 1984a; Toma, 1986).

3. Deer - *Y. pseudotuberculosis* is the primary cause of deer yersiniosis in New Zealand (Griffin, 1987). The disease causes considerable losses to the industry; consequently most reports of the disease relate to New Zealand. Of the *Y. pseudotuberculosis* serotypes, three are known to occur among deer in New Zealand (I, II, and III). It is interesting to note that serotype I (dominant in wildlife) is most commonly found in the South Island (Henderson, 1983) and serotype III (dominant in cattle and pigs) is most commonly found in the North Island (Hodges *et. al.*, 1984b). This implies that in the South Island, wild animals and birds are the reservoirs of *Y. pseudotuberculosis* infections for deer, while domestic animals may assume that role in the North Island.

More details of deer yersiniosis are discussed in the section dealing with yersiniosis in New Zealand.

Y. pseudotuberculosis infections in deer have also been reported from other countries. *Y. pseudotuberculosis* serotype IA has been isolated from free-living fallow deer in Great Britain (Chapman *et. al.*, 1979), and the organism has been incriminated in disease causing several deaths among farmed deer in that country (Fletcher, 1982). Toma (1986) also reported the isolation of *Y. pseudotuberculosis*, mostly serotype IB, from deer in Canada.

4. Pigs - *Y. pseudotuberculosis* has been incriminated in a few cases of disease in pigs, but most isolations of this organism have come from healthy animals.

Most of the observations on *Y. pseudotuberculosis* infections in pigs were made in Japan. In 1970, Tsubokura *et. al.* reported the

isolation of three *Y. pseudotuberculosis* strains. Two of the isolates belonged to serotype IVB, and one belonged to serotype IB. The results of a longitudinal study by Tsubokura *et. al.* (1976b) in Japan showed that the highest incidence of *Y. pseudotuberculosis* occurred during the winter months, and that most of the organisms isolated belonged to serotype III. Tsubokura *et. al.* (1984a) showed that serotype III was still the predominant *Y. pseudotuberculosis* strain found in Japanese pigs. In another study in 1989, Tsubokura *et. al.* indicated that although serotype III was still the most significant serotype recovered, increases in the isolation of serotypes IVB and IB have been recorded.

Toma and Deidrick (1975) were able to isolate 14 *Y. pseudotuberculosis* serotype III strains from healthy pigs sampled in Toronto abattoirs. Toma (1986) reported that during the period 1962-1985, 96.6% of all *Y. pseudotuberculosis* isolates taken from slaughtered swine in Canada belonged to serotype III.

An Australian survey on the prevalence of *Y. pseudotuberculosis* in healthy pigs resulted in the isolation of *Y. pseudotuberculosis* from 1.5% of the animals sampled. All the *Y. pseudotuberculosis* strains isolated in the study were found to be serotype III (Blackall, 1977).

The tendency for serotype III to be found in healthy swine has led to the conclusion that pigs are symptomless carriers for this strain. It is recommended, however, that further studies on the pathogenecity of *Y. pseudotuberculosis* serotype III isolated from these animals be made (Tsubokura *et. al.*, 1984a).

The question of the pathogenicity of *Y. pseudotuberculosis* strains isolated from pigs was studied by Shiozawa *et. al.* (1988), who found that the *Y. pseudotuberculosis* serotypes isolated from pork and swine throats were virulent using *in-vitro* and animal pathogenicity tests.

While most *Y. pseudotuberculosis* strains from pigs have been isolated from healthy animals, a few clinical cases have been reported. The organism has been recovered from a clinical case in Canada (Langford, 1972a), and from diarrhoeic pigs in Brazil (De Barcellos and De Castro, 1981). Hodges *et. al.* (1984a) reported seven isolates of *Y. pseudotuberculosis* from sick and dead pigs in New Zealand, all belonging to serotype III.

5. Cats and Dogs - *Y. pseudotuberculosis* has been shown to cause disease in cats by several authors. Mair *et. al.* (1967) reported two cases of pseudotubercular yersiniosis in cats characterised by general systemic involvement, with one cat showing jaundice as a result of hepatitis. *Y. pseudotuberculosis* serotype IIA was isolated from both cases. A 14-month old cat in the United States with a history of inappetence and chronic diarrhoea was shown to be infected with *Y. pseudotuberculosis* serotype IA (Hubbert, 1972). In Australia, O'Sullivan *et. al.* (1976) reported a case of yersiniosis caused by *Y. pseudotuberculosis* in a cat in quarantine, which had recently arrived from Papua New Guinea. The infection occurred concurrently with infection by the fluke *Platynosomum fastosum*. This was the first reported occurrence of both aetiologic agents in a cat in Australia. In 1979, Spearman *et. al.* reported another case of *Y. pseudotuberculosis* infection in a cat in Canada, characterised by anorexia, vomiting, and lethargy.

It has been shown that *Y. pseudotuberculosis* can be carried by healthy cats as well. Mackintosh and Henderson (1984) isolated mostly *Y. pseudotuberculosis* serotype I from healthy feral cats in New Zealand, as part of a survey for potential reservoirs of the infection for deer. Healthy cats also provided all the feline *Y. pseudotuberculosis* isolates reported from Japan, the majority of which were classified as serotype IVB (Tsubokura *et. al.*, 1989).

Dogs do not appear to be susceptible to disease caused by *Y. pseudotuberculosis*. All the Japanese isolates of *Y. pseudotuberculosis* recovered from dogs were isolated from healthy animals, and most of these isolates belonged to serotypes IVB and IB (Tsubokura *et. al.*, 1989). In one Japanese study however, most of the *Y. pseudotuberculosis* strains isolated belonged to serotype VA, with a few other serotypes (IVB, IVA, IIB and IB) recovered (Fukushima *et. al.*, 1984a). Tsubokura *et. al.* (1984b) described a *Y. pseudotuberculosis* isolate from a healthy dog in Japan which could not be classified into any of the established serotypes. It was then proposed by these authors that this isolate be placed in a new *Y. pseudotuberculosis* serotype, IIC.

6. Non-human primates - Mair (1965) reported 17 isolates of *Y. pseudotuberculosis* from monkeys in Great Britain. Eleven of these were serotype IA, with the remainder equally divided into serotypes IB and IIA. Of the five isolates of *Y. pseudotuberculosis* recovered from monkeys in Japan, three belonged to serotype IVB and two to IB

(Tsubokura *et. al.*, 1970). In another paper by Tsubokura *et. al.* (1984a), further strains of *Y. pseudotuberculosis* were reported from monkeys in Japan, with the majority (84.2%) belonging to serotype IB.

Isolated reports of *Y. pseudotuberculosis* infections in primates have appeared in recent years. Chang *et. al.* (1980) described a fatal *Y. pseudotuberculosis* infection in bushbabies (*Galago crassicaudatus*), characterised by diarrhoea, dehydration, and lethargy. Rosenberg *et. al.* (1980), and Strickland *et. al.* (1982) described cases of diarrhoeal conditions among monkeys (*Macaca fascicularis*) caused by *Y. pseudotuberculosis*. In addition to diarrhoea, Rosenberg *et. al.* (1980) reported accompanying abortion and stillbirths.

7. Small mammals and birds - Rodents and birds are considered to be the principal reservoirs of *Y. pseudotuberculosis* infections worldwide (Hubbert, 1972; Mair, 1973; Obwolo, 1976). Epidemics have been known to occur in birds, and yersiniosis due to *Y. pseudotuberculosis* is of much greater importance in these animals than in mammals (Mair, 1973). Large-scale outbreaks also occur in small mammals, especially in hares in Europe (Stovell, 1980). In Canada and the United States, chinchillas have been observed to be very susceptible to the infection (Langford, 1972a; Hubbert, 1972).

Rats and mice appear resistant to natural and experimental infections with *Y. pseudotuberculosis*; this characteristic enables these species to act as carriers of the organism without being adversely affected (Mair, 1973).

The distribution of *Y. pseudotuberculosis* serotypes in rodents and birds is very similar to the pattern of distribution in humans. Serotype I predominates, followed by serotype II (Mair, 1965; Hubbert, 1972; Bercovier *et. al.*, 1978; Hodges *et. al.*, 1984a; Mackintosh and Henderson, 1984; Toma, 1986; Weber *et. al.*, 1987).

The situation in Japan is rather different in this respect, for serotypes other than I and II predominate in small mammals, especially serotypes IV and V and their subtypes (Tsubokura *et. al.*, 1970; Tsubokura *et. al.*, 1984b; Kaneko *et. al.*, 1979; Tsubokura *et. al.*, 1989). The *Y. pseudotuberculosis* isolates that have been included in the new serotypes (VII and VIII) have been isolated from rats in Japan (Tsubokura *et. al.*, 1984b).

Y. pseudotuberculosis has never been isolated from birds in Japan (Tsubokura *et. al.*, 1989).

8. Other animals - Mair and Ziffo (1974) reported a case of *Y. pseudotuberculosis* infection from a foal in Scotland which exhibited signs of pneumonia prior to its death. Necropsy of the animal revealed numerous abscesses in the spleen, lungs, and the liver, which later yielded pure cultures of *Y. pseudotuberculosis* serotype IIA.

Yersinia enterocolitica infections. Compared with infections by *Y. pseudotuberculosis*, *Y. enterocolitica* infections in animals resulting in disease are rarely encountered. Mainly due to the interest created by human diseases caused by *Y. enterocolitica*, researchers have attempted to find possible sources and reservoirs of the infection, and the animal kingdom has long been suspected as a reservoir for *Y. enterocolitica* (Hurvell, 1981). As a consequence, several surveys on the carriage of *Y. enterocolitica* in domestic and wild animals have been undertaken in an attempt to identify specific animal hosts for the *Y. enterocolitica* serotypes common in human disease.

The recovery of *Y. enterocolitica* from animals was described for the first time in the 1960's, from swine, chinchillas, and hares (Hurvell, 1981). Subsequently, different *Y. enterocolitica* serotypes have been isolated from an increasing variety of animal species. Most strains isolated from animals usually differ from human *Y. enterocolitica* serotypes, both in biochemical and serological reactions. The search for *Y. enterocolitica* in pigs, dogs, and cats has shown, however, that these animal species can harbour *Y. enterocolitica* serotypes pathogenic for humans (Hurvell, 1981). Recent research has, with a few exceptions, continued to show the same results.

The following is a listing of *Y. enterocolitica* infections in both domestic and wild animals. They usually differ from human *Y. enterocolitica* strains, both biochemically and serologically.

1. Pigs - Pigs are the primary suspects as reservoirs of human *Y. enterocolitica* infections. Investigators in Europe (Pedersen, 1976; Pedersen, 1979; Pedersen and Winblad, 1979; Hurvell *et. al.*, 1979; Olsson *et. al.*, 1980), Japan (Tsubokura *et. al.*, 1973; Zen-Yoji *et. al.*, 1974; Tsubokura *et. al.*, 1976a; Asakawa *et. al.*, 1979; Fukushima and Tsubokura, 1985), and Canada (Toma and Deidrick, 1975; Schiemann, 1980; Schiemann and Fleming, 1981) have demonstrated that swine are the major reservoirs of the human strains of *Y. enterocolitica*, especially

serotype O:3. According to Hurvell (1981) South African workers were also able to show the same results.

Y. enterocolitica serotype O:8, which is very prominent in the United States, has also been recovered by Doyle *et. al.* (1981) from tongues of apparently normal pigs, the first report associating this serotype with a natural reservoir. Of all the *Y. enterocolitica* isolates recovered by the authors, serotype O:8 was the most predominant, followed by serotypes O:6,30 and O:3 phage type 9b.

Recent research has expanded our knowledge about the epidemiology of *Y. enterocolitica* infections in pigs. Christensen (1980) stated that the distribution of the predominant *Y. enterocolitica* strain in Danish pigs, serotype O:3, is dependent on the infection status of the herd concerned, and most of the *Y. enterocolitica* can only be isolated from tonsillar tissue of these animals. Contaminated pork products are thought to be the most common vehicles for transmission, and Tauxe *et. al.* (1987) determined that the most important factor influencing *Y. enterocolitica* infection in humans in Belgium was the practice of feeding raw pork to children. This may account for the extremely high incidence of *Y. enterocolitica* in that country, the highest incidence in the world.

The ability of pigs to carry and shed *Y. enterocolitica* is very important in the epidemiology of the infection. Studies have shown that most of the human pathogenic *Y. enterocolitica* are able to colonise the lymphoid tissue and gastrointestinal tracts of pigs without the animals producing significant antibodies against them (Fukushima *et. al.*, 1984b; and Schiemann, 1988). This condition results in the establishment of carrier animals, which have been shown to shed the organisms for up to nine weeks (Fukushima *et. al.*, 1983a).

A report from China (Zheng, 1987) recording the isolation of *Y. enterocolitica* from pigs with diarrhoea suggests that *Y. enterocolitica* may also be capable of producing illness in pigs, contrary to previous reports. The author reported a prevalence of 48.4% (60 out of 124 samples) in pig farms which had a history of diarrhoea or had pigs showing diarrhoea during the survey period. 96.66% (58 strains) of the *Y. enterocolitica* isolates were shown to belong to biotype 3 serotype O:3, with the remaining 2 isolates classified as biotype 3 serotype O:9. Whether *Y. enterocolitica* is the true causal agent in such cases remain to be determined.

Surveys on the carriage of *Y. enterocolitica* in pigs in other countries have shown variable results. In Australia, Blackall (1977) failed to isolate *Y. enterocolitica* from pigs, and Ho and Koh (1981) found *Y. enterocolitica* serotype O:3 in 4.87% of pigs sampled in Singapore. In Nigeria, serotypes O:3 and O:8 have been isolated from pigs (Adesiyun *et. al.*, 1986), and Okoroafor *et. al.* (1988) were able to isolate 4 strains of *Y. enterocolitica* from 746 pigs (0.53%), all of which were typed as biotype 1 serotype O:5.

Hunter *et. al.* (1983) found only 2 strains of *Y. enterocolitica* biotype 4 serotype O:3 out of 1931 pigs (0.10%) examined in the United Kingdom, although the authors were able to isolate a total of 112 *Y. enterocolitica* strains from these pigs, for an overall *Y. enterocolitica* prevalence of 5.8%. The most predominant serotypes encountered in this study were O:6 and O:5. The relatively low prevalence of *Y. enterocolitica* serotype O:3 in pigs in the United Kingdom is reflected in the low prevalence of this serotype in human disease (Lewis and Chattopadhyay, 1986; Greenwood and Hooper, 1987).

2. Dogs - Apart from the pig, the only other animal from which human pathogenic *Y. enterocolitica* has been isolated in considerable numbers is the dog. Cases of human yersiniosis have been reported where dogs have been considered as the prime source of the infection (Gutman *et. al.*, 1973; Wilson *et. al.*, 1976).

In a faecal survey for the presence of *Y. enterocolitica* in dogs, Tsubokura *et. al.* (1975) were able to isolate 2 (1.73%) *Y. enterocolitica* strains out of 115 dogs examined. One of the isolates was serotype O:3, and the other O:4. Pedersen (1976) was also able to isolate one strain of *Y. enterocolitica* serotype O:3 from a survey of dogs in Denmark.

Kaneko *et. al.* (1977) carried out monthly samplings for the presence of *Y. enterocolitica* from the gastrointestinal contents of dogs destroyed in a dog pound. In the course of the 13-month study, the authors discovered that the incidence of *Y. enterocolitica* was highest during the colder months of the year. There were, however, no significant differences in the monthly and seasonal incidences. Twenty-five of the 451 (5.54%) dogs examined were positive for *Y. enterocolitica*, the predominant strain of which was biotype 4 serotype O:3. The other significant isolates were serotypes O:5B, and O:9, the isolation of O:9

being the first in Japanese dogs. It was also found during the course of the study that dogs aged between four weeks and three years were the most commonly affected.

In a later survey of dogs in Denmark, Pedersen and Winblad (1979) were able to isolate two strains of *Y. enterocolitica* serotype O:3 from 117 dogs, although the most prominent *Y. enterocolitica* serotype isolated by these authors in dogs was serotype O:5a.

A prospective study of *Yersinia* spp. in dogs in Japan was undertaken from November 1980 to December 1981, and an overall *Y. enterocolitica* prevalence of 19.8% was demonstrated (Fukushima *et. al.*, 1984a). The predominant *Y. enterocolitica* strains encountered in this study were found to be biotype 1 serotypes O:5 and O:6, and among the human serotypes encountered, serotype O:3 was the most significant, followed by serotype O:5,27.

More recently, due to an outbreak of enteritis in an Italian kennel involving *Y. enterocolitica* serotype O:3, a survey of apparently healthy dogs in the same kennel was undertaken to determine the faecal carriage of *Yersinia* species (Fantasia *et. al.*, 1985). 19 out of 63 dogs (30.15%) were found to harbour *Yersinia* spp., with 15 of these isolates belonging to biotype 4 serotype O:3, showing a prevalence specific for this serotype of 23.8%. This figure indicates a higher prevalence of *Y. enterocolitica* in dogs than the previous Japanese studies.

In Nigeria, as a result of a survey for *Yersinia* spp. in dogs, an isolate of *Y. enterocolitica* serotype O:8 was described (Trimnell and Adesiyun, 1988). This is the first report of *Y. enterocolitica* serotype O:8 isolated from a dog outside North America.

Although *Y. enterocolitica* serotypes known to be pathogenic for man have been isolated from healthy dogs, there have also been reports of disease in dogs due to the same serotypes. Farstad *et. al.* (1976) described a case of chronic enteritis in a dog from which *Y. enterocolitica* biotype 4 serotype O:3 was isolated. Papageorges *et. al.* (1983) reported two cases of enteritis in dogs in Canada caused by the same biotype and serotype.

3. Cattle - Several surveys have been performed to determine *Y. enterocolitica* carriage by healthy cattle. Most of these attempts have been due to the desire to find animal reservoirs for

human *Y. enterocolitica* infections. Results of such surveys have revealed that most of the *Y. enterocolitica* serotypes isolated from these animals are not implicated in human disease, and reports of human pathogenic serotypes isolated from these animals have been infrequent.

Inoue and Kurose (1975) examined 115 samples of cattle intestinal contents at a Japanese slaughterhouse and found a prevalence of 7.9%, with the non-human serotypes making up the bulk of the isolates. The isolation of serotype O:5 from one sample was the most significant finding of this study, serotype O:5 being considered pathogenic for humans.

Cattle sources have also yielded serotype O:8 in the United States (Shayegani *et. al.*,1981) and in Nigeria (Adesiyun *et. al.*,1986). The other significant *Y. enterocolitica* serotypes isolated from these animals have been serotypes O:7,8 and O:6,30 (Shayegani *et. al.*, 1981; Davey *et. al.*, 1983). Results obtained by other workers showed mostly *Y. enterocolitica* serotypes considered non-pathogenic for humans (Wooley *et. al.*, 1980; Fukushima *et. al.*, 1983b).

While most *Y. enterocolitica* isolates have been recovered from apparently healthy animals, *Y. enterocolitica* has been reported to cause suppurative enteritis in cattle in New Zealand (Belton and McSporran, 1988).

4. Sheep - Sheep infected by *Y. enterocolitica* can either carry the organism in the gastrointestinal tract or show disease characterised usually by enteritis.

Y. enterocolitica has been isolated from an outbreak of diarrhoea in a flock of Romney hoggets in New Zealand (McSporran *et. al.*, 1984), characterised by a morbidity rate of 30%, a mortality rate of 0.88%, and a case-fatality rate of 3%. The most significant feature of the infection at necropsy was suppurative enteritis, a condition which is the most common characteristic of *Y. enterocolitica* enteritis in New Zealand sheep (Belton and McSporran, 1988).

In a survey to determine the carriage of *Yersinia* spp. of lambs at an abattoir in New Zealand, Bullians (1987) was able to isolate *Y. enterocolitica* from 13 out of the 66 lambs sampled. Of the isolates, four were *Y. enterocolitica* biotype 5, and the remainder fell within the

biotypes 1 to 4, but were not precisely biotyped. Three of these *Y. enterocolitica* isolates were serotyped and two were found to be O:3.

5. Deer - In New Zealand, disease in deer caused by *Y. enterocolitica* is not as common as disease caused by *Y. pseudotuberculosis*. Henderson (1983), in examining 350 routine deer cases by post-mortem examination in the South Island, encountered only 2 cases (0.6%) of yersiniosis due to *Y. enterocolitica* from 1979 to 1982, compared to 55 cases (15.7%) of *Y. pseudotuberculosis* enteritis diagnosed during the same period.

This study was followed by a faecal survey for *Yersinia* spp. from apparently normal deer (Henderson, 1984). In this survey, *Y. enterocolitica* was isolated from 176 out of 922 faecal samples (19%) and *Y. pseudotuberculosis* was isolated from 7 out of the total (0.76%).

The results of these two mentioned studies may infer that although *Y. enterocolitica* can be isolated more frequently from deer faecal samples, disease caused by this organism is quite rare in comparison with disease caused by *Y. pseudotuberculosis*. The localisation of *Y. pseudotuberculosis* in the lymph nodes or its sporadic shedding may account for its low isolation rate from faecal material (Henderson, 1984).

Y. enterocolitica has also been isolated from 18.6 % of 145 wild deer examined in the United States, with *Y. enterocolitica* serotype O:6,31 predominating. One other serotype O:5,27 was isolated (Shayegani *et. al.*, 1986).

6. Other ruminants - *Y. enterocolitica* has been isolated from cases of abortion in buffaloes in India (Das *et. al.*, 1986), and from healthy camels slaughtered in Nigeria (Kwaga *et. al.*, 1987).

7. Small mammals and rodents - Among the small mammals and rodents, chinchillas have been most commonly affected by *Y. enterocolitica* infections. Langford (1972b), in reviewing all the *Y. enterocolitica* strains isolated from dead or sick animals in British Columbia from 1959 to 1967, listed 18 cases of *Y. enterocolitica* infection in chinchillas, out of the total of 23 cases overall for that period. In the United States, the situation has been similar; a review of all the cases of *Y. enterocolitica* infection in mammals and birds up to 1972 showed

that the only report of *Y. enterocolitica* encountered was an outbreak of yersiniosis due to *Y. enterocolitica* among chinchillas during the winter of 1964 to 1965 (Hubbert, 1972).

Hares and rabbits have also been commonly affected by *Y. enterocolitica* infections, mostly in Europe (Hurvell, 1981). Bercovier *et. al.* (1978) isolated 14 *Y. enterocolitica* strains (4.08%) from 339 hares and 4 rabbits collected from a terrestrial ecosystem in France. Most of the *Y. enterocolitica* isolated from these animals were serotypes O:2a,2b; O:2a,2b,3; and O:7,8. The majority of the first two serotypes mentioned belonged to biotype 5.

Rats and small rodents have also been shown to harbour *Y. enterocolitica* (Tsubokura *et. al.*, 1975; Kapperud, 1975; Kapperud, 1977; and Kaneko *et. al.*, 1978). The predominant *Y. enterocolitica* strain isolated from these investigations was serotype O:6.

8. Primates and zoo animals - Clinical *Y. enterocolitica* infections have been reported in primates. An outbreak of gastroenteritis among bushbabies has been described by Mair *et. al.* (1970). McClure *et. al.* (1971) reported infections caused by *Y. enterocolitica* in a group of monkeys characterised by ulcerative enterocolitis, regional lymphadenopathy, and necrosis of the liver, spleen, and lymph nodes.

In Japan, *Y. enterocolitica* has been isolated from healthy monkeys. Otsuki *et. al.* (1973) were able to isolate *Y. enterocolitica* serotypes O:5, O:6, O:12, and O:14 from healthy monkeys, and Sasaki *et. al.* (1989) described the isolation of *Y. enterocolitica* serotypes O:5a and O:8 from macaques at the Tokyo Tama Zoo.

9. Birds - Birds have been shown to carry *Y. enterocolitica* strains. Kapperud and Olsvik (1982) characterised *Y. enterocolitica* strains isolated from crows and gulls in Norway, most of which were biotype 1, and two of these were serotyped as O:6. Weber *et. al.* (1987) isolated mostly environmental and non-pathogenic *Y. enterocolitica* strains from birds in Germany.

Japanese investigators have shown that the majority of *Y. enterocolitica* strains isolated from Japanese birds have been serotypes O:5a, O:6,30, and O:4,32 (Kato *et. al.*, 1985). These findings have been

confirmed in a subsequent survey, and it was also determined that these isolates were non-pathogenic for humans (Tanaka *et. al.*, 1987).

Isolation of *Yersinia* Species from Environmental Sources

Several species of *Yersinia* have been isolated from environmental sources, primarily water and soil. The presence of *Yersinia* species in these sources is an important aspect of the epidemiology of yersiniosis in humans and animals.

Water appears to be a major environmental source of *Yersinia* species. However, the majority of the *Yersinia* species isolated from water have been the "environmental" types, which have not been associated with human or animal disease. Lassen (1972) was able to isolate *Y. enterocolitica* serotypes O:7 and O:13 from drinking water. Serotype O:6 made up the majority of the *Y. enterocolitica* strains isolated by Kapperud (1977) and Caprioli *et. al.* (1978). Environmental species also made up most of the *Yersinia* strains isolated by Van Pee *et. al.* (1980) in Belgium.

Exceptions to these reports include the isolation of *Y. enterocolitica* serotype O:8 by Shayegani *et. al.* (1981) from surface water in New York, and the isolation of *Y. enterocolitica* biotype 3B serotype O:3 from water sources in Japan by Fukushima *et. al.* (1984d).

Attempts to isolate *Y. enterocolitica* from water sources suspected to be involved in outbreaks of gastroenteritis have shown variable success. Keet (1974) was able to isolate *Y. enterocolitica* serotype O:8 from river water suspected as the source of infection for a human patient affected by the same serotype. Highsmith *et. al.* (1977) reported the isolation of *Y. enterocolitica* biotype 1 from well water implicated in a water-borne outbreak of enteritis. Harvey *et. al.* (1976), following an outbreak of gastroenteritis in California, examined water samples from lakes and streams in the area of the outbreak and reported that most of the *Y. enterocolitica* strains they isolated were untypable; these authors were not able to isolate human pathogenic *Y. enterocolitica* bioserotypes.

Other *Yersinia* species have been isolated from water in significant numbers. Most of the recorded isolations of *Y. frederiksenii*, *Y. intermedia* and *Y. aldovae* have been from aquatic sources (Ursing *et. al.*, 1980; Brenner *et. al.*, 1980; Bercovier *et. al.*, 1984), and a considerable number of *Y. kristensenii* and *Y. rohdei* isolates have also come from water sources (Bercovier *et. al.*, 1980b; Aleksic *et. al.*, 1987).

The majority of *Y. kristensenii* isolates have come from soil samples (Bercovier *et. al.*, 1980b) and Botzler (1987) reported the first isolations of *Y. frederiksenii* from soil in Europe and *Y. enterocolitica sensu stricto* (serotype O:6,30) from soil in the Federal Republic of Germany.

Isolation of *Yersinia* Species from Foods

Y. enterocolitica and related species have been isolated from milk (Schiemann and Toma, 1978; Hughes, 1979; Hughes, 1980; Fukushima *et. al.*, 1984c; Franzin *et. al.*, 1984; Walker and Gilmour, 1986; Stone, 1987), and various food products (De Boer *et. al.*, 1982; Tacket *et. al.*, 1985; De Boer *et. al.*, 1986; Warnken *et. al.*, 1987).

Seasonality of Yersiniosis

Most cases of human yersiniosis appear to occur during the colder months of the year (Asakawa *et. al.*, 1973; Vandepitte and Wauters, 1979; De Groote *et. al.*, 1982; Mingrone *et. al.*, 1987). Fukushima *et. al.* (1985) mentioned that most *Y. enterocolitica* serotype O:3 isolations were reported between summer and autumn, conversely *Y. pseudotuberculosis* and *Y. enterocolitica* biotype 1 strains were usually isolated in winter or spring.

Age Prevalence of Yersiniosis

Y. enterocolitica infections have been shown to be most common among children, mostly in the 1-5 year age group (Vandepitte and Wauters, 1979; De Groote *et. al.*, 1982). Fukushima *et. al.* (1985) have shown that in cases of enteritis, the isolation of *Y. enterocolitica* serotype O:3 and *Y. pseudotuberculosis* was significantly higher in children under 3 years old. Isolations from appendices in cases of mesenteric adenitis were higher in children over 10 years old.

Yersiniosis in New Zealand

Yersiniosis in New Zealand has followed the worldwide trend, that is, the role of *Yersinia* organisms as human and animal pathogens has assumed importance only in the last three decades. However, reports of disease and of

isolations from healthy subjects have been sporadic, and active search for possible hosts of *Yersinia* organisms has only begun in earnest in recent years.

Human Infections

Y. pseudotuberculosis infections. The first report of human *Y. pseudotuberculosis* infection in this country was made by Henshall in 1963, when he described cases of mesenteric adenitis in three children with clinical signs resembling appendicitis. On surgery, however, he found the appendices to be normal, with only the mesenteric lymph nodes enlarged. *Y. pseudotuberculosis* was isolated from the lymph nodes of one patient. This was the first reported isolation of *Y. pseudotuberculosis* as a human pathogen outside of Europe.

Y. pseudotuberculosis was later associated with a case of reactive arthritis (Rose, 1976) in New Zealand, although this was only shown serologically.

More recently, another case of mesenteric lymphadenitis was reported by Malpass (1981) in a 19-year old soldier who also had symptoms resembling classical appendicitis. Laparotomy similarly revealed a normal appendix with only the mesenteric lymph nodes showing pathological changes. Biopsy of these nodes revealed abscesses which later yielded *Y. pseudotuberculosis*.

Y. enterocolitica infections. Human infections with *Y. enterocolitica* in New Zealand have also been reported sporadically. Watson *et. al.*, (1979), in a survey for *Campylobacter* and *Y. enterocolitica* in Palmerston North, failed to isolate any *Y. enterocolitica*, leading them to conclude that *Y. enterocolitica* is an infrequent cause of enteritis in the country.

Y. enterocolitica serotypes O:3, O:5, O:8, and O:9 have, however, been detected in New Zealand (Beeching *et. al.*, 1985). Clinical features of infection include diarrhoea, and reactive arthritis following enteritis (Jones and Bruns, 1987; Ameratunga *et. al.*, 1987).

Animal Infections

Infections with *Yersinia* species in animals were, again, only reported occasionally, until the late 1970's when the booming deer industry brought along some "new" (until then relatively unimportant) diseases, one of the most prominent of which was yersiniosis.

Y. pseudotuberculosis infections. The realisation that yersiniosis could cause considerable losses in the deer industry only came about when a series of reports appeared from the different animal health laboratories throughout the country (Anonymous, 1978a, 1978b; Anonymous, 1979a, 1979b, 1979c). As a result of these reports, several surveys on the prevalence and incidence of yersiniosis were undertaken. Results of such surveys showed that considerable variation existed in the prevalence and incidence of *Y. pseudotuberculosis* in different regions. Remarkable differences were also noted in the serotypes of the organisms isolated from the two main islands of the country.

Henderson (1983) summarised and analysed routine deer cases submitted to the Invermay Animal Health Laboratory (South Island) for the period 1979-1982. His results showed that there was a distinct seasonal trend in the number of *Y. pseudotuberculosis* isolations, mostly peaking in the winter months. The majority of these isolates (64%) came from animals under a year old. Serological typing revealed that more than half (57%) of these isolates were of serotype I, 27.86% were of serotype II, and 14.75% were of serotype III. Neither serotypes IV and V nor any other *Y. pseudotuberculosis* serotypes were encountered during the course of the study.

In contrast, the results of the survey conducted by Hodges *et. al.* (1984b) in the North Island showed that out of the 117 cases of deer yersiniosis diagnosed at the Ruakura Animal Health Laboratory, 75 (64%) belonged to serotype III, 9 (8%) belonged to serotype II, and 33 (28%) belonged to serotype I. When compared with the results of the South Island survey, the most apparent difference was the geographic distribution of the *Y. pseudotuberculosis* serotypes affecting deer: serotype I was more common in the South Island and serotype III more common in the North Island.

Together with this survey of deer cases, Hodges *et. al.* (1984a) also gathered data on yersiniosis cases from other domestic livestock, which included cattle, sheep, goats, pigs, rabbits, guinea pigs, and birds. The results showed that serotype III was predominant in other species of farm animals, whereas in smaller mammals (rabbits and guinea pigs) and birds, serotypes I and II were more prevalent.

In an attempt to explain the differences in the distribution of *Y. pseudotuberculosis* serotypes in the North and South Islands, a survey was undertaken in the South Island to determine the most probable sources for deer (Mackintosh and Henderson, 1984). The survey involved the collection of samples from apparently healthy small mammals (rabbits, hares, mice, rats, and cats) and birds (sparrows, gulls, starlings, ducks, and other bird species). The

results of the survey showed that among small mammals, feral cats had the highest prevalence of *Y. pseudotuberculosis* (27.8%). Among birds, ducks had the highest (5.3%). The distribution of the *Y. pseudotuberculosis* isolates recovered from these animals appeared similar to the distribution of *Y. pseudotuberculosis* strains from deer cases in a survey by Henderson (1983), where serotype I was the most predominant type encountered.

It would appear, as a result of the work by Mackintosh and Henderson (1984) and of the other studies previously mentioned, that the epidemiology of *Y. pseudotuberculosis* infections in the South Island is different from that of the North Island. Apparently, domestic animals assume the role of reservoirs of *Y. pseudotuberculosis* infections (serotype III predominant) in the North Island, and wild animals serve as reservoirs of the infection in the South Island, where serotype I has been shown to be predominant.

The overall picture of the infection is far from complete, however. Closer examination of the data presented in the surveys (Hodges *et. al.*, 1984a; Mackintosh and Henderson, 1984) shows that, in fact, serotype I was the predominant *Y. pseudotuberculosis* strain isolated from small mammals and birds from both islands. Thus the differences in serotype distributions in the North and South Islands may actually be due to several other factors which need to be elucidated, such as serotype specificities for different animal species and management practices in deer farming. A wildlife survey in the North Island and a domestic animal survey in the South Island may help explain some of the disparities mentioned.

Surveys of healthy deer in New Zealand have revealed the same contrasting results. South Island surveys showed very low *Y. pseudotuberculosis* prevalences: 0.13% (Henderson and Hemmingsen, 1983), 0.76% (Henderson, 1984), and 1.17% (Mackintosh and Henderson, 1985). These results indicate that very few carriers of *Y. pseudotuberculosis* occur in deer in that part of the country, and that infection in deer would most probably come from other species or from other deer already infected with *Y. pseudotuberculosis*.

A survey on the carriage of *Y. pseudotuberculosis* in healthy deer undertaken in the North Island by Hodges *et. al.* (1984b) showed an overall prevalence of 10.7%, which is considerably higher than the South Island results. Most of the isolates (91.48%) were determined to be serotype III. These results suggest that *Y. pseudotuberculosis* serotype III infection is quite common in healthy deer, at least in the North island. The authors of this study suggested that the differences in the results of this study compared to the South Island

results may be explained by the differences in the isolation techniques used by the researchers.

While it has caused tremendous problems for the deer industry, *Y. pseudotuberculosis* has also been shown to cause disease in other domestic animals in the country. Cases of yersiniosis have been reported in cattle (Hodges *et. al.*, 1984a), and in sheep, where the organism has been involved in cases of abortion, as reported by Hartley and Kater (1964).

Y. enterocolitica infections. *Y. enterocolitica* has been involved in a variety of clinical syndromes in domestic animals in New Zealand. The organism has been shown to cause diarrhoea among hoggets (McSporran *et. al.*, 1984), and suppurative enteritis in cattle and sheep (Belton and McSporran, 1988).

An interesting feature of *Y. enterocolitica* infections in New Zealand is the isolation of biotype 5 from domestic animals. *Y. enterocolitica* biotype 5 has only been previously isolated in Europe from hares and rabbits (Nilehn, 1969a; Bercovier *et. al.*, 1978) and goats (Krogstad *et. al.*, 1972). New Zealand isolations of this biotype have been reported in deer (Henderson, 1984), sheep (Bullians, 1987), and goats (Buddle *et. al.*, 1988).

Yersiniosis in Goats

Epidemics of yersiniosis have been known to occur in guinea pigs and turkeys, but only sporadic infections have been reported in goats (Mair and Harbourne, 1963). Most reports of *Yersinia* species infections in goats appear to involve either *Y. pseudotuberculosis* or *Y. enterocolitica*, which is probably due to the fact that most of these isolations were from clinical cases submitted to diagnostic laboratories, with only a few from surveys on healthy animals.

Yersinia pseudotuberculosis Infections

Yersinia pseudotuberculosis infections in goats have shown varied clinico-pathological signs, such as abortion and early neonatal death, mastitis, conjunctivitis, pneumonia, enteritis, and generalised infections. The first report of *Y. pseudotuberculosis* infection in goats was probably made by Baumann in 1927 (as cited by Watson and Hunter, 1960). A subsequent report of *Y. pseudotuberculosis* in a goat causing pneumonia and generalised infection was made in India by Rajagopalan and Sankaranarayanan in 1944. The authors

isolated pure cultures of *Pasteurella (Yersinia) pseudotuberculosis* serotype I from the spleen, liver, lungs, and some of the enlarged lymphatic glands of a goat that died after showing signs of high body temperature and pneumonia for 17 days. On necropsy most of the pathological changes were found in the liver and the lungs.

In a review of yersiniosis in mammals and birds in the United States, Hubbert (1972) mentioned a 1967 outbreak of yersiniosis in a goat flock in California. 150 of the flock of 400 died of the infection, which occurred during the winter of that year. *Y. pseudotuberculosis* serotype III was isolated from two dead goats available for necropsy. The most significant post-mortem findings from these two animals were emaciation, heavy louse infestation, and internal parasitism.

Tsubokura *et. al.* (1970) reported two *Y. pseudotuberculosis* strains in Japan isolated from goats by earlier workers. These strains belonged to serotype IB. Morita *et. al.* (1973) reported a case of *Y. pseudotuberculosis* serotype I infection in a goat in Japan characterised by generalised involvement of the internal organs. The animal was a two-year old female which gave birth to two kids that died shortly after birth. Thereafter, the animal showed signs of emaciation, and died two weeks later. Post-mortem examination revealed pathological changes in the liver, spleen, mesenteric lymph nodes, lungs, and the gastro-intestinal tract.

Y. pseudotuberculosis has also been implicated in cases of mastitis and abortion. Cappucci *et. al.* (1978) isolated cultures of *Y. pseudotuberculosis* serotype III from a composite milk sample taken from a doe with chronic mastitis. This report appears to be the first documented isolation of *Y. pseudotuberculosis* in association with mastitis. Jones *et. al.* (1982) also reported the isolation of *Y. pseudotuberculosis* serotype IA from milk samples taken from a goat in England showing mastitis. Both these reports stated that the does had aborted as well, but *Y. pseudotuberculosis* was only implicated in these abortions indirectly, for in neither case was *Y. pseudotuberculosis* isolated from the reproductive tracts of the does or the aborted fetuses.

Witte *et. al.* (1985), however, reported that *Y. pseudotuberculosis* can directly cause abortion and early neonatal death in goats. In their report, the researchers were able to isolate *Y. pseudotuberculosis* in pure culture from the placenta and the abomasal contents of the dead kids, which implied intrauterine infection by *Y. pseudotuberculosis*.

Toma (1986) reported the isolation of *Y. pseudotuberculosis* serotype III from a goat in Canada, but no details were given on the clinico-pathological signs of the infection.

Y. pseudotuberculosis has been isolated from a number of clinical conditions from goats in New Zealand. McSporran (1983) reported an outbreak of conjunctivitis in a group of 3 to 5 month-old kids from a large Kaipara goat farm, where the prevalence of infection was 8%. Conjunctival swabs taken from the affected animals yielded a pure growth of *Y. pseudotuberculosis*. Buddle *et. al.* (1988), in a goat mortality study in the southern North Island, recovered *Y. pseudotuberculosis* from one goat out of 21 (4.8% of cases) which had died of yersiniosis. In a survey of *Y. pseudotuberculosis* serotypes from domestic livestock in New Zealand, it was determined that out of the thirteen *Y. pseudotuberculosis* strains isolated from goats, eleven belonged to serotype III, and two to serotype I (Hodges *et. al.*, 1984a).

Yersinia enterocolitica Infections

The earliest instance of *Y. enterocolitica* infection in goats was reported by Krogstad *et. al.* in 1972. The authors described the occurrence of disease in a flock of goats in Norway, characterised by sudden death and diarrhoea. The outbreak occurred over a period of three months during the winter and spring of 1972 and affected one group of goats in the flock (the flock was divided into two groups as part of a feeding experiment). Forty-nine percent of the goats in the affected group became ill. Among those affected, most were kids (65%). 39% of the sick animals died, and again, the dead animals were made up mostly of kids (74%). The authors were able to isolate *Y. enterocolitica* serotype 2 from the intestinal contents of three of the dead animals. This serotype is usually associated with biotype 5 of *Y. enterocolitica* (Krogstad, 1975). During the outbreak, some of the goat handlers also became ill with abdominal pains and diarrhoea. Agglutinating antibodies against *Y. enterocolitica* serotype 2 were found in serum dilutions of up to 1/1250 in one of these persons. However, *Y. enterocolitica* serotype 2 could not be isolated from faecal samples of the person concerned.

Due to the interest stimulated by this outbreak, one of the authors (Krogstad, 1974) carried out a follow-up study on *Y. enterocolitica* infections in the same flock for 12 months, doing both serological and bacteriological examinations on sick and healthy goats. He also tried to infect goats with *Y. enterocolitica* serotype 2 experimentally, and surveyed the distribution of *Y. enterocolitica* serotype 2 in goats from eleven other parts of Norway. The results

showed that subclinical cases occurred in the infected flock during the outbreak of the disease, as evidenced by Widal-titres of ≥1/40 in both sick and healthy animals. The author, however, had difficulty in isolating *Y. enterocolitica* serotype 2 from faecal material. The serological survey on healthy goats in the other parts of the country revealed that subclinical infections of *Y. enterocolitica* serotype 2 (or exposure to the strain) may have occurred in the animals, but again, the author was not able to isolate any *Y. enterocolitica* serotype 2 from faecal material taken from these animals. Experimental infection only succeeded when intraperitoneal injection of *Y. enterocolitica* serotype 2 was used, but not when daily oral dosing for seven days of live *Y. enterocolitica* serotype 2 was employed. The results led the author to conclude that *Y. enterocolitica* serotype 2 was probably a ubiquitous bacterium in the environment of goats in Norway, and could induce diarrhoea and death only if predisposing factors were present.

In 1982 Sutherland reported that *Yersinia* species (unspecified) can contribute to some gastrointestinal disturbances in goat kids, particularly in Congested Bowel Syndrome (CBS).

An outbreak of yersiniosis involving *Y. enterocolitica* in goat weanlings in New Zealand was reported by Orr *et. al.* (1987) on an Otago farm. The weanlings were raised indoors from one week of age, and fed *ad lib* sheep milk substitute. The outbreak occurred just after weaning, when they were moved into another area previously occupied by pigs, which put them into very stressful conditions. One week after the move, four of the weanling began to scour, lost weight, and became weak. Three of the goats died within a few days of developing diarrhoea. The only change seen consistently on necropsy was a considerable enlargement of the mesenteric lymph nodes; otherwise no remarkable gross pathology was observed. Intestinal samples taken during the post-mortem examination yielded *Y. enterocolitica*. Attempts by the authors to isolate *Y. enterocolitica* from the bedding and drainage channels of the goats' shed were unsuccessful.

A goat mortality study in the southern North Island of New Zealand carried out by Buddle and his co-workers (1988) revealed some interesting facts about the epidemiology of yersiniosis in goats. Of the 324 goats autopsied in the study, 21 (6.48%) died of yersiniosis, and from 20 (95.23%) of these animals cultures of *Y. enterocolitica* biotype 5 were recovered, with the remaining animal providing the only *Y. pseudotuberculosis* isolate encountered in the course of the study, as mentioned earlier. Yersiniosis was found to be the most common cause of mortality among goats aged 7 to 19 months, and was the second most common cause in goats 10 weeks to 6 months of age. The infection did not appear to be very important in goats younger than 10 weeks or older than 19 months. The

authors also stated that yersiniosis was most commonly found in goat farms with flock sizes falling within the 201-to-500-animal range.

Y. enterocolitica has been recovered from goat's milk (Hughes and Jensen, 1981; Walker and Gilmour, 1986; Robinson, 1983).

Laboratory Isolation of *Yersinia* Species

Yersiniosis cannot be diagnosed by clinical signs alone (Wetzler, 1981); the disease is most often diagnosed at post-mortem (Henderson, 1983). Cultural identification is the only reliable diagnostic method currently available for use in animals (Baskin, 1980). This was shown by Krogstad (1974) and Hodges *et. al.* (1984a) when they were able to isolate *Y. enterocolitica* and *Y. pseudotuberculosis* respectively from faeces of infected animals. However, identification is also difficult, for these organisms may be mistaken for other non-pathogenic species of the family *Enterobacteriaceae* (Toma, 1986). The difficulty in isolating *Yersinia* species must be emphasized, due to the slower growth of these organisms when compared to some of the naturally occurring intestinal bacteria (Krogstad *et. al.*, 1972). A diagnosis of yersiniosis can be confirmed in some cases by the presence of characteristic histological lesions (Morita *et. al.*, 1973; Hodges *et. al.*, 1984a).

Isolation from Clinical Material and Faeces

Specimens submitted to the laboratory for the isolation of *Yersinia* species may include faecal material, blood, lymph nodes, biopsy materials from other parts of the body, water samples, and food specimens.

Clinical specimens such as blood, lymph nodes, and biopsy material from the liver or spleen do not pose any technical difficulty for the culture of *Yersinia* species, as they are normally sterile. These samples can usually be inoculated directly into trypticase-soy broth or brain heart-infusion broth and then subcultured onto blood agar plates (Mair and Fox, 1986).

The isolation of *Yersinia* species from faeces, food, and environmental samples, on the other hand, presents special problems to the investigator. The presence of other enteric bacteria in faeces and other bacteria in food and environmental samples complicate the isolation procedure and present a number of technical difficulties.

Enrichment Methods for *Yersinia* Species Isolation

The problems involved in isolating *Yersinia* species from faeces, food, or environmental material using conventional or routine laboratory techniques forced researchers to devise more effective methods for enriching *Yersinia* numbers in laboratory media, thereby increasing the recovery rate.

Cold enrichment methods. Greenwood *et. al.* (1975) were able to recover *Y. enterocolitica* from a stool sample taken from a clinical case of diarrhoea using buffered saline as the enrichment medium. Samples were incubated at 4°C for 21 days, and subcultures onto *Salmonella-Shigella* (SS) agar were made at 10 and 21 days. At the time of original enrichment, routine direct inoculations of the stool sample onto MacConkey, XLD, and Hektoen enteric agar plates were also performed. No enteric pathogens were recovered using the conventional procedure; 21 days of cold enrichment and plating onto SS agar with subsequent incubation at room temperature for 48 hours resulted in the recovery of *Y. enterocolitica* serotype 6. Eiss (1975) also found that selective culturing at 4°C using Rappaport's broth for 5 days increased the recovery rate of *Y. enterocolitica* by 44%.

Pai *et. al.* (1979) studied the comparative efficacy of cold enrichment for the recovery of *Y. enterocolitica* serotype O:3 from stools obtained from both diarrhoeic and convalescent or asymptomatic patients. Results of the study revealed that cold enrichment using PBS increased the recovery of *Y. enterocolitica* serotype O:3 considerably in convalescent and asymptomatic subjects but only minimally in patients with diarrhoea caused by the serotype.

Van Pee and Stragier (1979) found that at low temperatures (4°C) *Y. enterocolitica* serotypes O:3 and O:9 and *Pseudomonas fluorescens* showed distinct growth in cold enrichment media, while other *Enterobacteriaceae* failed to grow. Enrichment was maximal after 3 weeks of cold enrichment, although the largest increase was observed after 10 days. According to the authors, the use of PBS as a cold enrichment medium can be improved by adding 3% tryptone-soy broth (TSB).

Other authors attest to the efficacy of cold enrichment when compared with conventional methods (Weissfeld and Sonnenwirth, 1980; Van Noyen *et. al.*, 1981; Van Landuyt *et. al.*, 1981). The use of cold enrichment using PBS for the recovery of *Y. enterocolitica* serotypes O:3 and O:9 has been questioned (Pai *et. al.*, 1979; Van Noyen *et. al.*, 1980; Van Noyen *et. al.*, 1981), but this deficiency is overcome by the ability of this technique to increase the recovery of other

Yersinia species, such as *Y. frederiksenii*, *Y. kristensenii*, and *Y. intermedia* (Van Noyen *et. al.*, 1981). Furthermore, Oosterom (1979) has argued that the use of PBS is recommended if it is desirable to isolate other *Y. enterocolitica* serotypes which may be pathogenic to man, such as O:5,27 and O:6,30., and also *Y. pseudotuberculosis*, some strains of which may also be pathogenic for humans.

Alkali method of enrichment. Although cold enrichment methods are effective for the isolation of Yersinia species, the technique is time-consuming and may not allow for the completion of the culture (and therefore diagnosis) within a clinically relevant time frame. Taking this into consideration, Aulisio *et. al.* (1980) proposed the use of an alkali enrichment step using potassium hydroxide instead of the cold enrichment procedure for the recovery of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from foods. They found that the new method increased the yield of *Yersinia* species fourfold and the sensitivity 100-fold, shortened the incubation period, and decreased the growth of non-*Yersinia* bacteria. Assessments of this method, however, have produced conflicting reports as to its efficacy (Kachoris *et. al.* (1988); citing Ratnam *et. al.* (1983); and Weissfeld and Sonnenwirth (1982)), and at this point it has not been widely adopted to replace cold enrichment.

Selective Plating Media for the Recovery of *Yersinia* Species

Since *Y. enterocolitica* is very tolerant to high concentrations of bile salts, most of the common enteric plating media, such as MacConkey agar, SS agar, Levine's eosin-methylene-blue (EMB) agar, Tergitol-7 agar, lactose-sucrose-urea (LSU) agar, and desoxycholate-citrate agar have been used to isolate the organism (Feeley, 1981).

The wide spectrum of *Yersinia* strains recovered from human, animal, food, and environmental sources show a range of physiological attributes. Hence, some types may grow well in media which are inhibitory for others. An example is the growth of *Y. enterocolitica* serotypes O:3 and O:9 in SS agar with 2% desoxycholate (SS-D), which inhibits other serotypes and biotypes (Mair and Fox, 1986). This variability in physiological attributes resulted in the development of several selective media, in attempts to accommodate this diversity.

Bowen and Kominos (1979) proposed the use of modified pectin agar for the isolation of *Y. enterocolitica* from stool specimens, and claimed that the presumptive identification of *Y. enterocolitica* from mixed cultures is possible within 48 hours using this medium.

Schiemann (1979) proposed the use of a new agar medium for *Y. enterocolitica*, which he designated CIN agar (cefsulodin-irgasan-novobiocin). The formulation of this medium came as a result of studies by the author on the growth of *Y. enterocolitica* in response to selective agents, the details of which were published a year later (Schiemann, 1980a).

Evaluations of this medium by Schiemann (1979) showed that it was especially inhibitory to *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus mirabilis*. It did not, however, inhibit *Serratia* spp., and some species of *Citrobacter* and *Enterobacter*; other species of *Pseudomonas* and *Proteus* were also able to grow. Colony appearance of *Y. enterocolitica* on CIN was described as having a deep red centre with a rather sharp border, which was usually surrounded by a translucent outer zone.

At about the same time that Schiemann's CIN agar was described, another new medium for the isolation of *Y. enterocolitica* was proposed by Dudley and Shotts (1979). The authors called the new medium cellobiose-arginine-lysine (CAL) agar. A year later, Soltesz *et. al.* (1980) made use of the high selectivity of bile salts and sodium oxalate for *Y. enterocolitica* to develop a new medium of their own, which they called "Y" medium. The authors claimed that the new medium achieved better recovery of *Y. enterocolitica* from experimentally infected faecal specimens than did MacConkey agar, LSU agar, and desoxycholate-citrate agar.

Additional differential media were introduced later by several authors. Agbonlahor *et. al.* (1982) introduced the DYS medium. Weagant (1983) described a lysine-arginine-iron (LAIA) agar. In 1987 Fukushima introduced a new selective medium called VYE agar, aimed specifically at preferentially isolating virulent *Y. enterocolitica*.

Efficiency of *Yersinia* spp. recovery. With the proliferation of new selective media for the isolation of *Yersinia* species, comparisons and evaluations of these media were necessary, so as to provide guidance to potential users.

Weissfeld and Sonnenwirth (1980) used Y medium in their work and found that the medium was not very effective without cold enrichment.

Head *et. al.* (1982) compared the efficiency of pectin agar, CAL agar, Y medium, CIN agar, MacConkey agar and SS agar for the recovery of *Y. enterocolitica* strains most commonly associated with disease in Canada and the United States. The authors found that CIN agar was by far the most effective medium for the recovery of *Y. enterocolitica*.

Harmon *et. al.* (1983) also attested to the effectiveness of CIN agar for the isolation of *Y. enterocolitica* from fresh ground pork homogenate when compared with brain-heart infusion (BHI) agar, bismuth sulfite (BS) agar, CAL, desoxycholate-citrate (DC) agar, MacConkey agar, MacConkey with Tween 80, and SS agar. However, the authors found that CIN was slightly inhibitory to *Y. enterocolitica* serotype O:12,25. With this in mind, they recommended that it may be advisable to use one more selective agar in conjunction with CIN; BS agar was recommended.

Davey *et. al.* (1983), in their study on the isolation of *Y. enterocolitica* from the faeces of cows, tried three different selective media: CIN, MacConkey, and CAL, and found CIN to be the most effective medium.

The widespread use of CIN agar in research and diagnostic work in recent years points to the effectiveness of this medium in isolating *Yersinia* species (Lynch, 1986; Lewis and Chattopadhyay, 1986; Simmonds *et. al.*, 1987; Greenwood and Hooper, 1987; Bullians, 1987; Slee *et. al.*,1988; Buddle *et. al.*, 1988). A slight disadvantage of CIN, however, was pointed out by Fukushima and Gomyoda (1986) when they noticed that CIN agar was slightly inhibitory to *Y. pseudotuberculosis* and *Y. enterocolitica* biotype 3B serotype O:3. They reported that maximal recovery of *Y. pseudotuberculosis* using CIN occurred only after 48 hours, with a recovery rate of 91%. The same is true of *Y. enterocolitica* biotype 3B serotype O:3, with a recovery rate of 92%.

Nevertheless, Sato (1987), in his study of the epidemiology of *Y. pseudotuberculosis* in Japanese children, successfully used CIN agar as the plating medium, which showed that CIN can also be used for the isolation of *Y. pseudotuberculosis* with good results.

Incubation Temperature of Culture Media

Nilehn (1969b) studied the growth of *Y. enterocolitica* at different incubation temperatures. The results showed that at 25°C the growth of *Y. enterocolitica* was much better than when the organism was incubated at 37°C. Bercovier and Mollaret (1984) stated that the optimum temperature for the growth of *Yersinia* species ranges from 28°C to 29°C, and that more phenotypic characteristics are exhibited by these organisms at these temperatures than at 35°C to 37°C. Incubation at 37°C is recommended, however, for the demonstration of motility of *Yersinia* species, for it has been shown that these organisms are motile at 28°C and non-motile at 37°C (Bottone, 1977).

Recommendations for the Optimal Recovery of *Yersinia* Species

For the most efficient recovery of *Yersinia* species from clinical material, several authors recommend the combination of cold enrichment and subsequent plating onto CIN agar. Davey *et. al.* (1983) showed that cold enrichment in PBS for three weeks at 5°C followed by plating on to CIN agar produced the best results in isolating *Yersinia* species from cows' faeces. Working with human stool samples, Van Noyen *et. al.* (1987a) found that the best results were obtained by cold enrichment followed by plating on to CIN, not only for *Y. enterocolitica* serotypes O:3 and O:9, but also for other types and species. The same authors, in another paper (1987b), added that this procedure may replace double enrichment and potassium hydroxide treatment for all types of *Yersinia* species. Ratnam *et. al.* (1983) has also reported the excellent recovery of all types of *Yersinia* using the same combination.

Identification of *Yersinia* Species

The Genus *Yersinia*

Bergey's Manual of Systematic Bacteriology (Bercovier and Mollaret, 1984) characterises the genus *Yersinia* as follows:

> "Straight rods to coccobacilli, 0.5-0.8 *u*m in diameter and 1-3 *u*m in length. Endospores are not formed. Capsules are not present, but an envelope occurs in *Y. pestis* grown at 37°C or in cells from *in vivo* samples. Gram-negative. Nonmotile at 37°C, but motile with peritrichous flagella when grown below 30°C, except for *Y. pestis* which is always nonmotile. Growth occurs in ordinary nutrient media. Colonies in nutrient agar are translucent to opaque, 0.1-1.0 mm in diameter after 24 h. Optimum temperature, 28-29°C. Facultatively anaerobic, having both a respiratory and fermentative type of metabolism. Oxidase-negative. Catalase-positive. Nitrate is reduced to nitrite with a few exceptions in specific biovars. Glucose and other carbohydrates are fermented with acid production but little or no gas. Phenotype characteristics are often temperature-dependent, and usually more characteristics are expressed by cultures incubated at 25-29°C than at 35-37°C. The enterobacterial common antigen is expressed by all species investigated. Occur in a broad

spectrum of habitats (live and inanimate), with some species adapted to specific hosts. The mol% G + C of the DNA is 46-50 (T*m*, Bd).

Type species: *Yersinia pestis* (Lehmann and Neumann 1896) Van Loghem 1944, 15."

Differentiating characteristics of the genus *Yersinia* and other related genera are given in **Table 2.1**.

Table 2.1. Characteristics of Some Genera of *Enterobacteriaceae* (Ewing, 1986).

Test	A	B	C	D	E	F	G
Hydrogen Sulphide	-	+	+/-	+	-	+/-	-
Urease	-	-	(+^{w})/-	-	-/(+)	+/-	+
Indole	+/-	+	-/+	-	-	+/-	+/-
Methyl Red	+	+	+	+	-	+	+
Voges-Proskauer	-	-	-	-	+	-	-
Simmon' Citrate	-	-	+	+	+	d	-
KCN	-	-	+/-	-	+	+	-
Phenylalanine	-	-	-	-	-	+	-
Mucate	d	-	nt	d	+/-	-	nt
Mannitol	+/-	-	+	+	+	-/+	+

KEY: **A** = *Escherichia*; **B** = *Edwardsiella*; **C** = *Citrobacter*; **D** = *Salmonella*; **E** = *Klebsiella*; **F** = *Proteus*; and **G** = *Yersinia*.

+ = 90% or more positive within 1 or 2 days; (+) = positive reaction after 3 or more days; - = no reaction (90% or more) in 30 days; +/- = most cultures positive, some strains negative; -/+ = most strains negative, some cultures positive; +/(+) = most reactions occur within 1 or 2 days, some delayed; **d** = different reactions, +, (+), -; **w** = weakly positive reaction; **nt** = not tested.

Biochemical Characteristics of *Yersinia* Species

The biochemical reactions of the various species of *Yersinia* are so diverse and varied that it is necessary to tabulate them to facilitate thorough comparisons. **Table 2.2** shows the biochemical reactions.

Table 2.2. Comparative Biochemical Reactions of *Yersinia* Species.

TEST	*Y pstb*[a]	*Y ent*[b]	*Y fred*[c]	*Y kris*[d]	*Y int*[e]	*Y ald*[f]	*Y roh*[g]	*Y mol*[h]	*Y ber*[i]	*Y ruc*[j]
Voges-Proskauer	-	+[l]	+	-	+	+	-	-	-	-/(+)
Arginine	-	-	-	-	-	-	-	-	-	-
Citrate (Simmon's)	-	-	V	-	V	+	+[n]	-	-	(+)
Aeculin	+	V	+	-	+	+	-	+[n]	+	(+)
H_2S (Kligler's)	-	-	-	-	-	-	-	-[o]	-	-
Indole	-	V[m]	+	V	+	-	-	-	-	-
Lipase (Tween 80)	-	V[m]	V	V	V	+	-[p]	-	-	V[p]
Lysine	-	-	-	-	-	-	-	-	-	+/(+)
Malonate	V[k]	-	-	-	-	-	-	-	-	-
Methyl Red	+	+/(+)	+	+	+	+	+	+	+	+
Motility	+	+/(+)	+	+	+	+	+	+	+	+
Mucate	-	-	-	-	V	nt	-	+	+	-
Nitrate	+	+[l]	+	+	+	+	+	+	+	+/(+)
ONPG (37°C)	V	+[l]	+	+	+	+	nt	nt	nt	nt
Ornithine	-	+[l]	+	+	+	+	+[n]	+	+	+
Phenylalanine	-	-	-	-	-	-	-	-	-	-
Urease	+	+	+	+	+	+	+[n]	+	+	-
Acid Production from:										
Adonitol	-	-	-	-	-	-	-	-	-	-
L-Arabinose	+	+	+	+	+	+	+	+	+	-
D-Cellobiose	-	+	+	+	+	-	+	+	+	-
D-Fructose	+	+	+	+	+	+	nt	nt	nt	nt
L-Fucose	nt	nt	nt	nt	nt	nt	nt	-	+	nt
D-Galactose	+	+	+	+	+	+	+[n]	+	+	nt
D-Glucose	+	+	+	+	+	+	+	+	+	+
myo-Inositol	-	+/(+)[l]	+	V	+/(+)	+	-	V	-	-
Lactose	-	-	V	V	-	-	(+)	-	-	-/(+)
Maltose	+	+	+	+	+	-	(+)	+	+	+
Mannitol	+	+	+	+	+	+	+	+	nt	+
Melibiose	+	-	-	-	+	-	V	-	-	-
α-Methyl-D-glucoside	-	-	-	-	+	-	-	-	-	-
Raffinose	V	-	-	-	+	-	V	-	-	-
L-Rhamnose	+	-	+	-	+	+	-	-	-	-
Salicin	V	V	+	-	+	-	-	V	+[n]	-
D-Sorbitol	-	+[l]	+	+	+	+	+	+	+	-
L-Sorbose	-	+[l]	+	+	+	-	nt	nt	nt	nt
Sucrose	-	+[l]	+	-	+	-	+	+	+	-
D-Trehalose	+	+[l]	+	+	+	+	+	+	+	+
D-Xylose	+	V[m]	+	+	+	+	+	+	+	-

KEY: + = positive; (+) = 90% or more positive between 4 and 7 days; - = negative; V = 10.1% - 89.9% positive; and nt = not tested.

NOTE: All tests were performed at 28°C except for *Y. mollaretii*, *Y. bercovieri* and *Y. ruckeri*, which were performed at 25°C.

[a] Kapperud and Bergan (1984)
[b] Bercovier *et. al.* (1980)
[c] Ursing *et. al.* (1980)
[d] Bercovier *et. al.* (1980)
[e] Brenner *et. al.* (1980)
[f] Bercovier *et. al.* (1984)
[g] Aleksic *et. al.* (1987)
[h] Wauters *et. al.* (1988)
[i] Wauters *et. al.* (1988)
[j] Ewing *et. al.* (1978)

[k] Serogroup IV strains positive
[l] Most negative strains biotype 5
[m] Reaction varies between biotype
[n] May be delayed
[o] TSI agar
[p] Corn Oil

Biotypes of *Y. enterocolitica*

Y. enterocolitica was initially grouped into 5 biotypes by Nilehn (1969a). This classification was based on the reaction of *Y. enterocolitica* in the following biochemical tests: indole production, acetoin production, nitrate reduction, utilisation of aesculin and salicin, ONPG reaction, ornithine reaction, and the production of acid from trehalose, sucrose, xylose, sorbose, and sorbitol. This scheme was later modified by Wauters in 1970, when lecithinase was introduced as a test (Wauters *et. al.*, 1987). Bercovier *et. al.* (1978) made additional revisions to the groupings, when they added biotypes 3A and 3B. Modifications were again carried out in 1980, when the DNAse test was added to the typing scheme (Bercovier *et. al.*, 1980a).

The latest biotyping scheme was proposed by Wauters *et. al.* in 1987. The new proposal involved the addition of new biochemical tests: pyrazinamidase reaction, β-D-Glucosidase and proline peptidase. Biotype 1 was divided by these authors to differentiate the non-pathogenic environmental strains (1A) from the human pathogenic strains originating from North America (1B). The creation of biotype 6 was proposed by these authors to accommodate the non-pathogenic *Y. enterocolitica* strains within biotype 3, biotypes 3A and 3B.

Biotyping of *Y. enterocolitica* is closely correlated with the pathogenic properties of the biotypes (Bercovier *et. al.*, 1980a). Thus, while biotypes 2, 4 and 5 include only the pathogenic strains belonging to a small number of serogroups, biotypes 1 and 3 contain several strains which either have pathogenic or non-pathogenic ("environmental") characteristics (Wauters *et. al.*, 1987). The different biochemical reactions in this proposal are shown in **Table 2.3.**

Table 2.3. Biogrouping of *Y. enterocolitica* According to Wauters *et. al.* (1987).

BIOGROUPS	1A	1B	2	3	4	5	6
Lipase	+	+	-	-	-	-	-
Aesculin/Salicin	+/-	-	-	-	-	-	-
Indole	+	+	(+)	-	-	-	-
Xylose	+	+	+	+	-	V	-
Trehalose/Nitrate	+	+	+	+	+	-	+
Pyrazinamidase	+	-	-	-	-	-	+
β-D-Glucosidase	+	-	-	-	-	-	-
Voges-Proskauer	+	+	+	+	+	(+)	-
Proline peptidase	V	-	-	-	-	-	+

Pathogenesis of *Yersinia* Infection

According to Wormser and Keusch (1981), there is no reason to doubt that, like other enteric pathogens, transmission of *Y. enterocolitica* in man occurs via the oral route, and in goats this may also be the case (Krogstad, 1974). The principal natural route of infection of *Y. pseudotuberculosis* is also the oral pathway (Obwolo, 1980; Witte *et. al.*, 1985). Obwolo (1980) cited some points to support the oral route of transmission:

a. Some outbreaks have been found to be due to feed contaminated by faeces contaminated by *Y. pseudotuberculosis*.

b. *Y. pseudotuberculosis* has been isolated from faeces of naturally infected animals and persons.

c. Lesions have been seen in the intestines and mesenteric lymph nodes in natural cases of yersiniosis.

Other routes of infection, however, may be possible. Baskin (1980) cites one report wherein *Y. pseudotuberculosis* has been recovered from an infected bite wound. Krogstad (1974) also produced clinical illness in one goat by injecting *Y. enterocolitica* intraperitoneally.

It has been shown for a number of years that stresses are necessary to precipitate yersiniosis in domestic animals. In temperate climates, most outbreaks of the disease occur in winter when the susceptible animal may be under the stress of cold or starvation (Obwolo, 1980; Baskin, 1980; Rosenberg *et. al.*, (1980). A virulent strain of the bacterium is also necessary to cause illness (Witte *et. al.*, 1985). Other predisposing factors include overcrowding, feeding changes, poor feed quality, or other types of disease (Krogstad, 1974); the degree of external and internal parasitism (Wetzler, 1981; Morita *et. al.*, 1973); and the stress of pregnancy (Morita *et. al.*, 1973).

Following oral infection, local lesions develop in the intestine. The organisms seem to spread through the portal venous system and the lymphatic vessels. Bacteraemia is followed by the establishment of foci of infection in organs such as the liver, spleen, lungs, and intestine, resulting in lesions that are subject to considerable variation (Obwolo, 1976; Baskin, 1980). Others may be found in the placenta of pregnant animals (Witte *et. al.*, 1985) or the mammary gland (Jones *et. al.*, 1982; Cappucci *et. al.*, 1978).

The clinical signs presented by yersiniosis are variable and depend on the host species involved (Henderson, 1983). In goats, different clinical signs are presented, as mentioned earlier. In a report of a spontaneous *Y. pseudotuberculosis* infection in goats, Morita *et. al.* (1973) found diarrhoea to be the most obvious clinical symptom and the animal affected was emaciated when it died. Krogstad *et. al.* (1972), in their report of the *Y. enterocolitica* outbreak in goats, presented similar clinical signs; some of the goats had acute diarrhoea for a short time, although others in the herd died suddenly without any previous clinical symptoms.

Another clinical manifestation is the development of mastitis and abortion. In two separate cases, pregnant does which had mastitis aborted near the termination of pregnancy. However, the role of *Y. pseudotuberculosis* in these cases of abortion can only be implicated indirectly, since no *Y. pseudotuberculosis* was isolated from the foetuses or reproductive tracts of the does concerned. Further details of the cases mentioned are described in earlier sections of this chapter.

Pathology

Necropsy of fatal cases of yersiniosis usually reveals emaciated carcasses with the peritoneal fluid sometimes blood-tinged or fibrinous (Obwolo, 1976). In a *Y. pseudotuberculosis* infection in a goat, grayish-white foci of poppy-seed to miliary size were present in the liver, enlarged spleen, and swollen mesenteric lymph nodes (Morita *et. al.*, 1973). In addition, catarrhal bronchopneumonia and diphtheroid and catarrhal enteritis were observed in the same case. In a *Y. enterocolitica* infection, however, only catarrhal enteritis was found at necropsy (Krogstad *et. al.*, 1972).

Microscopically, *Y. pseudotuberculosis* lesions were classified by Morita *et. al.*(1973) into the following groups:

a. Central necrotic foci surrounded by granulation tissue and often accompanied by bacillary masses. These were found in the liver, spleen, mesenteric lymph nodes, and pancreatico-duodenal lymph nodes.

b. Foci with septic and hyaline thrombi. These were found in the liver, lungs, and mesenteric lymph nodes.

c. A granulomatous cell-nodule consisting principally of macrophages and a purulent cell nodule. This was found in the liver.

In mastitis cases, the main pathological findings were the clotting of milk and the presence of blood in the milk (Jones *et. al.*, 1982; Cappucci *et. al.*, 1978). An aborted foetus showed dark red blotches in the otherwise pale lungs. Microscopically, this was shown as suppurative pneumonia. The placentas showed discoloured cotyledons with whitish spots in them, shown microscopically as suppurative placentitis (Witte *et. al.*, 1985).

Treatment and Control of Yersiniosis

Most broad-spectrum antibiotics are effective against yersiniosis (Obwolo, 1976; Hodges *et. al.*, 1980; Wormser and Keusch, 1981). For the treatment of mastitis, Jones and his co-workers (1982) used the following regimen: Sulphadoxine and trimethoprim intramuscularly and a combination of novobiocin, neomycin, procaine penicillin G, dihydrostreptomycin, and prednisolone by the intramammary route. However, Hodges *et. al.* (1980) found that *Y.*

pseudotuberculosis is resistant to novobiocin *in vitro*, and Wetzler (1981) has suggested that penicillin should not be used, for resistant strains may be encountered.

Control measures are based on a knowledge of the epidemiology of the disease. Proper management of animal units is necessary, and this includes good hygiene, avoidance of feed contamination, decreasing stressful conditions, and making reasonably sure that newly introduced animals are not carriers. On the larger scale, control measures should be directed at exclusion of wild rodents and birds (Obwolo, 1976).

Public Health Significance of Yersiniosis

It must be emphasised that yersiniosis is a zoonosis, and human pathogenic species may be recovered from apparently healthy animals and their products, including milk. Animal reservoirs, such as pigs and pet dogs (for *Y. enterocolitica*) and various kinds of domestic and wild animals (for *Y. pseudotuberculosis*), have been linked with human yersiniosis, and full awareness of the danger presented by these potential sources of infection is recommended. Control measures for human yersiniosis must therefore be directed towards the avoidance of animals which are obviously diseased. The importance of contaminated food and water as sources for human yersiniosis cannot be overemphasised; the prevention of human infection with *Yersinia* species, as with any other enterobacterial pathogens, is essentially a matter of good hygiene.

CHAPTER 3

CHARACTERISTICS OF GOAT FARMS IN THE MANAWATU AS REPORTED BY FARMERS IN A POSTAL SURVEY

Introduction

In order to achieve a clearer understanding of the goat farming practices in New Zealand as they might influence the epidemiology of *Yersinia* species infection, a postal survey was considered to be a very important aspect of the project. Thirty goat farms in the Manawatu were chosen for the examination of their goat flocks for the presence or absence of infection with *Yersinia* species. Questionnaires were sent out to each of the participating farmers.

Materials and Methods

Selection of Goat Farms

The 30 goat farms included in this study were mostly obtained from the membership roll of the Manawatu branch of the Mohair Producers Association of New Zealand (MOPANZ). A few names were obtained from the records of the Veterinary Clinic and the Veterinary Microbiology Laboratory at Massey University, based on the involvement of these farms in clinical cases submitted for diagnosis.

Most of these farms were within a 50-kilometre radius of Palmerston North. The farms included in the study comprised almost all of the commercial goat farms in the Manawatu at the time.

Initial contact with these farmers was made through telephone interviews, the purposes of which were to obtain their cooperation for our survey on the prevalence of *Yersinia* species among goats. Those farmers who agreed were then included in the postal survey.

The Questionnaire

A questionnaire regarding farm characteristics and pertinent goat farming practices was prepared (Appendix I). This was designed to be easily understood by the farmers and structured in such a way that coding for analysis could readily be achieved. Copies were then sent to farmers who agreed to cooperate in this study. A stamped, addressed envelope was included with each questionnaire sent out.

Statistical Analyses

All data gathered were stored in a microcomputer using the PANACEA 2* database program. Where possible, coding of replies was made. Chi-squared analyses and the determination of normal distribution statistics were performed using the same program.

Results

Response Rate

Of the 30 questionnaires sent out, 29 were returned, giving a response rate of 97%.

General Farm Information

Farm and paddock sizes. The farm areas of the the 29 goat farms ranged from 2 to 1521 hectares (mean: 117), and the 25, 50 and 75 percentile farm sizes were 6.7, 21.0 and 88.5 hectares, respectively. All the farms were divided into paddocks, which ranged from 0.25 to 20.25 hectares in area, with the mean paddock size being 4 hectares. Electrified fences were used in most (90%) of the farms.

Types of stock on the farm. 26 (90%) of the farms in the survey had livestock other than goats, with sheep being the most common additional enterprise. Of these farms, 24 (92%) had sheep, 20 (77%) had cattle, and 8

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* PAN Livestock Services Ltd., Department of Agriculture, University of Reading, Reading, Berkshire, England.

(23%) had horses. 14 (54%) of the farms kept both sheep and cattle as well as goats, and 6 (23%) had horses as well, on top of the two previously mentioned animals. 4 farms (15%) had sheep only, and 2 (8%) had horses only, raised together with goats in the same premises.

Pasture usage. All the livestock on the farms surveyed used the same pasture (paddocks) as goats, either simultaneously (goats and other livestock species run together), or alternately. The most common livestock using the same pasture as goats were sheep, cattle or a combination of both.

There was no apparent pattern to the type of paddock usage among the farms. Ten farms (38.5%) rotated their paddocks between the different animal species, while an equal number of farms allowed the simultaneous use of the paddocks by all the livestock species. Six (23%) employed either alternate or simultaneous utilisation of pasture areas by goats and other livestock.

Goat flock size. **Table 3.1** shows the flock sizes on the goat farms surveyed. The farms had populations ranging from 18 to 1295 goats, with a mean flock size of 276. More than half (55%) of the farms had flock sizes ranging from 51 to 200 goats. 21% had more than 501 goats, and 14% fell into the 201 to 500 range. Three farms (10%) had less than 50 goats.

Population density. The overall density of the goat population on the farms averaged 10 goats per hectare. Subdividing farms into paddocks (mean: 30 paddocks per farm) resulted in an average of 25 goats per paddock per farm, from a range of 1 to 200 goats per paddock.

Breed distribution. The distribution of breeds depended upon the production objectives of the farmers concerned. 27 (93%) of the flocks surveyed were kept for fibre production, 1 (3.5%) for milk production, and 1 (3.5%) for both milk and fibre production.

Of the fibre-producing flocks, the majority (69%) had Angora/feral crosses ("grades") as their predominant breed. 5 flocks (17.84%) had pure Angoras comprising the bulk of their goat populations, and the rest had mostly feral goats raised for cashmere production. In almost half of these farms (46%) ferals made up the next predominant breed. The only pure milk-producing flock had Saanens, and the mixed fibre/milk-producing flock had a combination of Angoras and Saanens.

Age structure of the flocks. For the purposes of this study, the goats were classified into three age strata: kids (less than 1 year old), hoggets (1 to 2 years

old), and adults (more than 2 years old). Of all the goats in the 29 farms, 31% were kids, 22% were hoggets, and 47% were adults (**Table 3.1**).

Table 3.1. Flock Sizes of the 29 Goat Flocks Categorised by Age Group

FARM	KIDS	HOGGETS	ADULTS	TOTAL
1	20	20	60	100
2	250	70	370	690
3	20	18	35	73
4	12	10	40	62
5	10	30	26	66
6	60	27	120	207
7	32	17	28	77
8	35	5	80	120
9	130	55	60	245
10	24	15	12	51
11	40	20	60	120
12	62	58	76	196
13	15	5	11	31
14	250	200	200	650
15	23	12	16	51
16	508	334	453	1,295
17	15	10	17	42
18	100	40	180	320
19	45	35	66	146
20	60	none	97	157
21	30	20	120	170
22	30	24	105	159
23	80	30	70	180
24	6	21	30	57
25	35	none	190	225
26	200	120	450	770
27	none	150	450	600
28	8	none	10	18
29	400	400	400	1,200
TOTAL	2,500	1,746	3,832	8,078

Pasture use by goats. More than half (52%) of the farmers ran different age groups of goats in separate paddocks. 31% of the farmers, however, mixed goats of all ages in the same paddock, running these animals as a single mob. The remainder of the farmers (17%) used a combination of both methods, depending on paddock availability.

Goat shelter. 50% of the respondents had permanent goat sheds in addition to naturally occurring shelter areas such as scrub, gullies, and trees. 29% had permanent sheds only, and 21% did not have any permanent goat shed, but relied entirely on the presence of natural shelter found on the farm premises.

Goat Management Practices

Kid management. Kidding usually takes place during the months of August to October. To protect the does during kidding, sheds were provided by 28% of the farms. Kids are usually left with the does for an extended lactation, as practised by 93% of the farmers. The remainder either used a combination of hand-rearing and natural doe-rearing or just used the hand-rearing method exclusively, as practised in the milk-producing farm.

The majority of the farmers (63%) wean kids at the age of 4 to 5 months. Some wean kids as young as 2.5 to 3.5 months (30%), while only 7% of the farmers wean the kids at 6 months of age.

Shearing practices. Among the fibre-producing flocks, goats are usually first shorn just after weaning, at the age of 5 to 6 months. This is practised by 81% of the farmers. A minority of the farmers start shearing either at an earlier age, or at a later age. One farmer in the survey stated that shearing starts on his farm only when the goats reach one year old.

Most farmers (85%) shear goats twice a year, while the rest do it just once. The first shearing of the year is usually done during the months of December to March, with the majority of farmers shearing during the month of February. The next shearing period occurs during the months of July to October, with the bulk carried out during the months of July and August. This second shearing phase coincides with the kidding season.

After shearing, most of the farmers (93%) release the goats directly to pasture, while the minority (7%) rest the goats for a few hours in the shed before releasing them.

Supplementary feeding. The majority of the respondents (86%) give supplemental feed to the goats, and 91% of those following this practice do so during the colder months of the year. A few of the farmers give supplementary feeding the whole year round.

Forty percent of those farmers giving supplementary feeds give only hay to the goats, while the majority (52%) provide additional supplements such as grains, pelleted feed, and mineral supplementation in addition to hay. One farmer just gave pelleted feed and another gave muesli, taken from a nearby food-processing company.

Water supply. Bores and town-supply water provide water to the majority (62%) of the farms. Natural sources (streams, lakes, and ponds) provide water to 24% of the goat farms. The remainder of the farms in the survey have either bores or town-supply water supplemented by natural sources.

Replacement of stock. For stock replacement, 60% of the farmers surveyed practised a combination of homerearing and outside purchase, with the remaining 40% practising homerearing entirely. Those who purchase goats from outside sources do so mostly (67%) from sources outside the Manawatu such as the Wairarapa, Hawke's Bay, and Waikato.

Animal health practices. Common routine animal health activities on the farms include deworming, lice control treatments, and vaccination against *Clostridium* species infections.

All the farmers in the survey had a regimen for deworming goats, and the majority (97%) included animals in all age groups in this treatment program. A regular schedule of deworming every 4 to 6 weeks was practised by 69% of these farmers on all the animals. The other farmers were also following the same schedule but were selective on the animals dewormed: some farmers would perform monthly deworming only on kids and a less frequent schedule on the older animals, usually 2 to 4 times a year.

Ninety-seven percent of the respondents routinely applied medicaments for controlling goat lice, a procedure performed usually once (by 59% of those doing the procedure) or twice (by 33%) a year. The most favoured treatment used was the pour-on type (82%), followed by drenching and dipping methods (14%). One farmer (4%) used combinations of dips and pour-ons.

About 66% of the farmers vaccinate their goats against clostridial diseases. Depending upon the inclination of the farmer, different age groups were reported as being vaccinated on various of the farms.

Mustering goats. 69% of the farmers used dogs for mustering goats. The use of dogs is more closely associated with farms having flock sizes bigger than 200 ($p = .0092$). All farms with goat populations greater than 200 goats used

dogs for mustering, while only 53% of farms with 200 or less goats used this practice.

Farmers' Comments

When asked for their views on goat management and health, 41% of the respondents made comments covering several aspects of goat raising. Because the views expressed were quite disparate, they cannot be taken as representative of the goat farmers' shared opinion on these matters.

One farmer said that raising goats, especially on a small unit, is quite labour-intensive when compared with raising other livestock. Another complained about the low returns from goat fibre production, and one specifically mentioned that he may be forced to look closely at costs, especially drenching and feeding costs, due to such low returns.

41% of those who made comments see foot problems and worms as the most important health problems affecting goats. These views were especially common among owners of Angoras, which are, as one farmer put it, "definitely not suited to this climate and land." Ferals and grades, not only are better suited to the climate, but easier to handle and control as well, in the view of one farmer.

Discussion

Goat farming in New Zealand came about as a natural extension to the existing livestock industry, with the basic skills already in place (Rumble, 1985). Also, existing farm infrastructures, with slight modifications, were found suitable for goat raising. Some characteristics of goats, however, are quite different from sheep, as farmers soon learned.

The questionnaire sent to farmers was an attempt to identify some characteristics of goat farms and the management procedures employed on these farms. Emphasis was given to factors which may put undue stress on goats, for it has been shown that stress can precipitate disease conditions. Stressful conditions have been shown to precipitate yersiniosis outbreaks in goats (Krogstad, 1974; Obwolo, 1980; Wetzler, 1981).

The most stressful events in the life of goats occur during pregnancy and

kidding, food and water shortages, and overcrowding. Additional conditions imposed by farmers, such as shearing, forced weaning, and mustering for routine farming procedures put considerably more stress on goats. Some of these events, such as kidding, shearing, and food shortages coincide with the coldest months of the year, which aggravates the situation.

Means have been devised to counteract the adverse effects of these conditions, however. Sheds are provided to does during kidding and shearing. Supplementary feeds are given in times of drought or during winter food shortages. The effects of these, however, greatly depend on the actual practices imposed by the farmers on these animals.

Goats are very sensitive to changes in weather conditions, such as very cold and wet weather and very cold winds. Because of this, they have the natural tendency to avoid these conditions (Rumble, 1985). This is probably a result of the lack of an outer layer of body fat in goats, in contrast to sheep (Yerex, 1986). With this in mind, adequate shelter is necessary, especially during times of stress.

The farmers' practice of immediately releasing the goats to pasture after shearing, especially during the winter shearing, may make these animals particularly susceptible to disease brought about by stress. Although shearing is done mostly in sheds, the practice of resting goats for a few hours before releasing them to pasture is only done by 7% of the goat farmers in this survey.

From the standpoint of disease prevention, it is quite unfortunate that kidding takes place during the colder months of the year. It is thus imperative that shelter be provided for the kidding does as well as for the new kids. Again, for this purpose, only 28% of the farmers surveyed provided sheds, an indication that during kidding, the majority of does and kids are exposed to stressful conditions, especially cold and wet weather.

Farmers see worms as one of the most important health problems affecting goats. Young goats are most susceptible to gastrointestinal nematode infections, especially during autumn (Anon., 1987a). The most obvious sign presented in these cases is scouring, and as such, farmers tend to consider any scouring goat as having worms. This may be true, but most farmers do not realise that yersiniosis (which also affects young goats at similar times of the year) often occurs in conjunction with gastrointestinal parasitism (Anon., 1987b).

CHAPTER 4

SCREENING OF GOAT FARMS FOR THE CARRIAGE OF *YERSINIA* SPECIES

Introduction

This screening survey was the first stage undertaken in the study of the epidemiology of yersiniosis in goat flocks. Since no previous reports on variations in the level of *Yersinia* infection among goat flocks in New Zealand exist, a prevalence survey of the infection was planned. Before going directly to this prevalence survey, however, it was considered necessary to screen goat farms in the Manawatu for the presence or absence of yersiniosis.

To achieve a known degree of confidence concerning the presence or absence of yersiniosis in goat flocks, a sampling plan was worked out, which is described in this chapter.

Materials and Methods

Selection of Goat Farms

The goat farms mentioned in Chapter 3 were included in this survey. Most of these farms were in the Manawatu area, within a 50-kilometre radius of Palmerston North. **Figure 4.1** shows the location of these farms.

Determination of Sample Sizes

The numbers of faecal samples taken from each farm were determined by assuming a 25% prevalence of *Yersinia* infection in these farms. The selection of this value was just assumed, since there were no reliable figures for the prevalence of the infection in goats to rely on. It was believed that the prevalence would be less than 25%, in which case the sample size chosen would give a higher confidence level than the one required.

For the purpose of detecting the presence of *Yersinia* in at least one animal from each flock at this assumed level of prevalence, a table for the determination of sample sizes (at the 95% confidence level) was consulted (Cannon and Roe, 1982). By using this table (Appendix II), the sample sizes were determined by selecting a value corresponding to the nearest flock sizes. In this case, samples of 11 goats were taken from each of the flocks with populations greater than 140 (16 flocks), 10 each from goat flocks within the 40-120 size range (11 flocks), and 9 each from 2 farms with flock sizes less than 40. A sample of 11 goats was taken from one goat farm for which no population data were available.

Since the flocks were stratified into three age groups (kids - less than one year old, hoggets - one to two years old, and adults - two to five years old), these samples were taken randomly in proportion to the populations of these age groups in each flock, to adequately represent these strata.

Collection of Faecal Material

From October to December 1987 visits were made to the above-mentioned goat farms and faecal samples collected following the above scheme. Faecal material from goats was collected directly from the rectum. To avoid contamination, separate disposable vinyl gloves were used for each animal sampled. Manual collection was performed by inserting a finger into the rectum and manipulating faecal material out, depositing this directly into sterile 5 ml. disposable plastic containers (**Figure 4.2**). In cases where digital collection of samples was unsuccessful, swabs from the rectal wall were taken using sterile cotton swabs and inoculated directly into 10 ml. of sterile M/15 Phosphate-Buffered Saline. Sampling from very small animals, or from those which had very small anal sphincters was also performed using sterile cotton swabs.

Inoculation Into M/15 Phosphate-Buffered Saline (PBS)

The faecal samples collected were inoculated into PBS (Appendix IV). This was done as soon as possible after the arrival of the samples from the farm, to avoid the effects of dehydration. Approximately 1 gm. of faecal material was used per 10 ml of the PBS solution, except in cases where sterile cotton swabs had been used. Hard and pelleted faecal material was allowed to soak in these tubes for a few minutes, then broken up using a sterile cotton swab and mixed thoroughly to form a fine suspension of faecal material (**Figure 4.3**). Unpelleted

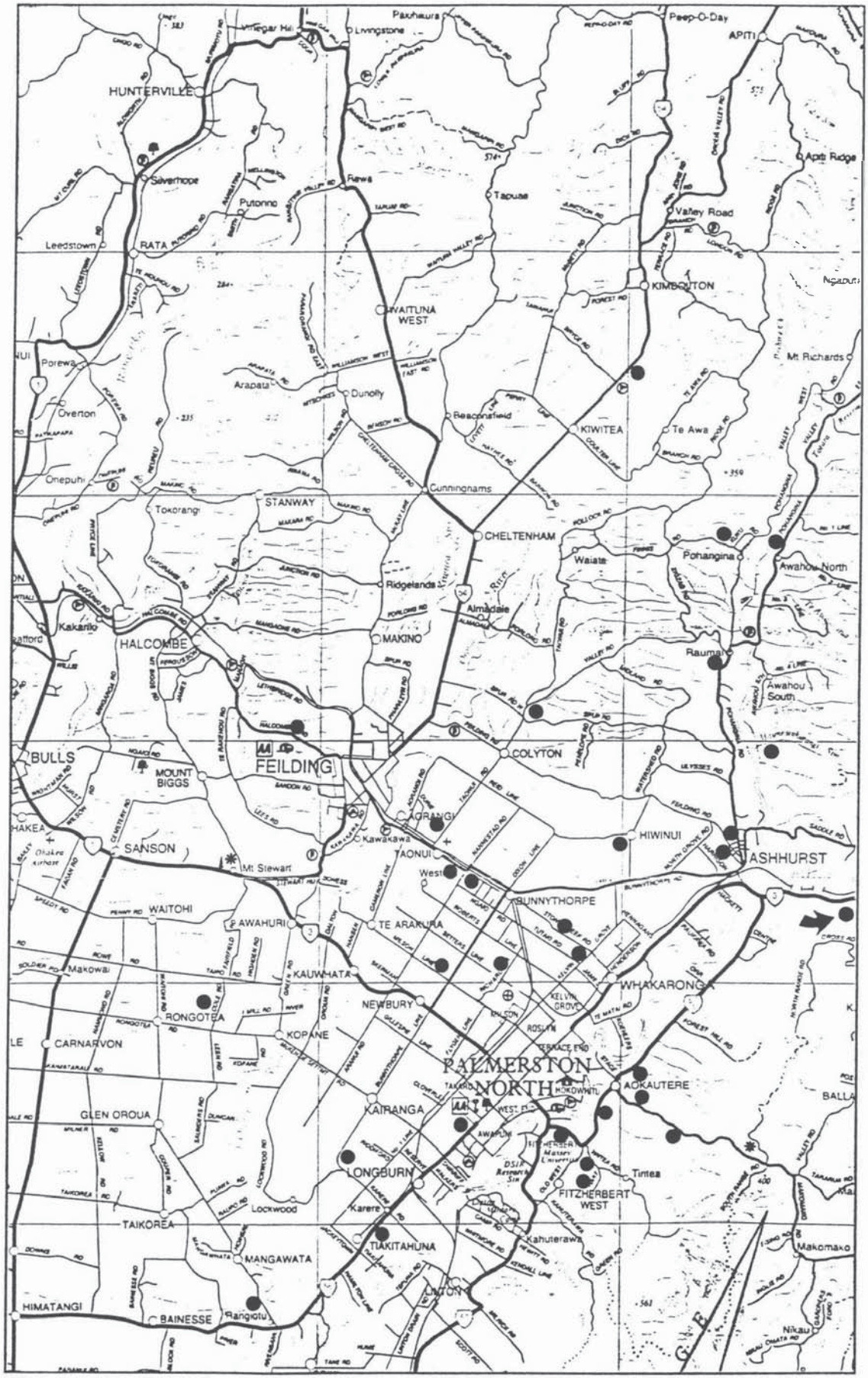

Figure 4.1. Location of Farms Screened for *Yersinia* Species Carriage

or unformed faecal material was transferred from the plastic collection tubes into the PBS solution using sterile cotton swabs, and then shaken to thoroughly disperse the faecal material throughout the buffer medium.

Figure 4.2. Disposable Plastic Container Used for Collecting Faeces

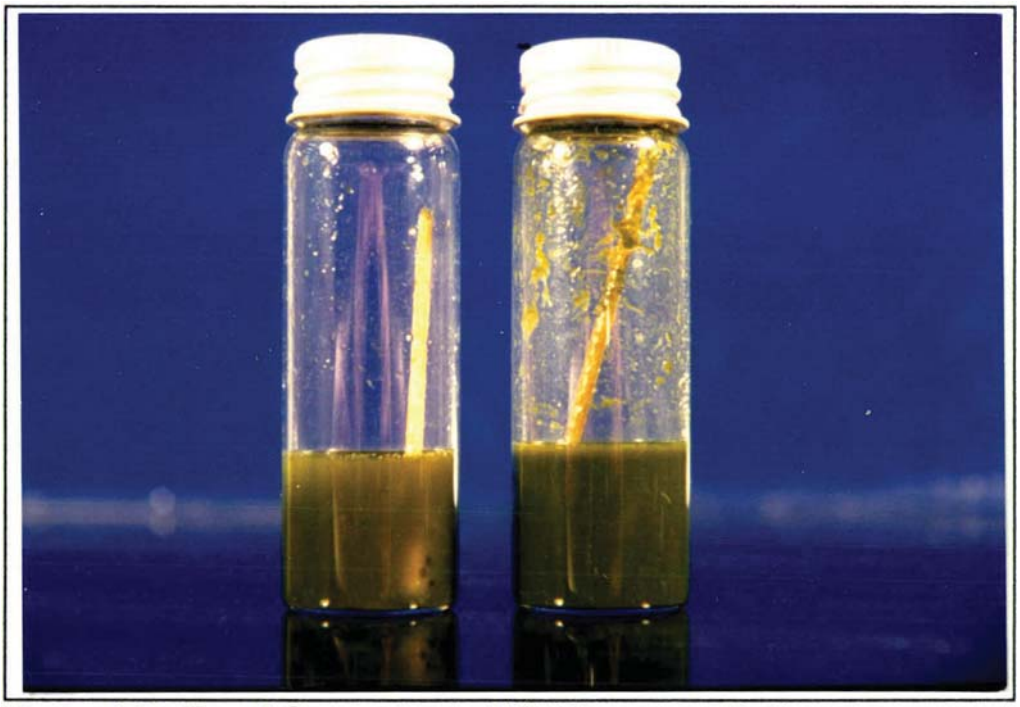

Figure 4.3. Faecal Suspension in M/15 Phosphate Buffer

Incubation of Faecal Suspensions

The faecal material suspended in PBS was cold-enriched at 4°C for 21 days, after which plating onto suitable solid media was performed.

Plating of Cold-Enriched Samples

After the cold enrichment period of 21 days, a loopful of suspension from each of the samples was plated onto *Yersinia* Selective Agar* (Difco) plates (Appendix IV). These plates were incubated at 29°C. Examination of the plates was carried out at 24 and 48 hours. Colonies of 0.5 to 1 mm in diameter at 24 hours or 2-5 mm in diameter at 48 hours which showed typical dark red "bullseye-like" features surrounded by a transparent border were considered as potential *Yersinia* species. Bacterial colonies which had dark red pigmentation but did not show the "bullseye" or the transparent border were also considered for screening.

Preparation of Media for Biochemical Tests

All media for biochemical tests were prepared following protocols from established microbiology texts and from the recommendations of the manufacturers of these media. Appendix IV gives details of the methods of preparation and the testing procedures.

Screening for *Yersinia* Species

After 48 hours incubation, suspect colonies from CIN plates were inoculated into 3 ml. tryptone water (TW). After 24 hours incubation at 29°C, the following media were then inoculated using growth in TW as the inoculum: Triple-Sugar-Iron agar (TSI) slant, Lysine-Iron-Agar (LIA) slant, and ornithine decarboxylase, lysine decarboxylase and arginine duhydrolase broths. These were incubated at 29°C for 24 to 48 hours. At this point, another bottle of tryptone water was inoculated and incubated at 29°C for 5 to 7 days for the determination of indole production. Cultures which gave at least one of the following reactions

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* Also known as Cefsulodin-Irgasan-Novobiocin (CIN) agar (Schiemann, 1979). Made by the manufacturer with slight modifications of the original formula.

were rejected: H_2S production (darkening on TSI), excessive gas production on TSI (more than a bubble), non-fermentation (TSI), and positive reactions in either or both the lysine and arginine broths.

Cultures which either gave an acid slant/acid butt (A/A) or an alkaline slant/acid butt (K/A) reaction on TSI and an alkaline slant/acid butt (K/A) reaction on LIA were considered as presumptive *Yersinia* species. Gram-reactions were performed on these isolates and those which were Gram-positive were rejected.

The next stage of screening involved the inoculation of urea agar and phenylalanine agar. Cultures showing positive reactions in phenylalanine were rejected.

Identification of *Yersinia* Species

Definitive identification of *Yersinia* species was achieved by performing the following additional tests: Methyl Red, Voges-Proskauer, indole, malonate, and Simmon's citrate. Likewise, acid production from the following carbohydrate media was determined: adonitol, D-cellobiose, lactose, maltose, mannitol, D-melibiose, D-raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, D-trehalose, and D-xylose. *Yersinia* species were then identified by using **Table 2.2** in Chapter 2.

Data Storage and Statistical Analyses

All data were stored in the PANACEA 2 database program. Farm data that were necessary for comparisons were taken from Chapter 3. Normal distribution statistics and chi-squared analyses were done using the same program. Analyses of continuous data were done using Student's t-test.

The Yates' correction procedure in the analysis of **2 × 2** tables was automatically applied when using the database program. Likewise, when expected frequencies were less than 5, the Fisher's exact test was also automatically implemented.

Results

Number of Animals Sampled

A total of 315 goats were sampled in this screening survey. 11 samples each were taken from 17 farms, 10 samples each from 11 farms, and 9 samples each from the remaining two farms.

Farms Positive for *Yersinia* Species

60% (18 out of 30) of the farms sampled had at least one animal positive for *Yersinia* species. From these farms 38 *Yersinia* strains were isolated from the total of 317 goats sampled, giving a prevalence of 12%. The number of *Yersinia* strains recovered from each farm ranged from 1 to 5. 44.4% of farms (8/18) had only 1 *Yersinia* isolate, 16.7% (3/18) had 2, and 27.8% (5/18) had 3 strains. 5.6% (1/18) had 4 *Yersinia* strains recovered and another 5.6% (1/18) had 5 strains.

Distribution of *Yersinia* Species Isolates

The distribution of *Yersinia* species isolated in this survey is shown in **Figure 4.4**. 21 (55.3%) of the 38 *Yersinia* isolates were *Y. frederiksenii*. There were 12 (31.6%) *Y. enterocolitica*, 3 (7.9%) *Y. kristensenii*, and 2 (5.3%) *Y. intermedia*. Neither *Y. pseudotuberculosis* nor any other *Yersinia* species was isolated.

Association Between *Yersinia* Infection and Flock Size

Figure 4.5 shows the distribution of the negative and infected flocks in relation to flock sizes. Flocks were classified into 2 categories: farms with less than or equal to 200 and farms with more than 200 goats. It was shown that there were significant differences betweeen infected and non-infected farms with regards to flock size ($p = 0.0267$). Farms with flock sizes greater than 200 were found to be more likely to have *Yersinia* infection than flocks with less than or equal to 200 goats (Relative Risk = 1.9).

Comparison between flock sizes of infected farms (mean: 380.5) and non-infected farms (mean: 111.7) revealed a significant difference ('t' = 2.212, $p = 0.0394$).

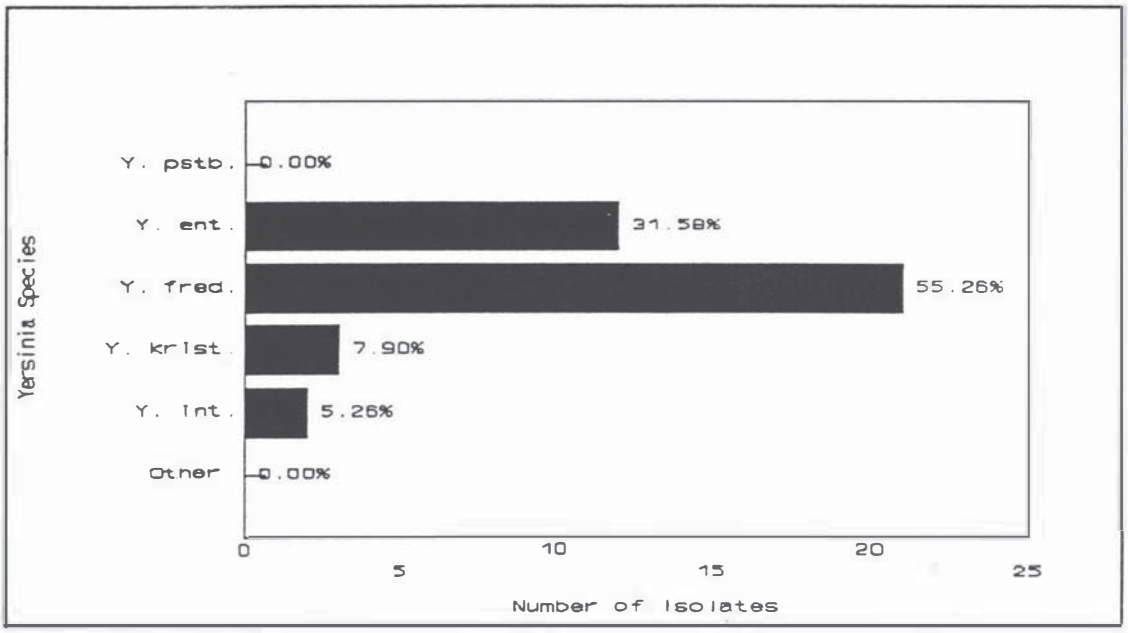

Figure 4.4. Distribution of *Yersinia* Species Isolated in the Screening Survey

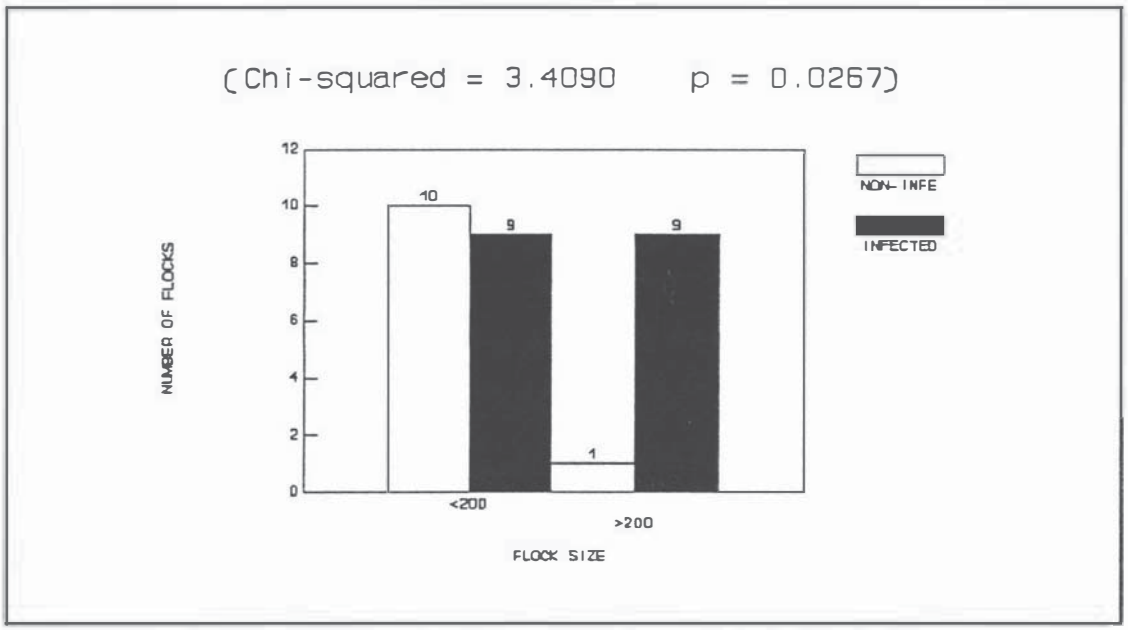

Figure 4.5. Association Between *Yersinia* Infection and Flock Size

Association Between *Yersinia* Species Infection and Shearing Frequency

As mentioned in the previous chapter, farmers usually shear goats twice a year, with only a few doing the practice once a year. Although no significant differences between shearing frequency and farm sizes were found, there was a greater tendency (p =0.0862) for *Yersinia* infection to occur in farms shearing goats twice a year, as shown in **Figure 4.6.** The risk of having *Yersinia* infection in flocks shorn twice a year is 2.95 times that in flocks shorn only once.

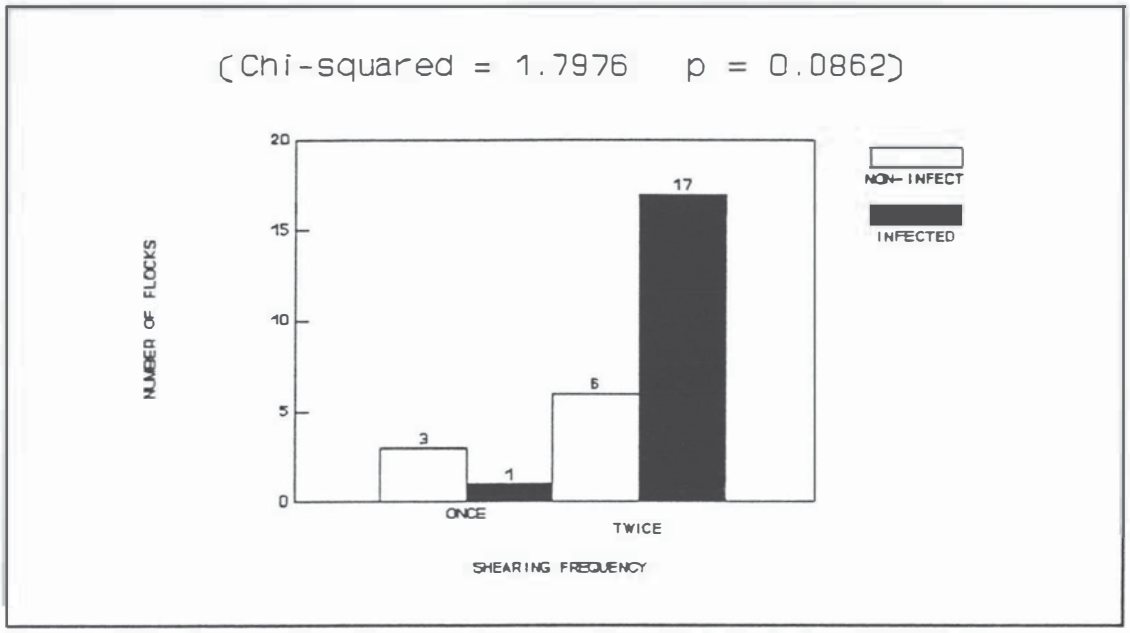

Figure 4.6. Association Between *Yersinia* Infection and Shearing Frequency

Association Between *Yersinia* Infection and Dog Use

It was shown in Chapter III that there was a significant association between the use of dogs for mustering and flock size, i. e., farms with bigger flocks tend to use dogs. In relation to this, a significant association was also seen between *Yersinia* infection and dog use (p = 0.0376), and the risk of having *Yersinia* infection in flocks which use dogs was found to be 2.27 times more than in flocks which do not use dogs (**Figure 4.7**).

Discussion

A screening survey of goat farms for the presence or absence of *Yersinia* infection was performed prior to the more detailed prevalence survey described in Chapter 5. This preliminary phase was undertaken for two main reasons. Firstly, although goat yersiniosis has been reported sporadically, specific knowledge on the infection status of goat flocks was lacking; thus it was deemed necessary to determine which flocks were infected and which were not. Secondly, as a direct benefit of this knowledge, considerable time, effort, and money were saved in the subsequent prevalence survey, by eliminating from the more intensive sampling procedure those farms which were found to be negative with regard to the infection at the time of sampling.

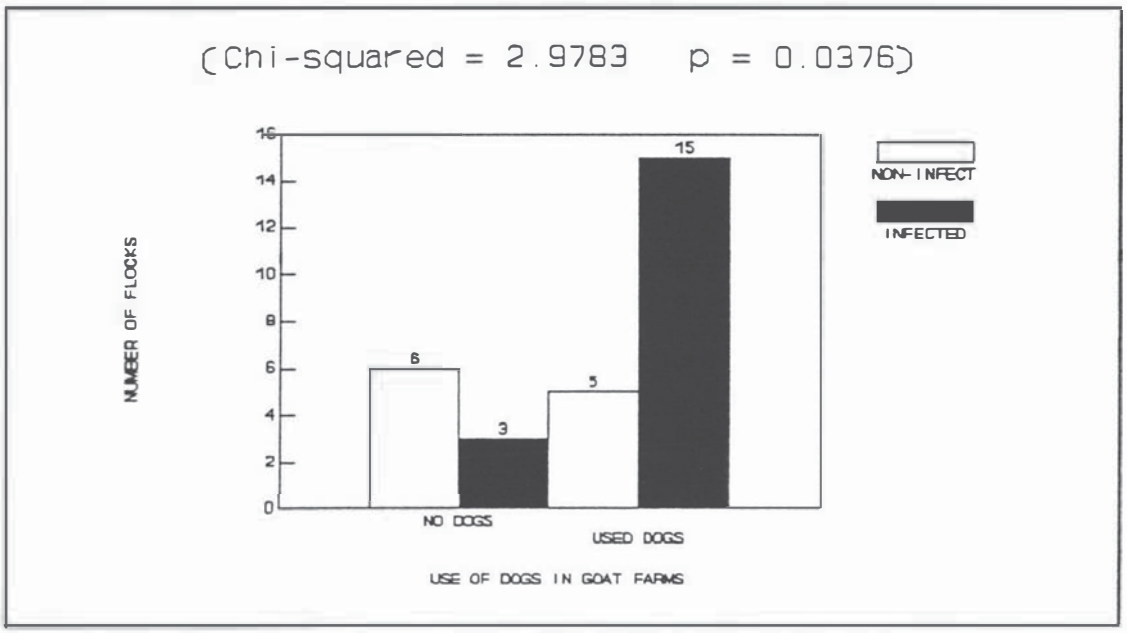

Figure 4.7. Association Between *Yersinia* Infection and Dog Use

The results showed that 60% of the farms surveyed carried the infection during the course of the survey. This figure, however, is almost certainly an underestimate of the number of farms infected, for it has been shown by several authors (see Chapter 3) that yersiniosis occurs more frequently during the colder months of the year. The collection period (October to December, 1987) of this screening survey, unfortunately, fell during the spring/summer season in New Zealand. Time constraints meant that only one screening procedure was possible, and one major objective of the screening survey was to ensure that the prevalence survey was limited to flocks which were infected, since the cost of culturing in this second phase was high. Prior to the screening, it was unclear whether infection at flock level was universal, common or rare. It is now clear that, for the study area at least, infection with *Yersinia* species is very common, and may be almost universal.

The greater tendency of bigger flocks (>200 goats) to have *Yersinia* infection has been shown in this study. This association has previously been shown for the disease yersiniosis by Buddle *et. al.*, who carried out a study of goat mortalities in the southern North Island of New Zealand in 1988. They were able to show that yersiniosis was most common in farms with flock sizes ranging from 201 to 500, and mentioned that in larger goat flocks the importance of microbial diseases increase, possibly due to an increased reservoir of infection. This hypothesis is supported by the present survey.

The close association of the presence of infection with management practices such as shearing frequency and the use of dogs for mustering may mean

that these practices exert considerable stress on goats, thereby making them more susceptible to microbial infections. The tendency of the occurrence of *Yersinia* infections in farms which use dogs for mustering may be a consequence of confounding, however. The presence of the infection may in fact be actually due to bigger flock sizes rather than dog use, since it has been shown in this survey that farms with bigger flock sizes use dogs more often than those with smaller flock sizes. The data from this study is too limited in scale to enable the effects of the two factors to be distinguished.

CHAPTER 5

PREVALENCE OF *YERSINIA* SPECIES INFECTION IN GOAT FLOCKS

Introduction

With the exception of sporadic clinical reports of yersiniosis outbreaks in goats, the only previous survey on the carriage of *Yersinia* species in healthy goats was carried out in Norway by Krogstad in 1974. In that study, a serological and faecal survey for *Y. enterocolitica* was undertaken as a result of an outbreak of clinical yersiniosis in a goat flock due to *Y. enterocolitica*. Although the author was able to demonstrate the presence of *Y. enterocolitica* serotype 2 among goats serologically, he was unable to isolate any *Y. enterocolitica* from faecal samples.

This present study therefore was an attempt to elucidate the distribution of *Y. enterocolitica* and of the other *Yersinia* species in apparently healthy goats in New Zealand. Our improved knowledge of the distribution of the various *Yersinia* species in goats is vital to our understanding of the epidemiology of the infection. With this information, the significance of *Yersinia* infections can be better assessed in goats, and this, in turn, can be related to infections in humans and other animal species.

Materials and Methods

Selection of Goat Farms

The goat farms found to be positive in the screening survey (Chapter 4) were all included in the prevalence survey. **Figure 5.1** shows the location of these farms.

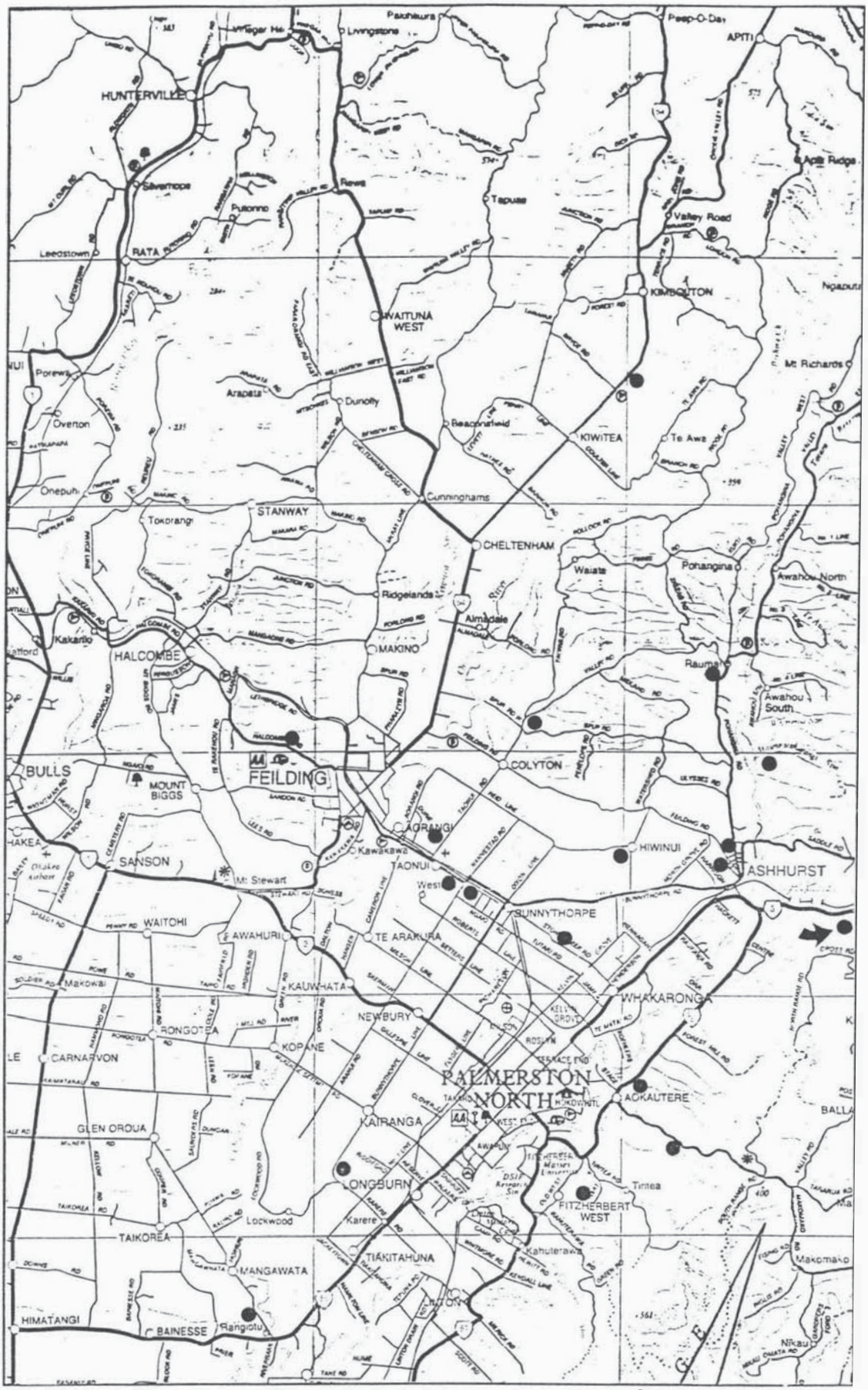

Figure 5.1. Location of Farms Included in the Prevalence Survey

Sampling Plan

From each of the 18 flocks found to be positive for *Yersinia* species in the screening survey, an age-stratified random sample of apparently healthy goats was taken from February to June 1988. The sample animals from each farm were stratified into 3 age groups: kids (less than one year old), hoggets (1 to 2 years old), and adults (2 to 5 years old).

The sample sizes were chosen according to flock size. For each flock, a hypothetical expected flock prevalence of 50% was assumed. This assumption was chosen since this is the prevalence which requires the largest sample size to estimate. The data would therefore satisfy the confidence requirements at any prevalence found. The estimates were made to within 10 ± percentage points at the 90% confidence level.

A table (based on the normal approximation to the binomial distribution) used for the estimation of disease prevalence in flocks with "infinite" populations (Appendix III) was then consulted in this regard. Since the populations of the goat flocks included in this survey were already known, adjustments were made for known populations by using the following formula:

$$1/n = 1/n_{\infty} + 1/N$$

where **n** = sample size required; n_{∞} = sample size required from an infinite population at a desired level of confidence and accuracy; and N = flock population size.

The value of n_{∞} at the assumed prevalence of 50% (with the estimate made to within 10% at the 90% confidence level) was determined to be 68. Thus the formula mentioned would look like this:

$$1/n = 1/68 + 1/N.$$

Further simplification of this formula (which made it easier to calculate sample sizes in the field without even a hand-held calculator) gave this result:

$$n = 68N/(68 + N).$$

Estimation of *Yersinia* Species Prevalence

For the estimation of confidence limits for the prevalence of *Yersinia* species infection in the goat flocks surveyed, the following formula was used (Cannon and Roe, 1982):

$$p \pm \left(v \sqrt{p(1-p)/n \times (1-n/N)} + 1/2n \right),$$

where **p** = proportion of positives in sample; **n** = sample size; N = population size; and **v** = corresponding normal percentage point (1.645 in 90% confidence).

Goat Characteristics Recorded

Several characteristics of the sample animals such as age group, sex, and faecal sample characteristics were recorded. All these data, as well as the prevalence and microbiological data, were stored in PANACEA 2 database computer program. Where possible coding of data was undertaken in the same program for statistical analyses.

Characterisation of Faecal Samples

The faecal samples collected from the sample animals were classified as either "formed" (pelleted) of "unformed" (unpelleted).

Laboratory Procedures for the Isolation of *Yersinia* Species

The methods employed for the isolation of *Yersinia* species from faecal material in this prevalence survey were essentially the same as those used in the screening survey (Chapter 4). Additional tests were performed on all the *Y. enterocolitica* isolates for the purpose of biotyping. For comparison purposes, these additional tests were also performed on the other *Yersinia* species encountered.

Biotyping of *Y. enterocolitica*

The biotyping scheme for *Y. enterocolitica* as described by Wauters *et. al.* (1987) was followed. In this scheme, the reactions of *Y. enterocolitica* in the

following tests or media were determined at 29°C: Lipase (tween esterase), aesculin, salicin, indole, xylose, trehalose, nitrate, pyrazinamidase, β-D-glucosidase, Voges-Proskauer, and proline peptidase. Most of the tests are routinely performed in microbiology. They were prepared by following instructions from established microbiology manuals, and are described in Chapter 4. The preparation of the special test media was carried out according to specific authors (Appendix IV). The reactions of the different *Y. enterocolitica* biotypes on these media and tests are shown in **Table 2.3** in Chapter 2.

Lipase. Lipase activity was detected by the precipitation of Tween 80 in nutrient agar by appropriate *Yersinia* biotypes.

Aesculin. The hydrolysis of aesculin was detected by the formation of a black colour in the presence of ferric ammonium citrate.

Pyrazinamidase. The detection of pyrazinamidase activity of *Y. enterocolitica* was based on the reaction of these microorganisms on the medium as described by Kandolo and Wauters (1985). Positive reactions are indicated by the formation of a pink colour change in the medium upon the addition of ferrous ammonium sulphate.

β-D-Glucosidase. Positive reactions are indicated by the development of a distinct yellow colour in a medium containing 4-nitrophenyl-β-D-glucopyranoside after overnight incubation at 29°C (Wauters *et. al.*, 1987).

Proline peptidase. The formation of a bright-orange colour in a medium containing L-Prolyl-β-naphtylamide upon the addition of Tris-laurylsulfate and Fast Blue after an overnight incubation at 29°C indicated the presence of free naphtylamine.

Serotyping of *Y. enterocolitica* Isolates

All *Y. enterocolitica* isolates were sent to the *Yersinia* Reference Laboratory in Melbourne, Australia in 1988. Unfortunately due to problems within the laboratory results have still not been received.

Storage of *Yersinia* Species

One set of all the *Yersinia* species isolates was stored on nutrient agar slopes at 4°C. Another set was stored in 15% glycerol broth placed in 2 ml. vials and frozen at -20°C (Appendix IV).

Statistical Analyses of Infection Rates

Differences in the prevalences within and between age cohorts were tested statistically using Chi-square tests in the PANACEA 2 database program. As mentioned in Chapter 4, the Yates' correction procedure in the analysis of 2×2 tables was automatically applied. Relative risks (MacMahon and Pugh, 1970) and the attributable risk rates (Martin *et. al.*, 1987) were also performed.

To determine the effects of several farm and flock characteristics (which were in the form of continuous data) on the level of *Yersinia* prevalence, multiple regression techniques in PANACEA 2 were performed. Several independent variables were tested to see if they had any influence on the dependent variable (prevalence). Initially, all the independent variables were included in the multiple regression equation, and checked by the use of "t" tests for the significance of their partial regression coefficients.

When the overall regression at the initial stage was found to be insignificant, a step-down procedure to select the "best" subset of independent variables for the equation was performed (Armitage, 1971). This procedure was implemented manually, that is, the independent variable which had the least significant partial regression coefficient was eliminated from (not entered into) the equation, and another multiple regression computation on the retained variables performed. After every subsequent computation, the least significant variable was dropped, and the computation performed again, and so on. This elimination process was stopped when all the retained partial regression coefficients were found to be significant ($p<0.10$).

Results

Location of Goat Farms

The 18 goat farms shown to be positive for *Yersinia* species carriage by the screening survey were included in this prevalence survey. The location of these farms is shown in **Figure 5.1.**

Flock Sizes of Sampled Farms

The goat populations of the individual sampled farms varied considerably from the population figures described in Chapter 3, since the prevalence survey was actually carried out at a different date than the questionnaire-interview. During the actual prevalence survey, the goat populations of the sampled farms ranged from 36 to 1,295, with a total of 6,770 goats under consideration. **Table 5.1** shows these figures.

Table 5.1. Flock Sizes of the 18 Goat Farms

FARM	Kids	Hoggets	Adults	TOTAL
A	505	337	453	1,295
B	41	29	63	133
C	198	55	297	550
D	56	22	42	120
E	40	40	120	200
F	49	32	69	150
G	48	12	70	130
H	300	96	804	1,200
I	14	19	3	36
J	26	11	18	55
K	20	20	48	88
L	132	50	58	240
M	102	32	186	320
N	12	56	56	124
O	228	156	216	600
P	254	66	376	696
Q	20	24	89	133
R	203	147	350	700
TOTAL	2,248	1,204	3,318	6,770

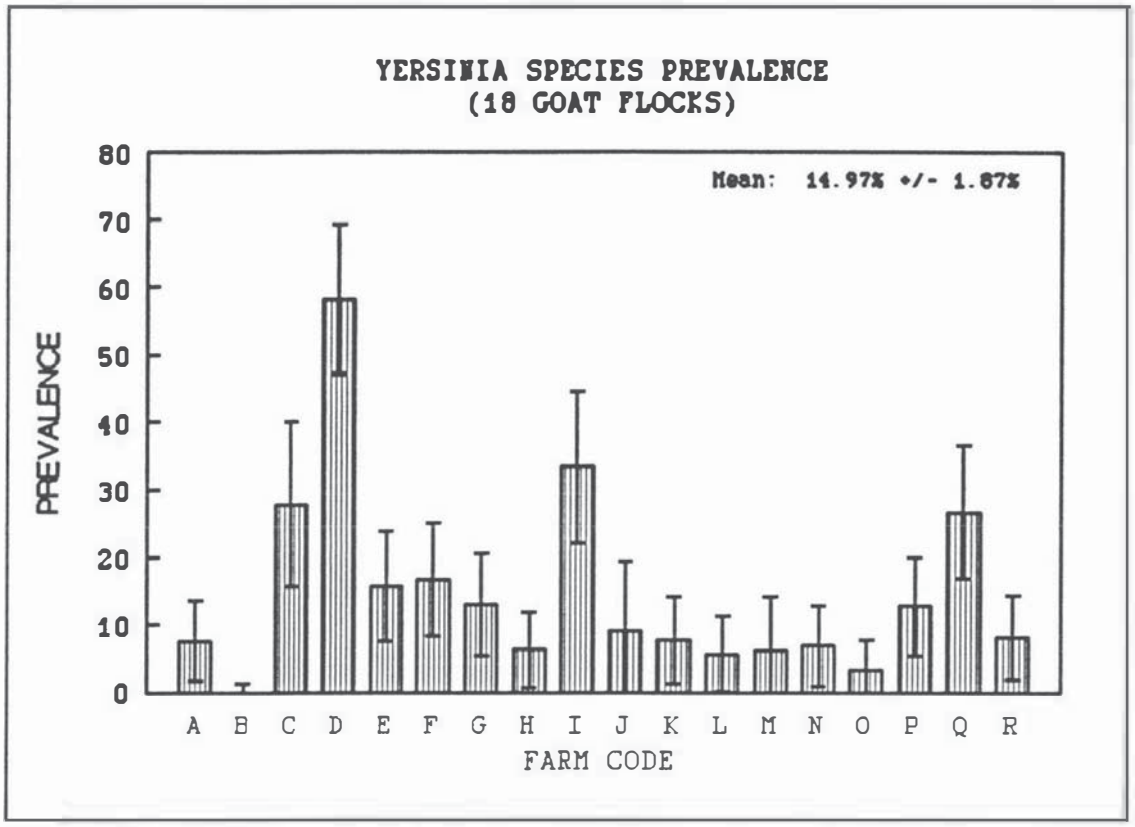

Figure 5.2. *Yersinia* Species Prevalence in 18 Goat Flocks

Number of Animals Sampled

The sample sizes taken from the 18 farms ranged from 24 to 66. Overall, a total of 902 goats were sampled from the 3 age groups. 296 faecal samples were taken from kids, 178 from hoggets, and 428 from adults. **Table 5.3** shows the results from the individual farms.

Overall *Yersinia* Species Prevalence

A total of 135 out of the 902 goats sampled from the 18 flocks were positive for *Yersinia* species, giving an overall prevalence of 14.97%. Individual farm prevalences ranged from 0 to 58.14%, and the 90% confidence limits are shown in **Table 5.3**. **Figure 5.2** shows a graphic presentation of the *Yersinia* species prevalence in the farms surveyed.

Population and Farm Effects on Prevalence

Multiple regression analysis was performed on the following independent variables to determine their influence on *Yersinia* species prevalence (**PREV**): Farm area in hectares (**AREA**), flock size (**FSIZE**), farm stocking rate for goats (**FSR**), paddock size in hectares (**PSIZE**), number of paddocks (**PAD**), and paddock stocking rate for goats (**PSR**). These mentioned variables were considered independent variables and the prevalence considered the dependent variable. Calculation of the regression proceeded with only 16 farms included, since 2 farms were automatically deleted by PANACEA 2 due to missing data.

The correlation coefficients of all the independent variables and that of the dependent variable are shown in **Table 5.2**. Results of the procedure showed that at the initial calculation (wherein all the six mentioned independent variables were included in the regression equation), only **FSR** was shown to have a significant partial regression coefficient, as shown by the "t" statistic. The rest were not significant, with **AREA** being the least significant ($p = .6450$). At this point **AREA** was dropped from the equation and the calculation for the regression estimates were performed again on the retained variables. Two other independent variables (**PSIZE** and **PSR**) were subsequently eliminated in the successive recalculation of the regression equation. The partial regression coefficients of the three remaining independent variables (**FSIZE**, **FSR**, and **PAD**) proved to be significant, thus proving that each of these three contributed separately to the effectiveness of the overall regression, which itself proved to be significant (**Table 5.8**).

Table 5.2. Correlation Matrix of **PREV** and All Independent Variables

	PREV	AREA	FSIZE	FSR	PSIZE	PAD	PSR
PREV	1.00	-0.16	-0.41	0.34	-0.44	0.09	-0.27
AREA		1.00	0.65	-0.44	0.62	0.55	-0.13
FSIZE			1.00	-0.07	0.87	0.32	0.55
FSR				1.00	-0.13	-0.47	0.42
PSIZE					1.00	0.09	0.64
PAD						1.00	-0.22
PSR							1.00

Table 5.3. Prevalence and Distribution of *Yersinia* Isolates in All Age Groups

Farm	Sample	*Y. ent.*	(%)	*Y. fred.*	(%)	*Y. int.*	(%)	*Y. krist.*	(%)	Total	Prev.	90% C. I.
A	66	3	4.55	1	1.52			1	1.52	5	7.58	± 5.96
B	45									0	0.00	± 1.37
C	61	9	14.75	7	11.48	1	1.64			17	27.87	± 12.14
D	43	15	34.88	10	23.26					25	58.14	± 11.07
E	51	2	3.92	2	3.92			4	7.84	8	15.69	± 8.20
F	48	5	10.42	3	6.25					8	16.67	± 8.32
G	46	5	10.87			1	2.17			6	13.04	± 7.62
H	64	3	4.69	1	1.56					4	6.25	± 5.61
I	24	6	25.00	1	4.17	1	4.17			8	33.33	± 11.20
J	31	8	25.81					1	3.23	9	9.03	± 10.43
K	39			2	5.13			1	2.56	3	7.69	± 6.50
L	53	1	1.89	2	3.77					3	5.66	± 5.52
M	56	8	14.29	1	1.79					9	6.07	± 8.12
N	44	3	6.82							3	6.82	± 5.95
O	61	1	1.64			1	1.64			2	3.28	± 4.37
P	63	8	12.70							8	12.70	± 7.35
Q	45			12	26.67					12	26.67	± 9.90
R	62	5	8.06							5	8.06	± 6.23
TOTAL	902	82		42		4		7		135		
MEAN			9.09		4.66		0.44		0.78		14.97	± 1.87

Note: **(%)** = Species-specific prevalence (Number of isolates of each species/TOTAL samples)

Table 5.4. Prevalence and Distribution of *Yersinia* Isolates in Kids

Farm	Sample	*Y. ent.*	(%)	*Y. fred.*	(%)	*Y. int.*	(%)	*Y. krist.*	(%)	Total	Prevalence
A	26	2	7.69							2	7.69
B	14									0	0.00
C	22	9	40.91							9	40.91
D	20	14	70.00	1	5.00					15	75.00
E	10	2	20.00							2	20.00
F	16	4	25.00							4	25.00
G	17	5	29.41							5	29.41
H	16	3	18.75							3	18.75
I	9	5	55.56							5	55.56
J	15	2	13.33					1	6.67	3	20.00
K	9									0	0.00
L	29	1	3.45	1	3.45					2	6.90
M	18	7	38.89							7	38.89
N	4	2	50.00							2	50.00
O	23	1	4.35			1	4.35			2	8.70
P	23	8	34.78							8	34.78
Q	7									0	0.00
R	18	4	22.22							4	22.22
TOTAL	296	69		2		1		1		73	
MEAN			23.31		0.68		0.34		0.34		24.66

Note: **(%)** = Species-specific prevalence (Number of isolates of each species/TOTAL samples)

Table 5.5. Prevalence and Distribution of *Yersinia* Isolates in Hoggets

Farm	Sample	*Y. ent.*	(%)	*Y. fred.*	(%)	*Y. int.*	(%)	*Y. krist.*	(%)	Total	Prevalence
A	17	1	5.88	1	5.88					2	11.76
B	10									0	0.00
C	6			1	16.67					1	16.67
D	8	1	12.50	4	50.00					5	62.50
E	10									0	0.00
F	10	1	10.00							1	10.00
G	4									0	0.00
H	5									0	0.00
I	13	1	7.69	1	7.69	1	7.69			3	23.07
J	6	5	83.33							5	83.33
K	9			1	11.11					1	11.11
L	11									0	0.00
M	6									0	0.00
N	20									0	0.00
O	16									0	0.00
P	6									0	0.00
Q	8			2	25.00					2	25.00
R	13	1	7.69							1	7.69
TOTAL	178	10		10		1		0		21	
MEAN			5.62		5.62		0.56				11.79

Note: **(%)** = Species-specific prevalence (Number of isolates of each species/TOTAL samples)

Table 5.6. Prevalence and Distribution of *Yersinia* Isolates in Adults

Farm	Sample	*Y. ent.*	(%)	*Y. fred.*	(%)	*Y. int.*	(%)	*Y. krist.*	(%)	Total	Prevalence
A	23							1	4.35	1	4.35
B	21									0	0.00
C	33			6	18.18	1	3.03			7	21.21
D	15			5	33.33					5	33.33
E	31			2	6.45			4	12.90	6	19.35
F	22			3	13.64					3	13.64
G	25					1	4.00			1	4.00
H	43			1	2.33					1	2.33
I	2									0	0.00
J	10	1	10.00							1	10.00
K	21			1	4.76			1	4.76	2	9.52
L	13			1	7.69					1	7.69
M	32	1	3.13	1	3.13					2	6.16
N	20	1	5.00							1	5.00
O	22									0	0.00
P	34									0	0.00
Q	30			10	33.33					10	33.33
R	31									0	0.00
TOTAL	428	3		30		2		6		41	
MEAN			0.70		7.01		0.47		1.40		9.57

Note: **(%)** = Species-specific prevalence (Number of isolates of each species/TOTAL samples)

The correlation coefficients of the dependent and the significant independent variables are shown in **Table 5.7**. There was negative correlation between prevalence and flock size, and positive correlation between prevalence and the two other independent variables. These figures mean that as flock sizes increase, the prevalence level decrease. Also, higher stocking rates and increased number of paddocks may mean higher levels of prevalence.

Table 5.7. Correlation Matrix of **PREV** and Significant Independent Variables

	PREV	**FSIZE**	**FSR**	**PAD**
PREV	1.00	-0.41	0.34	0.09
FSIZE		1.00	-0.07	0.32
FSR			1.00	-0.47
PAD				1.00

Table 5.8. Flock and Farm Effects on Prevalence*

FARM	PREV (%)	FSIZE	FSR	PAD
A	7.58	1295	0.85	76
C	27.87	550	1.15	192
D	58.14	120	30.00	7
E	15.69	200	20.00	5
F	16.67	150	18.75	13
G	13.04	130	18.57	2
H	6.25	1200	23.08	3
I	33.33	36	8.18	3
J	29.03	55	10.45	13
K	7.69	88	3.52	5
L	5.66	240	11.43	4
M	16.07	320	1.98	27
N	6.82	124	2.95	14
O	3.28	600	3.75	40
P	12.70	696	7.18	14
R	8.06	700	24.69	9
TOTAL	267.88	6054	186.5169426	427
MEAN	16.7425	406.50	11.6572520	26.6875

REGRESSION PARAMETER ESTIMATES

Regression Constant: 10.7423194

Partial Regression Coefficients	-.01897249	.8210128	.1551942
't'	2.3497234	2.2554679	2.0406548
p value	0.0367	0.0436	0.0639

ANALYSIS OF VARIANCE

	DF	SS	MS	F-RATIO	P
REGRESSION	3	1379.2894611	459.7631537	3.3038407	0.0575
RESIDUAL	12	1669.9224388	139.1602032		
TOTAL	15	3049.2119000			

Standard Deviation of Errors = 11.7966182968535
Multiple Coefficient of Determination (R^2) = .4523429352679934
Multiple Correlation Coefficient (R) = .672564446

* **Only 16 out of the 18 farms were included in the analysis because of missing data for farms B and Q.**

Age-Specific *Yersinia* Species Prevalence

73 of the 296 kids (24.66%) sampled were positive for *Yersinia* species (**Table 5.4**). In hoggets, 21 of the 178 sampled (11.97%) were positive (**Table 5.5**), and 41 of the 428 adults sampled (9.57%) were positive (**Table 5.6**). These figures indicate that *Yersinia* species carriage occurs more frequently in kids than in goats in the older age groups. Chi-squared analysis of the infection rates

within the cohorts showed that the prevalence figure in kids (Chi-squared: 18.59) accounted for more than half of the total chi-squared value (Chi-squared = 33.028), showing highly significant differences in *Yersinia* prevalence among the different age groups (p = 0.0000) (**Figure 5.3**).

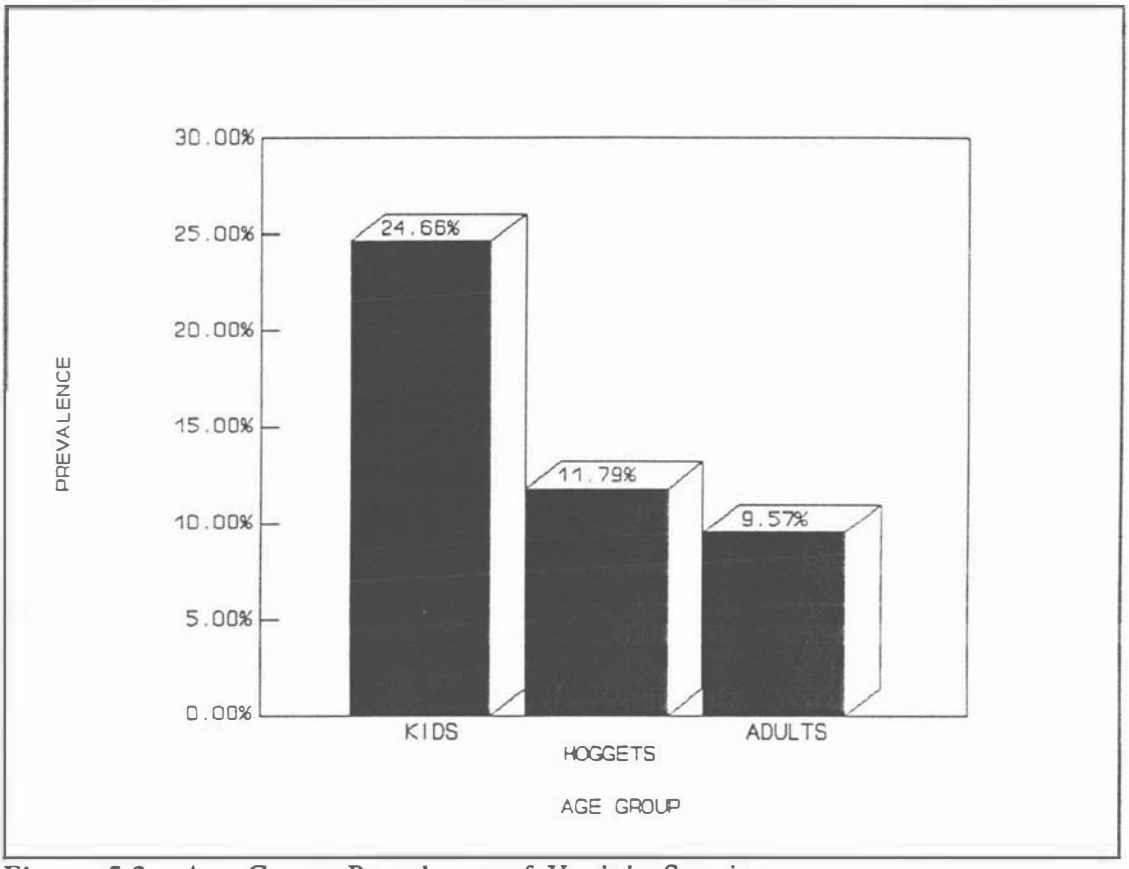

Figure 5.3. Age-Group Prevalence of *Yersinia* Species

Table 5.9 shows the differences in the infection prevalences of *Yersinia* species in the different age cohorts. Comparison of the infection rates between cohorts showed that there was a significant difference between the infection prevalences of kids and hoggets (Chi-squared: 10.7757; p = 0.001), with the risk of infection for kids being 2.09 times that for hoggets. There was also a significant difference between the infection prevalences of kids and adults (Chi-squared: 28.8792; p = 0.0000), with the risk of infection for kids being 2.57 times that for adults. There was, however, no significant difference between the infection prevalences of hoggets and adults (Chi-squared: 0.4537 p = 0.5006), with the risk of infection for hoggets being only 1.2 times that for adults.

Table 5.9. Comparison of Infection Levels Between Cohorts

BETWEEN:	Chi-squared*	p	RR**	AR***
KIDS	28.8792	0.0000	2.57	15.09
HOGGETS	0.4537	0.5006	1.23	2.22
ADULTS			1.00	1.00

* In comparison with the levels for adults.
** Relative Risk. Rate when compared with prevalence levels in adults as the standard.
*** Attributable Risk. Rate when compared to prevalence levels in adults.

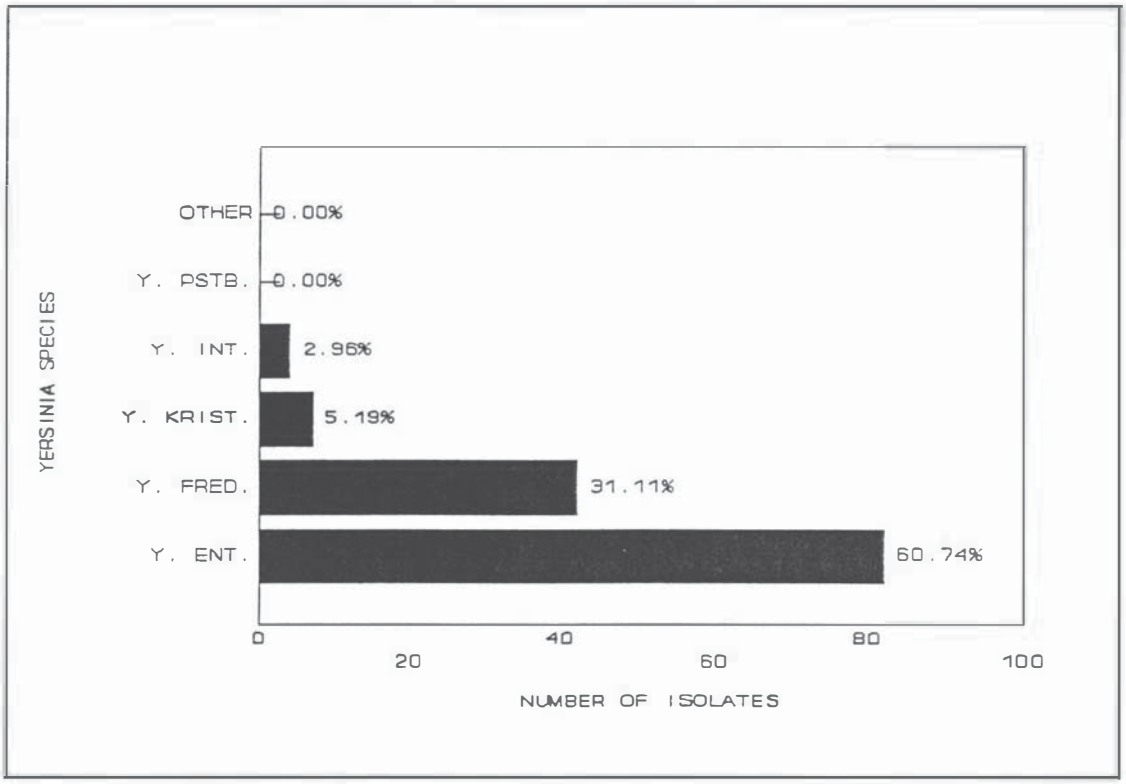

Figure 5.4. Species of *Yersinia* Isolated in the Prevalence Survey

Species of *Yersinia* Isolated

Of the 135 *Yersinia* species isolated in this survey, 82 (60.74%) were *Y. enterocolitica*, 42 (31.11%) were *Y. frederiksenii*, 7 (5.19%) were *Y. kristensenii*, and 4 (2.96%) were *Y. intermedia*. No isolates of *Y. pseudotuberculosis* nor of the other *Yersinia* species were recovered (**Figure 5.4**).

Age-Group Distribution of *Yersinia* Species

Figure 5.5 shows the age-group distribution of the *Yersinia* isolates. 73 of the 135 *Yersinia* isolates (54.1%) were recovered from kids, 21 (15.6%) from hoggets, and 41 (30.4%) from adults.

The age-group-specific distribution of the various *Yersinia* species showed contrasting results (**Tables 5.4, 5.5, and 5.6**). In kids, 69 of the 73 (94.5%) of the isolates were *Y. enterocolitica*, 2 (2.7%) were *Y. frederiksenii*, and 1 each (1.4%) were *Y. intermedia* and *Y. kristensenii*. In hoggets, 10 of the 21 (47.62%) were *Y. enterocolitica*, 10 (47.62%) were *Y. frederiksenii*, and 1 (4.76%) was *Y. intermedia*. In adults, 30 of the 41 (73.17%) *Yersinia* isolates belonged to *Y. frederiksenii*, 6 (14.63%) to *Y. kristensenii*, 3 (7.32%) to *Y. enterocolitica*, and 2 (4.88%) to *Y. intermedia* (**Figure 5.5**).

These results indicated that *Y. enterocolitica* was more commonly encountered in younger goats while *Y. frederiksenii* and other *Yersinia* species were more commonly found in older animals.

Biotypes of *Y. enterocolitica*

Eighty of the 82 (97.6%) isolates of *Y. enterocolitica* recovered from all the age groups belonged to biotype 5 (Wauters *et. al.*, 1987). One isolate (1.2%) was typed as biotype 3 (isolated from an adult goat) and another (1.2%) was typed as biotype 1A (isolated from a hogget).

Age-Group Distribution of "Pathogenic" and "Environmental" *Yersinia* Species

Wauters *et. al.* (1987), in their biotyping scheme for *Y. enterocolitica*, have classified biotypes 1A and 6 as non-pathogenic or "environmental" strains and the other biotypes (2 to 5) as potentially "pathogenic" strains, by virtue of their possession of a virulence plasmid. The other *Yersinia* species, such as *Y. frederiksenii*, *Y. intermedia* and *Y. kristensenii* have been mostly isolated from environmental sources (Ursing *et. al.*, 1980; Brenner *et. al.*, 1980; Bercovier *et. al.*, 1980b).

Taking these into consideration, the isolates in this study could be conveniently classified as being potentially "pathogenic" (*Y. enterocolitica* biotypes 3 and 5) or "environmental" (*Y. enterocolitica* biotype 1A, *Y. frederiksenii*, *Y.*

intermedia and *Y. kristensenii*) for the purpose of comparing their relative distributions in the different age groups (**Figure 5.6**).

The chi-squared test showed significant differences in the distribution of the isolates, with the "environmental" *Yersinia* species being more closely associated with adult goats, and the potentially "pathogenic" ones with the kids (Chi-squared = 86.2326 p = 0.0000).

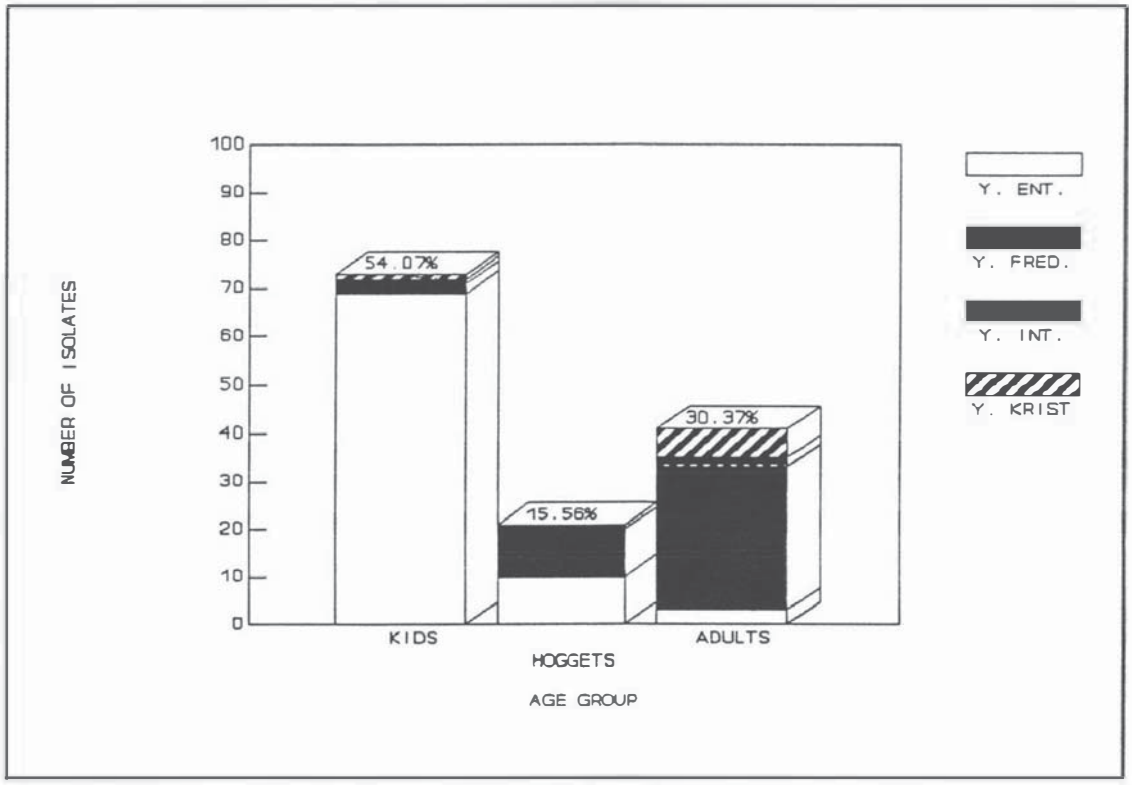

Figure 5.5. Age-Group Distribution of *Yersinia* Species

Association Between Faecal Sample Characteristics and *Yersinia* Infection

No significant association was found between faecal sample characteristics and *Yersinia* infection (Chi-squared = 1.1268 p = 0.2884).

Association Between Sex and *Yersinia* Infection

The sex of the animals was shown to be not significantly associated with *Yersinia* infection (Chi-squared = 0.4538 p = 0.5006).

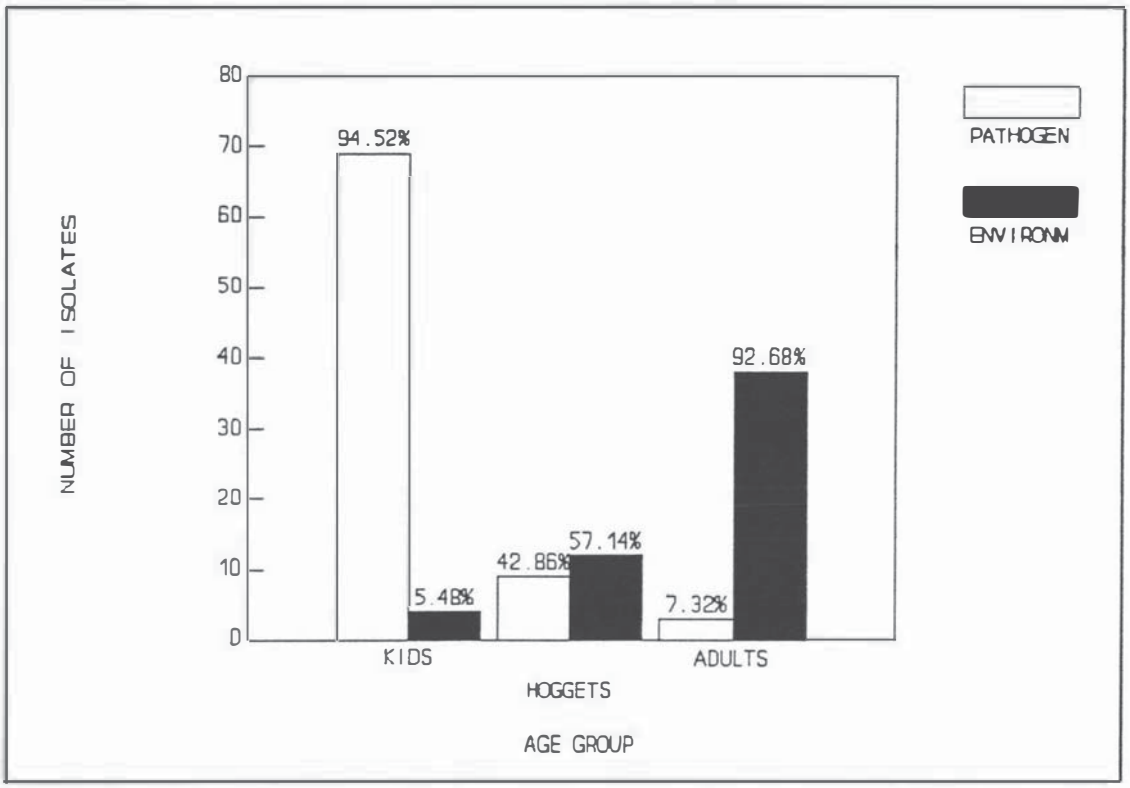

Figure 5.6. Distribution of "Pathogenic" and "Environmental" *Yersinia* Species

Discussion

The high variability in the farm prevalences (mean: 15.0%; range: 0% to 58.14%) of *Yersinia* species carriage in goats from farms already found infected in a screening study shows that under the conditions of the study infection was circulating, but at very variable levels both within and between age groups. However, the variation may also reflect the innate sampling error that came with this study. While it has been shown that *Yersinia* infections are mostly cold-weather events, constraints in the implementation of this study necessitated the collection of the faecal samples during the months of February to June 1988, which roughly corresponded to the summer-autumn period in New Zealand. The bulk of the collections were done during the early part of the stated period, with only one farm sampled during the month of June. Also, certain farm and flock characteristics might have influenced the levels of *Yersinia* species prevalences in the different goat flocks. Taking all of these factors into consideration, it is apparent that a single cross-sectional sampling of animals for *Yersinia* species gives only a partial picture of the actual state of infection in these animals. Collection of samples over an extended period of time, preferably for a year or more, is necessary to understand the epidemiology of seasonal

infections such as yersiniosis. In an attempt to clarify this situation, a cohort study (Chapter 6) was undertaken.

The higher prevalence of infection in kids reinforces the view that younger animals are more susceptible to *Yersinia* infection, particularly with *Y. enterocolitica*. This tendency has previously been demonstrated in humans (Vandepitte and Wauters, 1979; De Groote *et. al.*, 1982; Fukushima *et. al.*, 1985), dogs (Fukushima *et. al.*, 1984), and pigs (Fukushima *et. al.*, 1983). The study by Buddle *et. al.* (1988) clearly indicated that younger goats harbour more *Yersinia* organisms than older animals.

In this study, *Y. enterocolitica* comprised the majority (60.7%) of all *Yersinia* strains isolated from goats of all ages, with the rest made up mostly of the so-called "environmental" strains (*Y. frederiksenii*, *Y. intermedia* and *Y. kristensenii*). The preponderance of *Y. enterocolitica* in kids (94.7% of the strains isolated from this age group) showed that apart from being the most common carrier of *Yersinia* infections, this age group appears to be the most commonly infected with the potentially "pathogenic" *Yersinia* species (in this case *Y. enterocolitica*).

The predominance of biotype 5 strains (97.6%) among all the *Y. enterocolitica* isolates in this present study is very similar to the pattern reported by Buddle *et. al.* (1988). This may mean that, aside from hares and rabbits (Nilehn, 1969a; Bercovier *et. al.*, 1978), goats may serve as an important reservoir of this biotype. In fact, the first report of *Y. enterocolitica* infections in goats have been shown to be due to this biotype (Krogstad, 1975). On the other hand, the distribution of this biotype may actually be due to geographic considerations, for in New Zealand, this biotype has also been recovered from deer (Henderson, 1984) and sheep (Bullians, 1987), although in lesser proportions than the other *Y. enterocolitica* biotypes. In fact, this biotype has previously only been isolated from Europe in the 1960's and the 1970's (Nilehn, 1969a; Krogstad, 1972; Bercovier *et. al.*, 1978).

The failure to isolate *Y. pseudotuberculosis* in this study may reflect the difficulty in isolating this organism from faecal material (Mair and Fox, 1986), which, in turn, may be an indication of either sporadic shedding or localisation in the lymph nodes. Henderson (1984) forwarded a similar explanation when he was able to isolate surprisingly low numbers of *Y. pseudotuberculosis* in healthy deer, considering that this organism has been shown to be the most important cause of yersiniosis in these animals. On the other hand, the absence of *Y. pseudotuberculosis* in this present study may be an indication of the seasonality of infections with this organism.

CHAPTER 6

COHORT STUDY OF *YERSINIA* SPECIES INFECTION IN THREE GOAT FLOCKS

Introduction

After first evaluating the distribution of *Yersinia* species infection in goat flocks on a single sampling occasion (Chapter 5), the next step was to evaluate the pattern of *Yersinia* species infection in selected goat flocks throughout a year, in order to obtain a clearer picture of the temporal changes in infection. As *Yersinia* species infections are considered to be mostly cold weather events, it was considered necessary to monitor excretion of the various *Yersinia* species by different age cohorts for a year to determine whether there were seasonal or age influences on excretion.

The number of samples to be collected and processed per animal was quite large, so the study was limited to three flocks, with three cohorts per flock.

Materials and Methods

The Goat Flocks

Three of the goat flocks which were included in the prevalence survey for *Yersinia* species infection (Chapter 5) were chosen for this study (**Figure 6.1**). The selection of these flocks was based on several reasons, the most important of which was the full cooperation of the farm owners for a period of at least 13 months. The other reasons for the selection of these farms were that they were reasonably close to the diagnostic laboratory at Massey University, and that these three were relatively self-contained flocks, i.e., introduction of new stock from outside sources was rarely practised. The farmers concerned also agreed, if possible, to retain the animals chosen at the start of the study for the whole stated period.

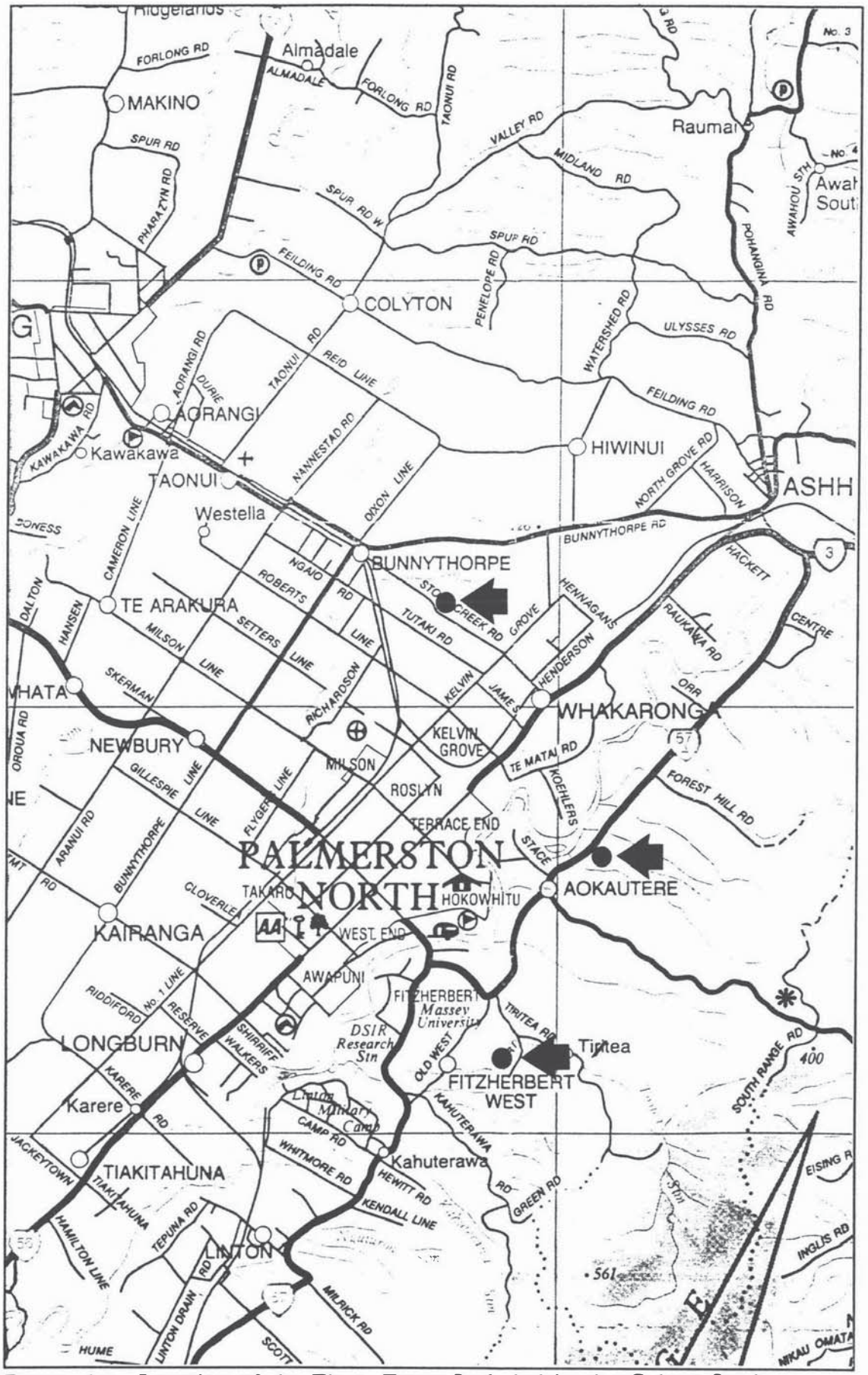

Figure 6.1. Location of the Three Farms Included in the Cohort Study

Farm A. This is a 4-hectare farm located in Aokautere, just outside Palmerston North. The pasture area was mostly situated on sloping hillsides, divided into paddocks by electrified fences.

Of the the total of 120 goats present at the start of this study, 56 were kids, 22 were hoggets and 42 were adults. These goats were mostly Angora grades (70% of flock) and ferals (30% of flock). The goats used paddocks alternately with a few sheep on the farm.

Kidding takes place during the months of August and September. The kids are weaned at 6 months of age, at which time they are separated from the does and run in another paddock. Shearing starts at about this age, and is carried out twice a year, during the months of February and September.

Farm B. This is a 4.4-hectare farm located in Harts Road, Palmerston North. Goats were raised on this farm as a hobby and to supplement the family income, as the owners were primarily involved in other jobs unrelated to farming. The farm has a relatively flat pasture area divided into 3 paddocks by electrified fences.

At the start of the study, there were 36 goats on the farm. Most of these were Angora grades (90% of flock) with the remainder (10%) being pure Angoras. These animals share the use of paddocks with cattle, sheep and horses on a rotational basis.

The does usually start kidding during the month of September and continue until November. Kiddings take place in specially-made kidding sheds which provide ample protection against adverse climatic conditions. The kids are weaned at the early age of 2.5 months, at which time they are separated from the does and run in another paddock.

Shearing on this farm is also carried out twice a year, in the months of February and August.

Farm C. This is a 7-hectare farm located at Stoney Creek Road, Bunnythorpe. This is another farm where goats were primarily raised as a hobby, for the owner was involved in another job elsewhere. However, additional income was derived from this flock, particularly from cashmere and cashgora production. The pasture area is divided into 2 paddocks by electrified fences.

When this study was started, there were a total of 130 goats on the farm. Of these, 48 were kids, 12 were hoggets, and 70 were adults. A slight majority

(55%) of these animals were classified as ferals, with the rest (45%) classified as Angora grades. These goats were usually run as a single mob, although the owner occasionally separated nursing does into a different paddock. The whole pasture area was used for goats, there being no other domestic livestock on the premises.

Kidding takes place during the months of August and September, and the kids weaned at 5 months of age. As with the other two farms, shearing was also carried out twice a year, during the months of February and August.

Flock History of Yersiniosis

Of the three farms included in this study, only Farm B had had any previous report of yersiniosis. As part of a clinical investigation of a diarrhoeal problem in young goats, specimens had been submitted to the veterinary microbiology laboratory at Massey University in the previous year, and *Y. enterocolitica* was isolated from faeces. The other two farms had had no confirmed reports of yersiniosis in their goat flocks.

Sampling Design

Stratification of the flocks by age. Before the actual sampling, the animals in each flock were stratified into three different age groups, with each age group being a cohort:

Cohort 1 = Kids (Less than one year of age)
Cohort 2 = Hoggets (From one year to two years)
Cohort 3 = Adults (From two years to five years).

Number of sample animals in each cohort. Where possible, a sample of ten goats were selected from each cohort in the three flocks at the start of the study. Thus, with three cohorts, the selection of thirty animals per farm was planned at the start of the study. They were then marked or tagged for identification in the subsequent samplings. These animals were then returned to the flock and were subjected to the normal management procedures employed on the respective farms.

Sample animals in each cohort that became permanently unavailable during the study for any reason were immediately replaced by randomly selecting

animals within the same cohort. These adjustments were made to keep the total number to thirty or so animals.

Sampling schedule. Each farm was sampled once a month for a period of at least 12 months (13 samplings). For convenience, samples were collected from Farm A on the first Tuesday of the month; from Farm B, on the second Tuesday of the month; and for Farm C, on the third Tuesday of the month. In cases where the sampling dates could not be adhered to, collections were done on the nearest date possible.

Collection of Faecal Samples

The procedures for the collection of faecal samples as outlined in Chapter 4 were followed in this study.

Isolation of *Yersinia* Species

The methods of cold-enrichment and selective plating of faecal samples for the detection of *Yersinia* species, as discussed in the preceding chapters were also used in this study.

Collection of Serum Samples

Blood samples from the sample animals in all the farms were taken on one occasion for future serological tests. Approximately 5 millilitres of blood were taken from each animal by jugular venipuncture using a sterile evacuated blood collection tube*. The blood was allowed to clot, and the serum sample drawn by the use of a sterile pasteur pipette and placed into a sterile 5-millilitre polycarbonate screw-capped tube. This was then stored at -20°C.

Biotyping of *Y. enterocolitica*

Biotyping of Y. enterocolitica isolates recovered during the course of this study were performed according to the scheme proposed by Wauters *et. al.* (1987).

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* Vacutainer® (Becton Dickinson and Company, Rutherford, New Jersey 07070, U. S. A.).

Serotyping of *Y. pseudotuberculosis*

Most of the *Y. pseudotuberculosis* strains encountered in this study were sent to Professor M. Tsubokura at Tottori University, Japan for serotyping.

Serotyping of *Y. enterocolitica*

All *Y. enterocolitica* isolates were sent to the *Yersinia* Reference Laboratory in Melbourne, Australia in 1988. Unfortunately due to problems within the laboratory results have still not been received.

Examination of Dead or Sick Sample Animals

At the start of the study, an understanding with the farm owners was reached, wherein they agreed to report any sick or dead goats on their farms, even if these animals were not chosen for the cohort study. It was also agreed that they would report in advance any cohort animal that was to be sold or culled.

The sample animals reported dead or clinically ill by the farmers were subjected to thorough clinical examinations or post-mortem inspections, as the case may be. Clinical samples from sick animals were submitted to the veterinary microbiology and parasitology laboratory at Massey University. Dead animals were likewise submitted to the pathology department of the same institution.

Meteorological Data

Meteorological data were taken from the monthly reports of the New Zealand Meteorological Service at the Grasslands Division, Department of Scientific and Industrial Research (DSIR), Palmerston North.** These included the following: **MAX** - mean daily maximum temperature, **MIN** - mean daily minimum temperature, **HUMID** - mean daily relative humidity, **GRASS** - mean daily minimum grass temperature, **RAIN** - total monthly amount of rain, **NRD** - number of rainy days, **NTRD** - number of days with more than 10 millilitres of rain, and **DEW** - number of dewy days per month.

** Observations taken at Latitude 40° 23' South and Longitude 175° 37' East.

Analysis of Infection Rates

The following values were calculated for each cohort, within and between the three goat flocks: number of animals under consideration at each testing time (**SAMP**), number of positive animals at each testing time (**TPOS**), point prevalence at each testing time (**PREV**), animal-days at risk (**ADR**), number of days between consecutive tests (**DT**), number of negative animals at the beginning of each test period (**NA**), number of positive animals at the end of each period (**PA**), and the 4-week incidence rate (**INC**).

Point prevalence of positive animals. This was calculated by dividing the number of animals tested positive (**TPOS**) by the total number of animals under consideration (**SAMP**) at each testing time. Thus:

$$\mathbf{PREV\ (\%) = (TPOS / SAMP) \times 100.}$$

The point prevalence figures were computed for the following classes: farm, age group (cohorts) and combinations of these. Under each class or combination, specific prevalences were computed for the different *Yersinia* species encountered, as well as for the potentially "pathogenic" and "environmental" types of *Yersinia*. The total prevalence of all *Yersinia* species under each class or combination were then calculated.

Animal-days at risk. The animal-days at risk (**ADR**) was calculated by multipying the number of days between consecutive tests (**DT**) and the number of negative animals present at the beginning of the period (**NA**). Thus:

$$\mathbf{ADR = DT \times NA.}$$

4-week percentage incidence. The 4-week percentage incidence (**INC**) was calculated by dividing the number of animals becoming positive during one inter-test period (**PA**) by the animal-days at risk (**ADR**), and then multiplying the quotient by 28. To get the value in percentage, the product was multiplied by 100. Thus:

$$\mathbf{INC = [(PA / ADR) \times 28] \times 100.}$$

Calculation of the percentage incidence was also performed using the classification described in the calculation of the point prevalence figures. Cumulative incidences were also calculated for each of the different seasons of

the year. In New Zealand, the year is divided into four "official" seasons: spring (September to November), summer (December to February), autumn (March to May) and winter (June to August).

Determination of Incidence Differences Between and Within Cohorts.

The differences in the incidence of *Yersinia* species infection between and within cohorts were tested using repeated measures regression analysis. The design of this study was basically a factorial with one within-subjects and one between-subjects variable (Pedhazur, 1982), with the three age groups (**COHORT**) representing the subjects. The three different flocks (**FARM**) represented the between-subjects factor and the four-yearly seasons (**SEASON**) represented the within-subjects factor. Coding of the factors and the creation of "dummy" variables were performed prior to the computation of the regression equations.

Calculations were carried out on the overall *Yersinia* species incidence, as well as on the incidence of the various species of *Yersinia* recovered during the course of the study.

Determination of Climatic Influences on *Yersinia* Species Incidence

To determine the influence of specific meteorological parameters on the incidence of *Yersinia* species infection in the different flocks and cohort, multiple regression techniques were performed using the meteorological parameters described on page 6 of this chapter as the independent variables. The effects of these variables were determined on the overall incidence of *Yersinia* species infection, as well as on the cohort-specific and the *Yersinia* species specific incidence levels.

Procedure for multiple regression analyses. The stepwise variable selection procedure for multiple regression was performed to evaluate the effects of the aforementioned meteorological data on the prevalence and incidence of *Yersinia* infections with respect to the different farms, age groups, and *Yersinia* species.

All the computations were performed in a microcomputer using multiple regression techniques in a statistical package.*** The forward selection

*** Statgraphics® v. 3.0 (STSC, Inc. and Statistical Graphics Corporation)

procedure within the program was initially chosen, since it allowed the entry of a single independent variable at a time, which thereupon selected the independent variables that best fit the model.

At the start of the procedure, the program calculates the partial correlation coefficients and the F-ratios that each independent variable would have if it were to be entered into the model in the next step. The program enters these variables into the model one after another, in the order of their F-ratios. The variable with the highest F-ratio was entered first, and those with lower F-ratios entered subsequently. The F-ratios, however, change when a variable is fitted into or removed from the model. As a result, the variable outside the model which had the highest recalculated F-ratio after the fitting of one variable into the model was fitted next.

The program set a limit of 4.00 for the F-ratio to enter into the model. Thus independent variables which had F-ratios lower than this value were considered not significant, and were, therefore, not included. In some cases, however, the independent variables which were excluded by the computer program were manually "forced" into the model to determine the significance of their contribution to the overall regression model. If proven significant, they were eventually included in the final model.

Thus, the final models judged to be the "best" for a particular dependent variable were chosen on the basis of their overall contribution to the regression equation, after a series of "forced" entries or eliminations from the model. The biological relationships of the independent variables with the dependent variables also put a lot of weight on the selection of the final "best" model.

Results

Sampling Results

The faecal samples were collected monthly on all the three farms, with most of the scheduled dates followed. In all, more than 13 samplings were undertaken from each of the farms, mostly because of the late entry of kids into the collection scheme. Thus from the projected 13 sampling per farm, the total samplings were extended to either 16 or 17 so that the late kids could be sampled at least 13 times.

Flock A. **Table 6.1** shows the results of the faecal sampling from Flock A. Sampling in this farm commenced on July 5, 1988 and lasted until October 3, 1989, for a total of 16 monthly samplings.

At the start of the study, only hoggets and adults were available for sampling, for at that time kidding had not commenced. Thus only Cohort 2 and Cohort 3 (20 animals in all) were sampled on the first three collection dates. When the new kids (born August and September, 1988) became available for the 4th collection time (October 10, 1988), the total number of animals sampled monthly from this collection date increased to thirty.

This number was maintained until the 13th collection time, after which the owner decided to dispose of the older animals (Cohorts 2 and 3). As a result, from the 14th to the 16th collection time only Cohort 1 was available for sampling.

Missing animals were reported on several occasions during the course of the sampling. In Cohort 1, missing animals were reported on the 7th (one animal), 11th (one animal), 12th (two animals), and 14th (one animal) collection time. None were missed in Cohort 2 during the course of the study, but in Cohort 3 missing animals were reported on the 2nd (one animal), 9th (one animal), and 12th (two animals) sampling. These missing animals either died of unknown causes or were culled by the farmer. On all these occasions replacement animals were chosen at random from the respective age groups.

Flock B. The results of the faecal collections from this flock are shown in **Table 6.1**. The first collection was undertaken on July 13, 1988, and the last on November 1, 1989, for a total of 17 collection dates.

At the beginning of the sampling, 25 animals were identified in this farm for the different cohorts. Ten of these were kids, 5 were hoggets, and 10 were adults. The 5 sample animals in Cohort 2 represented the whole hogget population on the farm at that time. This sample size was maintained until the 4th collection date, after which the new kids (born September to October 1988) became available for sampling.

Due to the availability of the new kids, the sample animals which were previously included in Cohort 1 were moved to Cohort 2, and the 5 animals previously in this classification were moved to Cohort 3, while still retaining the original 10 animals in this cohort. Thus from the 5th to the 15th collection dates there were 10 animals in Cohort 1, 10 animals in Cohort 2, and 15 animals in Cohort 3, for a total of 35 animals.

At about the time for the 16th collection date, the owner decided to dispose of the whole flock. This happened when there were two collections left to be made from the kids. Fortunately, the new owner of the kids in Cohort 1 was located. As a result, the last two samplings from kids were made on a different farm site.

On several occasions during the course of the study, sample animals were reported missing from the sample population. In Cohort 2 missing animals were reported during the 8th (one animal) and the 10th (one animal) collection dates. In Cohort 3, one animal was missing during the second collection date, and 2 were missing during the 15th collection date. For thse missing animals replacements were chosen at random from their respective age groups.

Flock C. Collection from this farm started on July 19, 1988 and continued until October 17, 1989, for a total of 16 samplings (**Table 6.1**). As in the case of Farm A, no kids were available for sampling at the start of the study. Consequently, from the first to the third sampling only Cohort 2 and Cohort 3 were sampled from a total of 20 goats.

The kids (born early August, 1988) became available for sampling only from the 4th collection date. From this date onwards, a total of thirty animals were sampled on every collection date until the end of the study.

Some sample animals were missed during the course of the study. In Cohort 1, 2 animals were missing during the 8th sampling. In Cohort 2, one animal was missing on the 6th sampling, and in Cohort 3, one animal each was reported missing on the 6th, 10th, 13th, and 15th samplings. Replacements for these missing animals were also selected at random from their respective age groups.

Fate of Missing Animals

Most of the missing animals were either culled by the farmer or were reported dead. Those animals reported dead were usually found in the morning, death having occurred overnight. In some cases, however, some of these animals were never found or discovered only after 24 to 48 hours. Although farmers had been asked to immediately notify us of deaths in cohort animals, most of these deaths were not reported, and our knowledge of these only came about at the next collection date. In such cases the causes of death were unknown.

On two occasions, however, sample animals were reported sick or dead by the farmers. On August 15, 1988, one adult goat from Farm B, which was part of Cohort 3, died of a condition characterised by a greenish, blood-tinged diarrhoea. This animal had just had its second faecal sampling 6 days earlier, without showing any apparent illness. A clinician from Massey University examined it and had faecal samples taken to the microbiology laboratory for examination, whereupon *Y. enterocolitica* biotype 3 was isolated. Interestingly, after 21 days of cold enrichment, the faecal material from the same goat collected 6 days prior to its death yielded the same organism. It was unfortunate that no post-mortem examination was undertaken on the dead goat, since the owner immediately disposed of it when its death was discovered.

Another case was reported by the owner of Farm A on June 7, 1989. This animal was a kid, and part of Cohort 1 on this farm. It was reported missing the day before, when the 12th faecal collection was performed. Because of this it was immediately replaced by another animal from its age group. Obviously, it was somewhere in the pasture during the collection time but failed to go with the flock during mustering. Apparently it was able to rejoin the flock later, for it was only found dead by the farmer the morning after, in the same paddock with the rest of the flock.

Necropsy was performed at the Department of Veterinary Pathology and Public Health at Massey University, and samples from the intestinal tract were sent to the microbiology laboratory for examination. *Y. enterocolitica* biotype 5 was isolated in almost pure culture from the small intestine, large intestine, and rectum. Although yersiniosis is one of the differential diagnostic possibilities in this animal, findings were not conclusive enough for death to be definitely attributed to *Yersinia*. A full report of the post-mortem examination is shown in Appendix V.

Flock Incidence of *Yersinia* Species

The incidence of *Yersinia* species in the different cohorts within the three farms was calculated for monthly inter-test periods, the incidence for the first inter-test period being the number of new isolations identified on the second collection, based on the previous sampling results. The sampling times and the inter-test periods were then classified into the different seasons of the year, to facilitate coding for subsequent statistical analyses. **Table 6.1** shows this seasonal classification.

Table 6.1. Seasonal Classification and Dates of Faecal Collections

			DATES (Day/Month)		
NUMBER	SEASON	YEAR	FLOCK A	FLOCK B	FLOCK C
1	Winter	1988	5/7	13/7	19/7
2	Winter	1988	2/8	9/8	16/8
3	Spring	1988	8/9	13/9	20/9
4	Spring	1988	4/10	11/10	18/10
5	Spring	1988	1/11	10/11	15/11
6	Summer	1988	6/12	15/12	20/12
7	Summer	1989	17/1	12/1	19/1
8	Summer	1989	7/2	22/2	22/2
9	Autumn	1989	7/3	15/3	21/3
10	Autumn	1989	4/4	11/4	18/4
11	Autumn	1989	2/5	9/5	19/5
12	Winter	1989	6/6	13/6	20/6
13	Winter	1989	4/7	11/7	18/7
14	Winter	1989	1/8	8/8	15/8
15	Spring	1989	5/9	12/9	19/9
16	Spring	1989	3/10	10/10	17/10
17	Spring	1989	-	1/11	-

Flock A. **Table 6.2** shows the incidence of various *Yersinia* species in Flock A. In this flock, the overall incidence of *Yersinia* infections, with all the cohorts combined, was highest during the colder months of the year, especially during winter in 1988 and autumn in 1989. During the 4 inter-test periods covering summer and early autumn in 1989 there were no isolations of *Yersinia* species from this flock. During winter of that year, however, no new positives appeared during two inter-test periods (13th and 15th).

From among the animals in Cohort 1 (kids) on this farm, new cases appeared only during 3 inter-test periods. The highest peaks occured during the 10th and 11th inter-test periods (autumn 1989) and the 14th inter-test period (winter 1989). No new cases occurred in this cohort during the other inter-test periods throughout the whole duration of the study.

In Cohort 2, the incidence started to increase during the late winter of 1988, and peaked during spring of that year (5th inter-test period). During summer and most of autumn 1989 (6th to 10th inter-test periods) no new cases appeared. The incidence again started to increase during late autumn and winter of that year, towards the end of the study.

In Cohort 3, the situation was very similar to that in Cohort 2, with the incidence peaking to a high of 50% during the first inter-test period (winter 1988) and decreasing in the subsequent inter-test periods. During the 5th to the 10th inter-test periods no new *Yersinia* infections were recorded, and during the last 2 inter-test periods (late autumn/early winter 1989) the incidence rose from 0 previously to 8% on the 11th and then to 30% on the 12th.

Table 6.2. *Yersinia* Species Incidence in Flock A

PERIOD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
COHORT 1																
Y. pstb.	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. ent. 5	-	-	-	0	0	0	0	0	0	10	48	0	0	0	0	-
Y. ent. 2/3	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. ent. 1a	-	-	-	0	0	0	0	0	0	10	0	0	0	0	0	-
Y. fred.	-	-	-	0	0	0	0	0	0	20	0	0	0	8	0	-
Y. int.	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. krist.	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. rohd.	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	-
PATH	-	-	-	0	0	0	0	0	0	10	48	0	0	0	0	-
ENVI	-	-	-	0	0	0	0	0	0	30	0	0	0	8	0	-
OVERALL*	-	-	-	0	0	0	0	0	0	40	32	0	0	8	0	-
COHORT 2																
Y. pstb.	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
Y. ent. 5	0	0	0	0	24	0	0	0	0	0	8	10	-	-	-	-
Y. ent. 2/3	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
Y. ent. 1a	0	0	10.8	0	0	0	0	0	0	0	0	0	-	-	-	-
Y. fred.	0	7.6	10.8	10	0	0	0	0	0	0	0	0	-	-	-	-
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
Y. krist.	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
PATH	0	0	0	0	24	0	0	0	0	0	8	10	-	-	-	-
ENVI	0	7.6	21.5	10	0	0	0	0	0	0	0	0	-	-	-	-
OVERALL*	0	7.6	10.8	10	24	0	0	0	0	0	8	10	-	-	-	-
COHORT 3																
Y. pstb.	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
Y. ent. 5	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
Y. ent.2/3	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
Y. ent. 1a	30	0	10.8	0	0	0	0	0	0	0	0	10	-	-	-	-
Y. fred.	10	22.7	0	10	0	0	0	0	0	0	8	10	-	-	-	-
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
Y. krist.	10	7.6	0	10	0	0	0	0	0	0	0	20	-	-	-	-
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
PATH	0	0	0	0	0	0	0	0	0	0	0	0	-	-	-	-
ENVI	50	30.3	10.8	20	0	0	0	0	0	0	8	40	-	-	-	-
OVERALL*	50	30.3	0	10	0	0	0	0	0	0	8	30	-	-	-	-
ALL COHORTS COMBINED																
Y. pstb.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. ent. 5	0	0	0	0	8	0	0	0	0	3.3	18.7	3.3	0	0	0	-
Y. ent. 2/3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. ent. 1a	15	0	10.8	0	0	0	0	0	0	3.3	0	3.3	0	0	0	-
Y. fred.	5	15.1	5.4	6.7	0	0	0	0	0	6.7	2.7	3.3	0	8	0	-
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. krist.	5	3.8	0	3.3	0	0	0	0	0	0	0	6.7	0	0	0	-
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
PATH	0	0	0	0	8	0	0	0	0	3.3	18.7	3.3	0	0	0	-
ENVI	25	18.9	16.2	10	0	0	0	0	0	10	2.7	13.3	0	8	0	-
OVERALL*	25	18.9	5.4	6.7	8	0	0	0	0	13.3	16	13.3	0	8	0	-

* Combined incidence of all *Yersinia* species isolates

Figure 6.2 shows a comparison of the incidence of *Yersinia* infection among the three cohorts in this farm.

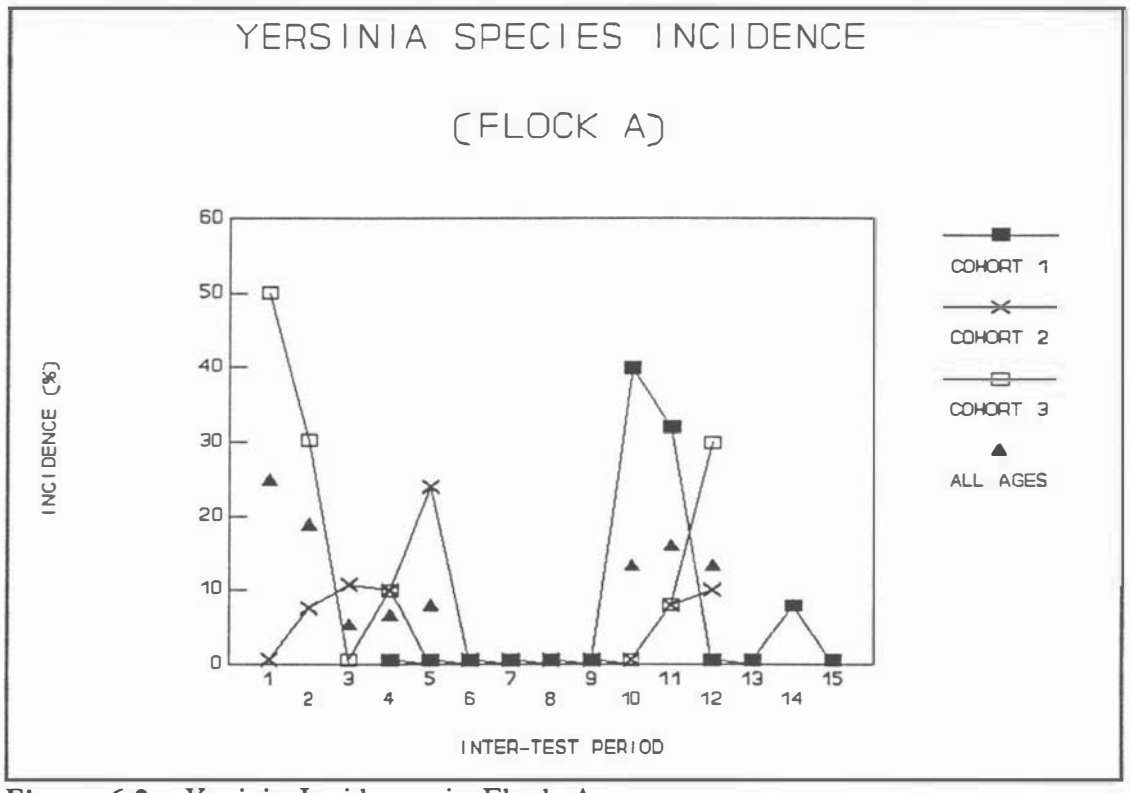


Figure 6.2. *Yersinia* Incidence in Flock A

Flock B. **Table 6.3** shows the incidence of *Yersinia* species in Flock B. In this flock, the overall *Yersinia* species incidence with all the cohorts combined was highest, as in Flock A, during the colder months of the year. During the first inter-test period (winter 1988) a peak percentage incidence of 58.07% was recorded. The 16th (spring 1989) inter-test period showed the next highest peak (26.67%). The inter-test periods in-between showed incidences ranging from 0 to 11.43% (13th inter-test period). From these, a pattern of incidences can be seen, increasing at the colder seasons of the year and decreasing when at the warmer seasons.

In Cohort 1 of this flock, the highest incidence (62.27%) was recorded during the first inter-test period (winter 1988), with the next highest (30%) recorded on the 13th inter-test period (winter 1989). In between these two inter-test periods the incidence varied from 0 to 10%, and during the 16th inter-test period (spring 1989) the incidence rose again to 26.67%. In all, no new cases were encountered in 10 inter-test periods distributed throughout the whole duration of the study.

In Cohort 2 of this flock the highest incidences were again recorded during the colder months of the year, with no new cases encountered during the summer of 1989.

In Cohort 3 the incidence pattern was similar to the other cohorts, with a peak incidence (51.85%) occurring during the first inter-test period (winter 1988), followed by 18.67% recorded during the 4th inter-test period (spring 1988).

Table 6.3. *Yersinia* Species Incidence in Flock B

PERIOD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
COHORT 1																
Y. pstb.	41.5	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y. ent. 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.3
Y. ent. 2/3	20.7	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0
Y. ent. 1a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y. fred.	10.4	0	10	0	0	0	0	0	0	10	0	0	20	8	0	0
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y. krist.	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.3
PATH	62.2	8	0	0	0	0	0	0	0	0	0	0	10	0	0	13.3
ENVI	10.4	0	20	0	0	0	0	0	0	10	0	0	20	8	0	13.3
OVERALL*	62.2	0	10	0	0	0	0	0	0	10	0	0	30	0	0	26.7
COHORT 2																
Y. pstb.	41.5	16	0	0	0	0	0	0	0	0	0	0	0	0	-	-
Y. ent.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-
Y. ent. 2/3	20.7	0	0	0	0	0	0	0	0	0	0	10	10	0	-	-
Y. ent. 1a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-
Y. fred.	0	0	20	9.3	0	0	0	0	0	0	0	10	0	0	-	-
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-
Y. krist.	0	16	0	0	0	0	0	0	0	0	16	0	0	0	-	-
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-
PATH	62.2	16	0	0	0	0	0	0	0	0	0	10	10	0	-	-
ENVI	0	16	20	9.3	0	0	0	0	0	0	16	10	0	0	-	-
OVERALL*	62.2	16	0	9.3	0	0	0	0	0	0	16	20	0	0	-	-
COHORT 3																
Y. pstb.	41.5	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-
Y. ent. 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-
Y. ent. 2/3	10.4	0	0	0	0	0	0	0	0	0	0	0	6.7	0	-	-
Y. ent. 1a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-
Y. fred.	0	0	0	18.7	0	0	0	8.9	0	0	5.3	0	0	0	-	-
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-
Y. krist.	10.4	0	0	0	0	0	0	0	0	0	0	6.7	0	0	-	-
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	6.7	0	0	-	-
PATH	51.9	0	0	0	0	0	0	0	0	0	0	0	6.7	0	-	-
ENVI	10.4	0	0	18.7	0	0	0	8.9	0	0	5.3	13.3	0	0	-	-
OVERALL*	51.9	0	0	18.7	0	0	0	8.9	0	0	5.3	6.7	6.7	0	-	-
ALL COHORTS COMBINED																
Y. pstb.	41.5	6.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y. ent. 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.3
Y. ent. 2/3	16.6	0	0	0	0	0	0	0	0	0	0	2.9	8.6	0	0	0
Y. ent. 1a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y. fred.	4.1	0	8	10.7	0	0	0	3.8	0	2.9	2.3	2.9	5.7	2.3	0	0
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y. krist.	4.1	3.2	4	0	0	0	0	0	0	0	4.6	2.9	0	0	0	0
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	2.9	0	0	0	13.3
PATH	58.1	6.4	0	0	0	0	0	0	0	0	0	2.9	8.6	0	0	13.3
ENVI	8.3	3.2	12	10.7	0	0	0	3.8	0	2.9	6.9	8.6	5.7	2.3	0	13.3
OVERALL*	58.1	3.2	4	10.7	0	0	0	3.8	0	2.9	6.9	8.6	11.4	0	0	26.7

* Combined incidence of all *Yersinia* species isolates

Figure 6.3 shows the comparative incidence patterns of the three cohort in this farm.

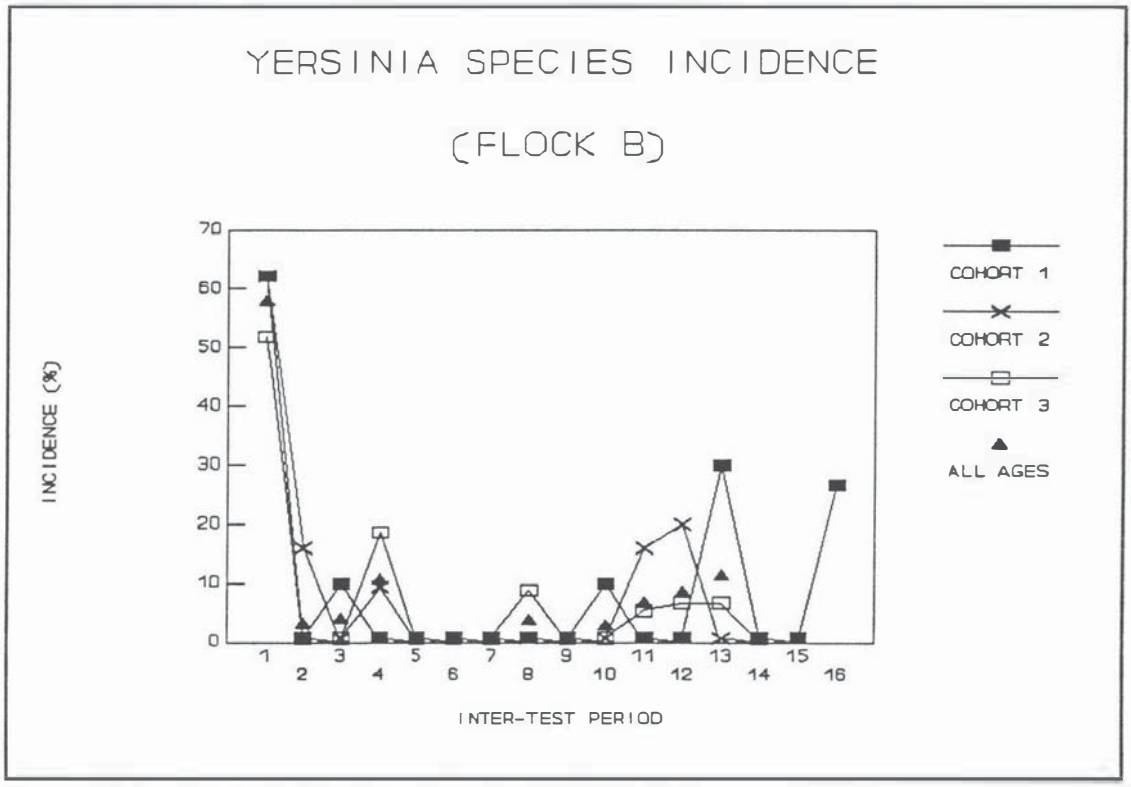


Figure 6.3. *Yersinia* Species Incidence in Flock B

Flock C. **Table 6.4** shows the incidence of *Yersinia* species in Flock C. The overall incidence for *Yersinia* species infection in this flock with all age groups combined showed a pattern which was quite unlike the pattern showed by the other two flocks. Although a peak of 48.89% was recorded on the 15th inter-test period (spring 1989), the next highest (38.02%) incidence was recorded during the 8th inter-test period (summer 1989). The cumulative incidence figures showed that, in fact, the highest incidence (66.06%) of *Yersinia* infection on this farm occurred during summer in 1989, followed by the incidence recorded in winter in 1989 (52.66%), and in autumn in 1989 (44.36%). However, the incidence figures for the winter of 1988 and the spring of 1989 were incomplete (only two and one inter-test periods were tested for each respectively), and proper comparisons with the other cumulative incidence figures cannot be made. The incidence figure recorded on the 15th inter-test period (48.89%) was comparable to the other figures mentioned, although it was taken from only one inter-test period.

Among the animals in Cohort 1, the highest incidence (65.88%) was demonstrated on the 7th inter-test period (summer 1989), and the next highest (53.33%) on the 15th inter-test period (spring 1989). The high peak in the 7th inter-test period was mostly due to *Y. enterocolitica* biotype 5 isolations.

In Cohort 2, the highest incidence (50%) was recorded on the 9th inter-test period (autumn 1989), with the next highest (40%) recorded on the 12th inter-test period (winter 1989). Cumulative incidence figures showed that the winter of 1989 had the highest incidence (82%), followed by the autumn 1989 incidence (67.5%). The incidence figure for summer 1989 was 49.35%.

In Cohort 3, the highest incidence (72.59%) was recorded on the 8th inter-test period (summer 1989), followed by a 53.33% incidence on the 15th inter-test period (spring 1989). Cumulative incidence figures within this cohort showed that summer 1989 had the highest (72.59%), as a result of the remarkably high incidence during the 8th inter-test period. The next highest incidence (53%) was recorded in the spring of 1989, but his cannot be properly compared with the other cumulative incidence figures since this was taken from only one inter-test period (15th). Next to this, the winter of 1989 had the highest figure (48%), followed by autumn 1989 (36.53%).

The high incidence levels during the summer period in the 2 older cohorts (hoggets and adults) were mostly due to the high level of environmental *Yersinia* species isolations, particularly that of *Y. frederiksenii*.

Table 6.4. *Yersinia* Species Incidence in Flock C

PERIOD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
COHORT 1																
Y. pstb.	-	-	-	0	0	0	0	0	0	0	8.8	20	0	0	0	-
Y. ent. 5	-	-	-	0	0	0	65.9	10.4	20	9	0	0	0	0	0	-
Y. ent. 2/3	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. ent. 1a	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. fred.	-	-	-	0	0	0	0	10.4	20	0	0	0	0	0	40	-
Y. int.	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. krist.	-	-	-	0	0	0	0	0	0	0	0	0	0	8	26.7	-
Y. rohd.	-	-	-	0	0	0	0	0	0	0	0	0	0	0	0	-
PATH	-	-	-	0	0	0	65.9	10.4	20	9	8.8	20	0	0	0	-
ENVI	-	-	-	0	0	0	0	10.4	20	0	0	0	0	8	53.3	-
OVERALL*	-	-	-	0	0	0	65.9	10.4	20	9	0	20	0	8	53.3	-
COHORT 2																
Y. pstb.	0	0	0	10	0	0	0	0	0	9	17.5	30	10	0	0	-
Y. ent. 5	0	0	0	0	0	10	8.2	0	0	9	0	0	0	0	0	-
Y. ent. 2/3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. ent. 1a	0	0	0	0	0	0	0	10.4	0	0	0	0	0	0	0	-
Y. fred.	0	8	20	10	0	0	0	31.1	60	0	0	0	10	8	26.7	-
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. krist.	10	0	0	0	0	0	0	0	0	0	8.8	10	10	32	26.7	-
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
PATH	0	0	0	10	0	10	8.2	0	0	18.1	17.5	30	10	0	0	-
ENVI	10	8	20	10	0	0	0	41.5	60	0	8.8	10	20	40	53.3	-
OVERALL*	10	8	20	10	0	10	8.2	31.1	50	0	17.5	40	10	32	40	-
COHORT 3																
Y. pstb.	0	0	0	0	0	0	0	0	0	0	8.8	10	0	0	0	-
Y. ent. 5	0	0	0	0	0	0	0	0	20	9	0	0	0	0	0	-
Y. ent. 2/3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. ent. 1a	0	8	0	0	0	0	0	0	0	0	0	0	0	0	13.3	-
Y. fred.	10	8	20	10	0	0	0	72.6	10	9	0	10	0	0	26.7	-
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. krist.	0	8	0	0	0	0	0	0	0	0	17.5	20	10	8	40	-
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
PATH	0	0	0	0	0	0	0	0	20	9	8.8	10	0	0	0	-
ENVI	10	24	20	10	0	0	0	72.6	10	9	17.5	30	10	8	80	-
OVERALL*	10	8	0	10	0	0	0	72.6	10	9	17.5	40	0	8	53.3	-
ALL COHORTS COMBINED																
Y. pstb.	0	0	0	3.3	0	0	0	0	0	3	11.7	20	3.3	0	0	-
Y. ent. 5	0	0	0	0	0	3.3	24.7	3.5	13.3	9	0	0	0	0	0	-
Y. ent. 2/3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. ent 1a	0	4	0	0	0	0	0	3.5	0	0	0	0	0	0	4.4	-
Y. fred.	5	8	20	6.7	0	0	0	38	30	3	0	3.3	3.3	2.7	31.1	-
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. krist.	5	4	0	0	0	0	0	0	0	0	8.8	10	6.7	16	31.1	-
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
PATH	0	0	0	3.3	0	3.3	24.7	3.5	13.3	12	11.7	20	3.3	0	0	-
ENVI	10	16	20	6.7	0	0	0	41.5	30	3	8.8	13.3	10	18.7	62.2	-
OVERALL*	10	8	10	6.7	0	3.3	24.7	38	26.7	6	11.7	33.3	33.3	16	48.9	-

* Combined incidence of all *Yersinia* species isolates

Figure 6.4 compares the *Yersinia* species incidence patterns exhibited by the three cohorts in this farm.

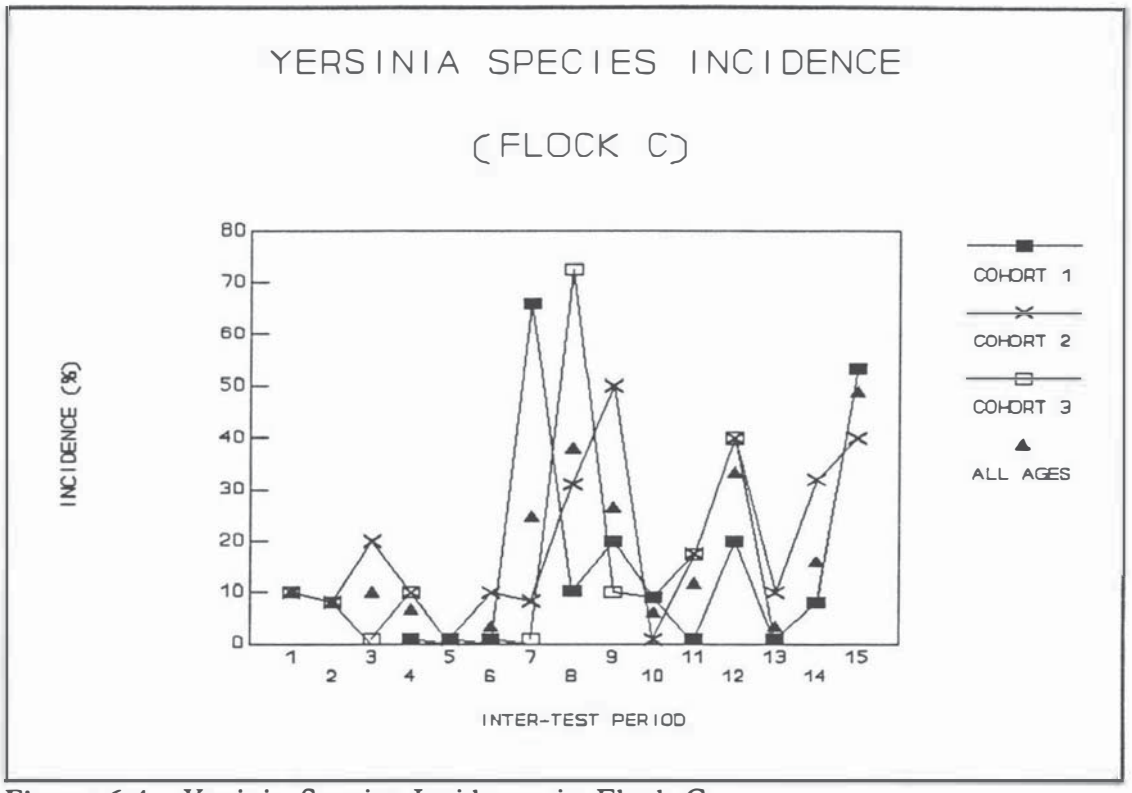


Figure 6.4. *Yersinia* Species Incidence in Flock C

Age Group Incidence of *Yersinia* Infection

The following incidence figures are averages derived from combining the same cohorts from the three goat flocks under study.

Cohort 1. **Table 6.5** shows the overall incidence of *Yersinia* species infection among kids from all the three goat flocks. The highest peak occurred during the first inter-test period (winter 1988) when a percentage incidence of 62.22% was recorded. The next highest was recorded on the 16th inter-test period (spring 1989). Other high peaks were observed during the 7th inter-test period (23.33%), and the 10th inter-test period (19.31%).

The incidence figures in the different seasons during the course of the study showed that the highest cumulative incidence occurred during the colder months of the year - in the winter of 1988 and the spring of 1989.

During the first three inter-test periods the incidence figures came only from *Yersinia* isolations in Flock B, because only this farm had kids available for sampling during these periods. The bulk of the new cases during the first inter-test period was made up of new *Y. pseudotuberculosis* cases. The high incidence encountered in summer (1989) was due to the unusually high incidence of *Y. enterocolitica* biotype 5 in Flock C, which in fact had the only new *Yersinia* cases among kids in the three goat flocks during this seasonal period. The incidence figure for the last inter-test period in this age group also came from incidence figures from Flock B alone.

Figure 6.5 shows the comparison of the 4-week incidences of *Yersinia* species infection in kids from the three goat flocks. The pattern of *Yersinia* species incidence in this age group shows that, overall, the pathogenic *Yersinia* species predominated, as indicated by the higher peaks of incidence throughout the whole period of the study. There was a tendency for the pathogenic *Yersinia* to peak during the colder months of the year, with the exception of the peak which occurred during the 7th inter-test period. This was almost totally due to the high incidence of pathogenic *Yersinia* species in flock C during this period.

It is also apparent, as shown in the graph, that *Yersinia* species incidence from both types peak every 4 to 5 inter-test periods regardless of the season of the year, although it can be seen that the peaks occurred more often during the colder inter-test periods.

Cohort 2. In this cohort, the incidence of *Yersinia* species infection was characterised by peaks during the colder months of the year, with the highest incidences recorded during the 15th (40%) and the 12th (23.33%) inter-test periods. A relatively high peak was also recorded during the first inter-test period (16.12%). An increase in the incidence was also observed in the warmer months of the year, however. During the 8th and 9th inter-test periods (late summer/early autumn 1989), percentage incidences of 11.05% and 16.87% were recorded respectively.

Cumulative seasonal incidences in this cohort showed that there were more new *Yersinia* species isolations during the winter of 1989 (44.33%), when compared with the other seasons of the year. The 40% incidence during the single inter-test period sampled during spring 1989 (15th) was already comaparable to the winter 1989 figures.

Figure 6.6 shows the comparison of the overall *Yersinia* species incidence patterns exhibited by the animals in this cohort from all the three farms. The

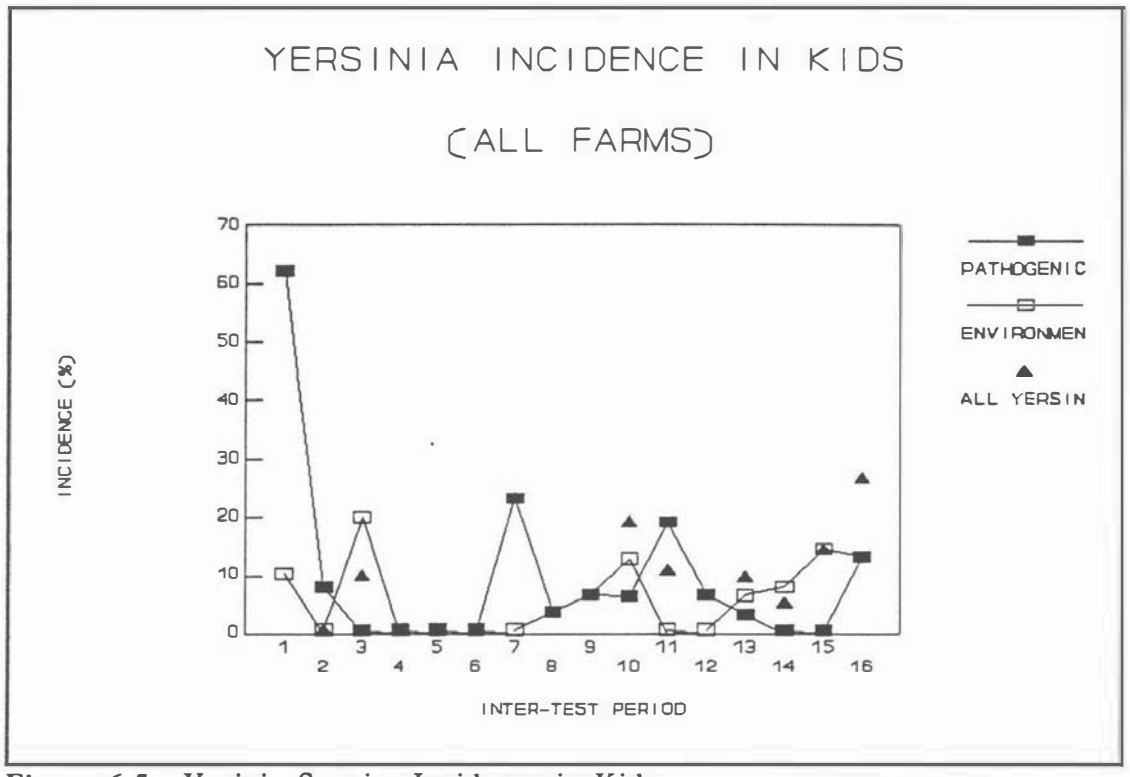


Figure 6.5. *Yersinia* Species Incidence in Kids

overall pattern of *Yersinia* incidence in this age group shows that the environmental *Yersinia* species had higher peaks, which means that there were more environmental than pathogenic *Yersinia* species recovered from this age group. It can be seen from the graph that pathogenic *Yersinia* species peaked highest on the winter months during the course of the study, with a slight peak occurring during late spring 1988. The environmental *Yersinia* species, on the other hand, were shown to peak during the spring and autumn periods. The peaks apparently occurred on both *Yersinia* types every 4 to 5 inter-test periods, after which the incidence declined and then rose again.

Cohort 3. As with the other cohorts, the highest incidences in this age group were recorded during the colder months of the study period, with the inter-test periods having the highest incidence being the 15th and the first (winters of 1989 and 1988 respectively). There was, however, a peak percentage incidence of 25.9% during the 8th inter-test period (summer 1989). This was mostly due to the incidence of environmental *Yersinia* species among animals of this cohort in flock C. Another peak in the incidence of the infection was recorded in the 4th inter-test period (13.86%).

Cumulative incidence figures in this cohort shows that the highest incidences were exhibited during winter in 1988 and spring in 1989, followed by

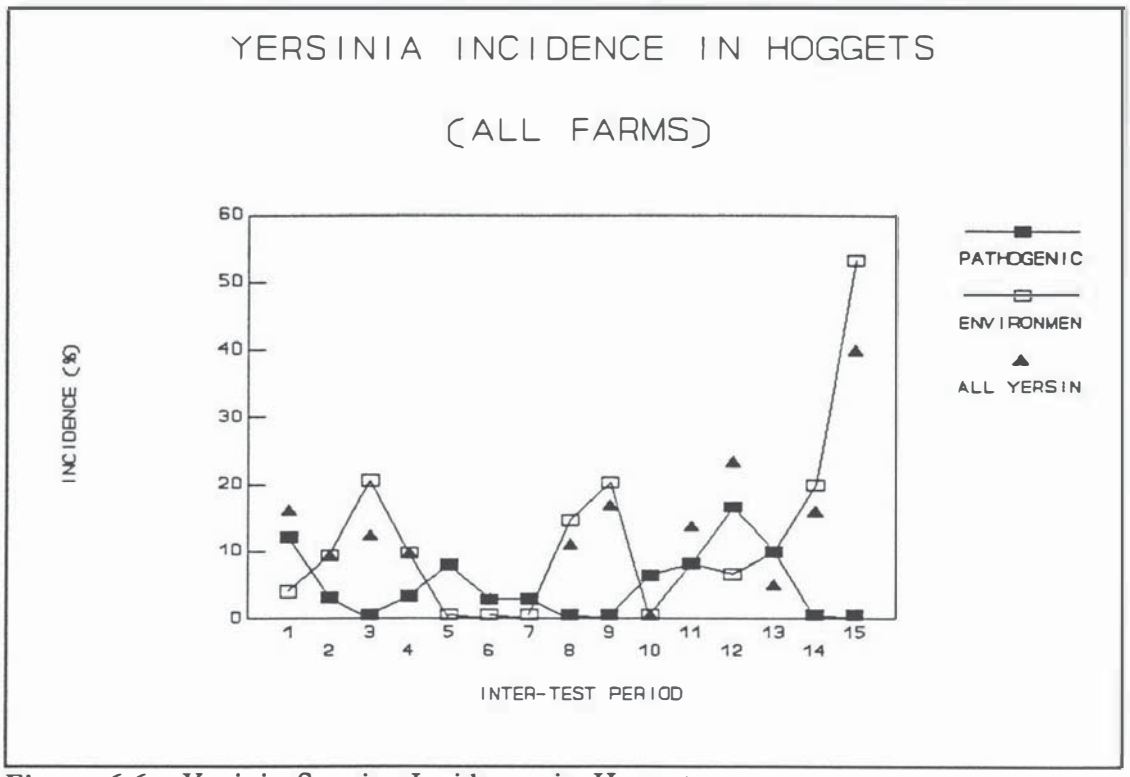


Figure 6.6. *Yersinia* Species Incidence in Hoggets

the incidence in winter 1989. The cumulative incidence during summer 1989 was, however, higher than the autumn figures for that year.

Figure 6.7 shows the comparative incidence levels of the overall *Yersinia* infections within the animals in this cohort from the three farms combined. The incidence patterns show that in this cohort, the peaks were mostly due to the environmental *Yersinia* species. In fact, no new pathogenic *Yersinia* species were isolated from the 2nd to the 8th inter-test periods, or on the 14th and 15th inter-test periods.

Table 6.5. Age Group Incidence of *Yersinia* Species (3 Flocks)

PERIOD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
COHORT 1																
Y. pstb.	41.5	8	0	0	0	0	0	0	0	0	2.7	6.7	0	0	0	0
Y. ent. 5	0	0	0	0	0	0	23.3	3.7	6.7	6.4	16.5	0	0	0	0	13.3
Y. ent. 2/3	20.7	0	0	0	0	0	0	0	0	0	0	0	3.3	0	0	0
Y. ent. 1a	0	0	0	0	0	0	0	0	0	3.2	0	0	0	0	0	0
Y. fred.	10.4	0	10	0	0	0	0	3.7	6.7	9.7	0	0	6.6	5.3	10.9	0
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y. krist.	0	0	10	0	0	0	0	0	0	0	0	0	0	2.7	7.3	0
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.3
PATH	62.2	8	0	0	0	0	23.3	3.7	6.7	6.4	19.2	6.7	3.3	0	0	13.3
ENVI	10.4	0	20	0	0	0	0	3.7	6.7	12.9	0	0	6.6	8	14.6	13.3
OVERALL*	62.2	0	10	0	0	0	23.3	3.7	6.7	19.3	11	6.7	9.9	5.3	14.6	26.7
COHORT 2																
Y. pstb.	8.1	3.1	0	3.3	0	0	0	0	0	3.2	5.5	10	5	0	0	-
Y. ent. 5	0	0	0	0	8	2.9	2.9	0	0	3.2	2.7	3.3	0	0	0	-
Y. ent. 2/3	4	0	0	0	0	0	0	0	0	0	0	3.3	5	0	0	-
Y. ent. 1a	0	0	4.1	0	0	0	0	3.7	0	0	0	0	0	0	0	-
Y. fred.	0	6.3	16.5	9.8	0	0	0	11	20.2	0	0	3.3	5	4	26.7	-
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. krist.	4	3.1	0	0	0	0	0	0	0	0	8.2	3.3	5	16	26.7	-
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
PATH	12.1	3.1	0	3.3	8	2.9	2.9	0	0	6.4	8.2	16.7	10	0	0	-
ENVI	4	9.4	20.6	9.8	0	0	0	14.7	20.2	0	8.2	6.7	10	20	53.3	-
OVERALL*	16.1	9.4	12.4	9.8	8	2.9	2.9	11	16.9	0	13.7	23.3	5	16	40	-
COHORT 3																
Y. pstb.	13.5	0	0	0	0	0	0	0	0	0	2.3	2.9	0	0	0	-
Y. ent. 5	0	0	0	0	0	0	0	0	5.8	2.8	0	0	0	0	0	-
Y. ent. 2/3	3.4	0	0	0	0	0	0	0	0	0	0	0	4	0	0	-
Y. ent. 1a	10.1	2.6	3.4	0	0	0	0	0	0	0	0	2.9	0	0	13.3	-
Y. fred.	6.7	10.5	6.8	13.9	0	0	0	25.9	2.9	2.8	4.7	5.7	0	0	26.7	-
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. krist.	6.7	5.2	0	2.8	0	0	0	0	0	0	4.7	14.3	4	3.2	40	-
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	2.9	0	0	0	-
PATH	16.9	0	0	0	0	0	0	0	5.8	2.8	2.3	2.9	4	0	0	-
ENVI	23.6	18.3	10.2	16.6	0	0	0	25.9	2.9	2.8	9.4	25.7	4	3.2	80	-
OVERALL*	37.11	13.1	0	13.9	0	0	0	25.9	2.9	2.8	9.4	22.9	4	3.2	53.3	-
ALL COHORTS COMBINED																
Y. pstb.	15.6	2.4	0	1	0	0	0	0	0	1	3.5	6.3	1.3	0	0	0
Y. ent. 5	0	0	0	0	2.5	1	8.2	1.2	4.3	4.1	6.1	1.1	0	0	0	13.3
Y. ent. 2/3	6.24	0	0	0	0	0	0	0	0	0	0	1.1	4	0	0	0
Y. ent. 1a	4.7	1.2	3.1	0	0	0	0	1.2	0	1	0	1.1	0	0	2.4	0
Y. fred.	4.7	7.3	11	8.2	0	0	0	14.1	9.6	4.1	1.7	3.2	4	3.2	16.5	0
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y. krist.	4.7	3.6	1.6	1	0	0	0	0	0	0	4.3	6.3	2.7	6.4	16.5	0
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	1.1	0	0	0	13.3
PATH	21.8	2.4	0	1	2.5	1	8.2	1.2	4.3	5.1	9.5	8.4	5.3	0	0	13.3
ENVI	14	12.1	15.7	9.2	0	0	0	15.3	9.6	5.1	6.1	11.6	6.6	9.6	32.9	13.3
OVERALL*	32.8	9.7	6.3	8.2	2.5	1	8.2	14.1	8.5	7.1	11.3	17.9	6.6	7.5	25.9	26.7

* Combined incidence of all *Yersinia* species isolates

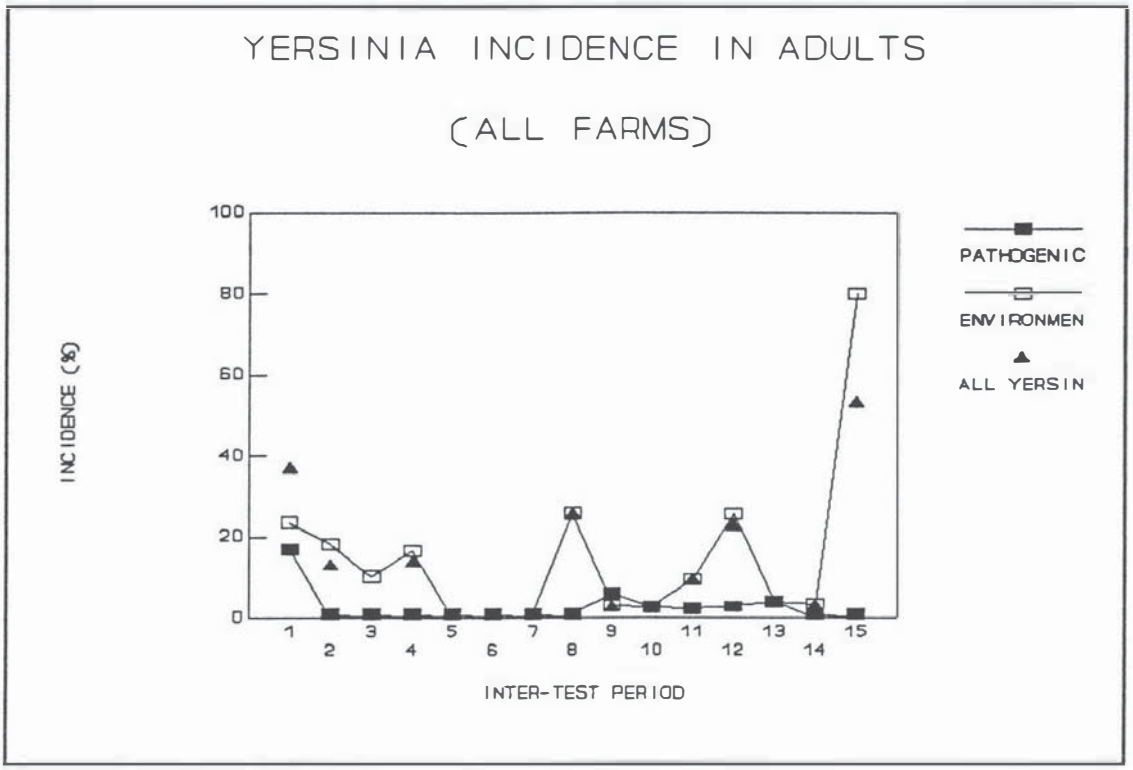


Figure 6.7. *Yersinia* Species Incidence in Adults

Overall Incidence

The 4-week percentage incidences of *Yersinia* species infection from all the cohorts on the three farms are shown in **Table 6.5**. From the table, it is apparent that there were seasonal changes in the incidence of *Yersinia* infection, with peaks occurring during the colder months of the year: the first inter-test period (winter 1988) and during the 12th and 16th inter-test periods (winter and spring 1989). There was, however, a lower peak during the 8th inter-test period (summer 1989).

The cumulative incidences in the different seasons of the year showed that the highest incidences occurred during winter in 1988 (42.44%) and spring in 1989 (52.55%), even though data from these seasons were each taken from only two inter-test periods.

Figure 6.8 shows the comparative incidence levels of Yersinia species in the different cohorts from all the three flocks combined. Examining the patterns reveals that all the three cohorts showed similar patterns in the levels of incidence throughout the whole period of the study, with the highest peaks occurring on the colder months of the year. The unexpected high incidence levels in Flock C (see **Table 6.4**) during the summer of 1988-1989, however, was mainly responsible for the peak incidence during that period.

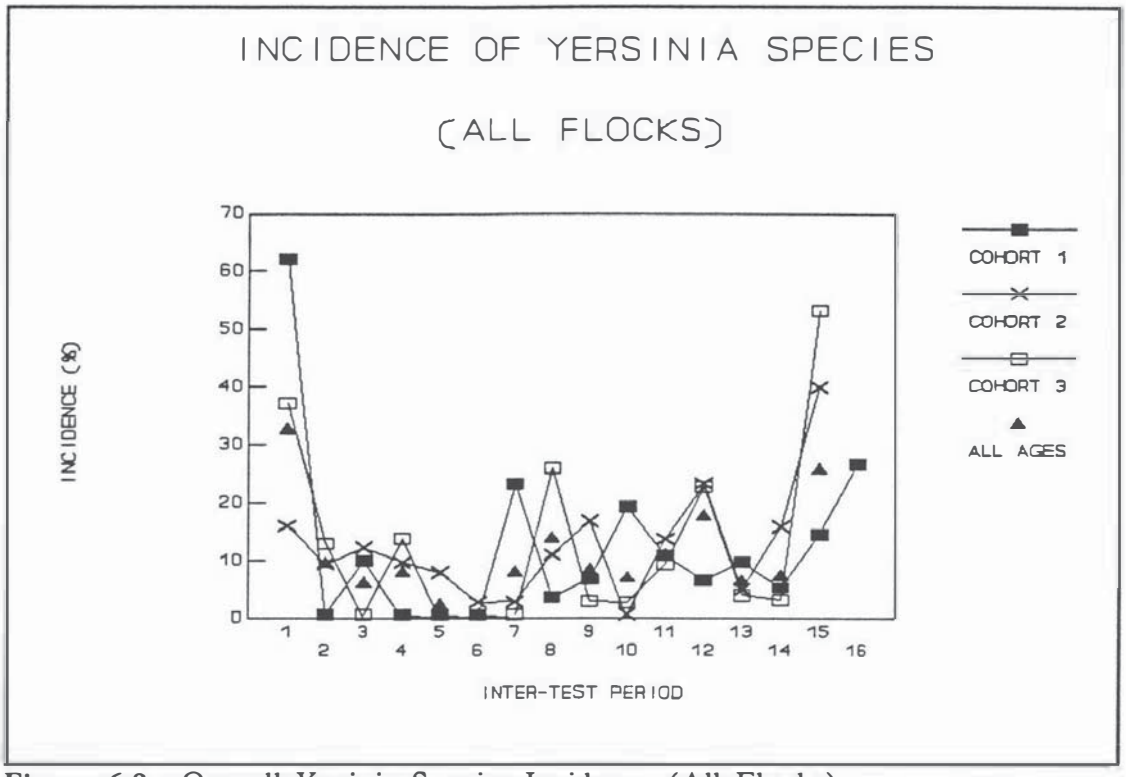


Figure 6.8. Overall *Yersinia* Species Incidence (All Flocks)

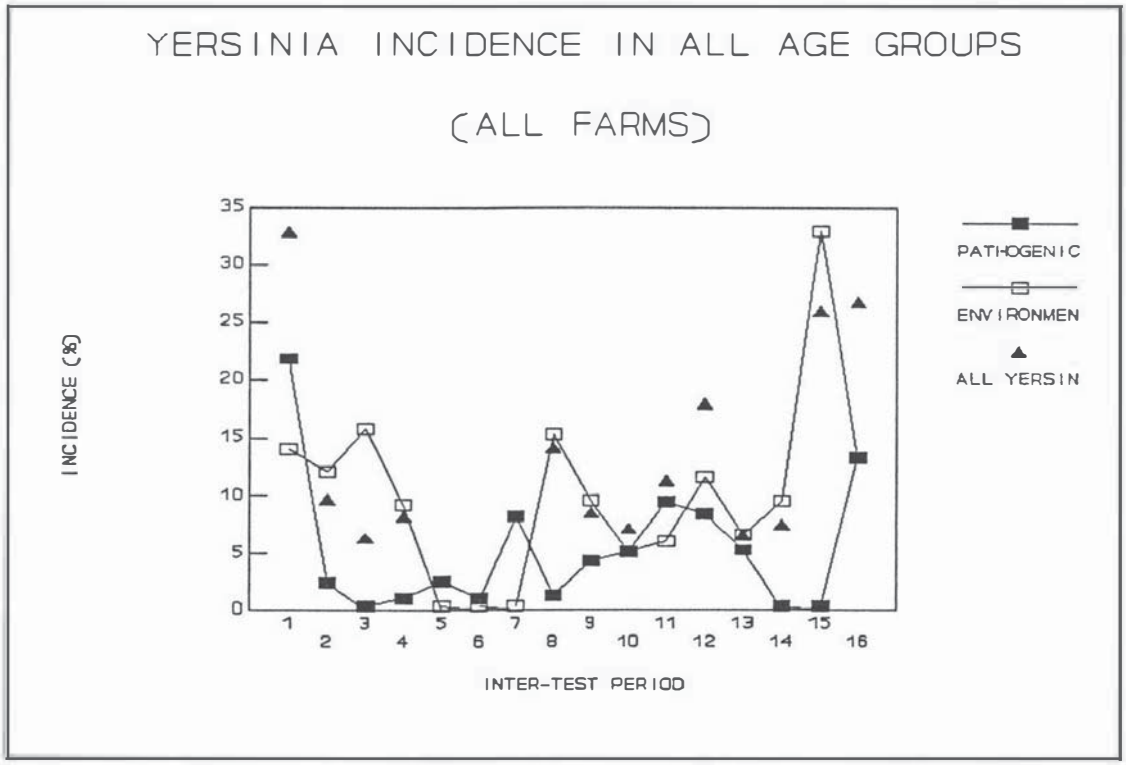


Figure 6.9. Comparison of the Overall Incidence Levels of the Pathogenic and Environmental *Yersinia* Species

Figure 6.9 shows the comparison of the incidence levels of the pathogenic and environmental species from all the cohorts and all the flocks combined. It can be seen from the graph that generally, seasonal peaks of incidence were higher for the environmental *Yersinia* species, the only exception being the high incidence of pathogenic *Yersinia* species (particularly *Y. pseudotuberculosis*) during the first winter of the study (1988). Also, it can be noticed that, as was shown in **Figure 6.8**, there was the unexpected peak during the summer of 1988-1989, with the environmental species mostly responsible. Again, it must be remembered that this peak was almost all due to the high incidence in Flock C.

Results of the Repeated Measures Regression Analysis

Repeated measures regression (also called repeated measures ANOVA or linear model with repeated measures) was employed to determine the differences in the incidence of *Yersinia* species within and between cohorts, with respect to flock and seasonal differences. Since repeated measures regression performed the analysis in a factorial design, matrices of "dummy" variables had to be created for the factors under consideration. Thus the following variables were incorporated into the regression equation: **A** (between-cohort variation due to flock influence), **AGE** (between-cohort variation due to cohort influence within groups), **B** (within-cohort variation due to seasonal influence), and **A × B** (interaction between **A** and **B**). These individual factors, as well as the overall regression results, were tested for significance using the F-test, with p-values $<.05$ considered being significant.

Analyses using these variables were performed independently on the incidence of the following *Yersinia* species: *Y. pseudotuberculosis*, *Y. enterocolitica* biotype 5, *Y. enterocolitica* biotypes 2 and 3 (combined), *Y. enterocolitica* biotype 1A, *Y. frederiksenii*, *Y. kristensenii*, and *Y. rohdei*. The incidence of all the *Yersinia* species combined, as well as those of the pathogenic and environmental classes of *Yersinia* were also tested.

Incidence of *Y. pseudotuberculosis*. The analysis of variance results of the individual independent variables used in the regression model for this species are shown in **Table 6.6**. From the table, it is apparent that seasonal influence on the incidence variation within cohorts was highly significant ($F = 4.42$ $p = .0058$), but variations between cohorts within flocks was found to be insignificant ($p > .05$). The full regression model was, however, found to be significant ($F = 1.78603$ $p = .0395$).

Incidence of *Y. enterocolitica* biotype 5. The incidence of this species was not significantly influenced by any of the individual factors ($p > .05$). The full regression model proved to be significant, however, as tested by the F-test ($F = 1.81619$ $p = .0353$).

Incidence of *Y. enterocolitica* biotypes 2 and 3 combined. The incidence of these biotypes (combined) was found to vary significantly between cohorts on the different farms ($F = 4.48$ $p = .0137$). Within the different cohorts, significant seasonal effects on the incidence of this species were also found ($F = 3.54$ $p = .0173$), as well as the interaction between farm and seasonal effects (**A × B** interaction, $F = 2.81$ $p = .0143$), which means that the seasonal variation at the three different farms did not follow the same pattern. The analysis of variance results of the overall regression equation for this dependent variable was also found to be significant ($F = 2.17029$ $p = .0090$).

Incidence of *Y. enterocolitica* biotype 1A. The only significant result found for the incidence of this species was the variation between cohorts on the different farms ($F = 3.14$ $p = .0474$), with the overall regression results not considered significant ($p > .05$).

Incidence of *Y. frederiksenii*. The only significant result for this species was the incidence variation between cohorts due to flock influence ($F = 5.67$ $p = .0046$), with the overall regression results also not considered significant ($p > .05$).

Incidence of *Y. kristensenii*. The most significant result for this species was the flock influence on the incidence between cohorts ($F = 5.91$ $p = .0037$). The incidence also differed significantly within cohorts due to seasonal influences ($F = 3.32$ $p = .0226$). The overall regression model was also found to be significant ($F = 2.00169$ $p = .0174$).

Incidence of *Y. rohdei*. The results of the regression analysis (individual and overall) for the incidence of the species proved to be insignificant ($p > .05$).

Incidence of pathogenic *Yersinia* species. The incidence of *Yersinia* species classified as pathogenic (*Y. pseudotuberculosis* and *Y. enterocolitica* biotypes 2, 3 and 5) showed significant within-cohort differences due to the interaction of flock and seasonal influences (**A × B** interaction, $F = 2.83$ $p = .0137$). Within-cohort variation was, however, found to be insignificant ($p > .05$). The overall regression model ($F = 1.79621$ $p = .0380$) was found to be significant.

Incidence of environmental *Yersinia* species. The only significant individual factor result of the analysis on the incidence of environmental *Yersinia* species was flock influence ($F = 7.38$ $p = .0010$). The overall regression model for this classification was also found to be significant ($F = 1.97479$ $p = .0193$).

Incidence of all *Yersinia* species combined. The only significant result obtained from this classification was the variation of the incidence between cohorts due to flock influence ($F = 3.99$ $p = .0213$). The overall regression result was found to be insignificant ($p > .05$).

Summary. The results of the repeated measures regression analysis on the differences in *Yersinia* species incidence between and within cohorts are summarised in **Table 6.6**. Where the result is significant ($p < .05$), the p-value is given; otherwise "ns" is placed instead.

From the table, it is apparent that the most common individual factor variation in the incidence of *Yersinia* species was the variation between age groups, with the differences in the environmental *Yersinia* species incidence (individual and combined) levels the most significant. The variation in incidence within cohorts due to seasonal influences was found to be most significant in the pathogenic *Yersinia* species, especially *Y. pseudotuberculosis* and *Y. enterocolitica* biotypes 2 and 3. The interaction between flock and seasonal influences was found to be significant only in the pathogenic *Yersinia* species (combined), as well as in *Y. enterocolitica* biotypes 2 and 3. A suprising result of this regression analysis was the absence of significant variations in the incidence of *Yersinia* species between cohorts within the different goat flocks.

Table 6.6. *Yersinia* Species Incidence Between and Within Cohorts

Yersinia Sp.	Between-Cohort Variation Due to Flocks (A)	Between-Cohort Variation Within Flocks (AGE)	Within-Cohort Variation Due to Seasons (B)	A X B Interaction	Overall
Y. pstb.	ns	ns	.0058	ns	.0395
Y. ent. 5	ns	ns	ns	ns	.0353
Y. ent. 2/3	.0137	ns	.0173	.0143	.0090
Y. ent. 1a	.0474	ns	ns	ns	ns
Y. fred.	.0046	ns	ns	ns	ns
Y. krist.	.0037	ns	.0226	ns	.0174
Y. rohdei	ns	ns	ns	ns	ns
PATHOGENIC	ns	ns	ns	.0137	.0380
ENVIRONMENTAL	.0010	ns	ns	ns	.0193
ALL *Yersinia* Sp.	.0213	ns	ns	ns	ns

Climatic Influences on *Yersinia* Incidence in the Different Cohorts

The following are the results of the stepwise regression procedure on the effects of climatic factors on specific *Yersinia* species incidence. The objective of this procedure was to select a smaller set of significant variables out of the original set, which can sufficiently explain the predicted values.

As can be recalled, the climatic factors used were the following: **DEW, GRASS, HUMID, MAX, MIN, NRD, NTRD** and **RAIN** (**Table 6.7**). The final regression models were decided mostly on the basis of the forward selection procedure for the inclusion of independent variables, but if the coefficient of determination (R^2) values for a particular model was low initially, additional independent variables were "forced" into the model in order to determine the best combination of independent variables for that model. If necessary, variables already in the model were removed if their F-ratios have become insignificant ($F < 4$) upon the forcing of a new variable. The "best" overall combination for the regression model was achieved when the highest coefficient of determination (R^2) value possible was reached while still retaining significant individual and overall F-ratios ($p < .05$).

Table 6.7. Values of Climatic Factors Used in the Stepwise Regression Analysis

PERIOD	DEW (d)	GRASS (°C)	HUMID (%)	MAX (°C)	MIN (°C)	NRD (d)	NTRD (d)	RAIN (dcl)
1	19	2.5	85	13.2	5.7	18	3	16.30
2	14	2.2	83	13.8	5.7	16	4	9.48
3	9	7.2	83	15.6	9.4	21	4	14.40
4	4	7.8	75	17.1	10.2	18	3	9.80
5	11	8.0	76	19.8	10.9	9	1	6.33
6	3	10.2	70	22.9	13.4	8	1	5.70
7	5	12.5	74	24.1	15.2	13	3	9.24
8	11	9.4	75	23.1	12.9	9	3	7.53
9	13	4.2	78	22.0	12.4	11	2	8.92
10	14	5.7	76	19.2	9.8	9	2	4.38
11	13	5.4	88	15.4	8.4	21	1	11.11
12	19	1.6	89	12.6	5.0	17	3	8.82
13	24	-0.7	86	12.3	3.1	10	2	5.34
14	22	1.4	84	14.2	5.3	11	2	5.31
15	18	5.2	78	16.5	8.3	13	0	2.53
16	13	7.2	78	18.0	9.6	23	6	12.31

KEY: d = Number of days
dcl = decilitres

While the individual contributions of some variables to the overall regression equation were insignificant (F-test, $p > .05$), these variables were retained in the model since their presence made the overall model highly significant.

Descriptions of the results are based on the specific *Yersinia* species incidence figures of the three different cohorts, and of the incidence of the three combined.

Incidence of *Y. pseudotuberculosis*. In Cohort 1 (kids), the independent variables significantly influencing the incidence of *Y. pseudotuberculosis* are **RAIN, MIN** and **NRD**, with the full regression model having an F-ratio of 6.44046 ($p = .0076$), an R^2 value of .616875. The other independent variables did not significantly influence the incidence levels of this species in this age group. In Cohort 2 (hoggets), **HUMID** was found to be the only significant predictor for *Y. pseudotuberculosis* incidence ($F = 11.7478$ $p = .0045$ $R^2 = .4747$), with the other variables being insignificant. Among the animals in Cohort 3, **RAIN, DEW, HUMID** AND **NRD** were found to be the most significant combination of the predictor variables for the incidence of *Y. pseudotuberculosis* in this age group ($F = 9.93521$ $p = .0012$ $R^2 = .783212$). When all the age groups were combined, the incidence of this species was significantly influenced only by the combination of **RAIN** and **DEW** ($F = 6.86686$ $p = .0092$). These results are summed up in **Table 6.8.**

Table 6.8. Climatic Effects on *Y. pseudotuberculosis* Incidence

	DEW	GRASS	HUMID	MAX	MIN	NRD	NTRD	RAIN	OVERALL
COHORT 1	-	-	-	-	.0249	.0420	-	.0028	.0076
COHORT 2	-	-	.0045	-	-	-	-	-	.0045
COHORT 3	.0431	-	.0103	-	-	ns	-	.0009	.0012
ALL AGES	.0365	-	-	-	-	-	-	.0129	.0092

Note: The meteorological factors included in the overall regression equation are indicated by the entries found in each cell. If significant, the p-value is displayed; otherwise "ns" is placed instead. A variable not included in the model is marked with a dash ("-").

Figure 6.10 shows a graphic comparison of the levels of **DEW** and **RAIN** with the overall incidence of *Y. pseudotuberculosis.*

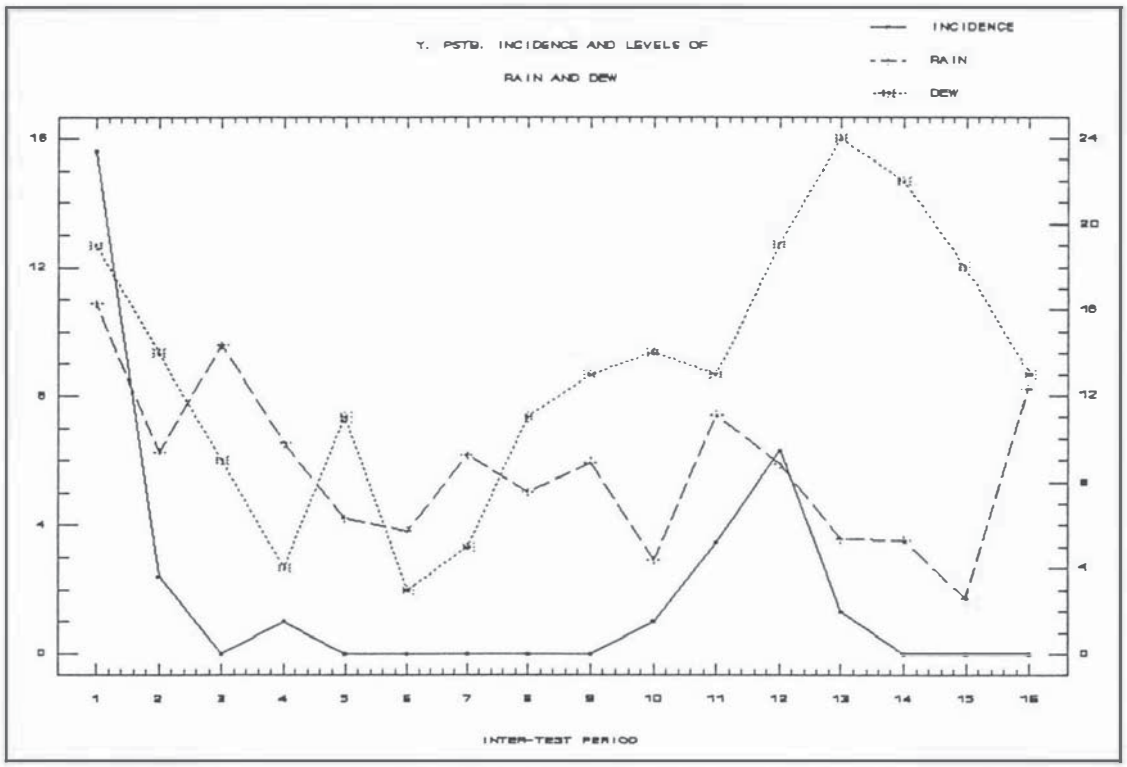


Figure 6.10. Comparison of levels of **DEW** and **RAIN** with *Y. pseudotuberculosis* Incidence

Incidence of *Y. enterocolitica* biotype 5. In kids, the combination of **HUMID, MAX** and **NRD** provided the best predictor model for the incidence of *Y. enterocolitica* biotype 5 ($F = 5.61643$ $p = .0122$ $R^2 = .58045$). All the predictor variables were not, however, enough to significantly predict or explain the incidence of this species among hoggets. In adults, **GRASS AND MIN** combined to form a highly significant predictor for *Y. enterocolitica* biotype 5

incidence (F = 37.4568 p = .0000 R^2 = .86193). The incidence of this species with all the cohorts combined was not significantly explained by any of the independent variables. **Table 6.9** shows the results.

Table 6.9. Climatic Effects on *Y. enterocolitica* biotype 5 Incidence

	DEW	GRASS	HUMID	MAX	MIN	NRD	NTRD	RAIN	OVERALL
COHORT 1	-	-	ns	.0054	-	.0485	-	-	.0122
COHORT 2	-	-	-	-	-	-	-	-	-
COHORT 3	-	ns	-	-	.0000	-	-	-	.0000
ALL AGES	-	-	-	-	-	-	-	-	-

Note: The meteorological factors included in the overall regression equation are indicated by the entries found in each cell. If significant, the p-value is displayed; otherwise "ns" is placed instead. A variable not included in the model is marked with a dash ("-").

Incidence of *Y. enterocolitica* bioytpes 2 and 3 (combined). **Table 6.10** shows the results of the regression analysis. The combination of **RAIN, HUMID, DEW, MIN** and **NTRD** combined significantly to produce the best model explaining the incidence of these species in kids (F = 12.5594 p = .0005 R^2 = .86263). In hoggets, **MIN** was the only significant variable included in the regression equation (F = 10.5189 p = .0059 R^2 = .42901), and in adults, **MIN** was again the only significant explanatory variable (F = 5.35551 p = .0377 R^2 = .29177). When all the age groups were combined, however, **RAIN, MIN** and **NRD** combined significantly to explain the incidence of *Y. enterocolitica* biotypes 2 and 3. A graphic comparison of these climatic factors with the incidence levels is shown in **Figure 6.11**.

Table 6.10. Climatic Effects on *Y. enterocolitica* biotypes 2 and 3 Incidence

	DEW	GRASS	HUMID	MAX	MIN	NRD	NTRD	RAIN	OVERALL
COHORT 1	.0008	-	ns	-	ns	-	.0016	.0013	.0005
COHORT 2	-	-	-	-	.0059	-	-	-	.0059
COHORT 3	-	-	-	-	.0377	-	-	-	.0377
ALL AGES	-	-	-	-	.0099	.0108	-	.0571	.0041

Note: The meteorological factors included in the overall regression equation are indicated by the entries found in each cell. If significant, the p-value is displayed; otherwise "ns" is placed instead. A variable not included in the model is marked with a dash ("-").

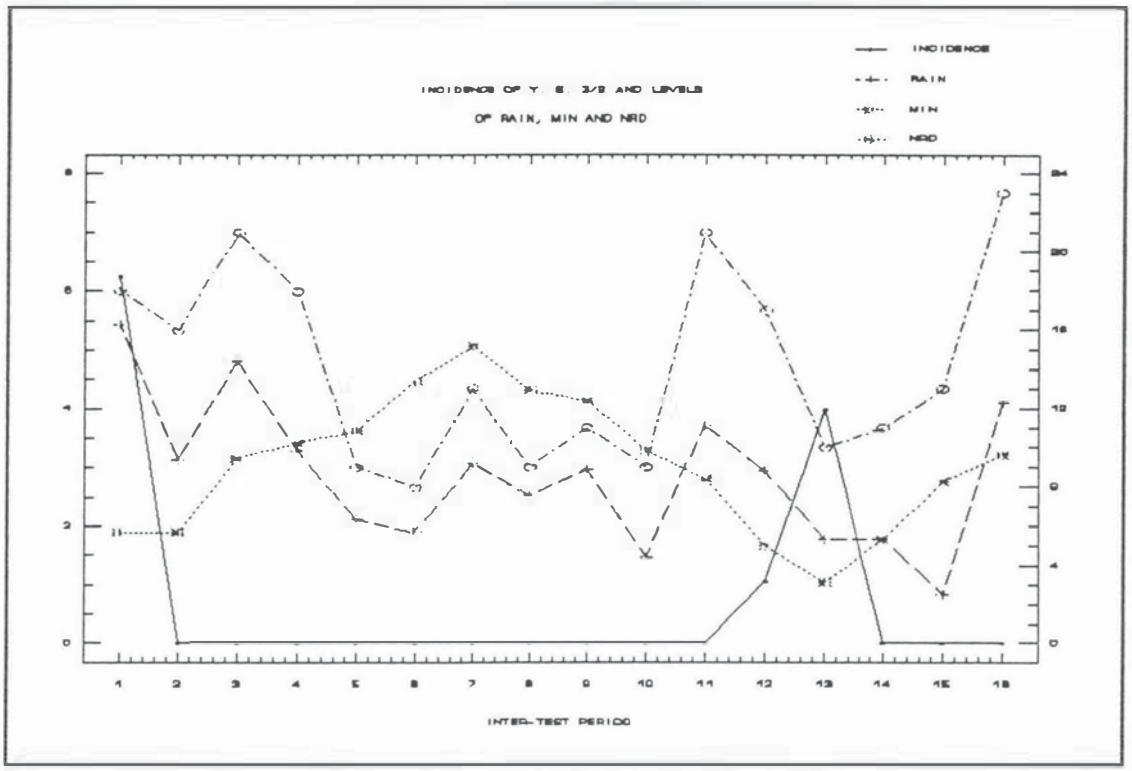


Figure 6.11. Comparison of the Levels of **RAIN**, **MIN** and **NRD** with the Incidence of *Y. enterocolitica* biotypes 2 and 3 (combined)

Incidence of *Y. enterocolitica* biotype 1A. There were no significant variables which influenced the incidence of this biotype in the different age groups and in the combined age groups.

Incidence of *Y. frederiksenii*. The incidence of *Y. frederiksenii* in adults was explained significantly by **NTRD** ($F = 5.70901$ $p = .0315$ $R^2 = .28967$). Climatic factors, however, have no significant influence on the incidence of this species on all the other age groups, as well as on the combined age groups. **Table 6.11** summarises the results.

Table 6.11. Climatic Effects on *Y. frederiksenii* Incidence

	DEW	GRASS	HUMID	MAX	MIN	NRD	NTRD	RAIN	OVERALL
COHORT 1	-	-	-	-	-	-	-	-	-
COHORT 2	-	-	-	-	-	-	-	-	-
COHORT 3	-	-	-	-	-	-	.0315	-	.0315
ALL AGES	-	-	-	-	-	-	-	-	-

Note: The meteorological factors included in the overall regression equation are indicated by the entries found in each cell. If significant, the p-value is displayed; otherwise "ns" is placed instead. A variable not included in the model is marked with a dash ("-").

Incidence of *Y. kristensenii*. It was only among hoggets and among the combination of the age groups that the climatic variables had any significant influences. In hoggets, **RAIN**, **HUMID**, **DEW** and **NRD** combined significantly to produce the best model for the incidence of this species ($F = 7.64914$ $p = .0043$ $R^2 = .75367$). In the combined age groups, **RAIN**, **DEW**, **MAX** and **MIN** contributed to form the best model for the incidence of *Y. kristensenii* ($F = 5.95276$ $p = .0084$ $R^2 = .68401$). **Figure 6.12** shows the comparative levels of the climatic factors in comparison with the incidence levels. The results summary is shown in **Table 6.12.**

Table 6.12. Climatic Effects on *Y. kristensenii* Incidence

	DEW	GRASS	HUMID	MAX	MIN	NRD	NTRD	RAIN	OVERALL
COHORT 1	-	-	-	-	-	-	-	-	-
COHORT 2	ns	-	.0166	-	-	.0034	-	.0276	.0043
COHORT 3	-	-	-	-	-	-	-	-	-
ALL AGES	.0143	-	-	ns	.0106	-	-	ns	.0084

Note: The meteorological factors included in the overall regression equation are indicated by the entries found in each cell. If significant, the p-value is displayed; otherwise "ns" is placed instead. A variable not included in the model is marked with a dash ("-").

Incidence of *Y. rohdei*. **Table 6.13** shows the results of the regression analysis. In kids, the incidence of this species was significantly influenced only by **NTRD** ($F = 9.66197$ $p = .0077$ $R^2 = .40833$). In the older age groups, climatic factors appeared not to have any significant effects on the incidence of

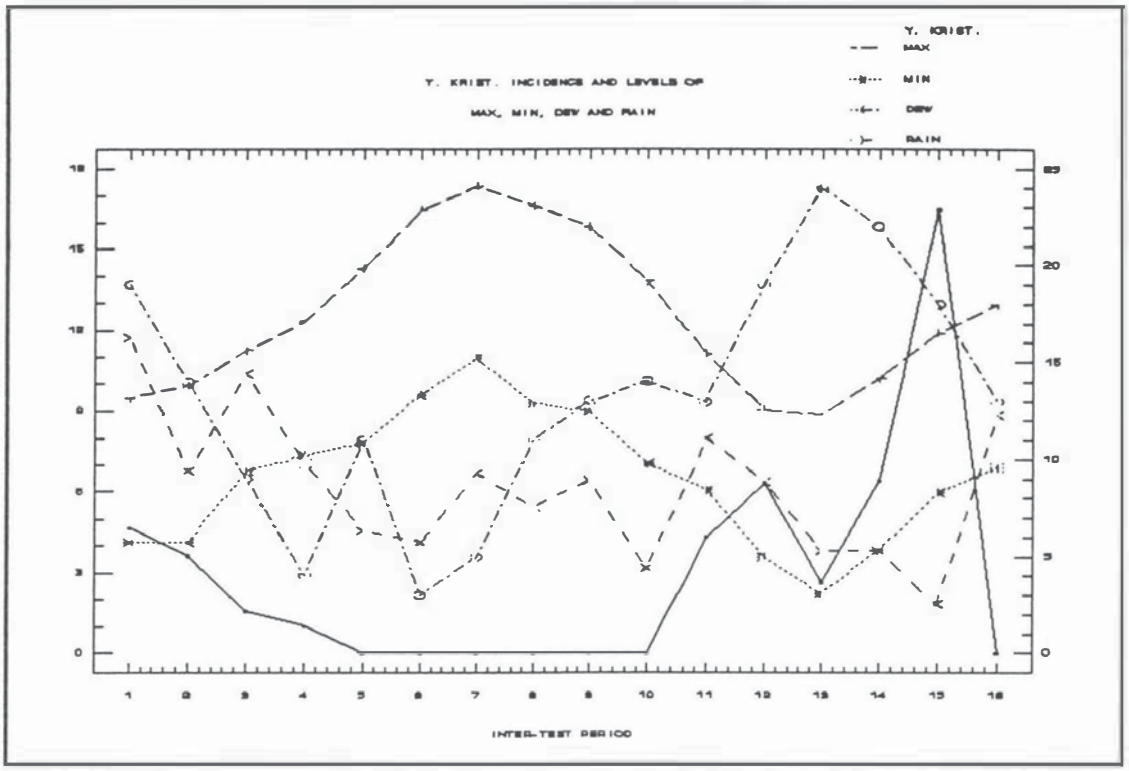


Figure 6.12. Comparison of the levels of **RAIN**, **DEW**, **MAX** and **MIN** with *Y. kristensenii* Incidence

Y. rohdei. However, the influence of **NTRD** on the incidence of this species in all the age groups combined was significant ($F = 10.1131$ $p = .0067$ $R^2 = .41940$). The **NTRD** levels are shown in comparison with incidence in **Figure 6.13.**

Table 6.13. Climatic Effects on *Y. rohdei* Incidence

	DEW	GRASS	HUMID	MAX	MIN	NRD	NTRD	RAIN	OVERALL
COHORT 1	-	-	-	-	-	-	.0077	-	.0077
COHORT 2	-	-	-	-	-	-	-	-	-
COHORT 3	-	-	-	-	-	-	-	-	-
ALL AGES	-	-	-	-	-	-	.0067	-	.0067

Note: The meteorological factors included in the overall regression equation are indicated by the entries found in each cell. If significant, the p-value is displayed; otherwise "ns" is placed instead. A variable not included in the model is marked with a dash ("-").

Incidence of pathogenic *Yersinia* species. The incidence of pathogenic *Yersinia* species in kids was greatly influenced by **RAIN** ($F = 9.75836$ $p = .0075$ $R^2 = .41073$), and in hoggets, it was the effects of **HUMID** that was significant

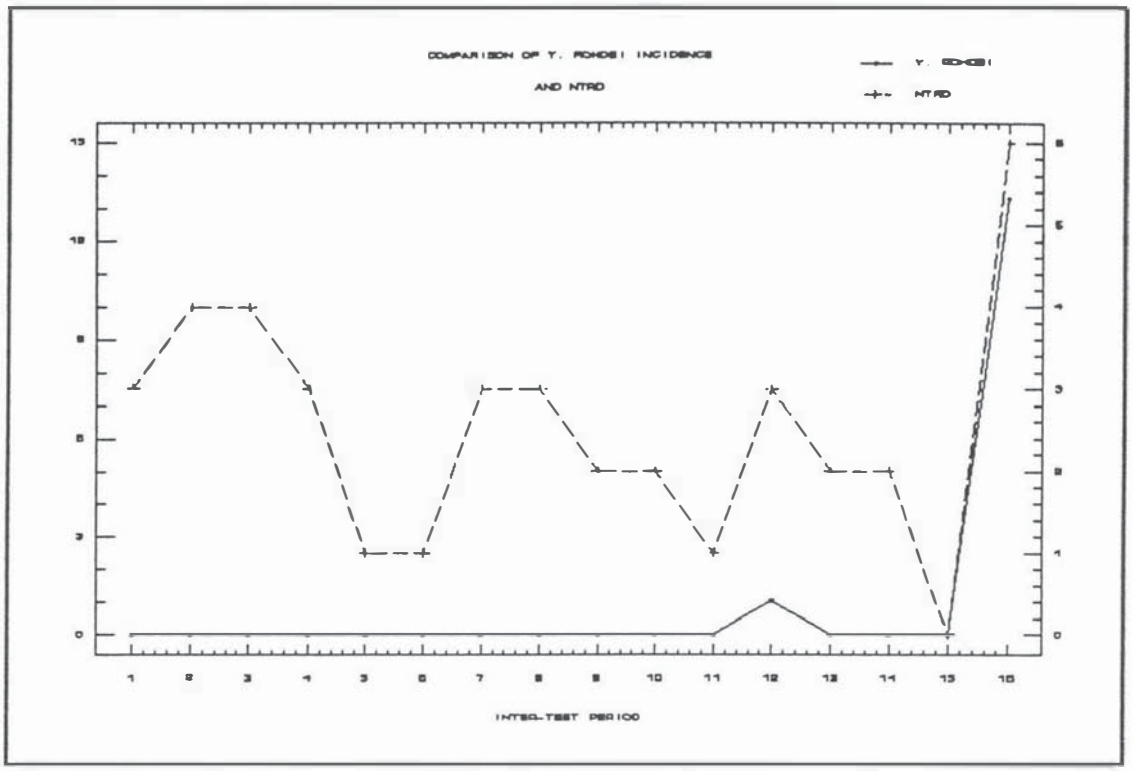


Figure 6.13. Comparison of **NTRD** levels with *Y. rohdei* Incidence

(F = 5.37877 p = .0373 R^2 = .292662). In adults, the combination of **RAIN, HUMID, DEW** and **NTRD** contributed significantly to the overall regression model (F = 10.9691 p = .0011 R^2 = .8134). When all the age groups were combined, however, only **RAIN** (**Table 6.14**) was significant in its contribution to the regression equation (F = 8.83961 p = .0101 R^2 = .38703).

Table 6.14. Climatic Effects on the Incidence of Pathogenic *Yersinia* Species

	DEW	GRASS	HUMID	MAX	MIN	NRD	NTRD	RAIN	OVERALL
COHORT 1	-	-	-	-	-	-	-	.0075	.0075
COHORT 2	-	-	.0373	-	-	-	-	-	.0373
COHORT 3	.0008	-	ns	-	-	-	ns	.0029	.0011
ALL AGES	-	-	-	-	-	-	-	.0101	.0101

Note: The meteorological factors included in the overall regression equation are indicated by the entries found in each cell. If significant, the p-value is displayed; otherwise "ns" is placed instead. A variable not included in the model is marked with a dash ("-").

Incidence of environmental *Yersinia* species. None of the climatic variables tested for their influence on the incidence of environmental *Yersinia* species in the different age groups, and in all the age groups combined were significant.

Incidence of all *Yersinia* species combined. In kids, **RAIN**, **HUMID** and **DEW** were all significant in their contribution to the regression model testing for the effects of climatic factors on the incidence of all *Yersinia* species combined ($F = 6.8964$ $p = .0059$ $R^2 = .63291$), while in hoggets and adults, no climatic factor was found to be significantly influencing the incidence of all *Yersinia* species in combination (**Table 6.15**). Testing the climatic factors on the overall incidence of all *Yersinia* species on all the age groups combined revealed that **DEW**, **HUMID** and **NRD** (**Figure 6.14**) had very significant contributions to the overall regression model ($F = 7.35165$ $p = .0047$ $R^2 = .647628$).

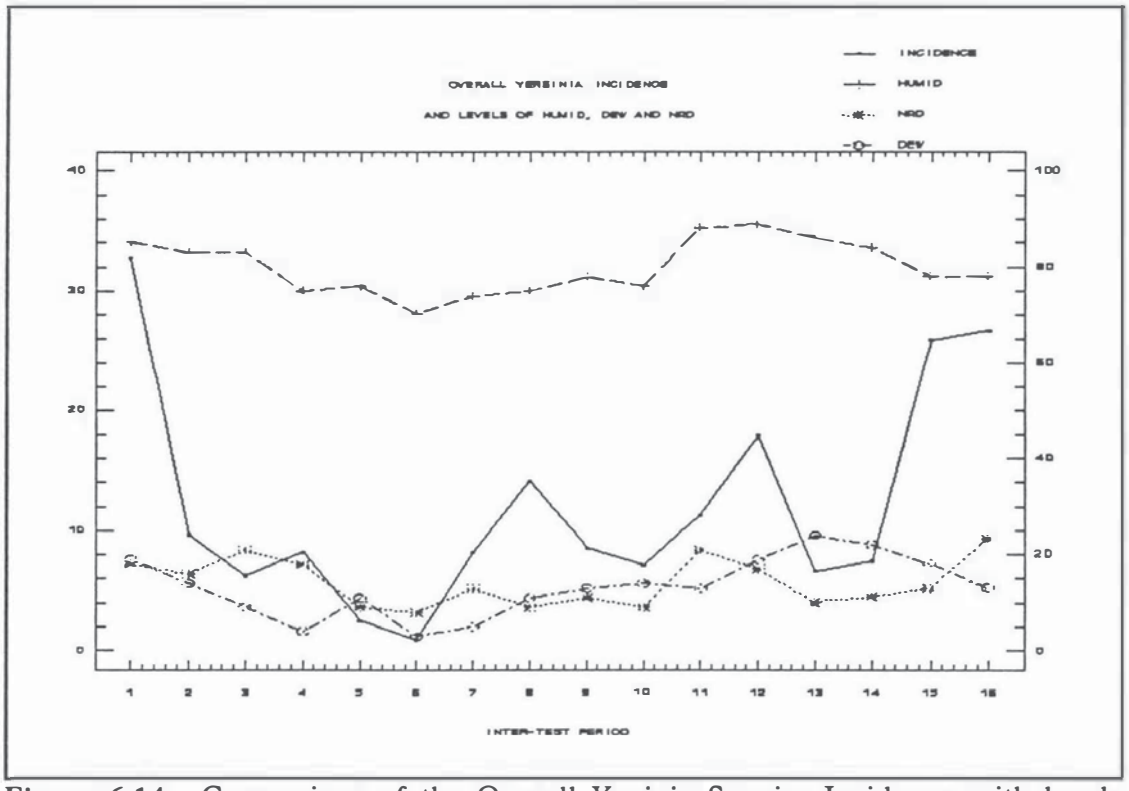


Figure 6.14. Comparison of the Overall *Yersinia* Species Incidence with levels of **DEW**, **HUMID** and **NRD**

Table 6.15. Climatic Effects on the Incidence of All *Yersinia* Species Combined

	DEW	GRASS	HUMID	MAX	MIN	NRD	NTRD	RAIN	OVERALL
COHORT 1	.0053	-	ns	-	-	-	-	.0105	.0059
COHORT 2	-	-	-	-	-	-	-	-	-
COHORT 3	-	-	-	-	-	-	-	-	-
ALL AGES	ns	-	ns	-	-	.0015	-	-	.0047

Note: The meteorological factors included in the overall regression equation are indicated by the entries found in each cell. If significant, the p-value is displayed; otherwise "ns" is placed instead. A variable not included in the model is marked with a dash ("-").

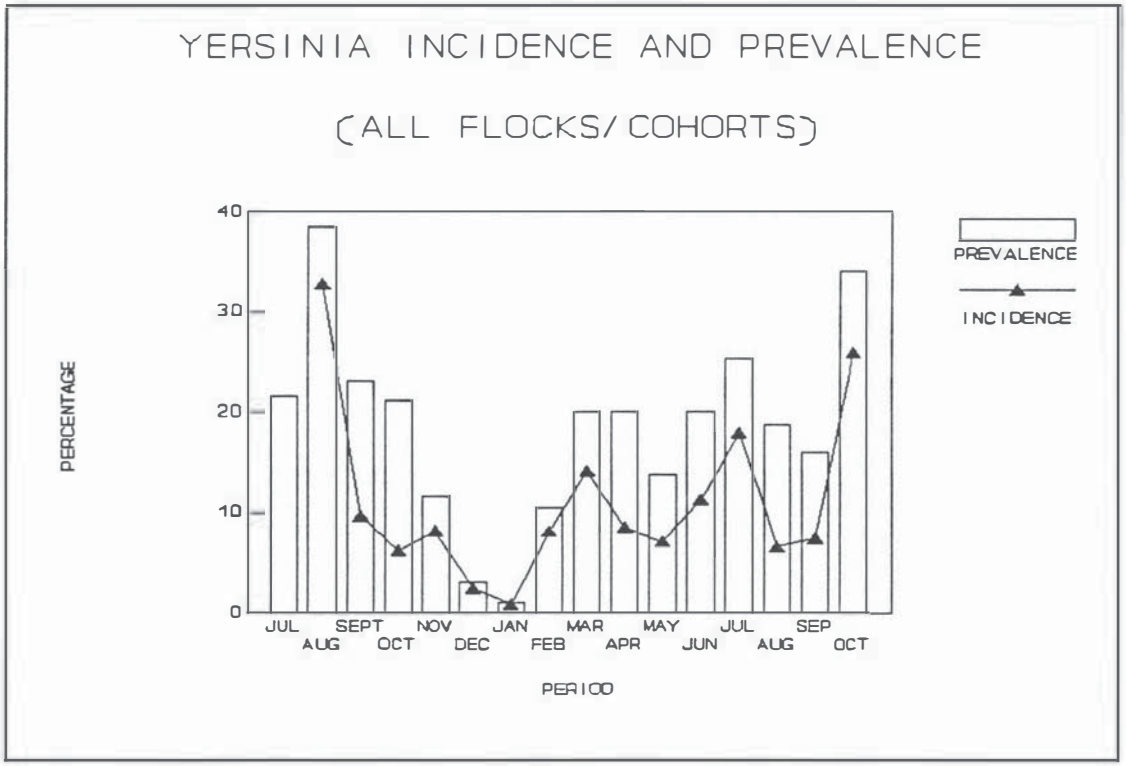


Figure 6.15. Comparison of the Overall Monthly Point Prevalence with Incidence Levels of *Yersinia* Species

Age Group Point Prevalence of *Yersinia* Species

Table 6.16 shows the monthly point prevalence of the different *Yersinia* species from the different age groups with all the three farms combined. From the table it can be seen that the highest overall point prevalence occurred during August 1988 (winter), with a prevalence of 38.5%, and October 1989 (spring), with a prevalence of 34%. A peak also occurred during the middle of winter 1989 (July), with a prevalence of 25.3%. From the pattern of the point prevalences it is apparent that *Yersinia* species carriage in faeces is more

prevalent during the colder months of the year. For the whole period of the study, an overall mean prevalence level of 17.55% was recorded.

Figure 6.15 shows a comparison between the overall monthly point prevalence levels and the incidence of *Yersinia* species. **Figure 6.16** shows the comparative point prevalences of the pathogenic and environmental *Yersinia* species.

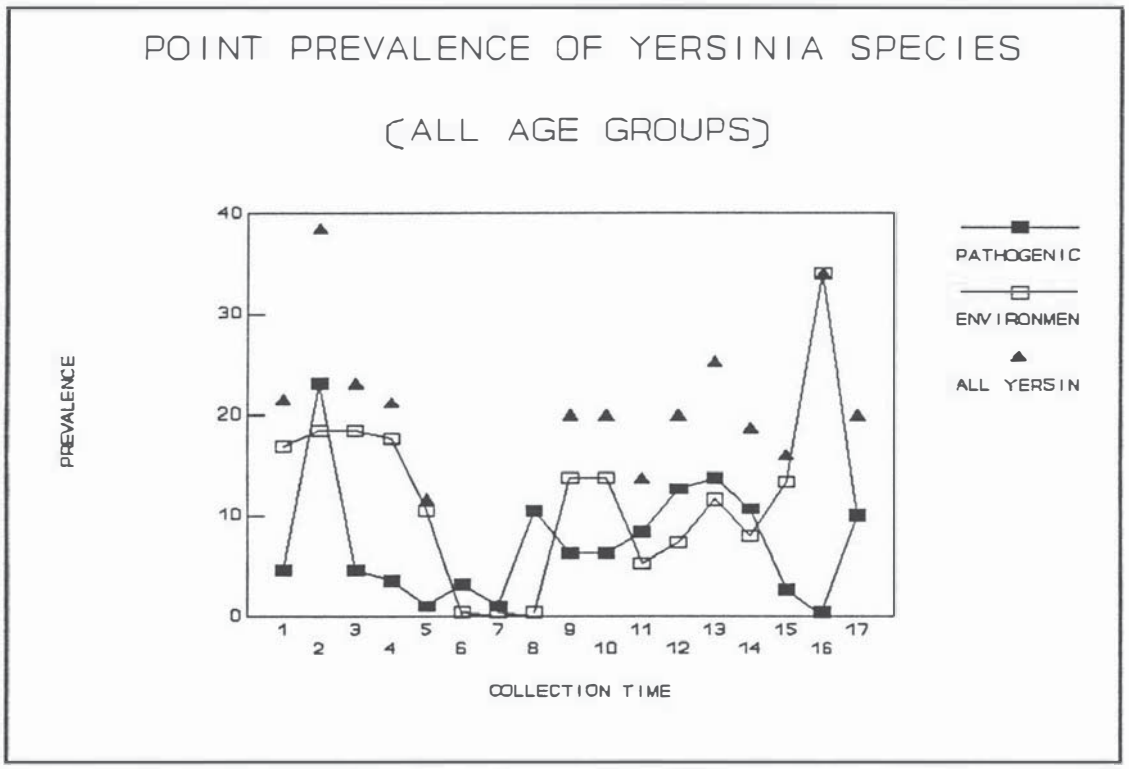


Figure 6.16. Comparative Point Prevalence Levels of the Pathogenic and Environmental *Yersinia* Species

Mean Overall Species-Specific Prevalence of *Yersinia* Infection

During the whole duration of the study, the following prevalence levels were determined for the specific *Yersinia* species encountered: *Y. pseudotuberculosis* = 2.45%; *Y. enterocolitica* biotype 5 = 3.79%; *Y. enterocolitica* biotypes 2 and 3 = 0.59%; *Y. enterocolitica* biotype 1A = 1.04%; *Y. frederiksenii* = 6.39%; *Y. kristensenii* = 3.35%; *Y. intermedia* = 0.07%; and *Y. rohdei* = 0.15%. All these summed up to a total mean species-specific prevalence of 17.83%. However, this figure is not a true total prevalence since there were instances where one animal carried multiple species, and this is just the sum of the species figures. The true total prevalence (number of animals positive for any *Yersinia* species) was calculated to be 17.55%.

Table 6.16. Age Group Point Prevalence of *Yersinia* Species (3 Flocks)

COLLECTION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
COHORT 1																	
Y. pstb.	0	40	20	0	0	0	0	0	0	0	0	3.3	10	6.7	0	0	0
Y. ent. 5	10	10	0	0	0	0	0	26.7	20	13.3	16.7	23.3	6.7	3.3	3.3	0	10
Y. ent. 2/3	0	20	0	0	0	0	0	0	0	0	0	0	0	3.3	0	0	0
Y. ent. 1a	0	0	0	3.3	0	0	0	0	0	0	3.3	0	0	0	0	0	0
Y. fred.	0	10	0	10	0	0	0	0	3.3	10	10	0	0	6.7	6.7	10	0
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y. krist.	0	0	0	6.7	0	0	0	0	0	0	0	0	0	0	3.3	10	0
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10
PATH	10	70	20	10	0	0	0	26.7	20	13.3	16.7	26.7	16.7	13.3	3.3	0	10
ENVI	0	10	0	10	0	0	0	0	3.3	10	13.3	0	0	6.7	10	16.7	10
OVERALL*	10	70	20	20	0	0	0	26.7	23.3	23.3	30	26.7	16.7	20	13.3	16.7	20
COHORT 2																	
Y. pstb.	0	8	4	0	3.3	0	0	0	0	0	3.3	6.7	13.3	5	0	0	-
Y. ent. 5	8	0	0	0	0	10	3.3	6.7	0	0	3.3	3.3	3.3	0	0	0	-
Y. ent. 2/3	0	4	0	0	0	0	0	0	0	0	0	0	3.3	5	0	0	-
Y. ent. 1a	0	0	0	4	0	0	0	0	3.3	0	0	0	0	0	0	0	-
Y. fred.	4	0	8	20	13.3	0	0	0	10	26.7	0	0	3.3	5	5	20	-
Y. int.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. krist.	4	4	4	0	0	0	0	0	0	0	0	10	3.3	5	20	50	-
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
PATH	8	12	4	0	3.3	10	3.3	6.7	0	0	6.7	10	20	10	0	0	-
ENVI	8	4	12	24	13.3	0	0	0	13.3	26.7	0	10	6.7	10	25	60	-
OVERALL*	16	16	16	24	16.7	10	3.3	6.7	13.3	26.7	6.7	20	26.7	20	25	60	-
COHORT 3																	
Y. pstb.	0	13.3	0	0	0	0	0	0	0	0	0	2.9	5.7	4	4	0	-
Y. ent. 5	0	0	0	0	0	0	0	0	0	5.7	2.9	0	0	0	0	0	-
Y. ent. 2/3	0	3.3	0	0	0	0	0	0	0	0	0	0	0	4	0	0	-
Y. ent. 1a	0	10	10	6.7	0	0	0	0	0	0	0	0	2.9	0	0	10	-
Y. fred.	20	13.3	13.3	13.3	14.3	0	0	0	22.9	5.7	2.9	5.7	5.7	0	0	20	-
Y. int.	3.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
Y. krist.	6.7	10	6.7	0	2.9	0	0	0	0	0	0	5.7	14.3	8	8	30	-
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	2.9	0	0	0	-
PATH	0	16.7	0	0	0	0	0	0	0	5.7	2.9	2.9	5.7	8	4	0	-
ENVI	30	33.3	30	20	17.1	0	0	0	22.9	5.7	2.9	11.4	25.7	8	8	60	-
OVERALL*	30	46.7	30	20	17.1	0	0	0	22.9	11.4	5.7	14.3	31.4	16	12	60	-
ALL COHORTS COMBINED																	
Y. pstb.	0	15.4	4.6	0	1.1	0	0	0	0	0	1.1	4.2	9.5	5.3	1.3	0	0
Y. ent. 5	4.6	1.5	0	0	0	3.2	1.1	10.5	6.3	6.3	7.4	8.4	3.2	1.3	1.3	0	10
Y. ent. 2/3	0	6.2	0	0	0	0	0	0	0	0	0	0	1.1	4	0	0	0
Y. ent. 1a	0	4.6	4.6	4.7	0	0	0	0	1.1	0	1.1	0	1.1	0	0	2	0
Y. fred.	10.8	6.2	9.2	14.1	9.5	0	0	0	12.6	13.7	4.2	2.1	3.16	4	4	10	0
Y. int.	1.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Y. krist.	4.6	4.6	4.6	2.4	1.1	0	0	0	0	0	0	5.3	6.3	4	9.3	22	0
Y. rohd.	0	0	0	0	0	0	0	0	0	0	0	0	1.1	0	0	0	10
PATH	4.6	23.1	4.6	3.5	1.1	3.2	1.1	10.5	6.3	6.3	8.4	12.6	13.7	10.7	2.7	0	10
ENVI	16.9	18.5	18.5	17.7	10.5	0	0	0	13.7	13.7	5.3	7.4	11.6	8	13.3	34	10
OVERALL*	21.5	38.5	23.1	21.2	11.6	3.2	1.1	10.5	20	20	13.7	20	25.3	18.7	16	34	20

* Combined prevalence of all *Yersinia* species isolates

Mean Cohort Prevalence of *Yersinia* Species

The mean prevalence levels of *Yersinia* species infection in the three cohorts for the whole duration of the study are shown in **Figure 6.17.** From the graph, it is apparent that the levels in the different cohorts were almost similar. The chi-squared test proved this (chi-squared = 0.0862 p = 0.9578).

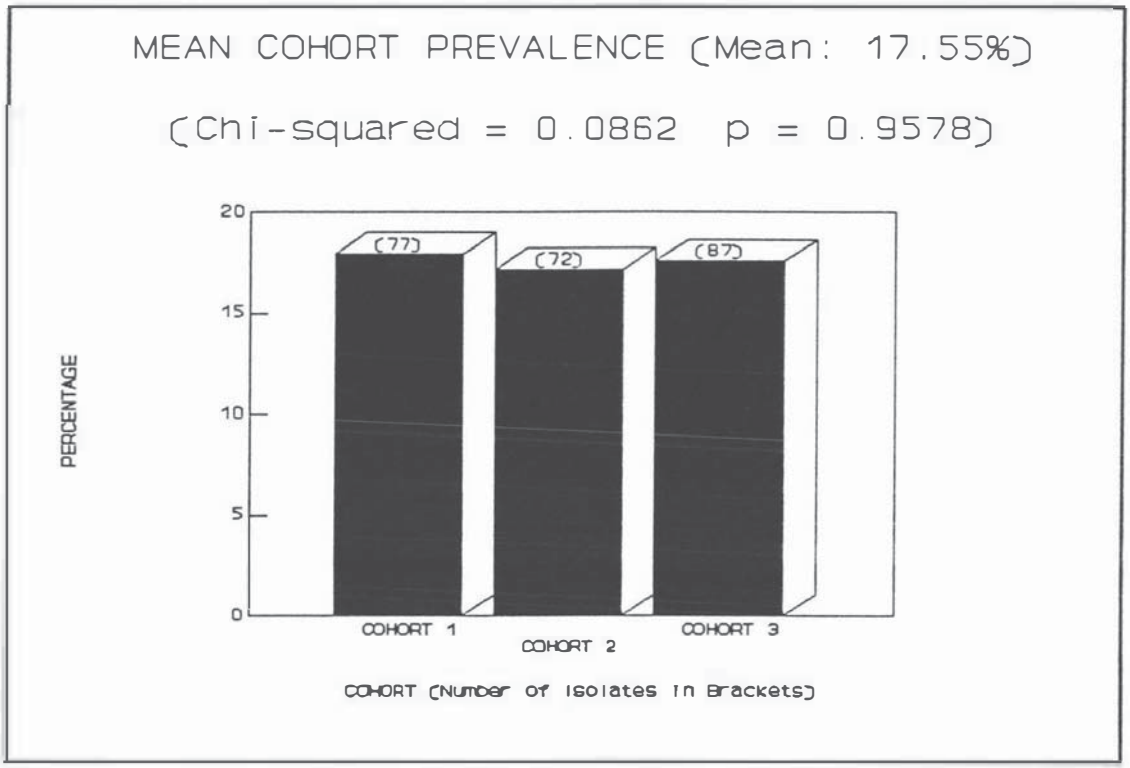


Figure 6.17. Mean Cohort Prevalence of *Yersinia* Species Infection (Whole Study Period)

Mean Flock Prevalence of *Yersinia* Species

Figure 6.18 shows the mean prevalence of *Yersinia* species for the whole duration of the study in the three flocks. Flock C had the highest overall mean prevalence (31.1%), followed by Flock A (13.3%) and Flock B (8.7%). The chi-squared test showed significant differences between the prevalences of the three flocks (chi-squared = 89.2533 p = 0.0000).

Mean Seasonal Prevalence of *Yersinia* Species

The overall mean prevalence levels of *Yersinia* species in the different seasons of the study are shown in **Figure 6.19.** The highest mean prevalence level was found during winter (24.3%), with the next highest during autumn

(17.9%). In spring, the mean level was 19.7%, followed by a low mean level of prevalence during summer (4.9%).

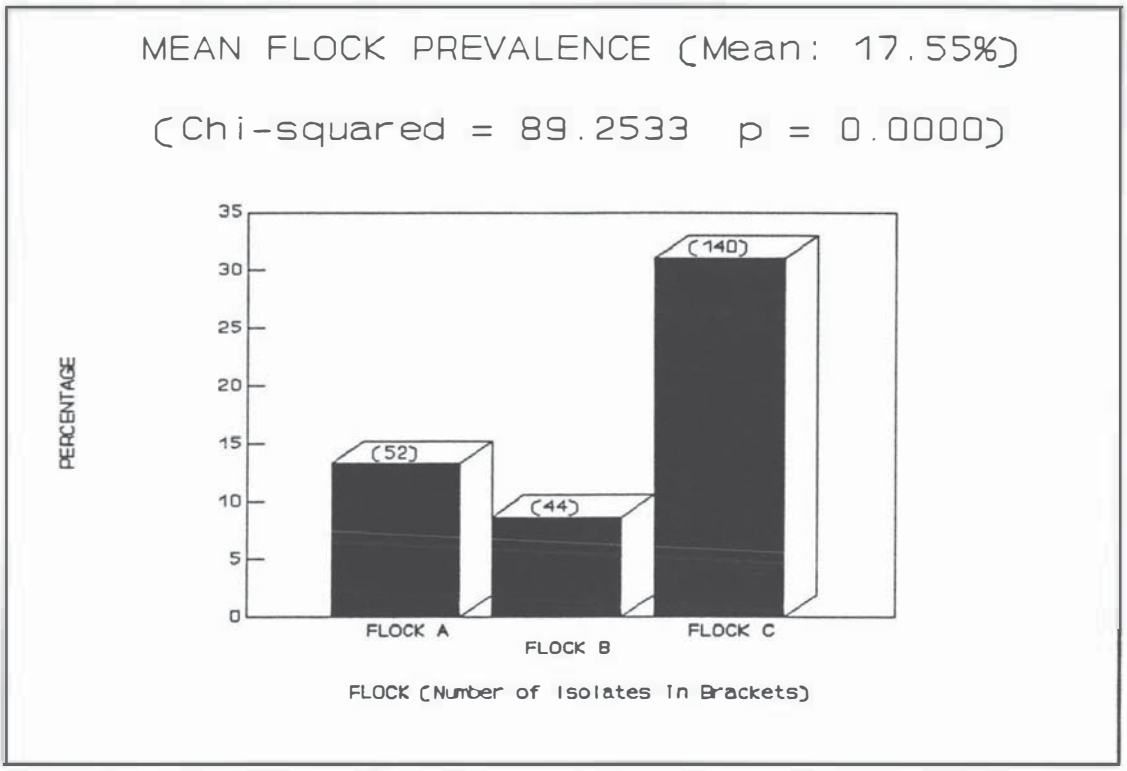


Figure 6.18. Mean Flock Prevalence of *Yersinia* Species Infection (Whole Study Period)

Distribution of *Yersinia* Species Isolated in the Cohort Study

Figure 6.20 shows the distribution of the various *Yersinia* species isolated from all cohorts in the different farms during the course of the study. Of the total of 244 strains isolated, 88 (36%) were *Y. frederiksenii*, 51 (21%) were *Y. enterocolitica* biotype 5, 47 (19%) were *Y. kristensenii*, 33 (14%) were *Y. pseudotuberculosis*, 14 (6%) were *Y. enterocolitica* biotype 1A, and 7 (3%) were *Y. enterocolitica* biotype 3. The remainder were made up of 2 isolates of *Y. rohdei* and one each of *Y. enterocolitica* biotype 2 and *Y. intermedia*. None of the other *Yersinia* species mentioned in Chapter 2 were isolated in the present survey.

While *Y. enterocolitica* biotype 5, *Y. frederiksenii* and *Y. kristensenii* were isolated from all the three flocks, the other *Yersinia* species have their own peculiar distributions with regard to the different flocks. *Y. pseudotuberculosis* was isolated only from Flocks B and C, and *Y. enterocolitica* biotype 1A was

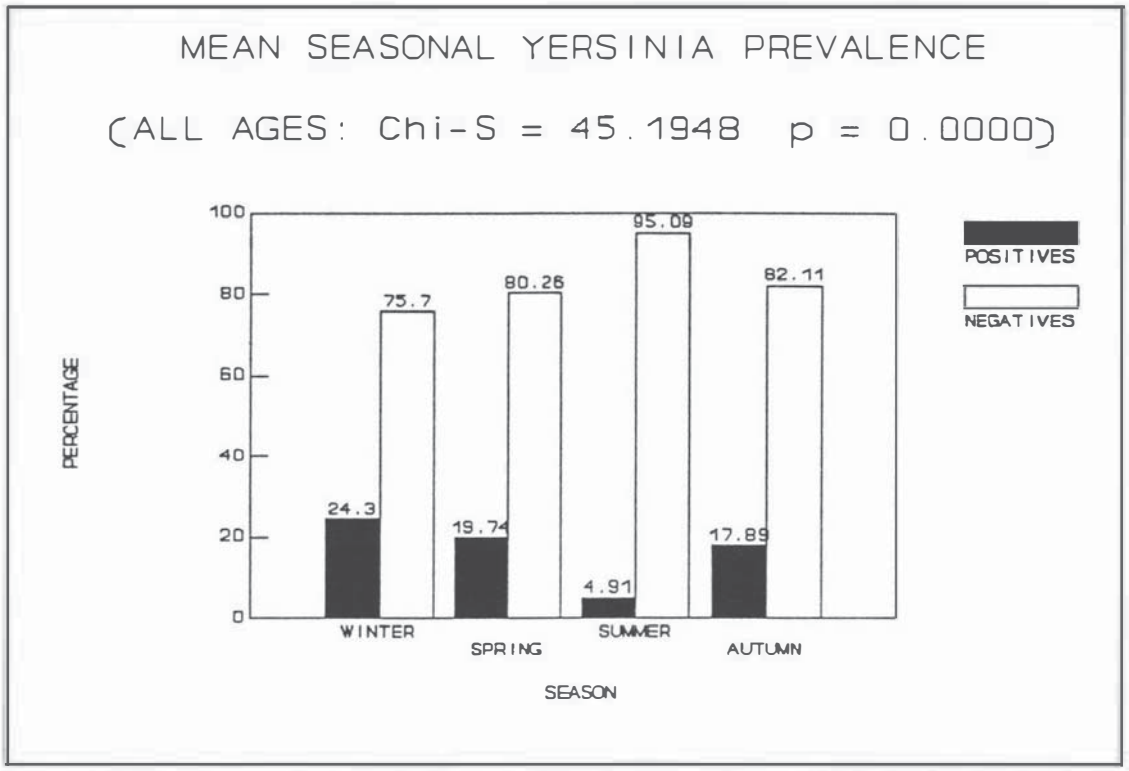


Figure 6.19. Mean Seasonal *Yersinia* Species Prevalence (Whole Study Period)

isolated only from Flocks A and C. Flock B was the sole source of all the *Y. enterocolitica* biotypes 2 and 3 isolates as well as of all the *Y. rohdei* isolates. *Y. intermedia* was isolated only from Flock A.

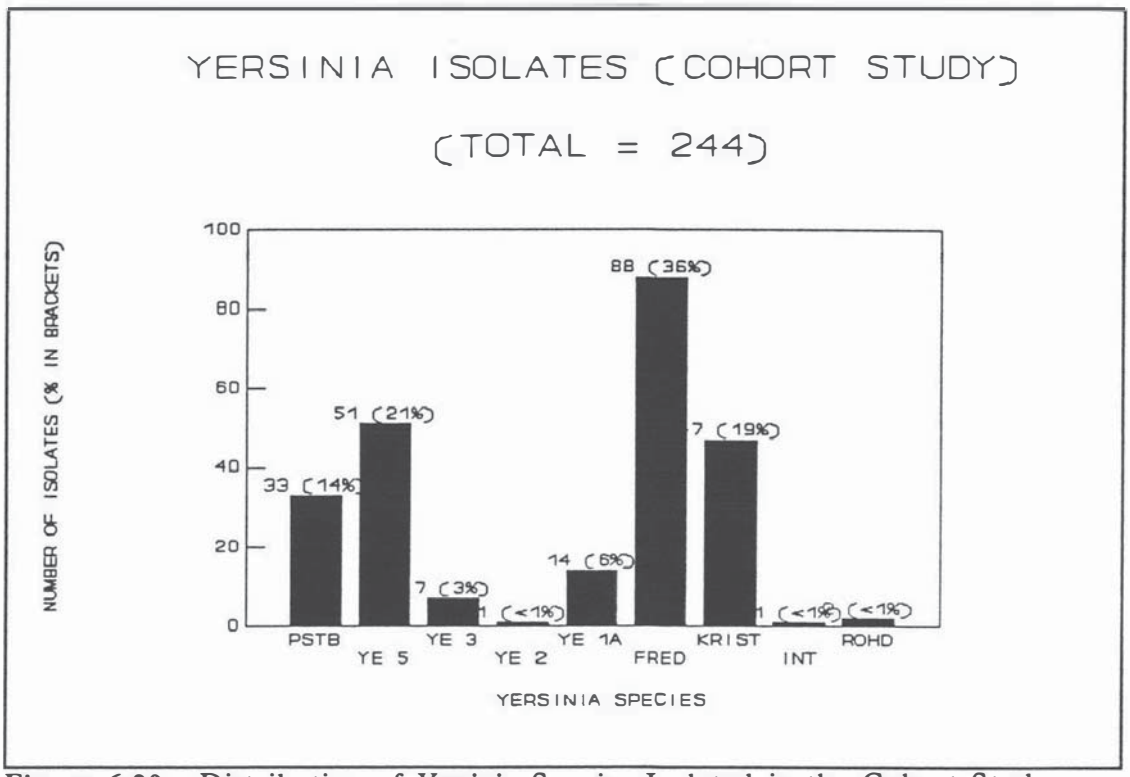


Figure 6.20. Distribution of *Yersinia* Species Isolated in the Cohort Study

Serotypes of *Y. pseudotuberculosis*. Of the 33 *Y. pseudotuberculosis* isolates recovered from goats during the course of the study, 32 were sent to Tottori University in Japan for serogrouping, and the only remaining isolate not sent to Japan was serogrouped at the microbiology laboratory at Massey University.

The results of the serogrouping showed that, of the total, 28 (84.8%) belonged to serogroup 3; 2 (6.1%) belonged to serogroup 2B; and 3 (9.1%) were untypable.

Flock Distribution of Pathogenic and Environmental *Yersinia* Species Isolates

Figure 6.21 shows the distribution of the different classes of *Yersinia* species within the three different goat flocks. As shown, Flock C had the largest number of *Yersinia* species isolates, the majority of which were environmental species. The two other flocks had fewer isolates, with Flock A having more environmental than pathogenic strains. Flock B was different; in this flock, the pathogenic *Yersinia* species predominated. Chi-squared analysis of this distribution revealed significant differences between the three farms with respect to the *Yersinia* species isolates recovered. In the analysis, the number of pathogenic *Yersinia* species in Flock B accounted for 40% of the total chi-squared value (Chi-squared = 6.810 p = 0.0334).

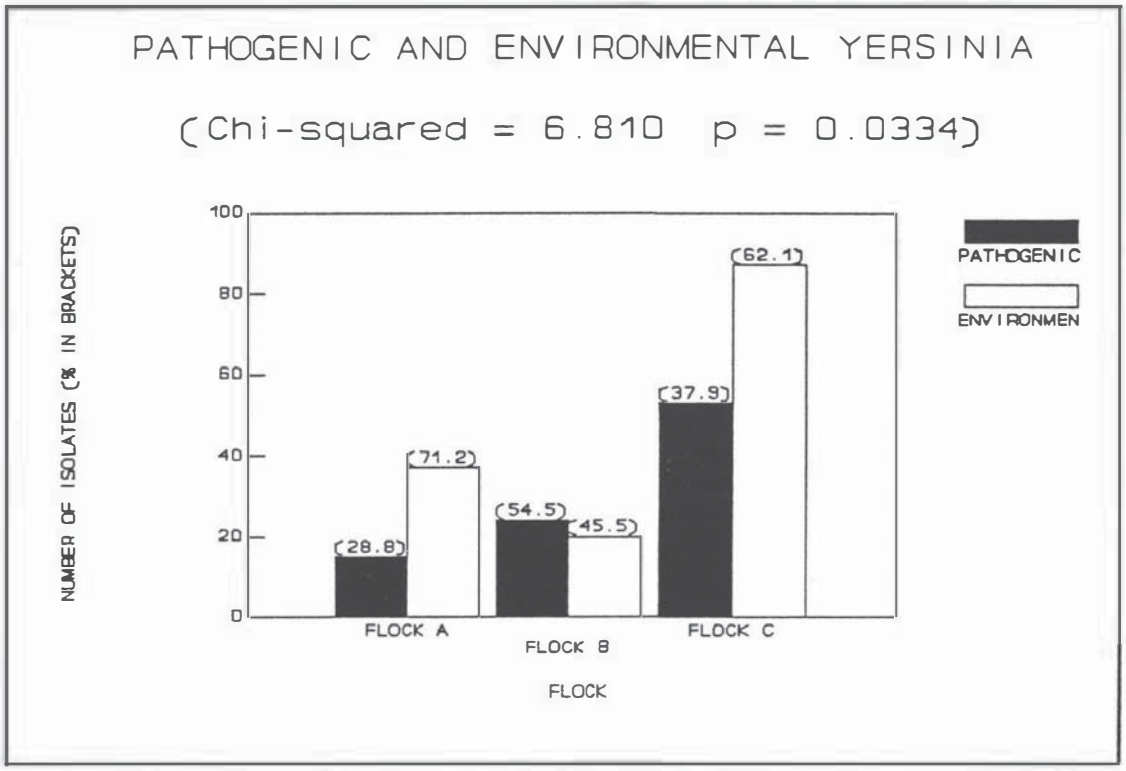


Figure 6.21. Flock Distribution of Pathogenic and Environmental *Yersinia* Species

Cohort Distribution of Pathogenic and Environmental *Yersinia* Species Isolates

Figure 6.22 shows the distribution of the pathogenic and environmental *Yersinia* species within the three different cohorts. In Cohort 1 (kids), the majority of the isolates (67.5%) belong to the pathogenic *Yersinia* species. In Cohort 2 (hoggets), the majority of the isolates turned out to be the environmental species. In Cohort 3 (adults), the environmental species were clearly predominant, constituting 83.9% of all the isolates in this cohort. As expected, the chi-squared analysis revealed highly significant differences in the distribution of the two different types of *Yersinia* in the different age groups (chi-squared = 45.8005 p = 0.0000).

Seasonal Isolations of *Yersinia* Species

Cohort 1. Among the animals in Cohort 1 from all the three flocks included in the study, a seasonal pattern can be seen in the distribution and number of *Yersinia* species isolated. In winter, the majority (92.6%) of all the isolates were the pathogenic species. This was the same picture presented during the autumn months, where 65.2% of the total isolates were pathogenic. In

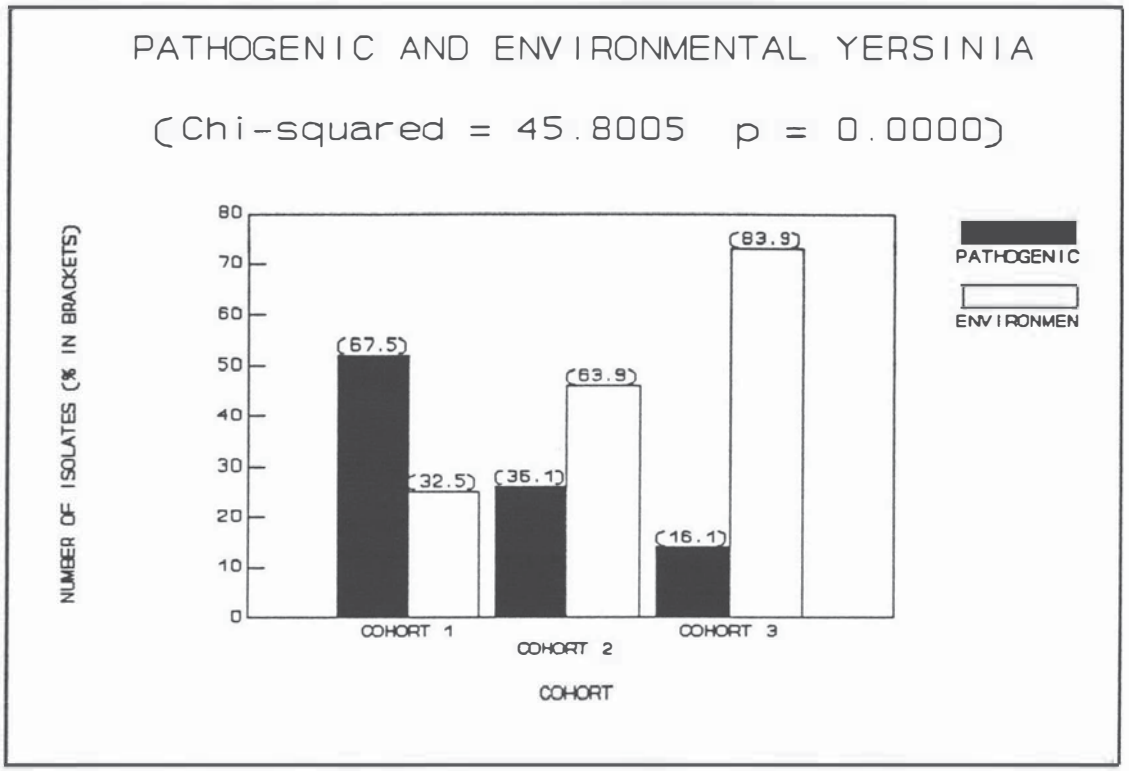


Figure 6.22. Cohort Distribution of Pathogenic and Environmental *Yersinia* Species

spring, however, the majority of the isolates (78.9%) were the environmental *Yersinia* species. During summer, only pathogenic species were isolated in this cohort. All these distributions revealed highly significant differences (chi-squared = 30.3564 p = 0.0000). **Figure 6.23** shows these distributions.

Cohort 2. During winter, the majority of the isolates (61.5%) belonged to the pathogenic *Yersinia* species. In spring, however, almost all (92.3%) of the isolates recovered belonged to the environmental species. Unlike Cohort 1, most of the autumn isolations (85.7%) were made up of the environmental *Yersinia* species, but the situation during summer in this cohort was the same - only pathogenic species were isolated. Again, the chi-squared analysis of the frequency distributions revealed very significant differences (chi-squared = 29.8939 p = 0.0000). **Figure 6.24** shows these distributions.

Cohort 3. In the adult animals from all the three flocks combined the environmental *Yersinia* species predominated in all the seasons of the year, showing insignificant seasonal differences in the distribution of the isolates (chi-squared = 5.564 p = 0.1359). A distinguishing feature in this cohort was the failure to isolate any *Yersinia* species during the summer season. **Figure 6.25** shows these distributions.

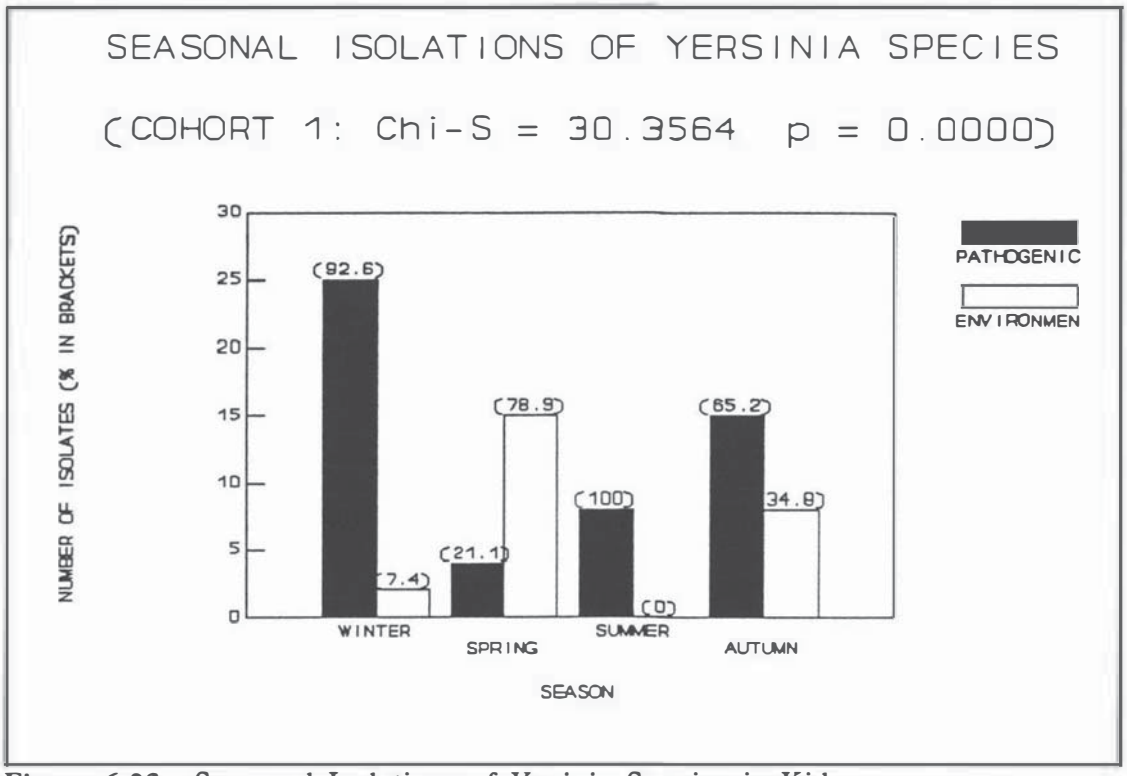


Figure 6.23. Seasonal Isolations of *Yersinia* Species in Kids

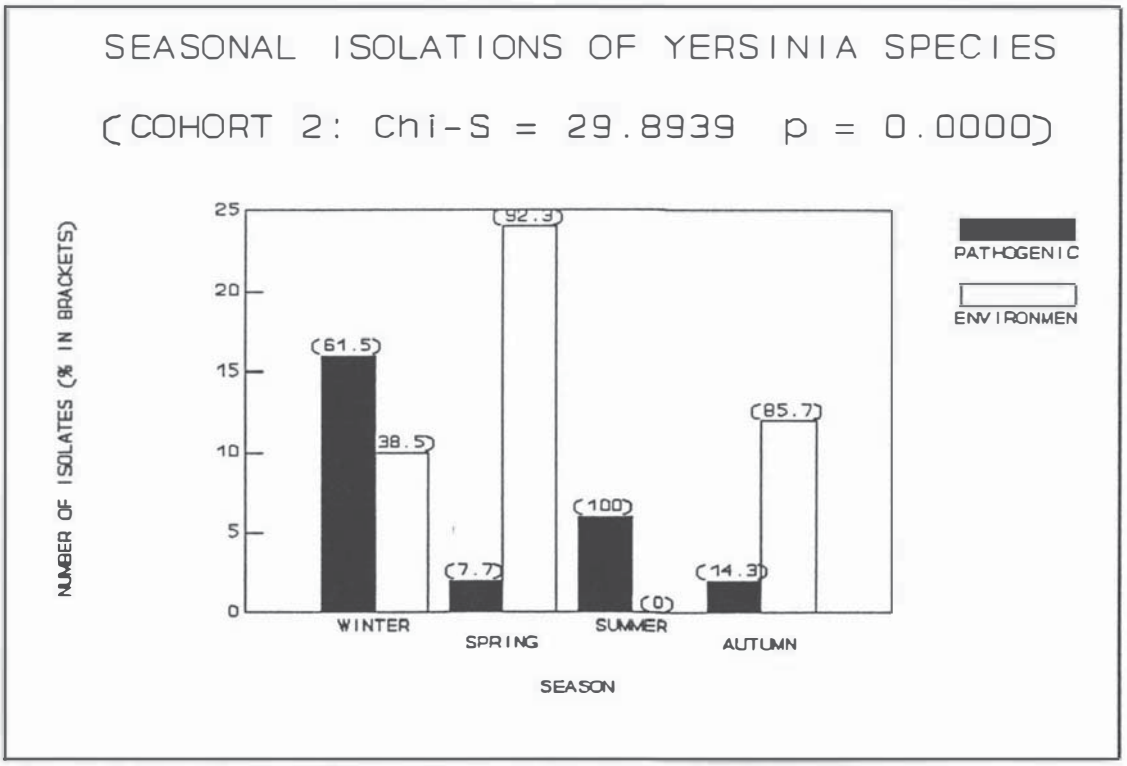


Figure 6.24. Seasonal Isolations of *Yersinia* Species in Hoggets

All ages combined. The overall distribution of the *Yersinia* species isolated with all the cohorts combined is shown in **Figure 6.26.** The combination of all

the age groups resulted in the pathogenic species being predominant only during winter, with the environmental species predominating during the other seasons of the year. The only exception was during the summer months, where no environmental *Yersinia* species were isolated from all the age groups. The chi-squared analysis revealed highly significant seasonal differences in the distribution of the isolates (chi-squared = 30.35.64 p = 0.0000).

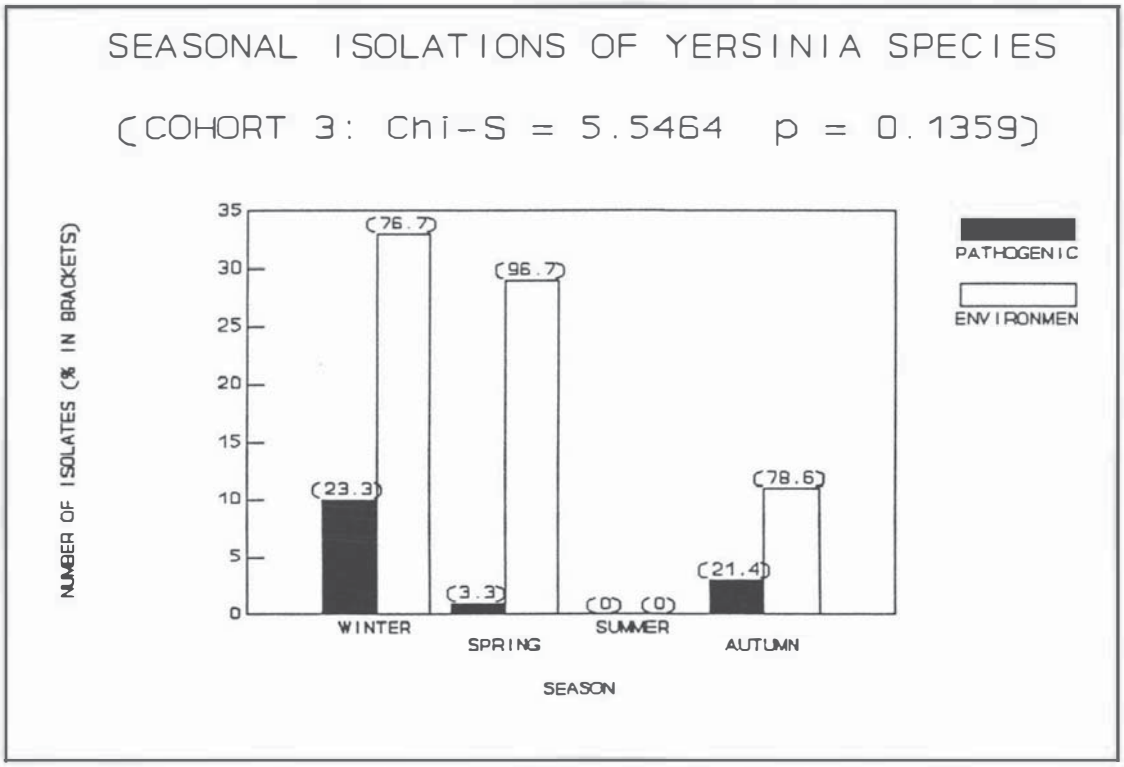


Figure 6.25. Seasonal Isolations of *Yersinia* Species in Adults

Apparent Duration of the Infection and Frequency of Reinfection

The durations of the infections with regard to specific *Yersinia* species were approximated by counting the number of consecutive collection times in which a particular animal had a particular *Yersinia* species recovered from its faecal material.

Y. pseudotuberculosis infection. Of the total of 33 isolations of this species throughout the course of the study, 16 (48.5%) were recovered by consecutive isolations from 7 individual animals, with the remainder coming from events in which there was at least one negative sampling before the animal became positive again (if ever). Six (85.7%) of these animals had *Y. pseudotuberculosis* isolated from faecal samples for 2 consecutive collection times and one shed the

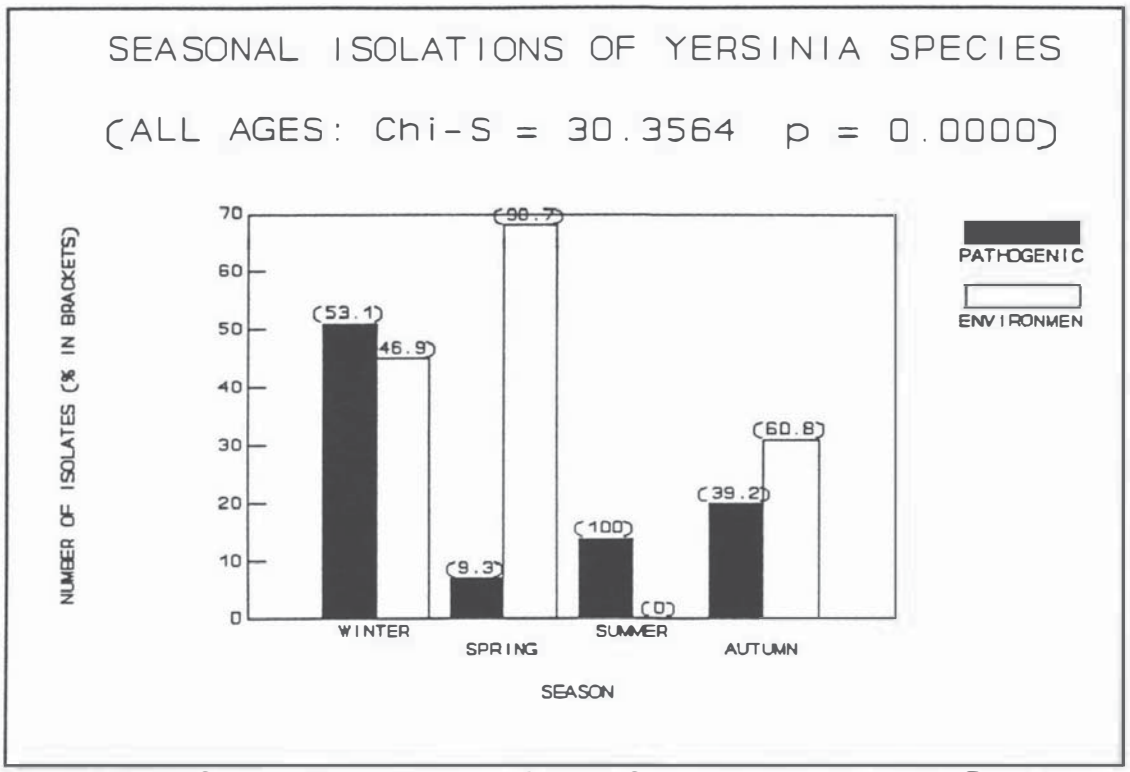


Figure 6.26. Seasonal Isolations of *Yersinia* Species from All Ages Combined

organism for 4 consecutive collection times. Thus from a total of 24 animals which became positive for this organism, 7 (29.2%) had maintained the infection for at least 28 days.

A significant feature of the infection of this species in goats was that, with the exception of the 7 mentioned animals, the other unconnected isolations occurred in these particular animals once only during the whole duration of the study. Thus, for example, if these animals were positive on the first collection time, they never became positive again on the succeeding collection times.

Y. enterocolitica biotype 5 infections. Twenty-five of the 51 (49%) isolations of this species from all ages of goats during the course of the study resulted from consecutive isolations from just 10 individual animals, while the remainder (51%) came from isolated events, the criterion for which was described in the preceding section. Eight of these 9 animals (88.9%) had 2 consecutive isolations of the organism from faecal material while another had 3. An exceptional case was described in one kid from flock C wherein *Y. enterocolitica* biotype 5 was isolated during 6 consecutive collection times. Thus of the total number of 38 animals which became positive for this species, 10 (26.3%) had maintained the infection for at least 28 days.

The significant feature described for *Y. pseudotuberculosis* infection with respect to the infection and subsequent reinfection of the animals also applied to infections of this species. In this case, the animals which became negative for the infection after being positive for a certain period never again became positive for the infection in the future collection times.

Y. frederiksenii infections. Twenty of the 83 isolations (24.1%) of this species resulted from two consecutive isolations from 10 animals, with the remainder (63.9%) coming from unconnected and isolated events in time either from these 10 animals or from others. The feature described for both *Y. pseudotuberculosis* and *Y. enterocolitica* biotype 5 infections, wherein positive animals were never found to be reinfected again, did not apply to the infections of this species. Animals which had become infected earlier became infected again during the other collection periods, although not consecutively.

Y. kristensenii infections. Eight of the 44 isolations (18.2%) came from repeated isolations from 4 animals, with the remainder coming from other unconnected events in time from different animals. All these 4 animals shed *Y. kristensenii* for 2 consecutive collection times. Again, as with *Y. frederiksenii*, animals which had been infected previously were found to have been reinfected again at later collection dates.

Other *Yersinia* species infections. The isolations of these species were too few to establish trends in infection and reinfection.

Discussion

The results of the prevalence survey (Chapter 5) were almost certainly minimum estimates, since the faecal samplings for that study were conducted during the warmer months of the year. This present study, therefore, attempted to find if variations in the incidence of *Yersinia* species infection do occur, and to identify factors which may influence these variations.

Variations in Incidence

To determine the presence of variations in *Yersinia* species incidence between and within cohorts in the three different goat flocks, the repeated measures regression procedure was chosen as the method of analysis for two main reasons. The first, as mentioned earlier, was that the design of this study

was basically a factorial with one factor measured repeatedly over time. The other reason was the non-orthogonality of the data, which precluded the use of standard analysis of variance.

Results of this analysis revealed distinct patterns and tendencies in the incidence of various *Yersinia* species in goats. It has been shown in this study that season, age and flock location all influence the incidence of *Yersinia* infection. The tendency of the pathogenic *Yersinia* species (particularly *Y. pseudotuberculosis* and *Y. enterocolitica* biotypes 2 and 3) to have distinct seasonal variations within the cohorts without significant between-cohort variation was shown as a result of this analysis, while there was, with the exception of the case of *Y. kristensenii*, no significant seasonal variations with significant between-cohort variation among the environmental types of *Yersinia*. These findings explain that while *Y. pseudotuberculosis* cases were distinctly seasonal (winter incidence mostly), infections occurred without regard to age (cohort) differences. These also explain why the incidence of *Y. frederiksenii* infections did not seem to have a significant seasonal pattern but then can mostly only be isolated from older animals.

The results of the monthly faecal collections from the three farms showed that, indeed, *Yersinia* species infection vary as the seasons change, with generally higher incidence levels during the colder months of the year. In the present study, such seasonal patterns were typically demonstrated in Flocks A and B. Flock C, however, had that peculiar peak of incidence in the summer months of 1988-1989, an event that was absent in the other two flocks. This then altered the typical cold-weather pattern of *Yersinia* infections in goats if the results of all these three flocks are taken together.

This minor aberration notwithstanding, the results of this study are congruent with parallel studies carried out on humans (Vandepitte and Wauters, 1979; De Groote *et. al.* 1982) as well as on other animal species. In a prospective study of *Yersinia* species infection in dogs, Fukushima *et. al.* (1984) reported that isolations were more frequent in winter and spring than they were in summer and autumn. In an earlier study among pigs, the same features of the infection were reported by Fukushima *et. al.* (1983), although seasonal variation was not reported by these authors in a highly contaminated farm.

Climatic Effects on Incidence

It is quite obvious that climatic factors are highly correlated with seasonal changes. This is particularly true in the Manawatu region of the North Island

in New Zealand, where winters are almost always associated with an abundance of rain. It was with the aim of identifying specific climatic factors that may influence the incidence of specific *Yersinia* species (or their combinations) that the stepwise regression procedure was performed using the climatic factors previously mentioned.

As mentioned earlier, the results of the repeated measures regression analysis showed distinct seasonal variations in the incidence of pathogenic *Yersinia* species, whereby infections usually occurred only during the cold season. This was explained by the results of the stepwise regression analysis, which showed why **MIN** was among the most important explanatory variables for the incidence of *Y. pseudotuberculosis*, *Y. enterocolitica* biotype 5 and *Y. enterocolitica* biotypes 2 and 3, either as the sole factor involved or in combination with others. While these species may prefer only cold conditions for proper growth, the rest of the *Yersinia* species usually have the other factors such as **RAIN**, **NRD**, **DEW** and other factors dealing with precipitation as the most significant explanatory variables. This may mean that these mostly "environmental" *Yersinia* are indeed environmental, that is, these are natural inhabitants of aquatic or terrestrial ecosystems which grow and thrive best when the conditions become ideal in the environment - wet, humid and cold. If this is true, then the detection of these environmental *Yersinia* species in the faeces may merely indicate transient passage through the gastrointestinal tract.

Mean Prevalence Levels

The overall mean prevalence level of 17.55% for the whole period of the study was comparable with the 14.97% level found during the prevalence survey.

Distribution of *Yersinia* Species Isolated in the Study

All the *Yersinia* species recovered in this study, with the exception of *Y. pseudotuberculosis*, *Y. enterocolitica* biotype 2 and *Y. rohdei*, were also previously recovered in the prevalence survey.

While the *Y. pseudotuberculosis* serogroup 3 isolates (melibiose-negative) are considered to be less virulent compared with the other serogroups of the species (M. Tsubokura, pers. comm.), evidence points to the ability of this serogroup to cause disease in goats (Hodges *et. al.*, 1984). This serogroup has also been found to be the most predominant *Y. pseudotuberculosis* strain isolated from cattle in New Zealand (Hodges *et. al.*, 1984), and has been incriminated in

cases of enterocolitis (Callinan *et. al.*, 1988; Slee *et. al.*, 1988) and abortion (Jerrett and Slee, 1989) in cattle in Australia.

Y. pseudotuberculosis serogroup 2B (melibiose-positive) is considered to be more virulent than serogroup 3. So far, it has been implicated only in a few cases of disease in cattle (actually serogroup 2) in New Zealand (Hodges *et. al.*, 1984) and has not, as yet been reported from goats in New Zealand.

The isolation of *Y. rohdei* appears to be the first isolation of this species from goats. The only previous isolations of this organism have been from human and dog faeces and surface water in Europe (Aleksic *et. al.*, 1987).

Approximation of the Duration of *Yersinia* Infection

The approximate estimation of the apparent duration of *Yersinia* infection in goats gave some insight into the carriage of these organisms by goats. The ability of some of these animals to shed *Yersinia* organisms in their faecal material for at least 28 days may mean, on one hand, that colonisation has occurred in the gastrointestinal tract of these animals and that determination of the presence of the organism can be achieved with high sensitivity by the ability to demonstrate these organisms in faecal material. On the other hand, these consecutive isolations from the same animals may have happened purely by chance, since some *Yersinia* species, especially the environmental strains, are natural inhabitants of aquatic and terrestrial ecosystems and may therefore exhibit only a transient passage through the gut. This aspect of the infection needs to be further elucidated, especially in the determination of the length of stay of these microorganisms in the gastrointestinal tract of these animals.

The failure to isolate *Y. pseudotuberculosis* and *Y. enterocolitica* biotype 5 from animals previously positive for these organisms for another time after they have become negative may mean that these animals have developed a degree of immunity against reinfection by these organisms. Such immunity would then be sufficient to inhibit intestinal colonisation by these organisms, resulting in the failure of their recovery from subsequent faecal material.

The ability of these two organisms to persist in the gastrointestinal tract may also mean that they have become established and have become capable of reproducing in sufficient numbers so as to be detected by faecal culture. The immune system of these animals may not, at the time of sampling, have been effective in countering the presence of these organisms.

These features as described for the pathogens cannot be said to apply to the infections caused by the environmental *Yersinia* strains. This is because some animals which were infected earlier again became positive for the same species at a later sampling date. Thus the presence of the microoorganisms in faecal material may only be a reflection of the status of these *Yersinia* species on pasture.

All these findings need to be further elucidated and proven by proper experimental methods, to clarify the mechanisms by which the epidemiological patterns described here are produced.

CHAPTER 7

CULTURAL, BIOCHEMICAL AND *IN VITRO* VIRULENCE CHARACTERISTICS OF *YERSINIA* SPECIES ISOLATED FROM GOATS

Introduction

In order to properly characterise members of a bacterial genus as heterogeneous as *Yersinia*, the growth of the various species on suitable media, as well as their biochemical characteristics must be fully described. In addition, to confirm their role in the pathogenesis of disease conditions, virulence tests must be performed. The following descriptions of growth and results of biochemical, as well as of the *in vitro* virulence tests are, therefore, attempts to achieve these ends.

Material and Methods

Bacterial Strains

A total of 379 strains of *Yersinia* species isolated from the prevalence survey (135) and from the cohort study (244) were tested biochemically using tests sufficient for the differentiation of the different *Yersinia* species and for the biotyping of *Y. enterocolitica*. Additional tests for *in vitro* determination of virulence characteristics were also performed.

In all, there were 33 strains of *Y. pseudotuberculosis*, 131 strains of *Y. enterocolitica* biotype 5, 8 strains of *Y. enterocolitica* bioytpe 3, 1 strain of *Y. enterocolitica* biotype 2, 15 strains of *Y. enterocolitica* biotype 1A, 130 strains of *Y. frederiksenii*, 54 strains of *Y. kristensenii*, 5 strains of *Y. intermedia*, and 2 strains of *Y. rohdei*. The strains from the prevalence survey were isolated during the period of February to June, 1988, and the strains from the cohort study were isolated during the period of July 1988 to November 1989. **Table 7.1** shows the distribution of the strains tested according to the source study.

Enrichment and Preliminary Screening Procedures

Cold enrichment of faecal samples and subsequent screening methods for the detection of *Yersinia* strains presented herein were described previously in Chapter 4. Additional tests, specifically tests for biotyping *Y. enterocolitica*, were described in Chapter 5.

In Vitro Virulence Tests

Testing for virulence markers was performed on all the *Yersinia* strains isolated using the following tests: autoagglutination at 37°C; calcium dependency at 37°C using magnesium oxalate agar (MOX); and combined calcium dependency and congo red binding using congo red-magnesium oxalate agar (CRMOX).

Autoagglutination. Testing for autoagglutination was carried out following the procedure described by Laird and Cavanaugh (1980), with slight modifications. Instead of the medium mentioned by these authors (RPMI-1640 medium with 10% calf serum and 25 nM HEPES), modified minimum essential Eagle medium (MEM, Flow Laboratories, U. K.) with 10% bovine foetal serum was used. Sterile tubes containing 2 ml. of the medium were inoculated with 48-hour growth of *Yersinia* strains grown on trypticase soy agar (TSA), and autoagglutination observed after 18 hours of incubation at 37°C.

Strains positive for this test showed irregular clumping at the sides of the tube, remaining clumped when the tube was shaken gently. Negative strains exhibited a uniform growth, characterised by settling at the bottom of the tube. When shaken gently, a uniform turbidity without distinct clump formation was seen (**Figure 7.1**). The full details of the procedure is outlined in Appendix IV.

Calcium dependency at 37°C. The requirement of calcium for the proper growth of some *Yersinia* strains has been shown to be associated with a virulence plasmid (Gemski *et. al.*, 1980). The demonstration of this characteristic is, however, dependent on temperature, and is usually only shown at 37°C. The procedure followed in the present study used magnesium oxalate agar (MOX) (Mair and Fox, 1986) for this test. Details of the test are given in Appendix IV.

Combined calcium dependency and congo red binding. The use of congo red as an indicator of potential virulence of *Yersinia* strains was proposed by Prpic *et. al.* (1983), where the authors were able to show that plasmid-bearing

Figure 7.1. Positive and Negative Reactions in the Autoagglutination Test (37°C)

colonies of *Y. enterocolitica* had the ability to bind congo red. Riley and Toma (1989) used this characteristic by modifying MOX agar to include congo red, thereby producing a medium (CRMOX agar) that was able to test for congo red binding and calcium dependency simultaneously.

The CRMOX plates were incubated at 37°C for 24 hours. Strains which were CRMOX-negative produced only large, colourless colonies (**Figure 7.2**). CRMOX-positve strains produced both small, red colonies and large, colourless colonies (**Figure 7.3**).

The full details of the procedure are outlined in Appendix IV.

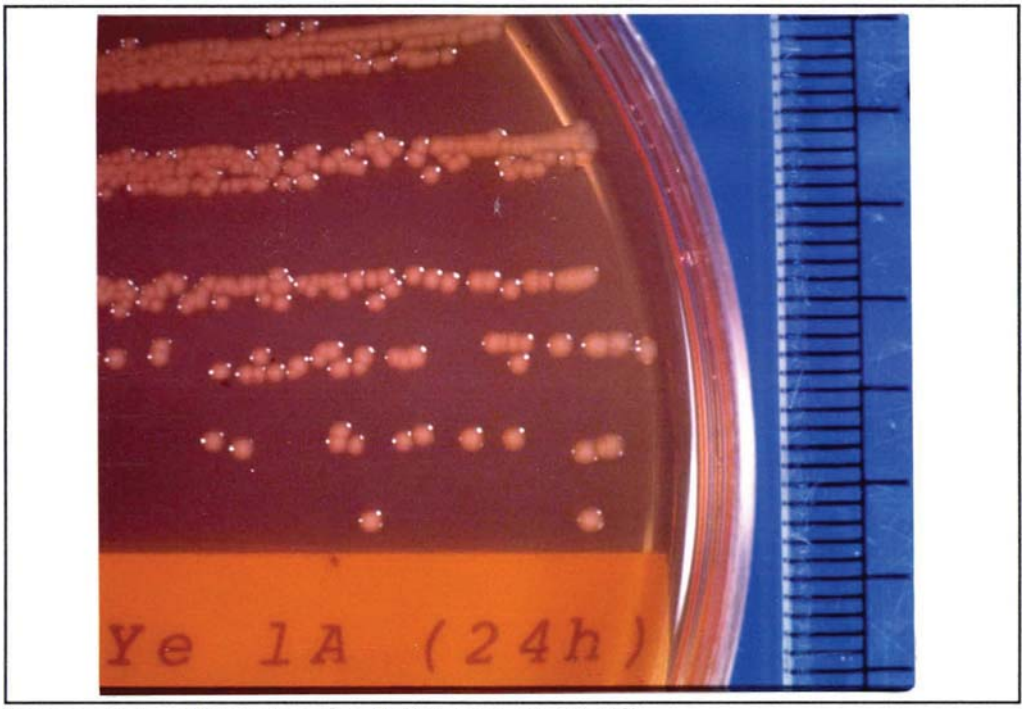


Figure 7.2. Negative CRMOX test at 37 °C

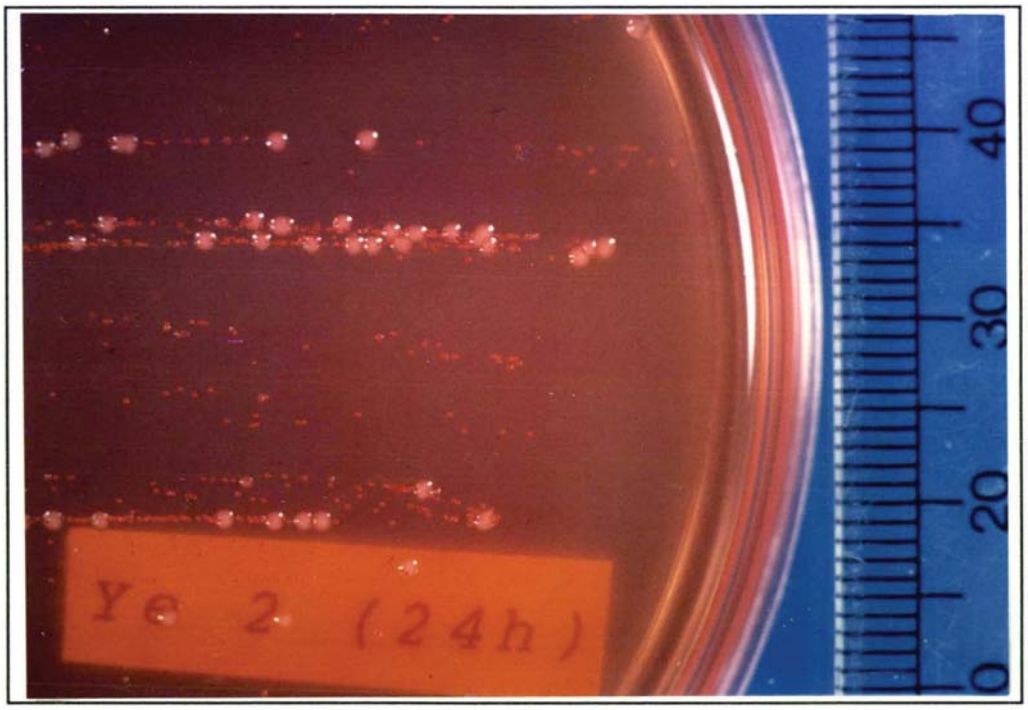


Figure 7.3. Positive CRMOX test at 37°C

Table 7.1. Numbers and Sources of *Yersinia* Strains Tested

Yersinia Species/Biotype	Prevalence Survey	Cohort Study	Overall
Y. pseudotuberculosis	-	33	33
Y. enterocolitica biotype 5	80	51	131
Y. enterocolitica biotype 3	1	7	8
Y. enterocolitica biotype 2	-	1	1
Y. enterocolitica biotype 1A	1	14	15
Y. frederiksenii	42	88	130
Y. kristensenii	7	47	54
Y. intermedia	4	1	5
Y. rohdei	-	2	2
TOTAL	135	244	379

Results

Growth on CIN Agar

All *Yersinia* species produce acid from mannitol, which is a constituent of CIN agar (Schiemann, 1979). As a result, *Yersinia* colonies growing on CIN appear typically reddish in colour due to the presence of neutral red as indicator in the medium. The different species and biotypes of *Yersinia* tested in this study, however, have some unique features with respect to their growth characteristics on CIN agar. It was thus possible, in many cases, to tell the different species of *Yersinia* apart by just examining their growth on this medium.

The following are the growth characteristics of the different *Yersinia* species encountered in both the prevalence survey and cohort study. Descriptions are of the 24- and 48-hour growth characteristics. In all such cases, the CIN plates were incubated at 29°C.

Colonies of *Y. pseudotuberculosis*. Twenty-four-hour colonies of *Y. pseudotuberculosis* were minute, from pinpoint to about 0.25 mm in diameter (**Figure 7.4**). The round colonies were coloured intense purplish red and had a rather dry appearance. When grown to 48 hours, the colonies got a little larger, with sizes ranging from 0.5 to 1 mm in diameter, and when incubated for a much

longer time, did not seem to grow much bigger when compared with their sizes at this incubation time.

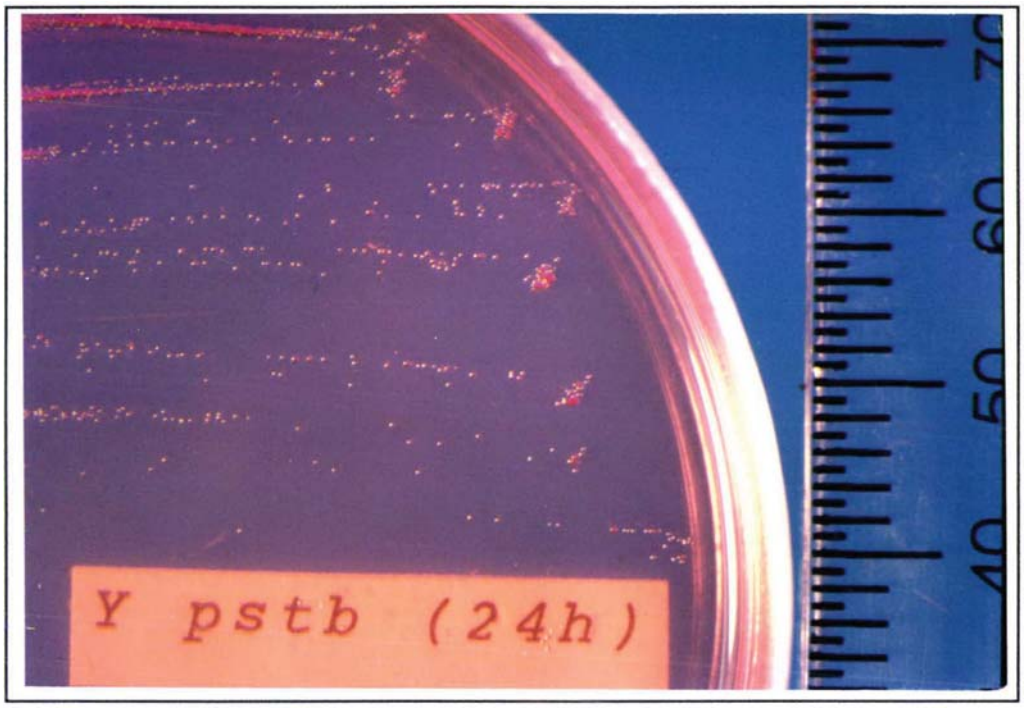


Figure 7.4. 24-hour Growth of *Y. pseudotuberculosis* on CIN (29°C)

The rather dry, leathery look was most pronounced at the 48-hour stage. The colonies were raised, flat, with entire borders. The transparent outer borders so commonly described in *Y. enterocolitica* were absent (**Figure 7.5**).

Colonies of *Y. enterocolitica* biotype 5. After 24 hours of incubation, colonies of *Y. enterocolitica* biotype 5 varied in size from about 0.25 to 1 mm in diameter, a little larger than the colonies of *Y. pseudotuberculosis* incubated for the same period of time (**Figure 7.6**). Colonies were coloured deep red, with

very thin translucent outside borders. These eventually disappear at 48 hours as the colonies grow larger, to about 1-2 mm in diameter (**Figure 7.7**). Unlike *Y. pseudotuberculosis* colonies, colonies of *Y. enterocolitica* biotype 5 were more moist and glistening.

Colonies of *Y. enterocolitica* biotypes 3, 2 and 1A. Colonies of these biotypes were very similar can be described as follows: After 24 hours incubation - small, about 0.5 to 2 mm in diameter, with raised, deep red centres surrounded by an outer zone of transparent border - the typical "bullseye-like" characteristic of *Y. enterocolitica* colony growth on CIN (**Figure 7.8**). Colony surfaces were usually glistening but not mucoid. After 48 hours of incubation

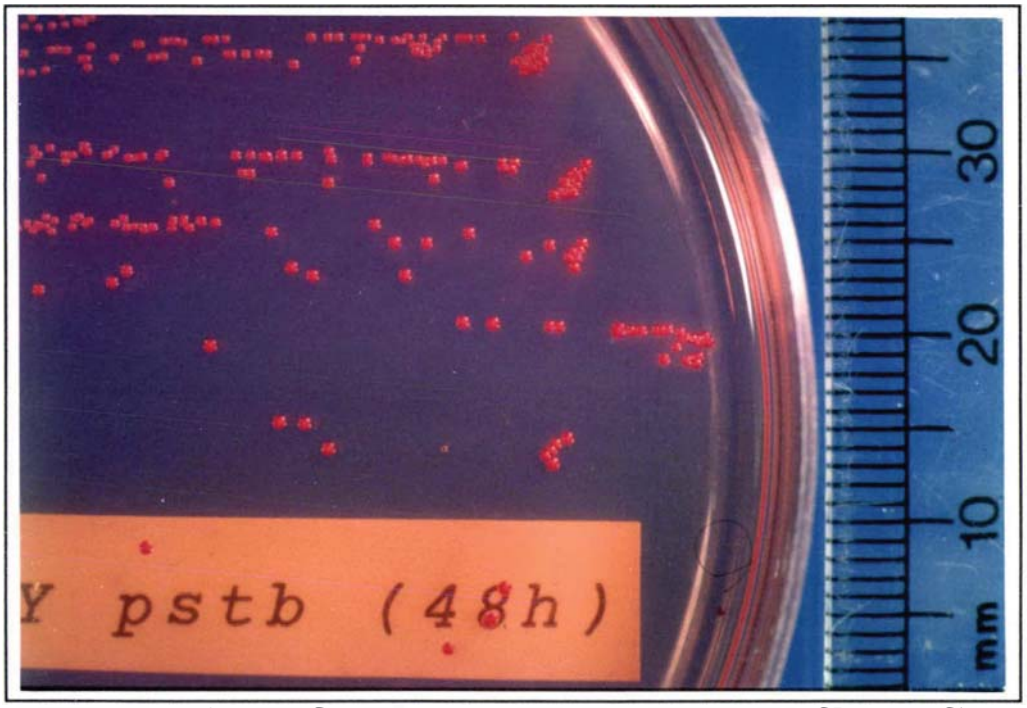


Figure 7.5. 48-hour Growth of *Y. pseudotuberculosis* on CIN (29°C)

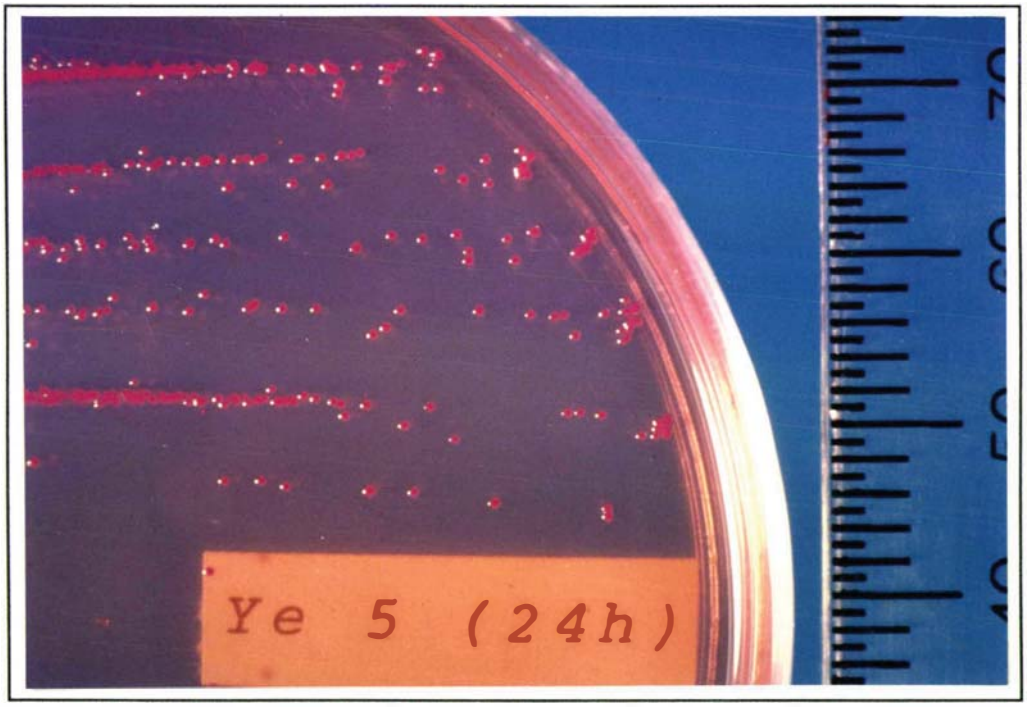


Figure 7.6. 24-hour Growth of *Y. enterocolitica* biotype 5 on CIN (29°C)

the colonies have increased in size considerably to about 2 to 5 mm in diameter (**Figure 7.9**). The same features (raised, deep red centres; transparent border) were retained, except for the pigmentation of the colonies, which tended to turn to yellow at this stage. In general, descriptions of the growth of these biotypes agree with growth features described elsewhere (Schiemann, 1979; Head *et. al.*, 1982).

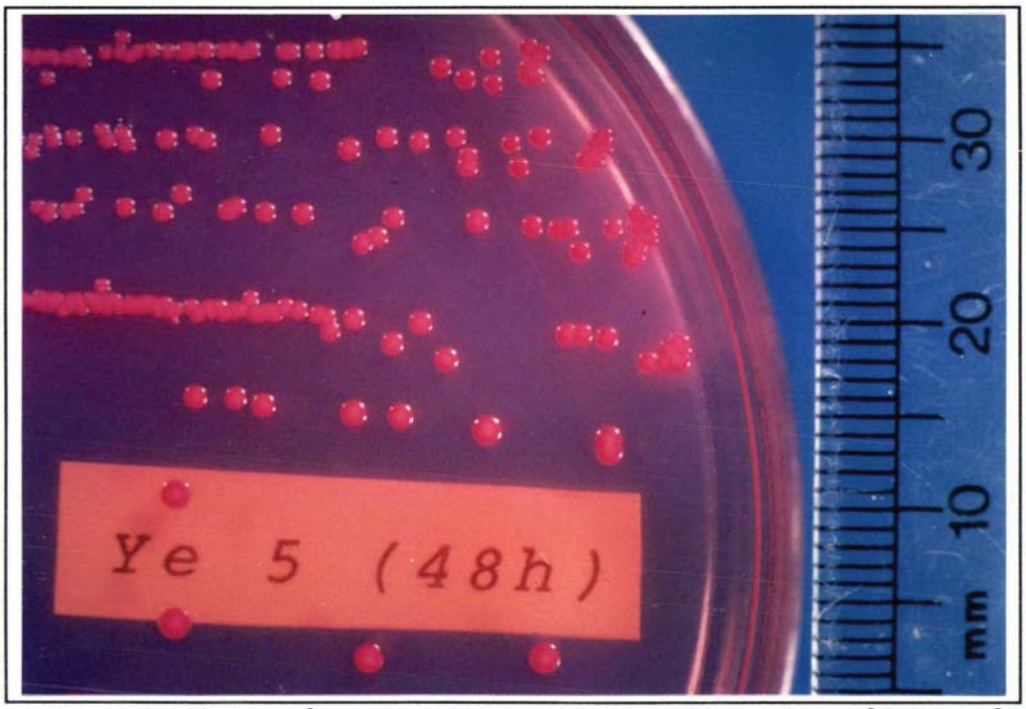


Figure 7.7. 48-hour Growth of *Y. enterocolitica* biotype 5 on CIN (29°C)

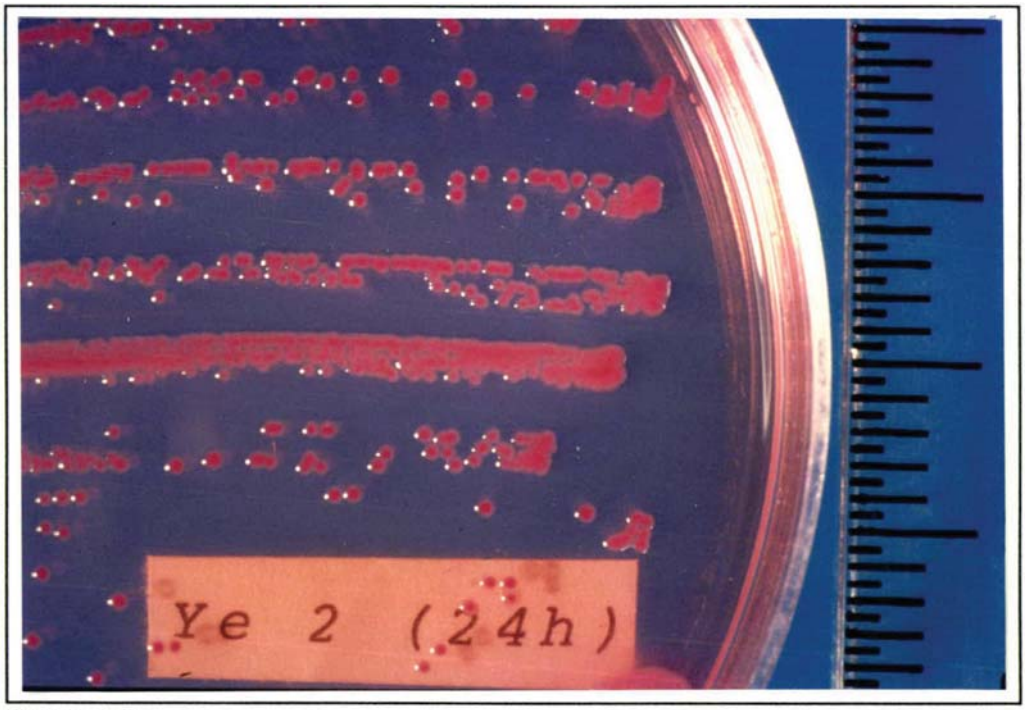


Figure 7.8. 24-hour Growth of *Y. enterocolitica* biotype 2 on CIN (29°C)

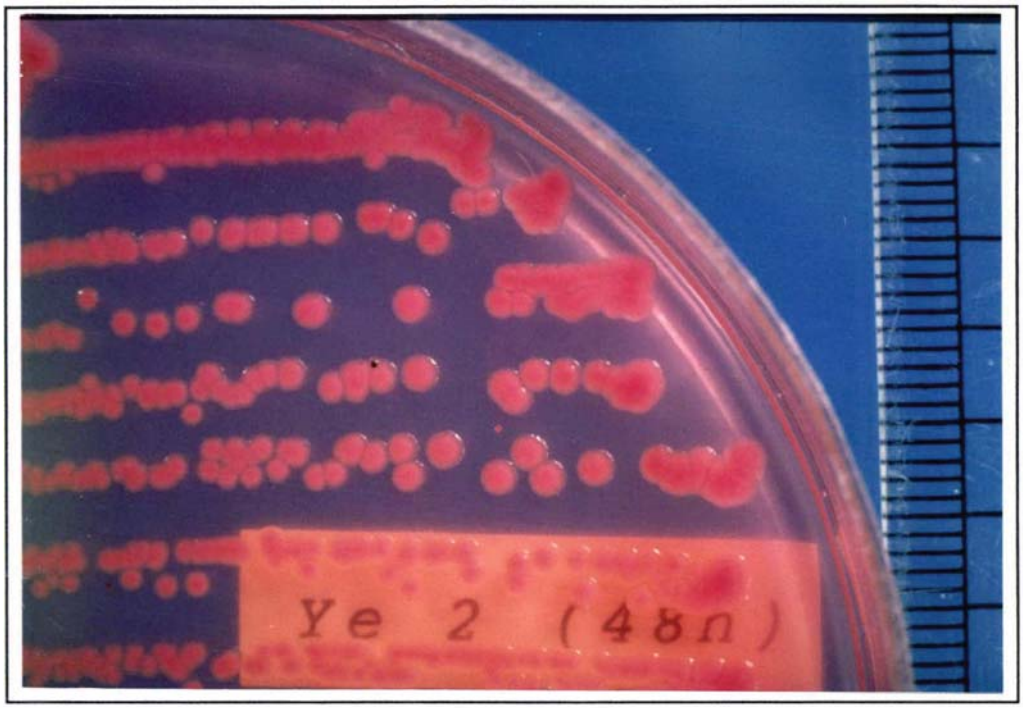


Figure 7.9. 48-hour Growth of *Y. enterocolitica* biotype 2 on CIN (29°C)

Colonies of *Y. frederiksenii*, *Y. kristensenii*, *Y. intermedia* and *Y. rohdei*. Colonies of these species tended to look similar. After 24 hours of incubation, colonies had almost the same features as the colonies of *Y. enterocolitica* biotypes 3, 2 and 1A, i. e., about 0.5 to 2 mm in diameter with deep red centres and transparent outer zones (**Figure 7.10**). Older colonies of these species had the same sizes as colonies of *Y. enterocolitica* biotypes 3, 2 and 1A at the same stage of incubation. After 48 hours of incubation, the red pigmentation of the colonies became more intense, with the transparent border disappearing. Often closer examination of the colonies revealed the presence of dark centre spots, much darker than the surrounding area.

Forty-eight hour colonies of these species were usually flat, unlike *Y. enterocolitica* biotypes 3, 2 and 1A colonies of the same age which usually had raised centres (**Figure 7.11**). The borders were also often irregular, as well as the overall shapes. Generally, colonies of these species were drier than colonies of *Y. enterocolitica* biotypes 3, 2 and 1A, although not as dry as colonies of *Y. pseudotuberculosis*. Zones in the medium on or near to the actual colony sites often had a characteristic reddish colouration or precipitate.

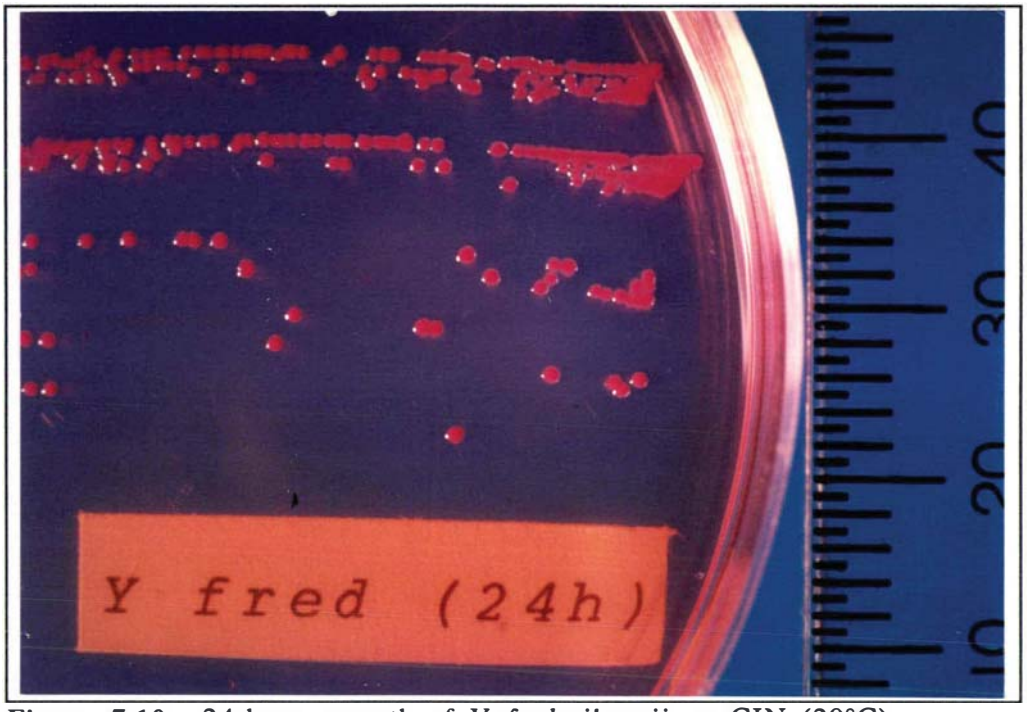


Figure 7.10. 24-hour growth of *Y. frederiksenii* on CIN (29°C)

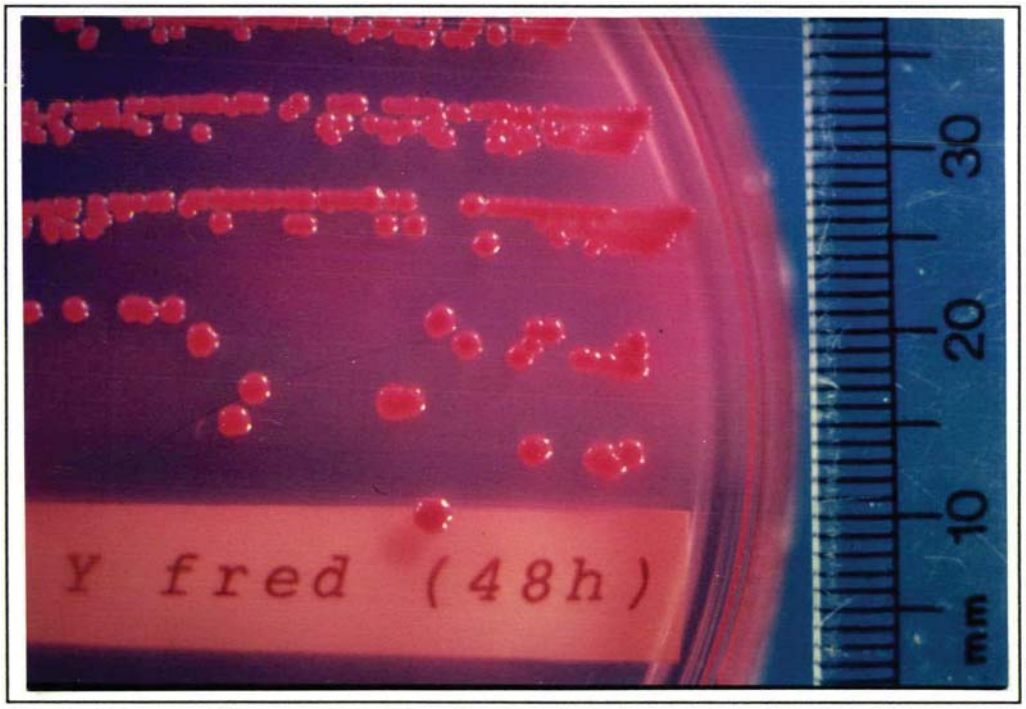


Figure 7.11. 48-hour Growth of *Y. frederiksenii* on CIN (29°C)

TSI Agar Reactions

Examination of 24- and 48-hour cultures of TSI agar inoculated with the above-mentioned *Yersinia* species revealed 2 distinct patterns. One pattern was the acid-slant/acid-butt reaction (A/A), with no gas or H_2S produced (**Figure 7.12**). This type of pattern was a characteristic of all strains of *Y. enterocolitica*

(all biotypes), *Y. frederiksenii*, *Y. intermedia* and *Y. rohdei*.

Another pattern exhibited was the alkaline-slant/acid-butt reaction (K/A), with no gas, no H_2S produced (**Figure 7.13**). This was the characteristic of all the *Y. pseudotuberculosis* and *Y. kristensenii* strains tested.

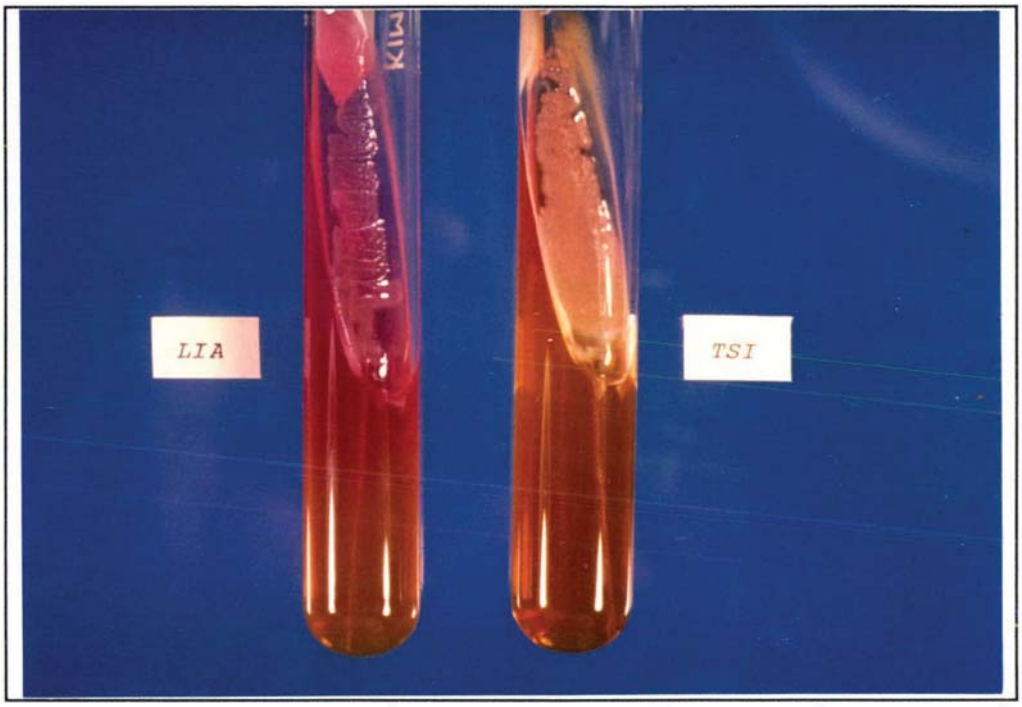


Figure 7.12. Reactions of *Y. enterocolitica* (all biotypes), *Y. frederiksenii*, *Y. intermedia* and *Y. rohdei* in TSI and LIA

LIA Agar Reactions

All the species and biotypes of *Yersinia* studied produced only one pattern on LIA - the alkaline-slant/acid-butt (K/A) reaction (**Figures 7.12 and 7.13**).

Biochemical Reactions

Y. pseudotuberculosis strains. The *Y. pseudotuberculosis* strains tested were grouped according to the serotypes to which they belong. Serotyping was performed, as mentioned in Chapter 6, by Professor Misao Tsubokura of Tottori University, Japan, on 32 of these isolates. The remaining isolate was serotyped at the veterinary microbiology laboratory at Massey University.

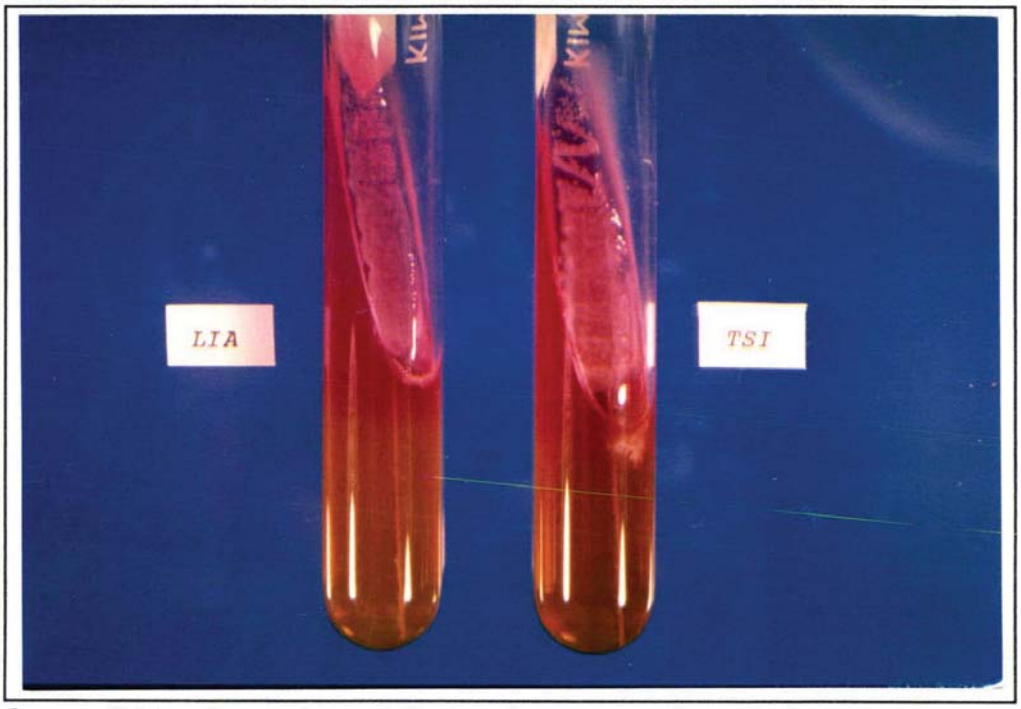


Figure 7.13. Reactions of *Y. pseudotuberculosis* and *Y. kristensenii* on TSI and LIA

Twenty-eight (84.8%) of the *Y. pseudotuberculosis* strains were shown to belong to serogroup 3, and 2 (6%) to serogroup 2B. Three (9%) of the strains were untypable. The results of the 34 cultural and biochemical tests (including *in vitro* virulence tests) for all of these strains are shown in **Table 7.2.**

Table 7.2. Biochemical Characteristics of *Y. pseudotuberculosis* Isolates

TEST	Serogroup 3 (28)	% +ve	Serogroup 2B (2)	% +ve	Untypable (3)	% +ve
Urea	+	100	+	100	+	100
Acetoin	-	0	-	0	-	0
Arginine	-	0	-	0	-	0
Citrate	-	0	-	0	-	0
β-D-Glucosidase	+	100	+	100	+	100
Aesculin	+	100	+	100	+	100
Indole	-	0	-	0	-	0
Lipase	-	0	-	0	-	0
Lysine	-	0	-	0	-	0
Malonate	-	0	-	0	-	0
Methyl Red	+	100	+	100	+	100
Motility	+	100	+	100	+	100
Nitrate	+	100	+	100	+	100
Ornithine	-	0	-	0	-	0
Phenylalanine	-	0	-	0	-	0
Proline	+	100	+	100	+	100
Pyrazinamidase	-	0	-	0	-	0
Adonitol	-	0	-	0	-	0
D-cellobiose	-	0	-	0	-	0
Lactose	-	0	-	0	-	0
Maltose	+	100	+	100	+	100
Mannitol	+	100	+	100	+	100
D-melibiose	-	0	+	100	-	0
α-Methyl-D-glucoside	-	0	-	0	-	0
D-raffinose	-	0	-	0	-	0
L-rhamnose	+	96	+	100	V	66
Salicin	+	100	-	0	-	0
D-sorbitol	-	0	-	0	-	0
Sucrose	-	0	-	0	-	0
D-trehalose	+	100	+	100	+	100
D-xylose	+	100	+	100	+	100
AA (37°C)	+	100	+	100	+	100
CD (37°C)	+	100	+	100	+	100
CRMOX (37°C)	+	100	+	100	+	100

Note: All tests were performed at 29°C unless otherwise specified. Data are calculated for 1-7 days incubation period. For further details see text. Number of strains tested in brackets.

Key: + = 90-100% positive; V = 26-75% positive; and - = 0-10% positive.

All the strains from all the serogroups tested showed positive reactions in the following tests: urease production, β-D-glucosidase, aesculin, methyl red, motility, nitrate reduction and proline peptidase, as well as acid production from maltose, mannitol, D-trehalose and D-xylose. All the strains were also positve for autoagglutination at 37°C, calcium dependency at 37°C, and congo red uptake at 37°C using CRMOX agar.

All the *Y. pseudotuberculosis* strains were negative for the following tests: acetoin production (Voges-Proskauer test), ornithine decarboxylase, arginine dihydrolase, citrate utilisation (Simmon's), indole production, lipase (Tween 80), lysine decarboxylase, phenylalanine, and pyrazinamidase. All were likewise negative for acid production from adonitol, D-cellobiose, lactose, α-methyl-D-glucoside, D-raffinose, D-sorbitol and sucrose.

Variable reactions between the different serogroups can be seen in the ability to produce acid from D-melibiose and salicin. All the serogroup 2B strains were positive for D-melibiose fermentation while all the strains in serogroup 3, as well as strains in the untypable group were negative. Only the serogroup 3 strains, meanwhile, were shown to ferment salicin, with the other serogroups unable to do so.

Variability between and within the serogroups occurred with respect to their ability to ferment L-rhamnose. While all the strains in serogroup 2B were positive in their ability to ferment this sugar, only 27 (96%) of the strains in serogroup 3 were positive for this reaction. Of the untypable *Y. pseudotuberculosis* strains, only 2 of the 3 (66%) were positive.

Y. enterocolitica strains. A total of 155 *Y. enterocolitica* strains were recovered from goats during the course of the 2 studies. Of these, 131 (84.5%) were *Y. enterocolitica* bioytpe 5, 8 (5.2%) were *Y. enterocolitica* biotype 3, 15 (9.7%) were biotype 1A, and one was *Y. enterocolitica* biotype 2. The results of the biochemical tests are shown in **Table 7.3**.

All the strains from all the biotypes of this species were found to be positive in the following reactions: urease production, motility, ornithine decarboxylase, and acid production from D-cellobiose and mannitol. Negative reaction for all strains rom all the biotypes were recorded in the following tests: arginine dihydrolase, citrate utilisation, lysine decarboxylase, malonate, phenylalanine, and acid production from adonitol, D-melibiose, α-methyl-D-glucoside, D-raffinose, and L-rhamnose. Almost all strains in all the biotypes were also negative for lactose fermentation, with the only exception being a single *Y. enterocolitica* biotype 5 strain recovered from Flock C in the cohort

study (12th sampling).

Y. enterocolitica biotypes 5, 3 and 2 are considered as potentially pathogenic biotypes, while *Y. enterocolitica* biotype 1A is considered an environmental biotype (Wauters *et. al.*, 1987). This can be readily seen in the reactions between the biotypes in the following biochemical tests, which are considered determinants of virulence in *Y. enterocolitica*, where negative reactions determine virulent strains: β-D-glucosidase, aesculin, lipase, proline peptidase, pyrazinamidase and salicin. The *in vitro* virulence tests at 37°C - autoagglutination, calcium dependency, and congo red uptake - provided further evidence to assist in the determination of potential pathogenicity.

Results of these pathogenicity tests on the different biotypes of *Y. enterocolitica* showed that, on one hand, almost all of the *Y. enterocolitica* biotype 1A strains studied were positive for the biochemical tests and negative for all the virulence tests, with exceptions in which one strain of bioytpe 1A failed to show positive reactions in proline peptidase and pyrazinamidase, and two strains failed to produce lipase. On the other hand, *Y. enterocolitica* biotypes 5, 3 and 2 were negative in all of the biochemical tests and positive in almost all of the *in vitro* virulence tests. The only exception in this case was the apparent inability of *Y. enterocolitica* biotype 5 to take up congo red (CRMOX agar), the uptake of which was considered indicative of potential virulence (Prpic *et. al.*, 1983; Riley and Toma, 1989).

All the strains in *Y. enterocolitica* biotypes 3, 2 and 1A were positive in their reactions in tests for nitrate reduction and in the production of acid from maltose, D-sorbitol and sucrose. *Y. enterocolitica* biotype 5 strains, however, were almost all negative (only 17% positive) in their ability to reduce nitrates to nitrites, and although the majority of strains in this biotype were able to ferment these three mentioned sugars, individual strain differences existed in their ability to do so.

The results of the Voges-Proskauer test (acetoin production) and the methyl red test varied between the individual strains of *Y. enterocolitica* biotype 5 (58% and 55% positive, respectively), while these tests were usually positive in the other biotypes of this species. Individual strain differences within the biotypes occurred, however, as shown by the negative Voges-Proskauer tests in 20% of the *Y. enterocolitica* biotype 1A strains and the negative methyl red tests in 14% of the *Y. enterocolitica* biotype 3 strains.

All the biotypes 2 and 1A strains were positive in their indole reaction, while biotype 5 (1% positive) and biotype 3 (0%) were usually negative. The

indole test is, in fact, used to differentiate biotype 3 (negative) and biotype 2 (positive) from each other, for these two have essentially the same reactions in all the other tests.

Y. frederiksenii strains. The reaction of *Y. frederiksenii* strains tested are shown in **Table 7.4**. The 130 strains identified as *Y. frederiksenii* were all positive for all of the following biochemical reactions: urease production, methyl red, motility, nitrate reduction, ornithine decarboxylase, proline peptidase, pyrazinamidase, and acid production from D-cellobiose, maltose, mannitol, L-rhamnose, salicin, D-sorbitol, sucrose, D-trehalose and D-xylose. Negative reactions for all the strains were recorded in the following tests: lysine decarboxylase, malonate utilisation, phenylalanine, and acid production from adonitol and D-melibiose.

Individual strain differences occurred within the species on the following tests: Voges-Proskauer (acetoin production), citrate utilisation, aesculin hydrolysis, indole and lipase production, and acid production from lactose. The Voges-Proskauer test showed very variable results (46% positive) in this species. The lipase test had a similar degree of variability (45% positive). All the *Y. frederiksenii* strains tested were, however, usually positive for both the aesculin and indole test (99% and 98% positive, respectively) and negative for citrate utilisation (14% positive). Also, lactose fermentation was not a feature of most of the *Y. frederiksenii* strains as only 2% were found positive in this test.

The *in vitro* virulence tests showed that this species is essentially lacking in virulence markers except for 4 strains which showed positive calcium dependency at 37°C using MOX agar. Examining the results of the other tests on these 4 strains revealed no significant deviations from the usual reactions of this species, except for negative lipase tests for 3 of these strains.

Table 7.3. Biochemical Characteristics of *Y. enterocolitica* Isolates

TEST	Biotype 5 (131)	% +ve	Biotype 3 (8)	% +ve	Biotype 2 (1)	% +ve	Biotype 1A (15)	% +ve
Urea	+	100	+	100	+	100	+	100
Acetoin	V	58	+	100	+	100	(+)	80
Arginine	-	0	-	0	-	0	-	0
Citrate	-	0	-	0	-	0	-	0
β-D-Glucosidase	-	0	-	0	-	0	+	100
Aesculin	-	0	-	0	-	0	+	100
Indole	-	1	-	0	+	100	+	100
Lipase	-	0	-	0	-	0	(+)	80
Lysine	-	0	-	0	-	0	-	0
Malonate	-	0	-	0	-	0	-	0
Methyl Red	V	55	(+)	86	+	100	+	100
Motility	+	100	+	100	+	100	+	100
Nitrate	(-)	17	+	100	+	100	+	100
Ornithine	+	100	+	100	+	100	+	100
Phenylalanine	-	0	-	0	-	0	-	0
Proline	-	0	-	0	-	0	+	93
Pyrazinamidase	-	0	-	0	-	0	+	93
Adonitol	-	0	-	0	-	0	-	0
D-cellobiose	+	100	+	100	+	100	+	100
Lactose	-	1	-	0	-	0	-	0
Maltose	(+)	77	+	100	+	100	+	100
Mannitol	+	100	+	100	+	100	+	100
D-melibiose	-	0	-	0	-	0	-	0
methylglucoside	-	0	-	0	-	0	-	0
D-raffinose	-	0	-	0	-	0	-	0
L-rhamnose	-	0	-	0	-	0	-	0
Salicin	-	0	-	0	-	0	+	100
D-sorbitol	+	97	+	100	+	100	+	100
Sucrose	+	95	+	100	+	100	+	100
D-trehalose	-	0	+	100	+	100	+	100
D-xylose	+	92	+	100	+	100	+	100
AA (37°C)	+	93	+	100	+	100	-	0
CD (37°C)	+	99	+	100	+	100	-	0
CRMOX (37°C)	-	1	+	100	+	100	-	0

Note: All tests were performed at 29°C unless otherwise specified. Data are calculated for 1-7 days incubation period. For further details see text. Number of strains tested in brackets.

Key: + = 90-100% positive; (+) = 76-89% positive; V = 26-75% positive; (-) = 11-25% positive; and - = 0-10% positive.

Y. kristensenii strains. **Table 7.4** shows the reactions of the *Y. kristensenii* strains tested. All of the 54 strains were shown to be positive in the following tests: urease production, methyl red test, motility, nitrate reduction, ornithine decarboxylase, and acid production from D-cellobiose, maltose, mannitol, D-sorbitol, D-trehalose and D-xylose. Negative reactions by all the strains studied were shown in tests for arginine dihydrolase, aesculin, lysine decarboxylase, malonate utilisation, phenylalanine, and acid production from adonitol, lactose, D-raffinose, L-rhamnose, and sucrose.

The reactions in the following tests were essentially positive (>90% positive) with a few individual strain variations: β-D-glucosidase, indole and pyrazinamidase. Reactions in the following tests were essentially negative (<10% positive): acetoin production, citrate utilisation, lipase, proline peptidase, and acid production from D-melibiose and salicin.

The *in vitro* virulence tests showed that *Y. kristensenii* strains were essentially devoid of plasmid-mediated virulence markers, except for 3 strains which showed calcium dependency at 37°C.

Y. intermedia strains. **Table 7.4** shows the test reactions of this species. All of the 5 *Y. intermedia* strains tested were positive in their reactions to the following tests: urease production, Voges-Proskauer test (acetoin production), β-D-glucosidase, aesculin, methyl red, motility, nitrate reduction, ornithine decarboxylase, proline peptidase, pyrazinamidase, and acid production from D-cellobiose, mannitol, D-melibiose, D-raffinose, salicin, D-sorbitol, sucrose and D-xylose. All the strains were negative for the following tests: indole, lipase, and acid production from lactose, maltose and L-rhamnose.

Variations in the ability of *Y. intermedia* to produce acid from L-rhamnose, D-melibiose, α-methyl-D-glucoside and D-raffinose, as well as the ability to utilise Simmon's citrate have been used to group *Y. intermedia* strains into, at first, 8 biotypes (Brenner *et. al.*, 1980), to which the addition of another biotype (biotype 9) has been suggested (Agbonlahor, 1986). Based on this biotyping scheme, 2 of the present *Y. intermedia* strains belonged to biotype 5. The remaining 3 could not be precisely fitted to any of the biotypes, but if these 3 had had positive citrate reactions they would have fitted nicely into biotype 3.

All the *Y. intermedia* strains studied were negative for virulence markers at 37°C.

Y. rohdei strains. The 2 Y. rohdei strains tested were isolated from goats in Flock B during the cohort study. Results of the tests showed that all these

strains were positive in their reactions in the following tests: urease production, β-D-glucosidase, methyl red, motility, nitrate reduction, ornithine decarboxylase, proline peptidase, pyrazinamidase, and acid production from D-cellobiose, maltose, mannitol, D-melibiose, D-raffinose, D-sorbitol, sucrose, D-trehalose and D-xylose. Negative reactions were exhibited by all strains in the following tests: Voges-Proskauer test (acetoin production), aesculin, indol, lipase, lysine decarboxylase, malonate, phenylalanine, and acid production from adonitol, lactose, L-rhamnose and salicin.

The only variable reactions encountered in this species were in their ability to utilise Simmon's citrate. One (50%) was found to be positive while the other was found to be negative.

The two strains of *Y. rohdei* were all negative for virulence markers at 37°C.

Table 7.4. Biochemical Characteristics of Other *Yersinia* Species Isolates

TEST	*Y. fred.* (130)	% +ve	*Y. krist.* (54)	% +ve	*Y. int.* (5)	% +ve	*Y. rohd.* (2)	% +ve
Urea	+	100	+	100	+	100	+	100
Acetoin	V	46	-	2	+	100	-	0
Arginine	-	0	-	0	-	0	-	0
Citrate	(-)	14	-	4	-	0	V	50
β-D-Glucosidase	+	100	+	94	+	100	+	100
Aesculin	+	99	-	0	+	100	-	0
Indole	+	98	+	91	(+)	80	-	0
Lipase	V	45	-	2	(+)	80	-	0
Lysine	-	0	-	0	-	0	-	0
Malonate	-	0	-	0	-	0	-	0
Methyl Red	+	100	+	100	+	100	+	100
Motility	+	100	+	100	+	100	+	100
Nitrate	+	100	+	100	+	100	+	100
Ornithine	+	100	+	100	+	100	+	100
Phenylalanine	-	0	-	0	-	0	-	0
Proline	+	100	-	4	+	100	+	100
Pyrazinamidase	+	100	+	93	+	100	+	100
Adonitol	-	0	-	0	-	0	-	0
D-cellobiose	+	100	+	100	+	100	+	100
Lactose	-	2	-	0	(-)	20	-	0
Maltose	+	100	+	100	(+)	80	+	100
Mannitol	+	100	+	100	+	100	+	100
D-melibiose	-	0	-	2	+	100	+	100
methylglucoside	-	0	-	0	-	0	-	0
D-raffinose	-	0	-	0	+	100	+	100
L-rhamnose	+	100	-	0	V	60	-	0
Salicin	+	100	-	7	+	100	-	0
D-sorbitol	+	100	+	100	+	100	+	100
Sucrose	+	100	-	0	+	100	+	100
D-trehalose	+	100	+	100	+	100	+	100
D-xylose	+	100	+	100	+	100	+	100
AA (37°C)	-	0	-	0	-	0	-	0
CD (MOX) (37°C)	-	3	-	6	-	0	-	0
CRMOX (37°C)	-	0	-	0	-	0	-	0

Note: All tests were performed at 29°C unless otherwise specified. Data are calculated for 1-7 days incubation period. For further details see text. Number of strains tested in brackets.

Key: + = 90-100% positive; (+) = 76-89% positive; V = 26-75% positive; (-) = 11-25% positive; and - = 0-10% positive.

Discussion

CIN Agar as the Plating Medium

In recent years, the use of CIN as a plating medium for the isolation of *Yersinia* species has become widespread, as can be seen by the numerous authors who have used CIN to isolate *Yersinia* species (Lewis and Chattopadhyay, 1986; Lynch, 1986; Simmonds *et. al.*, 1987; Bullians, 1987; Greenwood and Hooper, 1987; Buddle *et. al.*, 1988; Slee *et. al.*, 1988). This widespread use is certainly due to the effectiveness of this medium when compared with other specially formulated media for the recovery of *Yersinia* species (Head *et. al.*, 1982; Davey *et. al.*, 1983; Harmon *et. al.*, 1983).

There were however some disadvantages in the use of this medium. In the present study, the growth of other *Enterobacteriaceae* such as *Citrobacter*, *Enterobacter* and *Serratia* on the medium caused some confusion in the preliminary identification of *Yersinia* colonies as they were very similar to the appearance of certain *Yersinia* species colonies, with the exception of the colonies of *Y. pseudotuberculosis* and *Y. enterocolitica* biotype 5, which were much smaller. This necessitated the selection of many colonies from the primary inoculation plates for the screening procedure. This disadvantage was more apparent in 24-hour growths, since at this stage it was very difficult to tell the different *Yersinia* species from each other, as well as from the other enterobacterial species.

These difficulties often resulted in a further 24 hours incubation of the medium being necessary. This was because at 48 hours, differentiation of the different enterobacterial species from the *Yersinia* species was more readily accomplished, for the non-*Yersinia* species tended to show much larger colonies characterised by their reddish colour turning into whitish pink, and with the colony edges becoming irregular, unlike the *Yersinia* colonies which usually had entire edges. Nevertheless, it still took some experience to tell the colonies of the other *Enterobacteriaceae* apart from the *Yersinia* colonies, and reliance was never placed on visual examination alone. Even at this stage of incubation it was almost impossible to tell the other *Yersinia* species apart from each other, with the possible exceptions, again, of the *Y. pseudotuberculosis* and *Y. enterocolitica* biotype 5 colonies.

Another disadvantage of CIN was its slightly inhibitory effect on the growth of *Y. pseudotuberculosis*. The very small, pinpoint colonies exhibited by *Y. pseudotuberculosis* on CIN agar again necessitated another 24 hours of incubation

for proper characterisation. This was also the experience of Fukushima and Gomyoda (1986) who reported that maximal recovery of *Y. pseudotuberculosis* using CIN occurred only after 48 hours of incubation.

All these confusing colony characteristics resulted, in the present study, in the screening of about 3 or 4 colonies from each primary inoculation plate, usually taken from 48-hour colony growths. This involved a deal more time and effort, but usually avoided the frustrating situation of having a non-*Yersinia* strain being tested biochemically. Also, the necessity of using a large number of biochemical tests for the differentiation of *Yersinia* species and biotypes demanded that proper screening should have been done beforehand, to avoid wasting time and media.

Biochemical Characteristics of Yersinia Species

Y. pseudotuberculosis. According to Thal (1978), the "biochemical reactions of 1500 *Y. pseudotuberculosis* strains from the different serological groups from all over the world has proved to be homogeneous during a period of some 25 years." He, however, added that negligible deviations in a few reactions have been noted.

The most common deviations in the biochemical reactions of *Y. pseudotuberculosis* as found in this study were the inability of individual strains to ferment D-melibiose, L-rhamnose, and salicin. As shown in **Table 7.2**, serogroup 3 strains, as well as the untypable strains were non-fermenters of D-melibiose, and a few strains (one of each) of the same serogroups were non-fermenters of L-rhamnose. It was also shown that strains from the untypable group and serogroup 2B were non-fermenters of salicin.

The inability to ferment D-melibiose as a unique property of serogroup 3 has been pointed out in earlier studies. Tsubokura *et. al.* (1984a) reported that this characteristic was associated with non-virulence in mice, although Slee *et. al.* (1988) reported that the same serogroup, with the same inability to ferment D-melibiose, was capable of causing enterocolitis and diarrhoea among cattle in Australia. This serotype was also implicated in cases of illness and mortality in goats in New Zealand (Hodges *et. al.*, 1984a).

Y. enterocolitica. Biotyping schemes for *Y. enterocolitica* were developed due to the biochemical heterogeneity of the *Y. enterocolitica* strains. The biochemical properties of the 4 biotypes tested in the present study attested to this. Within the established biotypes, especially in biotypes 3, 2 and 1A, the

biochemical reactions were found to be quite homogeneous, with only a few individual strain variations in some of the biochemical tests. Thus, on the basis of these biochemical reactions, the delineation of the different biotypes was fairly straightforward.

Y. enterocolitica biotype 5 is considered an established biotype, having a well defined biochemical pattern (Bercovier *et. al.*, 1978). Bercovier *et. al.* (1980a) called strains belonging to this biotype the "hare" strains of Nilehn and Wauters, because they had been isolated only from hares in Europe and were considered to be biochemically inactive. The strains belonging to biotype 5 encountered in this study followed the reported biochemical reactions in most instances, with a few variations, as can be seen in **Table 7.3**.

The biochemical tests with the most variable reactions for this biotype were the Methyl Red test and the Voges-Proskauer test, wherein, respectively, only 55% and 58% of the strains studied were found to be positive. There were also a few strains which showed variable reactions in the nitrate reduction test (17% positive) and in the test for maltose fermentation (77% positive).

The variability of results in these four tests may possibly be due to their highly temperature-dependent nature, particularly the first two tests mentioned. For example, Krogstad (1975) reported that the *Y. enterocolitica* biotype 5 strains that he isolated from goats in Norway had varied reactions in the Voges-Proskauer test, where positive reactions were observed only at 22°C and not at 37°C. In the present study, the incubation temperature employed in the majority of test procedures was 29°C, about halfway between 22°C and 37°C.

Y. enterocolitica biotypes 3, 2 and 1A. The reactions of these biotypes were found to follow established reactions for the type species of *Y. enterocolitica* (Bercovier *et. al.*, 1980a) and for the reactions specific to the biotypes concerned (Wauters *et. al.*, 1987).

Y. frederiksenii. When compared with the type reactions for this species (Ursing *et. al.*, 1980), the biochemical reactions of the strains tested in this study were similar in almost all respects. Again, the only difference was in the reactions in the Voges-Proskauer test, where, unlike the reactions of the type species (98% positive), the results in the present study showed only 45% positive. This may, as in the case of *Y. enterocolitica* biotype 5, be a reflection of the temperature-dependence of this test.

Y. kristensenii. The *Y. kristensenii* strains tested in this present study agreed with almost all the biochemical reactions for this species (Bercovier *et.*

al., 1980b), the only difference being in the lipase test, where, in the present study, only 2% of the strains were positive. In the type reaction for this test, 74% of the strains tested were positive.

Y. intermedia. The major differentiating characteristic of this species, as shown previously was its ability to ferment α-Methyl-D-glucoside, although not all of the strains of this species were positive for this test (Brenner *et. al.*, 1980). As discussed earlier, this species, because of its heterogeneity, has been divided into several biotypes. Two of the strains tested in the present study were classified as biotype 5, but due to atypical reactions in the citrate test (negative), the other three cannot be fitted into any of the existing biotypes.

This variability in the reactions of *Y. intermedia* led Agbonlahor (1986) to propose the creation of another biotype of the species to accommodate the atypical strains he was able to isolate in Nigeria. Thus, the atypical strains tested in this study may belong to a new biotype.

Y. rohdei. The reactions of the 2 isolates of this species in the present study were typical of the type reactions for this species (Aleksic *et. al.*, 1987) except for variations in the citrate test and the fermentation of lactose, wherein the present strains were only 50% positive and 0% positive, respectively. In the type species reactions in these tests, 100% of the strains tested were positive for citrate utilisation after 7 days and 86% positive for lactose fermentation after the same period of time. In considering the type reactions in the other tests, however, the 2 strains in this study could be classified as belonging to biotype 2 of this species.

As mentioned in Chapter 6, the isolation of these strains appears to be the first isolation of this species from goats in New Zealand, or indeed worldwide. Further characterisation of these isolates, including DNA studies, are needed to firmly establish their identity, as atypical reactions in some tests were observed. Also, the significance of this species in goats needs to be further studied.

In Vitro Virulence Markers

The autoagglutination test (Laird and Cavanaugh, 1980), calcium dependency (Gemski *et. al.*, 1980), and congo red absorption (Prpic *et. al.*, 1983; Riley and Toma, 1989) were employed in the present study to determine potentially virulent strains. The results were consistent with the respective species and biotypes of *Yersinia*, except for a few variations. Thus *Y. pseudotuberculosis*, as well as *Y. enterocolitica* biotypes 3 and 2, were found to be

positive in all the three tests, and the majority of *Y. enterocolitica* biotype 1A and of the other *Yersinia* species were found to be negative.

Y. enterocolitica biotype 5, however, was found to be positive for autoagglutination and calcium dependency (MOX agar), but was apparently unable to absorb congo red. This inability to absorb congo red may be, on one hand, a natural characteristic of *Y. enterocolitica* biotype 5, which does not, in any way reduce its potential virulence. On the other hand, it may be an actual reflection of the general non-virulence of this biotype.

However, it has been mentioned earlier that this biotype has been shown to cause disease in goats (Krogstad, 1975; Buddle *et. al.*, 1988), and was shown, in this study, to have the property of autoagglutination at 37°C and calcium dependency at the same temperature. It is therefore necessary to do experimental studies on the virulence of this biotype to firmly establish its status as a pathogen, bearing in mind that in the present study, all of the isolates were taken from healthy animals.

CHAPTER 8

A NUMERICAL TAXONOMY STUDY OF *YERSINIA* SPECIES ISOLATED FROM GOATS

Introduction

The cultural and biochemical properties of the *Yersinia* strains included in this study formed the bases for the identification of these strains into species and/or biotypes. As there were numerous biochemical tests involved in the determination of these properties, it was often difficult to place individual strains into their proper categories, mainly due to the variability of individual strains in some tests. The use of a few key biochemical and *in vitro* virulence tests has always been the standard procedure for the identification and differentiation of the pathogenic and environmental species of *Yersinia* and has always been relied upon by diagnostic microbiologists.

Taxonomists, however, needed more than just a few key biochemical characteristics in order to classify the numerous *Yersinia* strains isolated from humans and animals. On top of that, techniques for handling large numbers of characteristics were needed to categorise these strains into logical groups. Numerical taxonomic methods were therefore employed for this purpose.

The demonstration of virulence *in vitro* has great epidemiological significance in bacterial infections. This is particularly true in the case of the genus *Yersinia*, not only because of the numerous species in the genus, but also because of the numerous biotypes and serotypes within each species which are heterogeneous in their biochemical reactions. It is extremely important, therefore, that the demonstration of *in vitro* virulence be considered in the classification of *Yersinia* species.

The aims of this study, therefore, were to determine if the various *Yersinia* strains isolated from goats could be properly placed into the species of *Yersinia* determined through DNA hybridisation techniques (see Chapter 2), and to determine if the various *Yersinia* strains from goats could be grouped according to their *in vitro* virulence properties.

Materials and Methods

Bacterial Strains Studied

A total of 388 strains of *Yersinia* species were examined in this study (**Table 8.1**). Of these, 244 were isolated during the cohort study and 135 were isolated during the prevalence survey. The remaining 9 strains were from clinical cases submitted to the veterinary microbiology laboratory at Massey University. The 388 strains were identified by standard classification methods (see Chapter 2) as *Y. pseudotuberculosis* (33 strains), *Y. enterocolitica* biotype 5 (140 strains), *Y. enterocolitica* biotype 3 (8 strains), *Y. enterocolitica* biotype 2 (1 strain), *Y. enterocolitica* biotype 1A (15 strains), *Y. frederiksenii* (130 strains), *Y. kristensenii* (54 strains), *Y. intermedia* (5 strains) and *Y. rohdei* (2 strains).

Microbiological Methods

The biochemical and *in vitro* virulence properties of the *Yersinia* strains derived from the prevalence survey and the cohort study were presented and discussed in Chapter 7, and the reactions of the remaining 9 strains were obtained from the veterinary microbiology laboratory at Massey University. Since some of the tests mentioned in Chapter 7 were not performed on the remaining 9 strains, the testing of the microbiological and *in vitro* virulence properties of these strains was repeated.

For numerical taxonomy purposes the reactions of the *Yersinia* strains on TSI and LIA were further characterised on the bases of the ability to produce the following reactions on TSI: acid slant, acid butt, alkaline slant, and alkaline butt; and on LIA: acid butt, alkaline slant, alkaline butt, and reddening of the slant.

All of the above-mentioned reactions, plus the biochemical and *in vitro* virulence reactions mentioned in Chapter 7 made up a total of 44 biochemical reactions which were analysed numerically.

Numerical Analysis

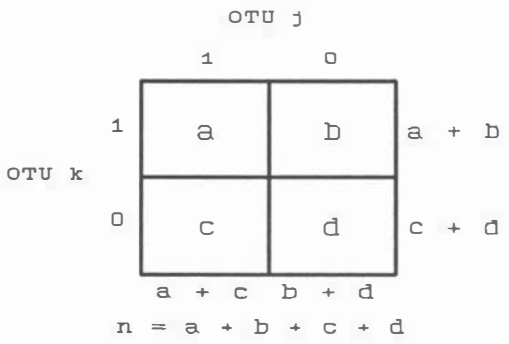
All the 44 biochemical characteristics were scored in binary form, using **1** for positive and **0** for negative. The data was arranged in a form of an **n × t**

matrix, where the **t** columns represented the different *Yersinia* strains studied and the **n** rows represented the **n** unit characters (biochemical properties). For the purposes of this study, each *Yersinia* strain studied represented a single operational taxonomic unit (**OTU**) (Sneath and Sokal, 1973). Thus, in all, 388 **OTUs** were studied for 44 characteristics.

Computation of the similarity matrix. All the characters were assigned equal weight. To estimate the degrees of similarity or dissimilarity between pairs of **OTUs**, the simple matching coefficient of Sokal and Michener (S_{SM}) was used (Sneath and Sokal, 1973). This coefficient is the ratio of the number of matches to the total number of characteristics in each pair of **OTUs**. S_{SM} has the following formula:

$$S_{SM} = \frac{a + d}{a + b + c + d}$$

where the letters **a**, **b**, **c** and **d** represent the traditional symbolism for **2 × 2** tables in statistics. An diagram of the **2 × 2** table is shown below.



The computations for this section were performed using the command **PROXIMITIES** in **SPSS^X** (Release 2.1)* and run in the Prime 9955 computer.

* SPSS Inc. 444 North Michigan Avenue, Chicago, Illinois 60611, U. S. A.

Table 8.1. List of *Yersinia* Strains Studied

Case Label	Species/Biotype	Source
1	*Y. intermedia*	Prevalence survey
2	*Y. enterocolitica* 5	Prevalence survey
3	*Y. enterocolitica* 5	Prevalence survey
4	*Y. enterocolitica* 5	Prevalence survey
5	*Y. enterocolitica* 5	Prevalence survey
6	*Y. enterocolitica* 5	Prevalence survey
7	*Y. enterocolitica* 5	Prevalence survey
8	*Y. enterocolitica* 5	Prevalence survey
9	*Y. enterocolitica* 5	Prevalence survey
10	*Y. enterocolitica* 5	Prevalence survey
11	*Y. enterocolitica* 5	Prevalence survey
12	*Y. enterocolitica* 5	Prevalence survey
13	*Y. enterocolitica* 5	Prevalence survey
14	*Y. enterocolitica* 5	Prevalence survey
15	*Y. enterocolitica* 5	Prevalence survey
16	*Y. enterocolitica* 5	Clinical case
17	*Y. frederiksenii*	Prevalence survey
18	*Y. enterocolitica* 3	Prevalence survey
19	*Y. enterocolitica* 5	Prevalence survey
20	*Y. enterocolitica* 5	Prevalence survey
21	*Y. enterocolitica* 5	Prevalence survey
22	*Y. frederiksenii*	Prevalence survey
23	*Y. enterocolitica* 5	Prevalence survey
24	*Y. enterocolitica* 5	Clinical case
25	*Y. kristensenii*	Prevalence survey
26	*Y. enterocolitica* 5	Prevalence survey
27	*Y. enterocolitica* 5	Prevalence survey
28	*Y. enterocolitica* 5	Prevalence survey
29	*Y. enterocolitica* 5	Prevalence survey
30	*Y. enterocolitica* 5	Prevalence survey
31	*Y. intermedia*	Prevalence survey
32	*Y. enterocolitica* 5	Prevalence survey
33	*Y. enterocolitica* 5	Clinical case
34	*Y. enterocolitica* 5	Prevalence survey
35	*Y. enterocolitica* 5	Prevalence survey
36	*Y. enterocolitica* 5	Prevalence survey
37	*Y. enterocolitica* 5	Clinical case
38	*Y. enterocolitica* 5	Prevalence survey
39	*Y. kristensenii*	Prevalence survey
40	*Y. enterocolitica* 5	Prevalence survey
41	*Y. enterocolitica* 5	Prevalence survey
42	*Y. enterocolitica* 5	Prevalence survey
43	*Y. enterocolitica* 5	Prevalence survey
44	*Y. enterocolitica* 5	Prevalence survey
45	*Y. enterocolitica* 5	Prevalence survey
46	*Y. enterocolitica* 5	Prevalence survey
47	*Y. frederiksenii*	Prevalence survey
48	*Y. enterocolitica* 5	Prevalence survey
49	*Y. frederiksenii*	Prevalence survey
50	*Y. enterocolitica* 5	Prevalence survey
51	*Y. enterocolitica* 5	Prevalence survey
52	*Y. enterocolitica* 5	Prevalence survey
53	*Y. enterocolitica* 5	Prevalence survey
54	*Y. enterocolitica* 5	Clinical case
55	*Y. enterocolitica* 5	Prevalence survey
56	*Y. frederiksenii*	Prevalence survey
57	*Y. frederiksenii*	Prevalence survey
58	*Y. frederiksenii*	Prevalence survey
59	*Y. frederiksenii*	Prevalence survey
60	*Y. frederiksenii*	Prevalence survey
61	*Y. kristensenii*	Prevalence survey

Table 8.1. (Continued. . .)

62	*Y. frederiksenii*	Prevalence survey
63	*Y. enterocolitica* 5	Prevalence survey
64	*Y. enterocolitica* 5	Prevalence survey
65	*Y. enterocolitica* 5	Prevalence survey
66	*Y. enterocolitica* 5	Prevalence survey
67	*Y. enterocolitica* 5	Prevalence survey
68	*Y. enterocolitica* 5	Prevalence survey
69	*Y. enterocolitica* 5	Prevalence survey
70	*Y. enterocolitica* 5	Prevalence survey
71	*Y. enterocolitica* 5	Prevalence survey
72	*Y. enterocolitica* 5	Prevalence survey
73	*Y. enterocolitica* 5	Prevalence survey
74	*Y. enterocolitica* 5	Prevalence survey
75	*Y. enterocolitica* 5	Prevalence survey
76	*Y. frederiksenii*	Prevalence survey
77	*Y. enterocolitica* 5	Prevalence survey
78	*Y. frederiksenii*	Prevalence survey
79	*Y. frederiksenii*	Prevalence survey
80	*Y. frederiksenii*	Prevalence survey
81	*Y. frederiksenii*	Prevalence survey
82	*Y. frederiksenii*	Prevalence survey
83	*Y. frederiksenii*	Prevalence survey
84	*Y. frederiksenii*	Prevalence survey
85	*Y. frederiksenii*	Prevalence survey
86	*Y. enterocolitica* 5	Prevalence survey
87	*Y. enterocolitica* 5	Prevalence survey
88	*Y. frederiksenii*	Prevalence survey
89	*Y. frederiksenii*	Prevalence survey
90	*Y. kristensenii*	Prevalence survey
91	*Y. kristensenii*	Prevalence survey
92	*Y. kristensenii*	Prevalence survey
93	*Y. kristensenii*	Prevalence survey
94	*Y. enterocolitica* 5	Prevalence survey
95	*Y. enterocolitica* 5	Clinical case
96	*Y. enterocolitica* 5	Prevalence survey
97	*Y. enterocolitica* 5	Prevalence survey
98	*Y. enterocolitica* 5	Prevalence survey
99	*Y. enterocolitica* 5	Prevalence survey
100	*Y. intermedia*	Prevalence survey
101	*Y. frederiksenii*	Prevalence survey
102	*Y. enterocolitica* 1A	Prevalence survey
103	*Y. enterocolitica* 5	Prevalence survey
104	*Y. enterocolitica* 5	Prevalence survey
105	*Y. enterocolitica* 5	Prevalence survey
106	*Y. enterocolitica* 5	Prevalence survey
107	*Y. enterocolitica* 5	Prevalence survey
108	*Y. enterocolitica* 5	Prevalence survey
109	*Y. enterocolitica* 5	Prevalence survey
110	*Y. enterocolitica* 5	Prevalence survey
111	*Y. enterocolitica* 5	Prevalence survey
112	*Y. frederiksenii*	Prevalence survey
113	*Y. frederiksenii*	Prevalence survey
114	*Y. frederiksenii*	Prevalence survey
115	*Y. frederiksenii*	Prevalence survey
116	*Y. intermedia*	Prevalence survey
117	*Y. frederiksenii*	Prevalence survey
118	*Y. frederiksenii*	Prevalence survey
119	*Y. frederiksenii*	Prevalence survey
120	*Y. enterocolitica* 5	Prevalence survey
121	*Y. enterocolitica* 5	Prevalence survey
122	*Y. enterocolitica* 5	Prevalence survey
123	*Y. frederiksenii*	Prevalence survey
124	*Y. enterocolitica* 5	Prevalence survey
125	*Y. enterocolitica* 5	Prevalence survey
126	*Y. enterocolitica* 5	Prevalence survey
127	*Y. enterocolitica* 5	Prevalence survey

Table 8.1. (Continued. . .)

128	*Y. enterocolitica* 5	Prevalence survey
129	*Y. frederiksenii*	Prevalence survey
130	*Y. frederiksenii*	Prevalence survey
131	*Y. frederiksenii*	Prevalence survey
132	*Y. frederiksenii*	Prevalence survey
133	*Y. frederiksenii*	Prevalence survey
134	*Y. frederiksenii*	Prevalence survey
135	*Y. frederiksenii*	Prevalence survey
136	*Y. frederiksenii*	Prevalence survey
137	*Y. frederiksenii*	Prevalence survey
138	*Y. frederiksenii*	Prevalence survey
139	*Y. frederiksenii*	Prevalence survey
140	*Y. frederiksenii*	Prevalence survey
141	*Y. kristensenii*	Cohort study
142	*Y. enterocolitica* 5	Cohort study
143	*Y. enterocolitica* 5	Cohort study
144	*Y. intermedia*	Cohort study
145	*Y. kristensenii*	Cohort study
146	*Y. enterocolitica* 1A	Cohort study
147	*Y. enterocolitica* 1A	Cohort study
148	*Y. frederiksenii*	Cohort study
149	*Y. enterocolitica* 1A	Cohort study
150	*Y. kristensenii*	Cohort study
151	*Y. kristensenii*	Cohort study
152	*Y. frederiksenii*	Cohort study
153	*Y. enterocolitica* 1A	Cohort study
154	*Y. frederiksenii*	Cohort study
155	*Y. frederiksenii*	Cohort study
156	*Y. enterocolitica* 1A	Cohort study
157	*Y. frederiksenii*	Cohort study
158	*Y. kristensenii*	Cohort study
159	*Y. kristensenii*	Cohort study
160	*Y. enterocolitica* 1A	Cohort study
161	*Y. frederiksenii*	Cohort study
162	*Y. frederiksenii*	Cohort study
163	*Y. enterocolitica* 1A	Cohort study
164	*Y. frederiksenii*	Cohort study
165	*Y. frederiksenii*	Cohort study
166	*Y. enterocolitica* 1A	Cohort study
167	*Y. enterocolitica* 1A	Cohort study
168	*Y. frederiksenii*	Cohort study
169	*Y. frederiksenii*	Cohort study
170	*Y. frederiksenii*	Cohort study
171	*Y. kristensenii*	Cohort study
172	*Y. enterocolitica* 5	Cohort study
173	*Y. enterocolitica* 5	Cohort study
174	*Y. enterocolitica* 5	Cohort study
175	*Y. enterocolitica* 1A	Cohort study
176	*Y. frederiksenii*	Cohort study
177	*Y. enterocolitica* 5	Cohort study
178	*Y. frederiksenii*	Cohort study
179	*Y. enterocolitica* 5	Cohort study
180	*Y. enterocolitica* 5	Clinical case
181	*Y. pseudotuberculosis*	Cohort study
182	*Y. enterocolitica* 3	Cohort study
183	*Y. pseudotuberculosis*	Cohort study
184	*Y. enterocolitica* 3	Cohort study
185	*Y. frederiksenii*	Cohort study
186	*Y. pseudotuberculosis*	Cohort study
187	*Y. pseudotuberculosis*	Cohort study
188	*Y. enterocolitica* 3	Cohort study
189	*Y. pseudotuberculosis*	Cohort study
190	*Y. pseudotuberculosis*	Cohort study
191	*Y. pseudotuberculosis*	Cohort study
192	*Y. enterocolitica* 3	Cohort study
193	*Y. pseudotuberculosis*	Cohort study

Table 8.1. (Continued. . .)

194	*Y. pseudotuberculosis*	Cohort study
195	*Y. kristensenii*	Cohort study
196	*Y. pseudotuberculosis*	Cohort study
197	*Y. pseudotuberculosis*	Cohort study
198	*Y. pseudotuberculosis*	Cohort study
199	*Y. pseudotuberculosis*	Cohort study
200	*Y. kristensenii*	Cohort study
201	*Y. kristensenii*	Cohort study
202	*Y. frederiksenii*	Cohort study
203	*Y. kristensenii*	Cohort study
204	*Y. frederiksenii*	Cohort study
205	*Y. frederiksenii*	Cohort study
206	*Y. frederiksenii*	Cohort study
207	*Y. frederiksenii*	Cohort study
208	*Y. frederiksenii*	Cohort study
209	*Y. frederiksenii*	Cohort study
210	*Y. frederiksenii*	Cohort study
211	*Y. frederiksenii*	Cohort study
212	*Y. frederiksenii*	Cohort study
213	*Y. frederiksenii*	Cohort study
214	*Y. frederiksenii*	Cohort study
215	*Y. frederiksenii*	Cohort study
216	*Y. kristensenii*	Cohort study
217	*Y. frederiksenii*	Cohort study
218	*Y. frederiksenii*	Cohort study
219	*Y. frederiksenii*	Cohort study
220	*Y. kristensenii*	Cohort study
221	*Y. frederiksenii*	Cohort study
222	*Y. frederiksenii*	Cohort study
223	*Y. frederiksenii*	Cohort study
224	*Y. frederiksenii*	Cohort study
225	*Y. kristensenii*	Cohort study
226	*Y. enterocolitica* 1A	Cohort study
227	*Y. frederiksenii*	Cohort study
228	*Y. frederiksenii*	Cohort study
229	*Y. frederiksenii*	Cohort study
230	*Y. frederiksenii*	Cohort study
231	*Y. frederiksenii*	Cohort study
232	*Y. frederiksenii*	Cohort study
233	*Y. frederiksenii*	Cohort study
234	*Y. kristensenii*	Cohort study
235	*Y. frederiksenii*	Cohort study
236	*Y. pseudotuberculosis*	Cohort study
237	*Y. frederiksenii*	Cohort study
238	*Y. frederiksenii*	Cohort study
239	*Y. enterocolitica* 5	Cohort study
240	*Y. enterocolitica* 5	Cohort study
241	*Y. enterocolitica* 5	Cohort study
242	*Y. enterocolitica* 5	Cohort study
243	*Y. enterocolitica* 5	Cohort study
244	*Y. enterocolitica* 5	Cohort study
245	*Y. enterocolitica* 5	Cohort study
246	*Y. enterocolitica* 5	Cohort study
247	*Y. enterocolitica* 5	Cohort study
248	*Y. enterocolitica* 5	Cohort study
249	*Y. enterocolitica* 5	Cohort study
250	*Y. enterocolitica* 5	Cohort study
251	*Y. frederiksenii*	Cohort study
252	*Y. enterocolitica* 5	Cohort study
253	*Y. enterocolitica* 5	Cohort study
254	*Y. enterocolitica* 5	Cohort study
255	*Y. enterocolitica* 5	Cohort study
256	*Y. enterocolitica* 5	Cohort study
257	*Y. frederiksenii*	Cohort study
258	*Y. enterocolitica* 1A	Cohort study
259	*Y. frederiksenii*	Cohort study

Table 8.1. (Continued. . .)

260	*Y. frederiksenii*	Cohort study
261	*Y. frederiksenii*	Cohort study
262	*Y. frederiksenii*	Cohort study
263	*Y. frederiksenii*	Cohort study
264	*Y. frederiksenii*	Cohort study
265	*Y. frederiksenii*	Cohort study
266	*Y. frederiksenii*	Cohort study
267	*Y. frederiksenii*	Cohort study
268	*Y. enterocolitica* 5	Cohort study
269	*Y. frederiksenii*	Cohort study
270	*Y. frederiksenii*	Cohort study
271	*Y. enterocolitica* 5	Cohort study
272	*Y. enterocolitica* 5	Cohort study
273	*Y. enterocolitica* 5	Cohort study
274	*Y. frederiksenii*	Cohort study
275	*Y. frederiksenii*	Cohort study
276	*Y. frederiksenii*	Cohort study
277	*Y. frederiksenii*	Cohort study
278	*Y. frederiksenii*	Cohort study
279	*Y. frederiksenii*	Cohort study
280	*Y. frederiksenii*	Cohort study
281	*Y. frederiksenii*	Cohort study
282	*Y. frederiksenii*	Cohort study
283	*Y. frederiksenii*	Cohort study
284	*Y. frederiksenii*	Cohort study
285	*Y. enterocolitica* 5	Cohort study
286	*Y. enterocolitica* 5	Cohort study
287	*Y. enterocolitica* 5	Cohort study
288	*Y. enterocolitica* 5	Cohort study
289	*Y. enterocolitica* 5	Cohort study
290	*Y. enterocolitica* 5	Cohort study
291	*Y. pseudotuberculosis*	Cohort study
292	*Y. enterocolitica* 5	Cohort study
293	*Y. enterocolitica* 5	Cohort study
294	*Y. frederiksenii*	Cohort study
295	*Y. enterocolitica* 5	Cohort study
296	*Y. enterocolitica* 5	Clinical case
297	*Y. enterocolitica* 5	Clinical case
298	*Y. enterocolitica* 5	Clinical case
299	*Y. enterocolitica* 5	Cohort study
300	*Y. enterocolitica* 5	Cohort study
301	*Y. enterocolitica* 5	Cohort study
302	*Y. enterocolitica* 5	Cohort study
303	*Y. enterocolitica* 5	Cohort study
304	*Y. enterocolitica* 5	Cohort study
305	*Y. enterocolitica* 5	Cohort study
306	*Y. frederiksenii*	Cohort study
307	*Y. kristensenii*	Cohort study
308	*Y. kristensenii*	Cohort study
309	*Y. frederiksenii*	Cohort study
310	*Y. pseudotuberculosis*	Cohort study
311	*Y. enterocolitica* 5	Cohort study
312	*Y. kristensenii*	Cohort study
313	*Y. pseudotuberculosis*	Cohort study
314	*Y. pseudotuberculosis*	Cohort study
315	*Y. kristensenii*	Cohort study
316	*Y. kristensenii*	Cohort study
317	*Y. pseudotuberculosis*	Cohort study
318	*Y. enterocolitica* 5	Cohort study
319	*Y. enterocolitica* 5	Cohort study
320	*Y. kristensenii*	Cohort study
321	*Y. kristensenii*	Cohort study
322	*Y. frederiksenii*	Cohort study
323	*Y. enterocolitica* 1A	Cohort study
324	*Y. frederiksenii*	Cohort study
325	*Y. enterocolitica* 3	Cohort study

Table 8.1. (Continued. . .)

326	*Y. rohdei*	Cohort study
327	*Y. kristensenii*	Cohort study
328	*Y. pseudotuberculosis*	Cohort study
329	*Y. enterocolitica* 5	Cohort study
330	*Y. pseudotuberculosis*	Cohort study
331	*Y. pseudotuberculosis*	Cohort study
332	*Y. pseudotuberculosis*	Cohort study
333	*Y. pseudotuberculosis*	Cohort study
334	*Y. pseudotuberculosis*	Cohort study
335	*Y. pseudotuberculosis*	Cohort study
336	*Y. kristensenii*	Cohort study
337	*Y. kristensenii*	Cohort study
338	*Y. kristensenii*	Cohort study
339	*Y. frederiksenii*	Cohort study
340	*Y. pseudotuberculosis*	Cohort study
341	*Y. pseudotuberculosis*	Cohort study
342	*Y. frederiksenii*	Cohort study
343	*Y. enterocolitica* 3	Cohort study
344	*Y. frederiksenii*	Cohort study
345	*Y. enterocolitica* 2	Cohort study
346	*Y. enterocolitica* 3	Cohort study
347	*Y. enterocolitica* 5	Cohort study
348	*Y. pseudotuberculosis*	Cohort study
349	*Y. pseudotuberculosis*	Cohort study
350	*Y. pseudotuberculosis*	Cohort study
351	*Y. kristensenii*	Cohort study
352	*Y. frederiksenii*	Cohort study
353	*Y. kristensenii*	Cohort study
354	*Y. pseudotuberculosis*	Cohort study
355	*Y. kristensenii*	Cohort study
356	*Y. frederiksenii*	Cohort study
357	*Y. frederiksenii*	Cohort study
358	*Y. enterocolitica* 5	Cohort study
359	*Y. rohdei*	Cohort study
360	*Y. kristensenii*	Cohort study
361	*Y. enterocolitica* 5	Cohort study
362	*Y. kristensenii*	Cohort study
363	*Y. kristensenii*	Cohort study
364	*Y. frederiksenii*	Cohort study
365	*Y. kristensenii*	Cohort study
366	*Y. kristensenii*	Cohort study
367	*Y. pseudotuberculosis*	Cohort study
368	*Y. kristensenii*	Cohort study
369	*Y. kristensenii*	Cohort study
370	*Y. kristensenii*	Cohort study
371	*Y. kristensenii*	Cohort study
372	*Y. kristensenii*	Cohort study
373	*Y. frederiksenii*	Cohort study
374	*Y. frederiksenii*	Cohort study
375	*Y. frederiksenii*	Cohort study
376	*Y. frederiksenii*	Cohort study
377	*Y. kristensenii*	Cohort study
378	*Y. kristensenii*	Cohort study
379	*Y. kristensenii*	Cohort study
380	*Y. kristensenii*	Cohort study
381	*Y. kristensenii*	Cohort study
382	*Y. kristensenii*	Cohort study
383	*Y. kristensenii*	Cohort study
384	*Y. frederiksenii*	Cohort study
385	*Y. frederiksenii*	Cohort study
386	*Y. enterocolitica* 1A	Cohort study
387	*Y. frederiksenii*	Cohort study
388	*Y. kristensenii*	Cohort study

Clustering procedure. The similarity matrix produced by the S_{SM} procedure was then analysed and grouped into clusters by hierarchical cluster analysis using the command **CLUSTER** in **SPSS**^{X}. The average linkage within groups (**UPGMA** - unweighted pair-group method using arithmetic averages) method of clustering was employed. The **UPGMA** method computed the average similarity or dissimilarity of a candidate **OTU** to an extant cluster, and weighted each **OTU** in that cluster equally, regardless of its structural subdivision (Sneath and Sokal, 1973).

Results

Characteristics of the Strains Tested

Of the the 44 characteristics tested for each of the 388 *Yersinia* strains included in this study, 6 were positive for all the strains at 29°C, and 11 were negative for all the strains at the same incubation temperature. **Tables 8.2** and **8.3** show these biochemical characteristics. This resulted in 27 biochemical characteristics in which one or more strains differed in their reactions.

Table 8.2. Characteristics Positive for All the 388 *Yersinia* Strains

Acid butt on TSI
Alkaline slant on LIA
Acid butt on LIA
Urease test
Motility
Mannitol fermentation

Table 8.3. Characteristics Negative for all the 388 *Yersinia* Strains

Alkaline butt on TSI
Alkaline butt on LIA
Reddening of the slant on LIA
H_2S production (TSI)
Gas production (TSI)
Arginine dihydrolase
Lysine decarboxylase
Malonate utilisation
Phenylalanine deaminase
Adonitol fermentation
α-methyl-D-glucoside fermentation

Results of Hierarchical Clustering

The hierarchical cluster analysis resulted in the construction of a dendrogram, shown schematically in **Figure 8.1**. Appendix VI shows the detailed computer printout of the dendrogram, indicating the clustering of the individual *Yersinia* strains. The dendrogram showed rescaled distances at which the different clusters and subclusters combined. At the rescaled distance of 16, the 388 strains have combined from the numerous subclusters to form 3 major clusters, designated here as clusters A, B and C. Clusters A and B then combined at the rescaled distance of 23, and this new combination combined with cluster C at the farthest possible distance of 25.

Cluster A. This cluster was composed of 206 *Yersinia* strains which could be subdivided into three subclusters (A1 to A3), which were combined at the rescaled distance of 16. Subclusters A2 and A3 combined at the rescaled distance of 14 and this new combination fused with A1 at the rescaled distance of 16 to form cluster A.

Subcluster A1 contained all the 54 strains of *Y. kristensenii*, and subcluster A2 contained the two strains of *Y. rohdei*. Subcluster A3 contained 150 strains of *Y. frederiksenii*, *Y. intermedia* and *Y. enterocolitica* biotype 1A. Although minor subclusters within subcluster A3 were combined at lower rescaled distances, the strains were not clearly clustered into distinct groups of *Yersinia* species. Thus *Y. enterocolitica* biotype 1A strains were found scattered among the minor subclusters, together with the *Y. frederiksenii* strains. *Y. intermedia* formed a definite cluster within subcluster A3 at a low rescaled distance of 6.

The fact that the *Y. kristensenii* strains (subcluster A1) combined only with subclusters A2 and A3 at the far rescaled distance of 16 meant that *Y. kristensenii* strains were relatively distantly related to the other *Yersinia* strains in cluster A.

Cluster B. This cluster contained 149 strains of *Y. enterocolitica* biotypes 5, 3 and 2. At the rescaled distance of 9, three definite subclusters were formed (B1 to B3). Subcluster B1 contained all the strains in *Y. enterocolitica* biotypes 3 and 2, and subcluster B2 contained all the *Y. enterocolitica* biotype 5 strains. Subcluster B3 contained a single strain which was biochemically identified as *Y. enterocolitica* biotype 5 but had the peculiar characteristic (for this biotype) of being indole-positive and positive for the uptake of congo red in CRMOX agar.

Cluster C. This cluster contained all the 33 strains of *Y. pseudotuberculosis*. At the rescaled distance of 5, however, 2 definite subclusters (C1 and C2) were formed. Subcluster C1 contained the 2 melibiose-fermenting serogroup 2B strains and subcluster C2 contained the melibiose-nonfermenting serogroup 3 and untypable strains.

Discussion

One of the most obvious features of the dendrogram resulting from the cluster analysis was the distinct difference between the *Y. pseudotuberculosis* strains and the other *Yersinia* strains. The dendrogram showed that the combination of the cluster containing *Y. pseudotuberculosis* (cluster C) with the other major clusters (A and B) occurred only at the farthest rescaled distance (25). This meant that *Y. pseudotuberculosis* strains, although related to the other *Yersinia* species, are only distantly so. This feature was shown in an earlier study by Moore and Brubaker (1975) wherein they found that *Y. pseudotuberculosis* and *Y. enterocolitica* were only 18-24% related to each other. In another DNA hybridisation study, a similar situation was shown by Brenner *et. al.* (1976) when they found out that *Y. pseudotuberculosis* was only 40-60% related to *Y. enterocolitica*.

The relative homogeneity of the biochemical reactions exhibited by all the *Y. pseudotuberculosis* strains was also obvious. From the dendrogram, it can be seen that subclusters under cluster C appear only below the rescaled distance of 5, unlike the heterogeneous reactions shown by the other *Yersinia* strains. This homogeneity was apparently the reason why, as in previous numerical taxonomy studies (Kapperud *et. al.*, 1981; Kaneko and Hashimoto, 1982), *Y.*

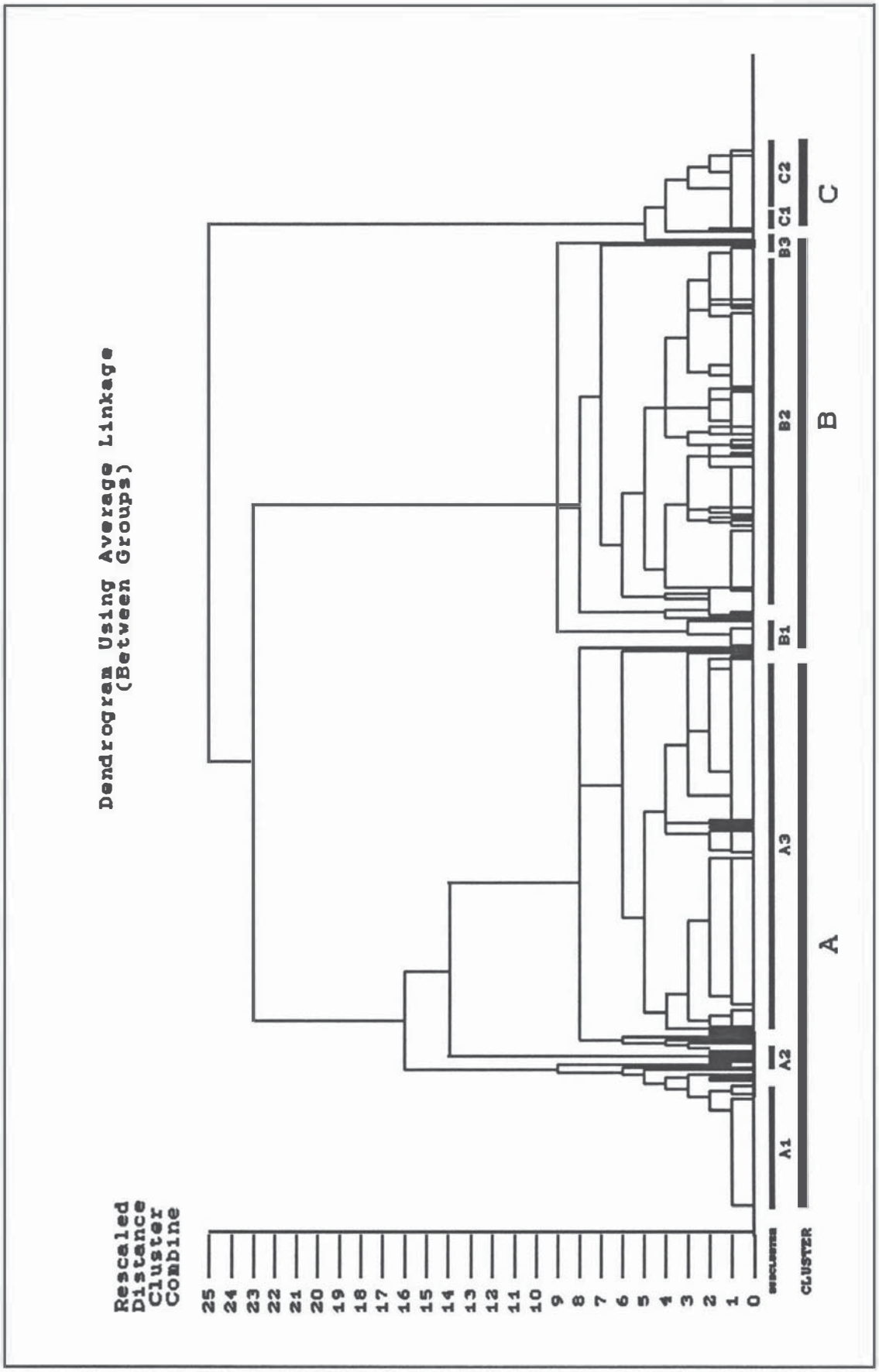


Figure 8.1. Dendrogram of 388 *Yersinia* Strains Obtained by Hierarchical Cluster Analysis Using UPGMA

pseudotuberculosis strains tended to be found in one cluster only and not dispersed into other clusters.

Another distinct result of the cluster analysis was the partitioning of the strains into, on one side, the potentially pathogenic strains and on the other, the environmental strains, with cluster A containing the environmental strains and clusters B and C containing the pathogenic strains. This was particularly obvious in the case of *Y. enterocolitica* biotype 1A, which is considered by Wauters *et. al.* (1987) as an environmental biotype. Although classified as *Y. enterocolitica* on the basis of their biotyping reactions, the goat strains of this biotype were clustered together with strains of *Y. frederiksenii*, probably due to the shared features of the two species as being positive for aesculin, salicin, pyrazinamidase and β-D-glucosidase, and negative for the *in vitro* virulence markers. These characteristics may mean that *Y. enterocolitica* biotype 1A is better regarded as a species distinct from *Y. enterocolitica*. DNA hybridisation techniques, however, signified otherwise. The new *Yersinia* species (see Chapter 2) and *Y. enterocolitica sensu stricto* (Bercovier *et. al.*, 1980a) have been characterised by these procedures, and *Y. enterocolitica* biotype 1A has been shown to part of *Y. enterocolitica* (Bercovier *et. al.*, 1980a). Nevertheless, the differences in *in vivo* virulence between biotype 1A and the other biotypes lends support to the findings from this study that biotype 1A has relatively low affinity with the other biotypes of *Y. enterocolitica*.

In their numerical taxonomy study of *Y. enterocolitica* and *Y. enterocolitica*-like bacteria, Kapperud *et. al.* (1981) were able to show that *Y. enterocolitica* biotype 5 strains were clustered outside the *Y. enterocolitica sensu stricto* (classification by Bercovier *et. al.*, 1980a) strains which were, in that study, mostly from environmental sources. It was also interesting to note that the *Y. enterocolitica* biotype 5 strains in that study were in the same cluster as the pathogenic *Y. enterocolitica* serotypes derived from clinical cases. In this present study, essentially the same results were obtained. While placed in different subclusters, all the *Y. enterocolitica* biotypes showing positive reactions for virulence markers (Biotypes 5, 3 and 2) were clearly placed in one cluster.

The apparent disparities in the results between DNA hybridisation studies and numerical methods in the classification of *Yersinia* species could be explained by the way classifications of species were obtained. While DNA hybridisation studies took only the genotypic characteristics into consideration, numerical taxonomic procedures usually considered the phenotypic characteristics of the individual OTUs (strain, species, genus, etc.), which, in the case of bacterial strains, were the biochemical and virulence activities demonstrated through laboratory tests. DNA studies did not usually take ecological considerations as

well. For example, Bercovier *et. al.* (1980a) were able to show that the *Y. enterocolitica sensu stricto* strains they studied were all in the same species but were derived from diverse ecosystems and showed different phenotypic characteristics. From the point of view of a diagnostic microbiologist or a clinician, for example, a *Yersinia* strain isolated from a goat which is negative for autoagglutination is substantially different in practical importance from a strain isolated from a human clinical case positive for autoagglutination, even though these may belong to the same species based on a few key biochemical properties.

The question of which method to use for the classification of *Yersinia* species should therefore be settled according to the purpose of such classification. In clinical microbiology it is quite obvious that phenotypic classification is the method of choice, since the clinician is interested mainly in the identification of strains. A person interested in broader relationships, such as the ecology of species in different ecosystems, may want to use the DNA hybridisation techniques for classification. In such a way he can determine phenotypic variations and changes within the same genotype in different ecological situations.

The taxonomic positions of the other *Yersinia* strains, as determined by the present study, were similar to the results shown in studies mentioned earlier (Kapperud *et. al.*, 1981; Kaneko and Hashimoto, 1982) and fell within the *Yersinia* species described in Chapter 2.

This present study therefore shows that while most of the *Yersinia* strains isolated from goats were grouped into their proper and established species, some exceptions were notable, especially the clustering together of *Yersinia* strains which did not possess virulence markers at 29°C. Such expressions of variability were only shown phenotypically, and therefore were taken into account by the numerical taxonomic procedure employed in this study.

CHAPTER 9

GENERAL DISCUSSION

In studying the epidemiology of *Yersinia* species infection in goats, several factors had to be considered prior to the actual sampling of goats for the presence or absence of *Yersinia* species. First, consideration was given to ascertaining the state of goat farming in the Manawatu, where the study was conducted. Next, attention was given to the methods to be employed in the actual study - the sampling method, the farms to be sampled, and the microbiological protocol to be followed. Also, consideration was given to the question of when to start, and the length of time necessary for sampling the animals from farms chosen for the study. Finally, decisions were made on the sampling designs and methods of statistical analyses to be followed throughout the different phases of the study.

The first consideration was tackled by conducting a mail questionnaire-study with 30 goat farmers in the Manawatu, which comprised the majority of the goat farmers in the area at the time. The questionnaire was intended to provide a broad picture of the actual state of goat farming in the area, including problems encountered and the approach each farmer followed to solve these problems. Specific questions regarding management practices and routine animal health practices on the farm were also included in the questionnaire. Finally, the farmers were asked about their views and attitudes concerning goat management and health.

The 96.7% response rate to the questionnaire was not surprising, considering the fact that constant reminders by telephone were made to the farmers starting 2 weeks after sending out the questionnaires. Analysis of the questionnaires produced some interesting results. It was apparent that most farmers' approach to raising goats was essentially the same as the traditional approach to sheep farming, especially in the management methods used. Maybe this is because goat farming in the Manawatu (and in New Zealand as a whole) started relatively late, as an offshoot of sheep farming. As a result, most farmers thought that goats were basically similar to sheep, having the same problems with regard to production and health management.

The farmers' growing realisation that goats were different from sheep was evident in the answers that they gave to questions on goat health and management. Some of the farmers mentioned that goats were not suited for New Zealand conditions and that these animals were very difficult to handle with regard to health management, particularly the Angoras. It was surprising to learn that the farmers' knowledge of bacterial infections causing gastrointestinal disturbances was virtually non-existent; most of them considered diarrhoeic conditions in these animals to be caused wholly by nematode infections, while in fact some may have been caused by other aetiological agents, including *Yersinia* species.

When it came to considering the plans for the actual samplings from goats, the decision to carry out faecal samplings for the diagnosis of *Yersinia* species infection was made for 2 reasons: firstly, *Yersinia* infections are usually manifested by gastrointestinal involvement (see Chapter 2) so that it was considered more likely to encounter these bacteria in faeces; secondly, no reliable serological test has been developed yet for the rapid, correct and efficient diagnosis of *Yersinia* infection, and cultural identification has been the only reliable diagnostic method available for use in animals (Baskin, 1980). Nevertheless, blood samples were taken once from goats during the cohort study, and the sera stored for future use.

One disadvantage of culturing faecal samples for *Yersinia* species was that cold enrichment for 21 days was necessary for the proper isolation of these bacteria, since it has been shown by earlier studies (see Chapter 2) that *Yersinia* species grow better than other bacteria at refrigeration temperatures (about 4°C). Thus 21 days at this temperature would selectively allow *Yersinia* species to grow sufficiently to be recovered by plating, and at the same time inhibit the growth of other bacteria. Although it was shown in the present work that cold enrichment in conjunction with the use of CIN plates (Schiemann, 1979) yielded cultures of *Yersinia* species, a minor disadvantage was the growth of *Enterobacter*, *Serratia*, and *Citrobacter* on CIN which often caused confusion in the selection of primary colonies for testing.

The screening phase of the study was considered necessary since the status of *Yersinia* species infection in goat flocks was unclear, with no indication as to whether infections at flock level were common or rare. This screening survey also identified farms that were included in the prevalence survey (those found positive), a phase which involved more intensive sampling from goats. The selection of only those farms found positive worked to our advantage, since the cost of culturing *Yersinia* species from goats from all the farms sampled would have been astronomical.

The prevalence survey emphasised the shortcomings of a single cross-sectional survey for seasonal infections such as those caused by *Yersinia* species. The results of this study, therefore, were actually minimum estimates of the infection, since, as was seen from the prevalences on individual farms, variability was very high. For example, one farm found positive in the screening survey was negative in the prevalence survey. Also, the timing of the prevalence survey (summer-autumn in New Zealand) probably reduced the isolation of *Yersinia* species which were later found to be more commonly isolated during the colder months.

The cohort study was then undertaken because it was necessary to explain the underestimates made in the prevalence survey and the extreme variability of the prevalence of *Yersinia* infection on the individual farms. This final study satisfactorily clarified several factors considered to be important in the epidemiology of *Yersinia* infections in goats. One was that the incidence of *Yersinia* species infection was indeed higher during the colder months of the year and that infection, especially of the pathogenic *Yersinia* species (*Y. pseudotuberculosis*, *Y. enterocolitica* biotypes 5, 3 and 2) was more common in younger animals. Another was that the most significant climatic factor affecting the incidence of pathogenic *Yersinia* species was the minimun daily temperature, and that the factors affecting the incidence of environmental species were related to the "wetness" of the environment, that is, rain, humidity and dew days.

It was also shown that once an animal has recovered (has become negative) from infection with a pathogenic *Yersinia* species (where infections were shown, on a few occasions, to last more than 2 sampling dates), it was not reinfected with the same species again, at least during the course of the observation period. This characteristic, however, was not a feature of infections with the environmental strains. These observations implied that the virulent nature of the pathogenic *Yersinia* species enabled them to persist in the animals for a longer time and that some degree of immunity was conferred on these animals once they had recovered from the infection. This was evidenced by the non-isolation of these species from recovered animals during the subsequent samplings. This may also explain why older animals showed a lower incidence of pathogenic *Yersinia* species carriage when compared with animals in the younger age groups.

The environmental species of *Yersinia*, as shown in earlier studies, have been isolated mostly from environmental ecosystems, with only occasional isolations from clinical cases, where the role of these species in the pathogenesis of such cases was doubtful. *Y. frederiksenii* and *Y. intermedia* have been isolated mostly from aquatic ecosystems (Ursing *et. al.*, 1980; Brenner *et. al.*, 1980) and

Y. kristensenii from soil (Bercovier *et. al.*, 1980). All these reports then, may indicate that the infections with these species were merely transient passages through the gut and were actually just a reflection of the status of the bacterial population on the pasture. Since these species are well suited to a wet environment, this may explain the significance of rain, dew and other precipitation factors on the incidence of these species in goats.

The sources of infection of the pathogenic *Yersinia* species for goats remain questionable, however. While it is considered that wild animals are the primary reservoirs of *Y. pseudotuberculosis*, there appears to be a variation in the geographical distribution of the bacteria in New Zealand. The propensity of *Y. pseudotuberculosis* serogroup 3 among domestic animals in the North Island (Hodges *et. al.*, 1984) may explain why the majority of the *Y. pseudotuberculosis* strains isolated from goats in this study belonged to that serogroup, which was rarely found in domestic animals in the South Island (Mackintosh and Henderson, 1984). A survey of wildlife for the carriage of *Y. pseudotuberculosis* may eventually prove if they also serve as sources of infection for domestic animals in the North Island.

The source of *Y. enterocolitica* biotype 5 infections for goats remains to be studied further. As this biotype has in the past only been isolated from hares (Nilehn, 1969a; Bercovier *et. al.*, 1978) and goats (Krogstad, 1972) in Europe, its occurrence in domestic animals in New Zealand is quite unique. Most significant, however, is the fact that in reports from New Zealand, goats appear to be the principal animal species carrying *Y. enterocolitica* biotype 5, with deer and sheep only occasionally being implicated (Henderson, 1984; Bullians, 1987; Buddle *et. al.*, 1988). The results of the present study support the findings of previous workers.

The significance of reports of this biotype's isolation in New Zealand is that goats may serve as a natural reservoir of the strain, at least in this country. Studies on the carriage of *Y. enterocolitica* biotype 5 by wildlife may eventually find new reservoirs, but for the moment no other animals, apart from those mentioned above, have been shown to harbour this biotype.

The role of the pathogenic *Yersinia* species as a cause of disease in goats has been demonstrated in earlier studies (Krogstad, 1972; Hodges *et. al.*, 1984a; Buddle *et. al.*, 1988). In the present study, however, no cases of disease definitely attributable to these strains were reported. Mention was made in Chapter 6 about 2 separate cases of diarrhoea in goats from the farms surveyed (apparently caused by *Y. enterocolitica* biotypes 3 and 5) but in both cases the investigations necessary to confirm these biotypes as the causes of deaths were

not adequate. In the first case (diarrhoea and eventual death apparently due to *Y. enterocolitica* biotype 3 infection), no post-mortem examination was performed on the dead animal and in the second case involving *Y. enterocolitica* biotype 5, the post mortem was performed only after some autolysis had occurred in the internal organs of the body, making it difficult to obtain histological corroboration.

The two cases mentioned emphasised the importance of the isolations of not only these two biotypes of *Y. enterocolitica* but also of *Y. pseudotuberculosis* from goats. Their presence in apparently healthy animals, as found in this study, meant that infection had indeed occurred, and that these animals were probably reacting to the infection by developing immunity against subsequent reinfection. The presence of the infection in these apparently healthy animals also meant that disease conditions could have developed in these animals if the right combination of predisposing factors were present, such as undue stress due to cold, feed shortages, rain, etc., with the actual development of such disease conditions almost entirely dependent on the balance between the individual animals' overall resistance and the ability of the bacteria to overcome this. Unfortunately, the natural resistance of healthy animals to the potentially pathogenic species prevented the observation and study of an actual disease outbreak in goats due to *Yersinia* species infection.

The heterogeneity of the biochemical reactions of the various *Yersinia* strains, particularly within *Y. enterocolitica*, has been shown in Chapter 7. An attempt to group these strains using numerical taxonomic techniques was performed (Chapter 8), mainly to determine if the various *Yersinia* strains isolated from goats would fall within definite clusters of *Yersinia* species, as defined in earlier studies (see Chapter 2). As it turned out, the clustering of the different strains was dependent principally on the *in vitro* virulence characteristics of the strains tested. Thus *Y. enterocolitica* biotype 1A strains, which did not possess any of the virulence markers at 29°C, were clustered with the group of environmental strains and not within the group containing *Y. enterocolitica*. This result then showed that genotypic characteristics differ significantly from phenotypic characteristics, often resulting in different classification schemes.

This present study on the epidemiology of *Yersinia* species infections in goats, therefore, confirmed several findings from earlier studies (see Chapter 2). It confirmed that infections, especially those by pathogenic species, were mostly cold-weather events and that the "wetness" of the environment also contributed to the incidence of these species, although to a lesser degree. The tendency of the infections to occur in younger age groups was also substantiated in this study,

particularly in cases of infections by *Y. pseudotuberculosis* and *Y. enterocolitica* biotypes 5, 3 and 2.

New insights into the epidemiology of the infections were, however, also shown by this study. It was revealed that infections with environmental species of *Yersinia* did occur in goats, but were most common only in the older age groups. Infections with these species were also shown not to be significantly influenced by climatic changes, although they were shown to be more likely to be influenced by precipitation changes than by temperature changes.

The apparent immunity to reinfection found in animals which had recovered from infections with the pathogenic *Yersinia* species was particularly significant in view of the eventual formulation of control measures for yersiniosis. Since this feature was not shown in infections with the non-pathogenic species, isolations of the non-pathogenic strains from the goats were not thought to be significant, and were considered only as a transient passage of the bacteria through the gut, and a reflection of the status of the growth of these organisms in the environment.

The finding that *Y. enterocolitica* biotype 5 appeared to predominate in goats in this study was a very significant finding, and implies that this biotype probably has a unique position as a potential pathogen for goats in New Zealand.

All these findings, however, need to be corroborated, and further experimental studies are necessary to confirm the results of this study on the epidemiology of *Yersinia* species infections in goat flocks.

APPENDICES

APPENDIX I

A SURVEY OF YERSINIOSIS IN GOAT FLOCKS

Conducted by: **Eugene B. Lañada**
Address:Department of Veterinary Clinical Sciences
Massey University
Palmerston North

PLEASE NOTE: **All individual flock information will be treated as strictly confidential. Only combined data from cooperating flocks will be used in the research.**

1. Farm Owner: _____ Code: _____

2. Address: _____ Date: _____

3. Telephone: _____

4. Farm area (hectares): _____

5. Flock size: Kids: (less than one year old) _____

   Hoggets:(one to two years old) _____

   Adults: (more than two years old) _____

   TOTAL _____

6. Flock production type: **(please tick)**

   Fibre:_____

   Milk:_____

   Mixed:_____

   Other:_____ Please specify: _____

7. Breed/s: **(please tick)**

   a. Fibre goats: Angora: _____ % of flock

   Grades: _____ % of flock

   Feral : _____ % of flock

Other : _____ % of flock (please specify) _____

b. Dairy goats: Saanen :_____ % of flock

Toggenburg :_____ % of flock

Alpine :_____ % of flock

Anglo-Nubian:_____ % of flock

Other :_____ % of flock

(please specify)_____

TOTAL:100%

8. At what age do you start shearing your goats? _____

9. How often do you shear your animals? (if applicable) _____

10. Please state time/s of year shorn: _____

11. After shearing, do you: **(please tick)**

a. Return the goats immediately to pasture: _____

b. Rest them in the shed for a few hours before releasing them: _____

12. Farm features:

a. Farm shelter: **(please tick)**

1) Permanent shed/s: _____

2) Natural shelter: _____

(gullies, hedges,

banks, scrub)

3) Other: _____ please specify _____

b. Please state average size of paddock: _____

c. Do you use electrified fencing? **(please tick)**

YES: _____

NO: _____

d. System of pasture allocation: **(please tick appropriate boxes)**

1) All age groups mixed in pasture? YES: _____ NO: _____

2) Different paddocks for different ages YES: _____ NO: _____

3) Separation of different breeds into different paddocks YES: _____ NO: _____

e. Please state the mating season of your goats _____

f. Please state the number of kiddings per year on your farm _____

g. Do you have kidding sheds? **(please tick)**

YES: _____

NO: _____

h. Kid rearing systems: **(please tick)**

1) Left with does until weaning: _____

2) Removed from does after birth and hand-reared: _____

3) Other: _____ please specify _____

i. At what age do you wean the kids? _____

j. Do you give supplemental feeding to your goats? **(please tick)**

YES: _____

NO: _____

k. If "YES", please specify: **(please tick)**

Hay : _____

Lucerne : _____

Grains : _____

Other : _____ please specify _____

l. When do you give supplemental feeding? Please state times/ seasons of year _____

_____

_____

_____

m. Water supply: **(please tick)**

1) Natural sources (streams, ponds, springs) : _____

2) Water troughs (town supply water) : _____

3) Water troughs (bore) : _____

4) Other: _____ please specify _____

13. Replacement policy: **(please tick)**

a. Entirely homereared: _____

b. Entirely purchased from outside sources: _____

c. Combination homerearing/outside purchase: _____

d. Other: _____(please specify) : _____

14. If outside purchase is practised, state location of source: **(please tick)**

a. Within the Manawatu area : _____

b. Outside the Manawatu : _____

15. If outside the Manawatu, please state location/s:
(Please rank sources according to degree of importance by numbering appropriate boxes, that is, 1, 2, ... etc.)

a. North Island:

1) Northland : _____

2) Auckland : _____

3) Waikato/King Country : _____

4) Bay of Plenty : _____

5) East Cape/Gisborne : _____

6) Hawke's Bay : _____

7) Taranaki : _____

8) Wanganui : _____

9) Wellington : _____

10) Wairarapa : _____

b. South Island:

11) Nelson : _____

12) Marlborough : _____

13) West Coast : _____

14) Canterbury : _____

15) Otago : _____

16) Southland : _____

c. Other: _____ (please specify) : _____

16. Are there any other goat farms within a 5-kilometre radius of your farm? **(please tick)**

YES: _____

NO: _____

17. If "YES", how many? _____

18. Please give reasons for culling animals _____

19. Flock health:

a. Do you drench your goats for worms? **(please tick)**

YES: _____

NO: _____

b. If "YES", how often? _____

If possible, indicate times (or seasons) of year _____

c. Types of animals drenched: **(please tick)**

Kids only : _____

Hoggets only : _____

Kids and Hoggets : _____

Adults : _____

All Animals : _____

Other combinations (please specify) : _____

d. Please state procedure for lice control:**(please tick)**

Dipping : _____

Spraying : _____

Pour Ons : _____

Other : _____ Please specify _____

e. How often is this procedure done? _____

If possible indicate times (or seasons) of year _____

f. Do you vaccinate your goats? **(please tick)**

YES: _____

NO: _____

g. If "YES", please specify vaccination procedures done:

1) _____ Age: _____

2) _____ Age: _____

3) _____ Age: _____

4) _____ Age: _____

20. Do you have other stock on the farm apart from goats? **(please tick)**

YES: _____

NO: _____

21. If "YES", please specify: **(please tick appropriate boxes)**

Sheep : _____

Cattle : _____

Horses : _____

Deer : _____

Other : _____ Please specify _____

22. Which species commonly use the same pasture as goats? _____

23. Please describe the system of use: **(please tick)**

Grazed with goats (simultaneously): _____

Alternate usage of pasture: _____

Other: _____ (please specify): _____

24. Do you use dogs for handling goats? **(please tick)**

YES: _____
NO: _____

HAVE YOU ANY COMMENTS YOU WOULD LIKE TO MAKE ON GOAT MANAGEMENT AND HEALTH?

Thank you very much for your cooperation in this survey.

The completed questionnaire should be sent to:

EUGENE B. LAÑADA
Department of Veterinary Clinical Sciences
Massey University
PALMERSTON NORTH

Telephone: **(063) 69-089 Ext. 8030**

APPENDIX II

SAMPLE SIZE REQUIREMENTS FOR DETECTING DISEASE [1]
(95% Confidence)

Population Size	Percentage of Diseased Animals in Population											
	50%	40%	30%	25%	20%	15%	10%	5%	2%	1%	0.2%	0.1%
10	4	5	6	7	8	10	10	10	10	10	10	10
20	4	6	7	9	10	12	16	19	20	20	20	20
30	4	6	8	9	11	14	19	26	30	30	30	30
40	5	6	8	10	12	15	21	31	40	40	40	40
50	5	6	8	10	12	16	22	35	48	50	50	50
60	5	6	8	10	12	16	23	38	55	60	60	60
70	5	6	8	10	13	17	24	40	62	70	70	70
80	5	6	8	10	13	17	24	42	68	79	80	80
90	5	6	8	10	13	17	25	43	73	87	90	90
100	5	6	9	10	13	17	25	45	78	96	100	100
120	5	6	9	10	13	18	26	47	86	111	120	120
140	5	6	9	11	13	18	26	48	92	124	139	140
160	5	6	9	11	13	18	27	49	97	136	157	160
180	5	6	9	11	13	18	27	50	101	146	174	180
200	5	6	9	11	13	18	27	51	105	155	190	200
250	5	6	9	11	14	18	27	53	112	175	228	250
300	5	6	9	11	14	18	28	54	117	198	260	300
350	5	6	9	11	14	18	28	54	121	201	287	350
400	5	6	9	11	14	19	28	55	124	211	311	400
450	5	6	9	11	14	19	28	55	127	128	331	450
500	5	6	9	11	14	19	28	56	129	225	349	500
600	5	6	9	11	14	19	28	56	132	235	379	597
700	5	6	9	11	14	19	28	57	134	243	402	691
800	5	6	9	11	14	19	28	57	136	249	412	782
900	5	6	9	11	14	19	28	57	137	254	437	868
1000	5	6	9	11	14	19	29	57	138	258	450	950
1200	5	6	9	11	14	19	29	57	140	264	471	1102
1400	5	6	9	11	14	19	29	58	141	269	487	1236
1600	5	6	9	11	14	19	29	58	142	272	499	1354
1800	5	6	9	11	14	19	29	58	143	275	509	1459
2000	5	6	9	11	14	19	29	58	143	277	517	1553
3000	5	6	9	11	14	19	29	58	145	284	542	1895
4000	5	6	9	11	14	19	29	58	146	288	556	2108
5000	5	6	9	11	14	19	29	59	147	290	564	2253
6000	5	6	9	11	14	19	29	59	147	291	569	2358
7000	5	6	9	11	14	19	29	59	147	292	573	2437
8000	5	6	9	11	14	19	29	59	147	293	576	2498
9000	5	6	9	11	14	19	29	59	148	294	579	2548
10000	5	6	9	11	14	19	29	59	148	294	581	2588
∞	5	6	9	11	14	19	29	59	149	299	598	2995

[1] The number of animals to be sampled in order to be 95% confident of detecting at least one infected animal if infection is present in the flock. Table adapted from Cannon and Roe (1982).

APPENDIX III

SAMPLE SIZE FOR ESTIMATION OF PREVALENCE [2]

	Level of Confidence								
	90%			95%			99%		
Expected	Desired Accuracy			Desired Accuracy			Desired Accuracy		
Prev.	10	5	1	10	5	1	10	5	1
10%	24	97	2435	35	138	3457	60	239	5971
20%	43	173	4329	61	246	6147	106	425	10616
30%	57	227	5682	81	323	8067	139	557	13933
40%	65	260	6494	92	369	9220	159	637	15923
50%	68	271	6764	96	384	9604	166	663	16587
60%	65	260	6494	92	369	9220	159	637	15923
70%	57	227	5682	81	323	8067	139	557	13933
80%	43	173	4327	61	246	6147	106	425	10616
90%	24	97	2435	35	138	3457	60	239	5971

[2] The number of animals to be sampled at different confidence levels for specific expected prevalences of "infinite" populations. Table adapted from Cannon and Roe (1982).

APPENDIX IV

MEDIA PREPARATION, BIOCHEMICAL AND VIRULENCE TEST PROCEDURES

Aesculin Hydrolysis

Preparation of the Medium (Wauters *et. al.*, 1987)

The following materials were mixed and dissolved by boiling together: 1 gm polypeptone (Oxoid), 0.1 gm aesculin, 0.1 gm ferric ammonium citrate, 0.5 gm agar, and 100 ml distilled water. After thorough mixing, 3 ml amounts were dispensed into bijoux bottles and sterilised by autoclaving at 120°C for 15 minutes. The bottles were slanted and cooled, and stored at 4°C.

Testing for Aesculin Hydrolysis

The slants were inoculated with 1-2 drops of a bacterial culture grown overnight in tryptone water at 29°C. Incubation was carried out at 29°C for 10 days. Positive aesculin hydrolysis was indicated by blackening of the medium.

Autoagglutination Test

Preparation of the Medium (Adapted from Laird and Cavanaugh, 1980)

9.72 gm of Modified Minimum Essential Medium Eagle (MEM)[3] was mixed with one litre distilled water and mixed for 30 minutes. One gm of sodium bicarbonate was added, and the final pH adjusted to 7.0 - 7.5. The solution was then filter-sterilised, and 100 ml of bovine foetal serum was added to make a 10% solution. The medium was stored in 200 ml amounts in sterile bottles at 4°C.

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[3] Flow Laboratories, U. K.

Testing for Autoagglutination

2 ml of the medium was dispensed into a small tube and inoculated with an isolated colony grown on trypticase soy agar. The tube was then incubated at 37°C for 24 hours. Agglutination-positive strains formed a layer of irregularly-edged flocculent growth at the bottom of the tube, and the medium was often clear. Strains negative for autoagglutination showed a different growth characteristic. In such cases, most of the bacteria were suspended in the medium, making a homogeneous, cloudy suspension.

β-D-Glucosidase Activity

Preparation of the Medium (Wauters *et. al.*, 1987)

4-nitrophenyl-*β-D*-glucopyranoside (Merck) was dissolved in 0.066 M phosphate buffer (pH 6) at a concentration of 0.1% (wt/vol) and dispensed in 0.25 ml amounts in small sterile tubes as necessary.

Detection of *β-D*-Glucosidase Activity

An equal volume of a 24-hour bacterial culture grown overnight in tryptone water at 29°C was added to a prepared tube of the medium and incubated overnight at 29°C. The formation of a distinct yellow colour (free nitrophenol) indicated a positive reaction.

Calcium Dependency Test

Preparation of the Medium (Mair and Fox, 1986)

Magnesium oxalate agar (MOX) was used for this test. To prepare the medium, 2.68 gm of sodium oxalate, 4.06 gm of magnesium chloride and 39 gm of Columbia base agar were mixed in 1 litre of distilled water. This mixture was then boiled and sterilised by autoclaving at 121°C for 15 minutes. When cooled, the medium was poured into sterile petri dishes.

Inoculation of the Medium

An isolated colony grown on blood agar for 24 hours at 29°C was plated onto the medium. After 24-hours incubation at 37°C, MOX-positive strains yielded pinpoint colonies often together with large colonies, whereas MOX-negative strains yielded only large colonies 0.5 to 2.0 mm in diameter.

Congo Red-Magnesium Oxalate Agar (CRMOX)

Preparation of the Medium (Riley and Toma, 1989)

40 gm of tryptic soy agar was mixed with 825 ml of distilled water and autoclaved for 15 minutes at 121°C. The molten medium was cooled to 55°C and the following solutions were added: 80 ml of 0.25 M magnesium chloride, 80 ml of 0.25 M sodium oxalate, 10 ml of 20% D-galactose and 5 ml of 1% congo red. All these solutions were sterilised by autoclaving for 15 minutes at 121°C, except for the D-galactose solution, which was filter-sterilised. After thorough mixing, the medium was dispensed into sterile petri dishes and stored at 4°C.

Inoculation of the Medium

The strains tested were first grown on blood agar at 29°C for 24 hours. From this, an isolated colony was plated onto CRMOX and incubated at 37°C for 24 hours. CRMOX-negative strains produced only large, colourless colonies while CRMOX-positive strains produced both small, red colonies and large, colourless colonies.

Lipase Activity

Preparation of the Medium (Mair and Fox, 1986)

28 gm of nutrient agar powder (Oxoid CM3) were suspended in 1 litre of distilled water and boiled to dissolve the powder completely. This mixture was then sterilized by autoclaving at 120°C for 15 minutes. It was then cooled down to 50°C, after which 10 ml of Tween 80 were added. After thorough mixing, approximately 20 ml of the medium were poured into sterile petri dishes.

Inoculation of the Plates

The organisms to be tested were inoculated on to the surface and spread over an area approximately 1 cm by 0.5 cm. Several tests were carried out on one plate. The plates were then incubated at 22°C for up to 4 days. The presence of precipitation around and under the inocula indicated a positive reaction. Some precipitates were barely visible after 24 hours, and a hand lens was necessary for their detection.

Lysine Iron Agar

1. Suspend 34.5 gm in distilled or deionised water and heat to boiling to dissolve completely.

2. Dispense as required.

3. Sterilise in the autoclave for 12 minutes at 15 lbs pressure (121°C).

4. Allow medium to cool in a position that will provide a short slant and a deep butt.

5. Inoculate with a straight needle by stabbing to the base of the butt and streaking the slant.

6. Caps of the tubes should be replaced loosely, allowing aerobic conditions to develop.

M/15 Phosphate-Buffered Saline, pH 7.6 (Mair and Fox, 1986)

1. Prepare Solution A by dissolving 9.07 gm of potassium dihydrogen phosphate (KH_2PO_4) in 1 litre of water.

2. Prepare Solution B by dissolving 11.87 gm of disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$) in 1 litre of distilled water.

3. Mix 128 ml of Solution A and 872 ml of Solution B, and add 9.0 gm of sodium chloride.

4. Dispense 10 ml amounts in universal bottles.

Proline Peptidase Activity

Preparation of the Medium (Wauters *et. al.*, 1987)

L-Prolyl-β-naphtylamide hydrochloride was filter-sterilised and added aseptically to autoclaved peptone water at a final concentration of 0.01% (wt/vol), and the medium was dispensed in 0.5 ml amounts in small sterile tubes.

Detection of Free Naphtylamide

Each of the sterile tubes containing the medium was inoculated with one drop of a bacterial culture grown overnight in tryptone water at 29°C. After incubating overnight at 29°C, free naphtylamide was detected by adding one drop of each of the following reagents: Tris-laurylsulfate (Zym A, API System) and Fast Blue (Zym B, API Ssytem). The tubes were allowed to stand for 5 minutes, and a bright-orange colour indicated a positive reaction.

Pyrazinamidase Test

Preparation of the Medium (Adapted from Kandolo and Wauters, 1985)[4]

The following materials were mixed and dissolved by boiling: 30 gm tryptic soy agar (Difco), 3 gm yeast extract (Difco), 1 gm pyrazinecarboxamide (Merck), and 1 litre 0.02 M Tris-maleate buffer. After boiling, the medium was dispensed in 5-ml amounts into screw-capped 160 by 16-mm polycarbonate tubes, and sterilised by autoclaving at 120°C for 15 minutes, after which they were slanted for cooling.

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[4] The only change made in the preparation of this medium was the substitution of 0.02 M Tris-maleate buffer for the original 0.2 M Tris-maleate buffer described by these authors. The original formula resulted, in our experience, in a medium that had difficulty in solidifying and supporting the growth of *Yersinia* species.

Inoculation of Tubes

The slants were inoculated with 1-2 drops of a bacterial culture grown overnight in tryptone water at 29°C. After incubating for 48 hours at 29°C, the presence of pyrazinoic acid was detected by flooding the inoculated slant with 1 ml of freshly-prepared 1% (wt/vol) ferrous ammonium sulfate (aqueous) solution. Readings were made after 15 minutes; the formation of brownish-pink colour indicated a pyrazinamidase-positive reaction, while negative cultures remained colourless.

Storage of Cultures at -20°C

Preparation of the Medium (Adapted from from Park, 1976)

Trypticase soy broth containing 15% glycerol (glycerol broth) was placed in 3 ml amounts in bijoux bottles and sterilised by autoclaving at 120°C for 15 minutes. The bottles were then cooled and stored at 4°C.

Storing Cultures at -20°C

Yersinia colonies actively growing on blood agar were scraped off the surface using sterile cotton swabs and suspended in bijoux bottles containing the glycerol broth. Approximately 2 ml of the suspension was then drawn up using a sterile pasteur pipette and deposited into sterile 2-ml screw-capped tubes.[5] The tubes were immediately placed in a deep freezer at -20°C.

Triple Sugar Iron Agar (Difco)

1. To rehydrate, suspend 65 gm in 1 litre distilled or deionised water and heat to boiling to dissolve completely.

2. Dispense into tubes and sterilise in the autoclave for 15 minutes at 15 lbs pressure (121°C).

3. Allow the tubes to solidify in a slanting position so that a generous butt

[5] Wheaton® 2-ml vials (Cat. No. 224881)

is formed.

4. Inoculate with a straight needle by stabbing to the base of the butt and streaking the slant.

5. Caps of the tubes should be replaced loosely, allowing aerobic conditions to develop.

Urea Agar (Difco)

1. To rehydrate, suspend 29 gm Bacto Urea Agar Base in 100 ml distilled or deionised water and mix thoroughly to dissolve completely.

2. Filter sterilise this concentrated base. Do not boil or autoclave concentrated base.

3. Dissolve 15 gm of Bacto Agar in 900 ml distilled or deionised water by boiling and sterilise in the autoclave for 15 minutes at 15 lbs pressure (121°C).

4. Allow to cool to 50-55°C and aseptically add 100 ml of the filter-sterilised concentrated Bacto Urea Agar Base to the cooled Bacto Agar.

5. Mix thoroughly and distribute in sterile tubes. Slant the tubes so as to have a butt about 2 cm in depth and a slant about 3 cm in length.

6. Inoculate slants with 1-2 drops of a bacterial culture grown overnight in tryptone water at 29°C. Incubate slants at 29°C for a minimum period of 48 hours.

Yersinia Selective Agar (Difco)

This medium is based on the Cefsulodin-Irgasan-Novobiocin (CIN) Agar formulation of Schiemann (1979). Complete preparation of this medium is achieved by the combination of the *Yersinia* Selective Agar Base and the *Yersinia* Antimicrobic Supplement CN.

Ingredients (per litre) of *Yersinia* Selective Agar Base

Bacto Yeast Extract	2 gm
Bacto Peptone	17 gm
Proteose Peptone (Difco)	3 gm
Mannitol	20 gm
Sodium Deoxycholate	0.5 gm
Sodium Cholate	0.5 gm
Sodium Chloride	1 gm
Sodium Pyruvate	2 gm
Magnesium Sulfate Heptahydrate	10 mg
Bacto Agar	13.5 gm
Bacto Neutral Red	30 mg
Bacto Crystal Violet	1 mg
Irgasan	4 mg

Ingredients of *Yersinia* Antimicrobic Supplement CN

Cefsulodin	4 mg
Novobiocin	2.5 mg

Preparation of the Medium

1. To rehydrate the base, suspend 59.5 gm *Yersinia* Selective Agar Base in 1 litre distilled or deionised water and heat to boiling to dissolve completely.

2. Sterilise in the autoclave for 15 minutes at 15 lbs pressure (121°C).

3. Cool to 45-50°C.

4. Aseptically add 10 ml rehydrated *Yersinia* Antimicrobic Supplement CN.

5. Mix thoroughly, avoiding the formation of air bubbles, and dispense into sterile 90-100 mm Petri dishes, approximately 20 ml per dish.

APPENDIX V

PATHOLOGY REPORT ON A DEAD GOAT

MASSEY UNIVERSITY
Palmerston North
New Zealand
Telephone (063) 69-099

PATHOLOGY REPORT

Department of Veterinary Pathology and Public Health

SUBMITTERS REF:

ACCESSION NO. 20175

SUBMITTER
E. Lanada

DATE SENT: 8.6.89

SPECIES: Caprine
SEX: Female
AGE: 8 months
BREED: Grade
IDENTIFICATION:

OWNER
Ken Beagley
Aokautere, R.D. 1
Palmerston North

NO.AT RISK :
NO.AFFECTED :
NO.DEAD :
PREVIOUS ACCESSION NO: #8018

HISTORY : Weakness, loss of condition, diarrhoea for 7 days. **Probable time of death, about 8.30am or a little later 8.6.89.** Suspect Yersiniosis.

GROSS FINDINGS : This 8 month old female Angora cross goat was in very thin body condition. Soiling with faecal matter extended from the perianal area over the hindquarters and down the hindlimbs.

There was very little omental, perirenal and pericardial fat reserves and these were undergoing serous atrophy.

PARASITOLOGY: Total nematode count

Abomasum	400
Small intestine	600

Moderate numbers of Eimeria sp oocysts were seen in mucosal scrapings of the ileum and ascending colon.

HISTOPATHOLOGY : Autolytic changes were advanced in the stomach, ileum and ascending colon.

In liver sections there was a diffuse pattern of periportal necrosis of hepatocytes accompanied by a localised infiltration of neutrophils.

BACTERIOLOGY: See attached Photocopy

DIAGNOSIS : ? Yersiniosis
Diffuse periportal hepatic necrosis.

COMMENT : The culture of a moderate growth of Yerisinia enterocolitica from the rectum and Yerisinia sp from both the small and large intestines is suggestive of Yersiniosis. However this organism is recognised as part of the normal flora in upto 50% of goats. In order to substantiate a diagnosis of Yersiniosis following a necropsy, it would be necessary to demonstrate the characteristic histopathological changes the follow invasion of this organism into the lamina propria of the intestines and later the mesenteric lymph nodes and subsequently the visceral organs.

The generalised periportal necrosis accompanied by a neutrophil infiltration suggest a very recent (during the past 12 hours) toxic insult.

HISTO FILE NO: 6800/3

Postgraduates:
Undergraduates: K. Woodley, K. Thompson

Pathologist : L. M. Badcoe
Date : 6 July 1989

DEPARTMENT OF VETERINARY PATHOLOGY AND PUBLIC HEALTH
MASSEY UNIVERSITY

MICROBIOLOGY REPORT

Case No: 20175 Date Taken: 8.6.89

Animal: Caprine Owner: Beagley

Submitter: L. Badcoe Lab No: 894436

Specimen: Rect/S

Microscopic Examination Gram ..... Zn ..... MZN ..... UV ..... Giemsa ..... KOH ..... CF .....

Gram +ve cocci ..... Gram +ve bacilli .....

Gram – ve cocci ..... Gram – ve bacilli .....

Culture Aerobic ..... Anaerobic ..... 10%CO_2 ..... Mycology .....

++ Yersinia enterocolitica

Specimen: Lg. Intest/S

Microscopic Examination Gram ..... Zn ..... MZN ..... UV ..... Giemsa ..... KOH ..... CF .....

Gram +ve cocci ..... Gram +ve bacilli .....

Gram – ve cocci ..... Gram – ve bacilli .....

Culture Aerobic ..... Anaerobic ..... 10%CO_2 ..... Mycology .....

+ Yersinia sp. (from M1/S)

Specimen: Small Intest/S

Microscopic Examination Gram ..... Zn ..... MZN ..... UV ..... Giemsa ..... KOH ..... CF .....

Gram +ve cocci ..... Gram +ve bacilli .....

Gram – ve cocci ..... Gram – ve bacilli .....

Culture Aerobic ..... Anaerobic ..... 10%CO_2 ..... Mycology .....

++ Yersinia sp. (from M1/S)

Specimen: .....

Microscopic Examination Gram ..... Zn ..... MZN ..... UV ..... Giemsa ..... KOH ..... CF .....

Gram +ve cocci ..... Gram +ve bacilli .....

Gram – ve cocci ..... Gram – ve bacilli .....

Culture Aerobic ..... Anaerobic ..... 10%CO_2 ..... Mycology .....

Specimen: .....

Microscopic Examination Gram ..... Zn ..... MZN ..... UV ..... Giemsa ..... KOH ..... CF .....

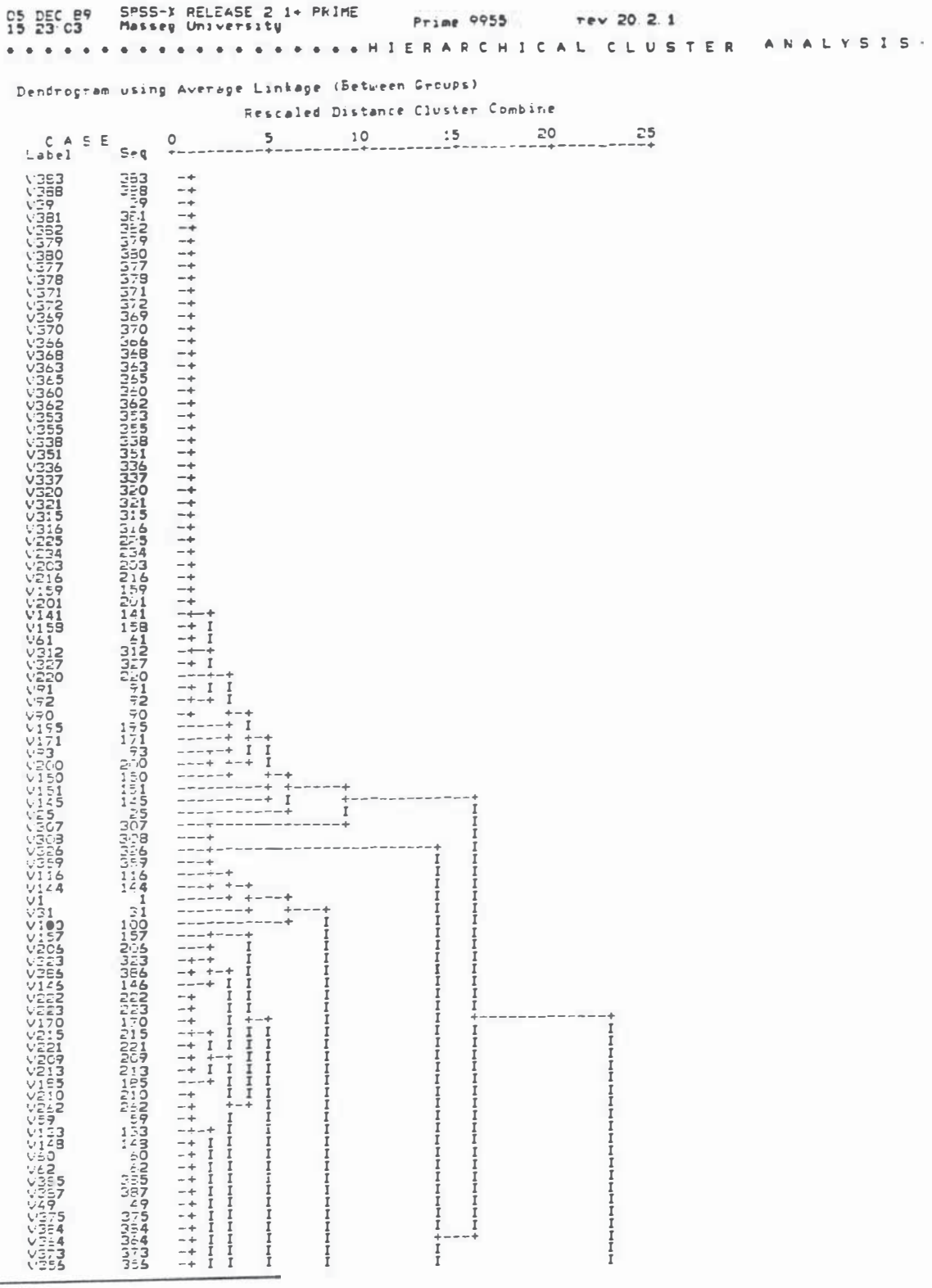
Gram +ve cocci ..... Gram +ve bacilli .....

Gram – ve cocci ..... Gram – ve bacilli .....

Culture Aerobic ..... Anaerobic ..... 10%CO_2 ..... Mycology .....

APPENDIX VI

DENDROGRAM [6]



[6] Refer to Table 8.1 for details of strains

05 DEC 89 SPSS-X RELEASE 2 1+ PRIME
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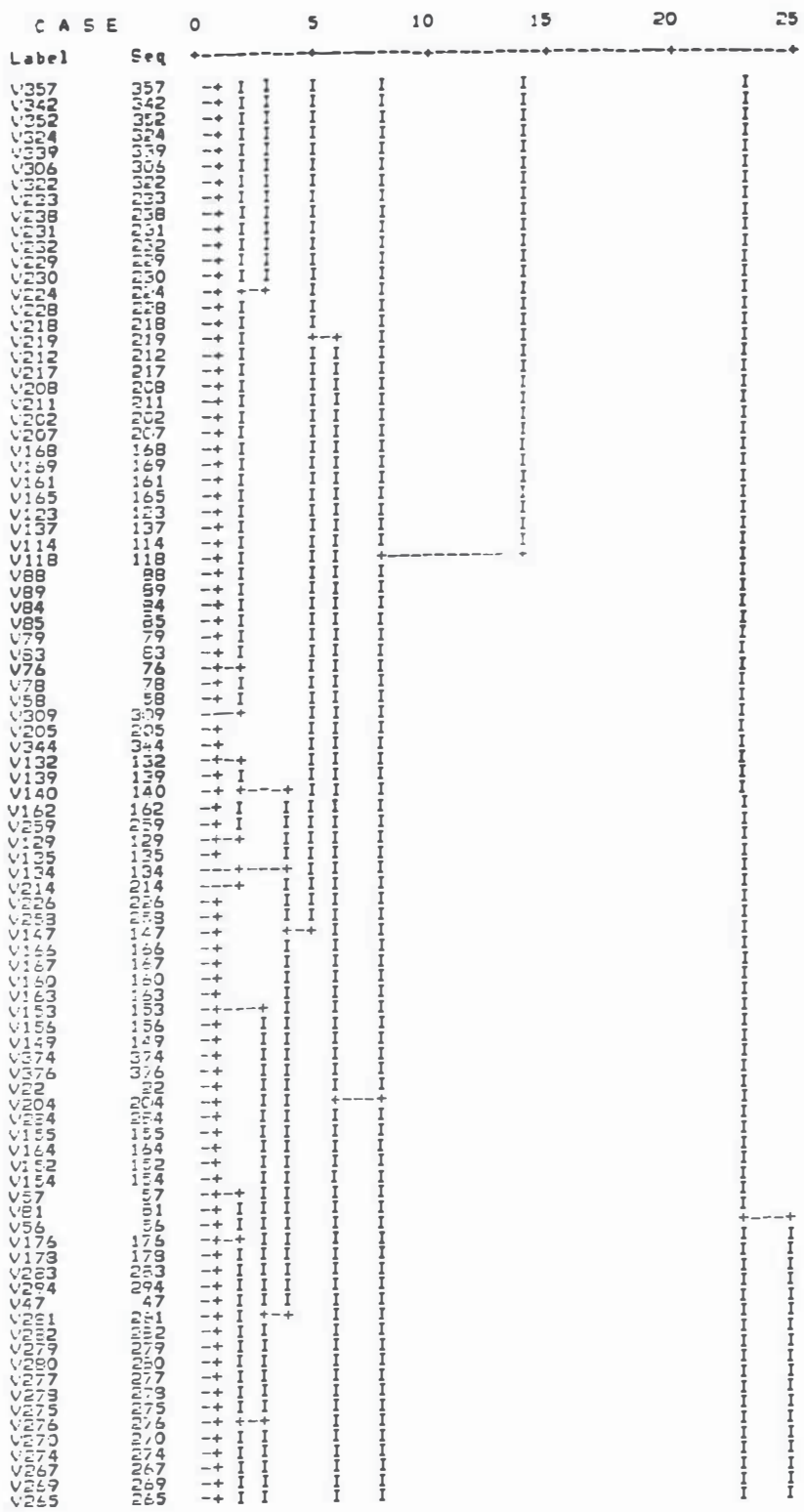

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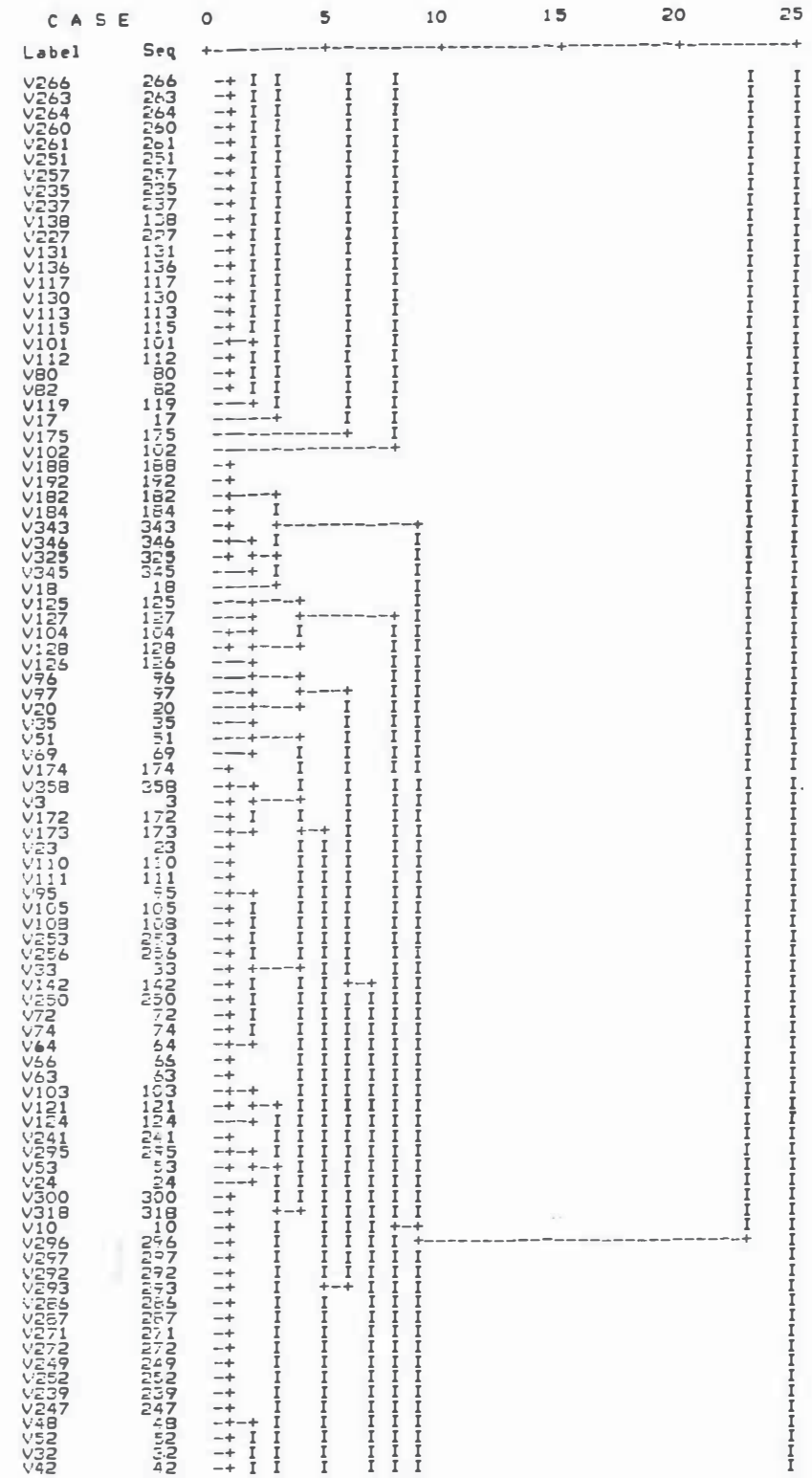

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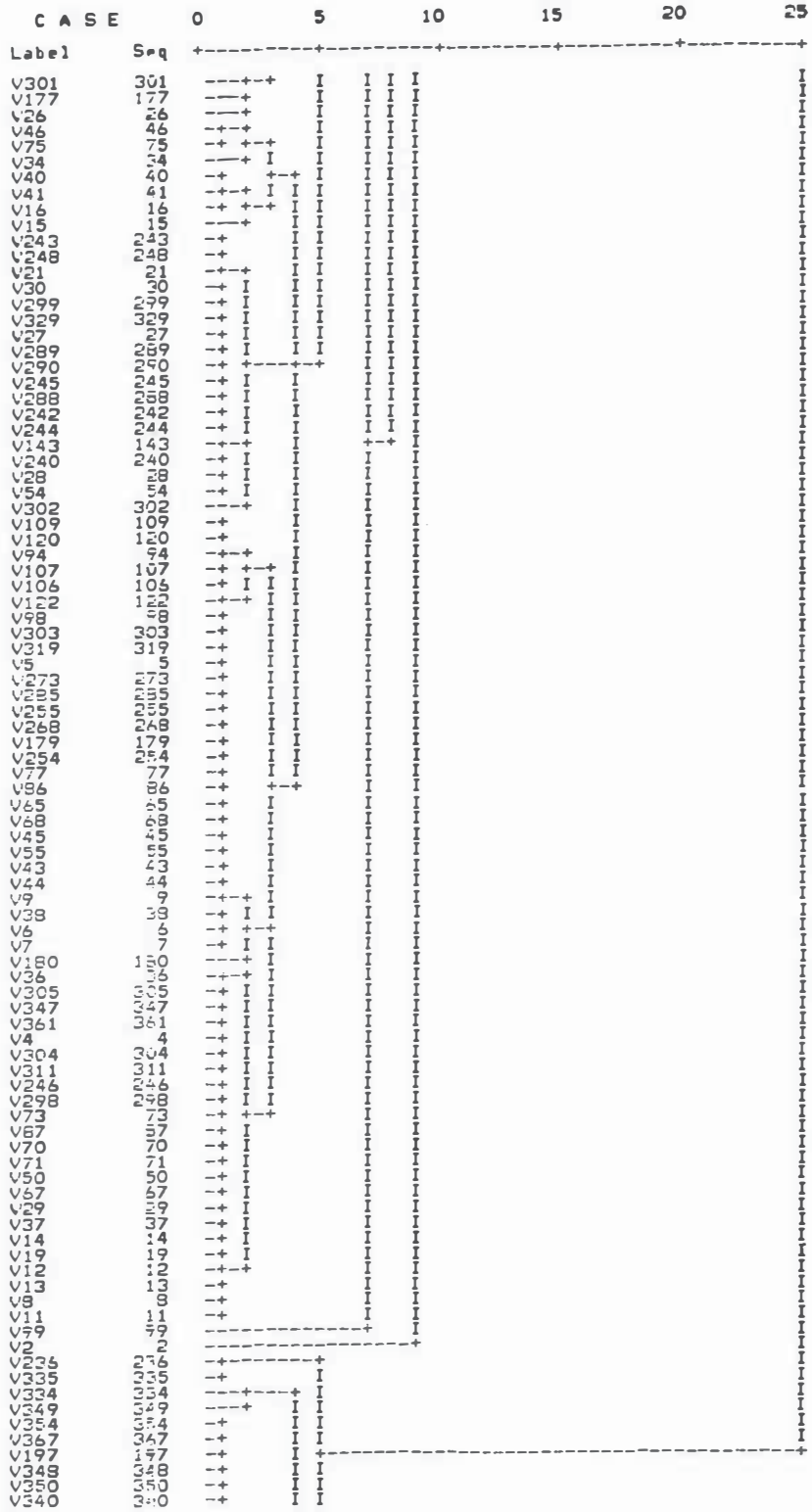

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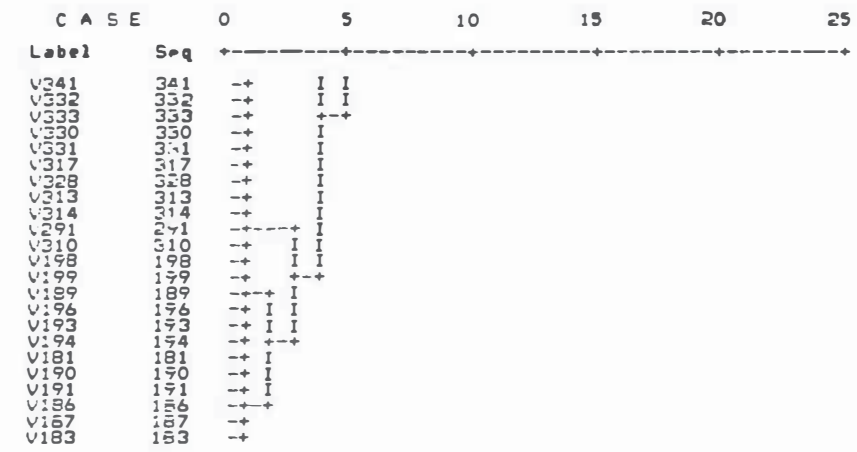

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