THE TONSILLAR CARRIAGE OF *YERSINIA* SPECIES BY PIGS

A THESIS PRESENTED IN PARTIAL FULFILMENT (40%)
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"Let those who labour hold the reins"

M.R. Bishop

In memory of a Grenadian Hero and Martyr
who endeavoured to uplift the standards
of the working class through mass
education and social reforms.
His spirit lives on!

To my family and children Maurice and Yenifer.

ABSTRACT

The impetus for this study arose due to the increasing isolation of species of *Yersinia* from people, with pigs being suspected as major reservoirs of human pathogenic strains of the organism in New Zealand. The general aims of the study, conducted in two phases, among pigs from several herds sent for slaughter at an abattoir in Palmerston North were:

(i) to determine the presence of human pathogenic strains of *Yersinia* in the tonsils of slaughtered pigs and their distribution among selected herds,

(ii) to determine the seasonal effect on prevalence of isolation and type of organism isolated, and

(iii) to determine the *in vitro* virulence characteristics of strains of the organism isolated from the tonsils of slaughter pigs, and their potential public health implications.

The first phase involved a cross-sectional study, conducted between August and September, 1993. Tonsils were collected from 124 pigs from eight farms and were examined for the presence of species of *Yersinia*. A total of 77 (62.1%) strains of *Yersinia* were isolated, this consisted of 42 (33.9%), 27 (21.8%), 7 (5.6%) and 1 (0.8%) strains of *Y. enterocolitica*, *Y. pseudotuberculosis*, *Y. frederiksenii* and *Y. kristensenii* respectively. *Yersinia enterocolitica* serotypes 0:3, 0:5,27 and *Y. pseudotuberculosis* comprised 26 (33.8%), 12 (15.6%) and 27 (35.1%) of the total number of isolates respectively. Yersiniae were isolated from all eight farms with individual farm prevalences ranging from 20% to 100%, while the number of species per farm ranged from 1 to 3. The pyrazinamidase activity test correctly identified 48 of the isolates as pathogenic or non-pathogenic yersiniae, (a specificity of 96%).

The second phase, a longitudinal study, was conducted over a period of twelve months (February 1993 - January 1994), among pigs from four farms, selected according to the particular strain of *Yersinia* prevailing in the herd. A total of 705 pigs were examined for the carriage of species of *Yersinia* in their tonsils. A total of 264 isolates were obtained, consisting of 198 (75%), 55 (20.8%), 5 (1.9%), and 1 (0.4%) strains of *Y. enterocolitica*, *Y.*

pseudotuberculosis, *Y. intermedia*, *Y. frederiksenii* and *Y. kristensenii* respectively. *Yersinia enterocolitica* serotypes 0:5,27 and 0:3 comprised 105 (39.8%) and 78 (29.5%) of the total number of isolates respectively. *Yersinia pseudotuberculosis* comprised 55 (20.9%) with serotype III, 39 (14.8%) the most consistently isolated serotype.

Yersiniae were isolated throughout the year particularly in the colder months. *Yersinia enterocolitica* serotypes 0:3 and 0:5,27 were found throughout the year with the lowest prevalence in the warmer months. However, a seasonal variation existed among serotypes of *Y. pseudotuberculosis*, with serotypes I and II found only in the winter and spring. Serotype III was found throughout the year, except for February.

During phase two of the study, 150 isolates of *Yersinia* were tested for *in vitro* virulence-associated characteristics. The autoagglutination test, CR-MOX agar, and the pyrazinamidase assay, coupled with salicin and aesculin tests, were highly successful in separating pathogenic from non-pathogenic strains of *Y. enterocolitica*. Likewise, the three assays successfully identified virulence activity in the majority of strains of *Y. pseudotuberculosis* with specificity among the three assays ranging between 90-100% for both *Y. pseudotuberculosis* and *Y. enterocolitica*.

The study also revealed marked variation in prevalence and type of *Yersinia* species isolated from pigs from different farms. The fact that particular serotypes predominate and persist on specific farms strongly suggest that there are factors such as source of pigs, management parctices or contact with other animals which determine their status. Identification of these determinates could lead to control or eradication of important yersiniae from pig farms.

The overall prevalence of 41.1% ranks New Zealand among countries with reported high isolation rates of the organism and further emphasises the fact that pigs constitute major reservoirs for human pathogenic strains of *Yersinia* worldwide. The infection among slaughter pigs in New Zealand may be of human health concern and this warrants further investigation particularly to determine whether the strains isolated from pigs are identical to those involved in human disease.

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GENERAL INTRODUCTION

Yersiniosis, a zoonotic disease caused by *Yersinia enterocolitica* and *Y. pseudotuberculosis*, is now recognised worldwide. The species *Y. enterocolitica* is an important cause of gastroenteritis in humans, especially in temperate countries (Mollaret *et.al.*,1979; WHO, 1981, 1987). *Yersinia enterocolitica* is considered to be a foodborne pathogen, despite the fact that attempts to isolate the bacterium from foods implicated in cases of disease in humans have rarely proved successful. However, some large foodborne outbreaks caused by *Y. enterocolitica* have been reported in the U.S.A., Canada and Japan (see Table 1.7). Pork products are considered to be the most likely source of infection (Hurvell, 1981; Lee *et.al.*, 1981; Morris and Feeley, 1976), although some aspects of the epidemiology still remain to be clarified.

Pigs appear to constitute an important reservoir for *Y. enterocolitica* infection (Hurvell, 1981; Kapperud, 1991; Schiemann, 1989), and are the only food animal which regularly harbours pathogenic *Y. enterocolitica*. Pigs are often healthy carriers of *Y. enterocolitica* biotype 4/0:3 and biotype 2/0:9 strains which cause disease in humans. Biotype 1B/0:8, the predominant human pathogen in the U.S.A., appears to be rare in pigs. This serotype may have entirely different reservoirs and ecology (Schiemann, 1989). Serotype 0:3 and 0:9 are both faecal commensals and inhibitants of the oral cavity of pigs, especially the tonsils and tongues. Serotype 0:3 is also frequently encountered as a surface contaminant on freshly slaughtered pig carcasses (Andersen, 1988; Nesbakken, 1988; Nesbakken *et.al.*, 1985).

Many surveys, reviewed elsewhere (Table 1.6) have demonstrated the common occurrence of *Y. enterocolitica* and related microbes in the intestinal tract and oral cavity of healthy slaughter pigs. The earlier reports of *Y. enterocolitica* in pigs were based on examination of faeces or intestinal contents. It was later demonstrated that the isolation frequency of these bacteria was approximately ten times greater from the tongues or tonsils than that obtained from faeces (Pedersen, 1979; Schiemann, 1980; Wauters, 1979). The reported isolation rates range up to and in excess of 56.0% (Table 1.6) depending on the type of

samples examined (tongues, tonsils, throat swabs), geographical origin, and efficacy of the isolation methods.

In Belgium, which is the country with the highest reported incidence of *Y. enterocolitica* infection of people, a case control study has shown that the infection was strongly associated with eating raw pork (Tauxe *et.al.*, 1987). The apparent rareness of *Y. enterocolitica* infection in Moslem countries (Samadi *et.al.*,1982) also supports the potential role of pork as the vehicle of *Y. enterocolitica* infection.

In New Zealand, reports of human disease due to yersiniosis, and of isolations from healthy subjects have been sporadic (Henshall, 1963; Lello and Lennon, 1992; Malpass, 1981; McCarthy and Fenwick, 1990; Rose, 1976), and an active search of possible hosts of species of *Yersinia* has only begun in earnest in recent years.

Prior to this study, only one published report of isolation of yersiniae from pigs in this country existed (Hodges *et.al.*,1984). However, unpublished data by Fenwick (*pers.comm* 1989) suggested that pigs may be carriers of human pathogenic yersiniae in their tonsils.

This study was therefore conducted as a follow up to the former, with the aim of confirming the findings and establishing some epidemiological aspects of the occurrence of yersiniae in the tonsils of slaughtered pigs.

The study was conducted in two phases. The first phase involved a cross-sectional study to determine the presence of species of *Yersinia* in the tonsils of slaughtered pigs and their distribution among farms supplying pigs for slaughter. The second phase, a longitudinal study, which was based on findings from the first, involved a selection of farms in relation to their particular carriage of species of *Yersinia*. Abattoir sampling was carried out on a monthly basis for twelve months, with the objective of investigating the seasonal effects on the occurrence of species of *Yersinia* in the tonsils of slaughtered pigs. During this phase, isolates were tested for possible virulence-associated characteristics with the aim of determining the role of pigs as possible reservoirs for human infection with yersiniae and thus the potential public health significance of pathogenic strains which may be harboured as free-living commensals in the tonsils of slaughtered pigs.

CHAPTER ONE

REVIEW OF THE LITERATURE

Section A: Yersiniosis

INTRODUCTION

The genus Yersinia belongs to the family *Enterobacteriaceae*, and is composed of at least 11 different species. *Yersinia pestis*, the cause of bubonic plague (the Black Death), *Y. pseudotuberculosis*, an animal pathogen which occasionally infects humans, and *Y. enterocolitica*, a cause of substantial morbidity and occasional mortality in humans and animals are the three most important yersiniae (Robins-Browne and Hartland, 1991). Strains previously termed "*Y.enterocolitica*-like organisms" have now been classified as seven separate species: *Y. frederiksenii; Y. kristensenii; Y. intermedia; Y. aldovae; Y. rohdei; Y. mollaretii* and *Y. bercovieri* (Aleksic *et.al.* 1987; Bercovier *et.al.*,1984; Brenner *et.al.*,1980; Wauters *et.al.* 1988b). These are all widespread in the environment and are usually not associated with disease. *Yersinia ruckeri* is an important fish pathogen responsible for red mouth disease in rainbow trout and some other fishes (Alecksic *et.al.*,1987). However its classification in the genus Yersinia is controversial.

With the acceptance of the new generic name *Yersinia* for *Pasteurella pestis, P. pseudotuberculosis* and *Yersinia enterocolitica* (Hubbert, 1972; Mair, 1973; Mollaret and Thal, 1974; Obwolo, 1976) the term yersiniosis now refers to infections caused by either *Y. pseudotuberculosis, Y. enterocolitica* and the new *Yersinia* species. Both *Y. pseudotuberculosis* and *Y. enterocolitica* in man and animals produce similar clinical signs and pathological lesions.

Porcine and human strains of yersiniae have been found indistinguishable by biochemical, serological and phage-typing methods (Hurvell, 1981; Wauters, 1979) therefore, pigs are suspected of being a major source of infection for man. Published data strongly supports the association between human yersiniosis and consumption of raw pork (Tauxe *et.al.*,

1987). A strong correlation has been demonstrated between the serogroups of the strains isolated from humans and pigs in the same geographical area (Bottone, 1977; Wauters, 1979; Schiemann and Fleming, 1981). Furthermore, typing methods based on detection of heterogenicity in plasmid and chromosomal DNA have so far been unable to distinguish between human and porcine strains (Andersen and Saunders, 1990; Kapperud *et.al.*,1990; Nesbakken *et.al.*,1987).

HISTORICAL AND GEOGRAPHICAL ASPECTS

The first description of *Y. pseudotuberculosis* was made by Malassez and Vignal in 1883 (Bissett, 1981). Since then investigators have studied the properties of the organism and sought methods to distinguish it from closely related members of the genus *Pasteurella* and other yersiniae (*Y. pestis* and *Y. enterocolitica*) as well as from other members of the family *Enterobacteriaceae*. Dickinson and Macquot (1961) isolated *Y. pseudotuberculosis* from the alimentary contents of healthy pigs. *Yersinia pseudotuberculosis* is a well recognised animal pathogen which causes zoonotic infections (Mair, 1975; Paff *et.al.*,1976; Saari and Triplett, 1974). Studies done in Europe, Canada and Japan have shown that pigs are an important reservoir of *Y. pseudotuberculosis* (Narucka and Westendoorp, 1977; Toma and Deidrick, 1975; Tsubokura *et.al.*,1976; Zen-yoji *et.al.*,1974).

When Frederiksen in 1964 (cited by Schiemann, 1982) suggested the name "*Yersinia enterocolitica*" for a bacterium that had first been described by Schleifstein and Coleman in 1939 (cited by Sonnenwirth, 1974) and had variously been referred to as *Bacterium enterocoliticum, Pasteurella pseudotuberculosis b,* Pasteurella X and even "Germe X" he could not have predicted that, within the next decade, reports of human infections would increase from a few dozen to thousands, and that there would be by 1977 three international symposia devoted to this organism (Schiemann, 1982). Within that short span of time *Y. enterocolitica* had become a well-established bacterial species in the family *Enterobacteriaceae*. Since then the organism has been isolated with increasing frequency from man and animals (including dogs and pigs) and from some human foods. By 1976 it had become a cause for some concern in both human and veterinary medicine (Morris and Feeley, 1976).

Yersinia enterocolitica has become recognised as a major cause of diarrhoea in people in much of the industrialised world. In Scandinavia, Japan, Canada and parts of Europe, the isolation rates of *Y. enterocolitica* from patients with gastroenteritis rival those of *Salmonella* (Christensen, 1987; de Groote *et.al.*, 1982; Hiroshi, 1981; Marks *et.al.*, 1980; van Noyen *et.al.*, 1981). In these countries *Yersinia enterocolitica* 0:3 is the predominant serotype and appears to have an important reservoir in pigs (Christensen, 1987; Narucka and Westendoorp, 1977; Schiemann, 1980; Shiozawa *et.al.* 1987). First isolated in Belgium in 1963, now the country with the highest reported incidence of yersiniosis, *Y. enterocolitica* 0:3 infections have been linked to the ingestion of raw pork (Tauxe *et.al.*, 1987). The reported isolation rate increased to 12/100,000 in 1984 with the highest incidence in children aged 1 to 4 years (van Noyen *et.al.*, 1981).

Laboratory reports of infections due to *Y. enterocolitica* increased in England and Wales from 45 in 1980 to a provisional number of 591 in 1989; reports also increased in Scotland (Cover and Aber, 1989). Laboratory confirmation of infections is difficult and takes longer than for most other gastro-intestinal pathogens so that the reported cases probably underestimate the true incidence of the disease. A study of faecal samples of patients in London (Lewis and Chattopadhyay, 1986) and another in Poole (Greenwood and Hooper, 1987) gave isolation rates of *Yersinia* spp. of 6.1% and 3.5% respectively, but the organisms isolated did not include the serotypes of *Y. enterocolitica* usually recognised as pathogenic to humans in the U.K. These studies do not imply, therefore, a substantial under-diagnosis of yersiniosis and the incidence of disease in the U.K. would appear to be much lower than in other countries such as Belgium, the Netherlands, Canada and Australia (Cover and Aber, 1989).

In the United States the ingestion of contaminated foods has been implicated as the mode of transmission in the infrequent outbreaks (Black *et.al.*, 1978; Morse *et.al.*, 1984; Tacket *et.al.*, 1985) and one family outbreak has been attributed to contact with sick dogs (Gutman *et.al.*, 1973). Unlike those in other industrialized countries, the reported isolation rates of *Y. enterocolitica* in clinical laboratories in the U.S.A. have generally been low (Dajani and Maurer, 1980; Kachoris *et.al.*, 1988; Marynout *et.al.*, 1982; Weissfeld and Sonnenwirth, 1980) and until recently the 0:3 serotype has been uncommon (Bissett *et.al.*, 1990; Kay *et.al.*, 1983). In November 1988, the first reported outbreak of *Y. enterocolitica* 0:3 infections in the United States was detected in black children in Atlanta (Lee *et.al.*, 1990).

Investigations of this outbreak identified indirect exposure to contaminated raw pork and found evidence of a widely distributed reservoir in the American pig population (Lee *et.al.*, 1990).

In Australia, the distribution of human cases of yersiniosis is patchy. Although meaningful comparisons of case rates are not possible, because the requirement to notify cases differs between the states, it appears that most human infections occur in South Australia, Queensland and New South Wales. The crude rate of yersiniosis in South Australia (14.5/100,000) is similar to that reported in Belgium (Robins-Browne, 1992).

Yersiniosis is uncommon in tropical and developing countries (Robins-Browne, 1992), however a few reports exist on the isolation of *Y. enterocolitica* from animals (Adesiyun *et.al.*,1986), pigs and humans (Agbonlahor *et.al.*,1985; Ikheloa *et.al.*,1992; Lombin *et.al.*, 1985; Okoroafor *et.al.*, 1988; Samadi *et.al.*, 1982) in tropical and sub-tropical regions. Adesiyun *et.al.*(1992) reported the isolation of *Y. enterocolitica* from livestock in Trinidad. To the author's knowledge this is the first report of the isolation of this organism in Trinidad and possibly the West Indies. In Latin America, yersiniosis has been reported from Brazil and Argentina involving both *Y. pseudotuberculosis* and *Y. enterocolitica* infections (Falcoa, 1981; 1987; Mollaret *et.al.*,1979).

The serogroups commonly involved in pathological processes in man or animals belong to distinct serogroup-biovar-phagevar combinations (Mollaret *et.al.*,1979; Table 1.1).

Table 1.1: Interrelationships between serogroup, biovar, phagevar, and geographical distribution of the most common human pathogenic strains of *Y. enterocolitica*.

Serogroup	Biovar	Phagevar	Location
3	4	VIII	Europe, Japan, others
3	4	Ixa	South Africa
3	4	Ixb	Canada
3	3	11	Japan
9	2	X3	Europe
8	IB	Xz	USA, Canada

Some biogroups of *Y. enterocolitica* are associated with particular geographic areas but not as closely as was once thought (Robins-Browne, 1992). Biotype 1B yersiniae were once thought to be restricted to the USA, but these strains have now been identified in several other countries, including Australia (Robins-Browne, 1992), Canada (Toma and Lafleur, 1981) and Europe (Chiesa *et.al.*,1991). Although *Y. enterocolitica* has been classified into approximately 60 serogroups on the basis of O antigens (Wauters, 1981), the strains associated with disease in man or animals belong to only a few serogroups. Thus 0:3, 0:5,27, 0:8 and 0:9 are the most important causative agents of *Yersiniosis* in man (See Table 1.2).

Table 1.2: Relationship between biotype, serogroups, pathogenicity and geographic distribution of *Y. enterocolitica*.

Biotype	Serogroup(s)	Geographic distribution
1A	0:4; 0:5; 0:6; 0:7,8; 0:10; 0:14; 0:16; 0:21; 0:22; 0:25; 0:37; 0:41; 0:46; 0:47; 0:57; NT[a]	Worldwide, including New Zealand
1B	0:4,32; **0:8***; **0:13[a],13[b]***; 0:16; 0:18; **0:20***; **0:21***; 0:25; 0:41,42; NT	USA, Canada, Australia, Europe
2	**0:5,27***; **0:9***; 0:27	Europe, Japan, New Zealand
3	**0:1,2,3***; **0:3***; **0:5,27***	Europe, Japan, Australia, New Zealand
4	**0:3***	Worldwide including New Zealand (but uncommon in Southern and Western USA)
5	**0:2,3***	Europe, Australia, New Zealand
6	0:3, 0:7; 0:7,19; 0:8; 0:10; 0:16; 0:18; 0:19,36; 0:19,43; 0:47; NT	Worldwide

[a]NT not typable
* indicates strains associated with gastrointestinal infection of humans. Other strains occasionally may cause extraintestinal or opportunistic infection. Adapted from: Robins-Browne and Hartland, 1991; Robins-Browne, 1992.

GENERAL CHARACTERISTICS OF YERSINIA SPECIES

The bacterium

According to Bercovier and Mollaret (1984), yersiniae are straight rods to coccobacilli, 0.5-0.8*u*m in diameter and 1-3*u*m in length, Gram-negative asporogenous bacteria. Pleomorphism occurs depending on the type of medium and incubation used. Capsules are not present but an envelope occurs in *Y. pestis* grown at 37 °C or in cells from *in vivo* samples. Yersiniae are facultatively anaerobic, having both a respiratory and fermentative type of metabolism. They are 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 characteristic 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. Yersiniae occur in a broad spectrum of habitats (live and inanimate) with some species adapted to specific hosts. They are nonmotile when cultured at 37 °C but motile when cultured at 22-29 °C except for *Y. pestis* which is always nonmotile. Fresh isolates may require a few subcultures to express their motility. Mobile cells have 2-15 peritrichous flagellae characterised by a long wave length (Nilehn, 1969).

Growth requirements

Enteropathogenic yersiniae grow fairly rapidly on simple media such as nutrient agar with confluent growth in 24 hours. They grow well on MacConkey or desoxycholate agar, multiply well at low temperatures perhaps down to 0 °C. The upper limit of growth for some strains is 42 °C - optimum range 27 ° to 30 °C and between pH 6.3 and 7.3 (Barton, 1992), however, Falcao *et.al.* (1979) suggested a pH range between 7.6-7.9. Growth of *Y. enterocolitica* at 0 °-2 °C in milk after 20 days has been observed. Growth at 0 °-1 °C on pork and chicken has been observed (Leistner *et.al.*,1975) and three strains were found to grow on raw beef held for 20 days at 0 °-1 °C (Hanna *et.al.*, 1977). In milk at 4 °C, *Y. enterocolitica* grew and attained up to 10^7 cells per ml in 7 days and competed well with the background flora (Amin and [illegible]ughon, 1987). *Yersinia pseudotuberculosis* serotype 4b did not grow but survived in experimentally inoculated raw pork at 6 °C and 25 °C (Fukushima, 1987).

The addition of NaCl to growth media raises the minimum growth temperature. In brain heart infusion (BHI) broth containing 7% NaCl, growth did not occur at 3 °C or 25 °C after 10 days. At pH 7.2, growth of one strain was observed at 3 °C and very slight growth at pH 9.0 at the same temperature; no growth appeared at pH 4.6 and 9.6 (Stern *et.al.*, 1980). Although 7% NaCl was inhibitory at 3 °C, growth occurred at 5% NaCl. With no salt, growth was at 3 °C over the pH range 4.6-9.0 (Stern *et.al.*,1980; Swaminathan *et.al.*,1982). Clinical strains were less affected by these parameters than were environmental isolates.

Yersinia enterocolitica is destroyed in 1 to 3 minutes at 60 °C (Hanna *et.al.*, 1977). It is rather resistant to freezing, with numbers decreasing only slightly in chicken after 90 days at -18 °C (Leistner *et.al.*,1975). Recent studies indicate that *Y. enterocolitica* can proliferate in blood donated for transfusion and stored for above three weeks (Ardiuno *et.al.*, 1989; Jones *et.al.*, 1993).

Most human isolates of *Y. entercolitica* produce a heat-stable enterotoxin (YST), with properties similar to those produced by enterotoxigenic *Escherichia coli* (ETEC) (Boyce *et al*, 1979). This YST can survive 100 °C for 20 minutes (Okamoto *et al* 1981).

For establishment of the role of the heat stable enterotoxin (YST) in the virulence of human isolates of *Y. enterocolitica*, more information is needed on nutritional factors and growth conditions that regulate the synthesis of YST. Recently a defined medium containing four amino-acids (L-methionine; L-glutamic acid, glycine and L-histidine) that supported the growth of and synthesis of YST at levels equivalent to those observed in a complex trypticase soy broth containing 0.6% yeast extract medium was developed (Amirmozafari and Robertson, 1993).

Isolation

Biological samples for laboratory isolation of yersiniae can include food and environmental samples (surface swabs from dairies, throat and rectal swabs from pig carcasses) (Greenwood and Hooper, 1985); palatine tonsils from pigs (De Boer *et.al.*, 1986; Hanna *et.al.*, 1980; Hunter *et.al.*, 1983; Nesbakken and Kapperud, 1985); drinking water (Langeland, 1983); free-living and wild animals (Kapperud 1975, 1977, 1981); pathological products (abscess, blood, lymph node, stools) (Carniel and Mollaret, 1990).

The recovery rate of yersiniae is low in most clinical laboratories. Most strains of *Y. enterocolitica* will grow on selective enteric media and will appear as small, lactose-negative colonies on MacConkey and Salmonella Shigella (SS) agars in 48 hours. In some laboratories, plates of MacConkey agar inoculated with stool specimens suspected of harbouring yersiniae are routinely incubated at room temperature. *Yersinia enterocolitica*, in particular, can best be recovered from stool specimens that are incubated at 25 °C. Cold enrichment of highly contaminated specimens, such as faeces, by incubating cultures at 4 °C for 1 to 3 weeks in phosphate-buffered saline (PBS) prior to subculture onto enteric media also enhances the recovery of *Y. enterocolitica* (Pai *et.al.*, 1979). Weissfeld and Sonnenwirth (1982), reported that pre-treatment of stools with 0.5% potassium hydroxide at a ratio of 1:2 for 2 minutes, followed by plating onto enteric media, resulted in the recovery of the highest number of *Yersinia* isolates. The superiority of cefsulodin-irgasan-

novobiocin (CIN) agar for recovery of *Y. enterocolitica* from stool suspensions containing 10^2 colony forming units or less has been reported by Head and colleagues (1982).

Nesbakken and Kapperud (1985) compared three different isolation procedures for their relative efficacy with regards to the recovery of naturally occurring yersiniae from porcine tonsils.

(i) Direct plating on CIN agar (DP)
(ii) Pre-enrichment in PBS + 1% sorbitol and 0.5% bile (PSB) for eight days at 4 °C, followed by selective enrichment in modified rappaport broth (MRB) for four days, at 20 to 25 °C (PSB-MRB) and
(iii) Cold enrichment in PSB (three weeks, 4 °C).

The results indicate that PSB cold enrichment should be included in any combination regardless of the type of yersiniae sought. This procedure was the most effective single method for recovery of *Y. enterocolitica* 0:3/biotype 4, *Y. enterocolitica* biotype 1 and *Y. kristensenii*.

Several investigators (Davey *et.al.*, 1983; Doyle and Hugdahl, 1983; Greenwood and Hooper, 1985; Hunter *et.al.*, 1983; Okoroafor *et.al.*, 1988; Ratnam *et.al.*, 1983, van Noyen *et.al.*, 1987a, 1987b) investigating human and animal specimens have recommended the combination of cold enrichment in M/15 phosphate buffered saline (PBS) pH 7.6, with subsequent plating onto CIN agar. This combination was extremely efficient not only for the recovery of *Y. enterocolitica* serotype 0:3 and 0:9 but also for other types and species of the genus *Yersinia* from a range of samples.

Identification

Suspicious colonies from CIN (Bull's eye, red to pink, pinpoint colony), MacConkey or other enteric media should be picked for complete identification or screened in biochemical media. Presumptive identification is achieved by inoculating the colony onto Triple-sugar-iron agar (TSI) slant and urea agar (Wauters, 1973). A third test is a modification of Edward and Fife's (1961) Lysine-Iron agar (LIA), to Lysine-Arginine-Iron agar (LAIA) (Weagant, 1983). Typical reactions of *Yersinia* spp. on LAIA are alkaline slant (purple),

acid butt (yellow) (K/A), no H_2S production (no darkening of butt) or gas formation after 24 hours at 28 to 29 °C.

Urease-positive strains that give (TSI) acid butt plus alkaline or acid slant (A/A or K/A) no gas or no blackening after 24 hours at 28 to 29 °C are verified by Gram staining and motility testing at 28 °C and 37 °C (Christensen, 1980; Lassen, 1975). For differentiation of *Yersinia* spp. the organism are inoculated into biochemical test media (sugars, urea, aesculin, methyl red (MR), voges-proskauer (VP) at 29 ° and 37 °C, Simmons citrate, ornithine, arginine, lysine). The major biochemical tests that differentiate *Y. pseudotuberculosis* from *Y. enterocolitica* are ornithine decarboxylase, sucrose and sorbitol. *Yersinia pseudotuberculosis* is negative for all three, whereas *Y. enterocolitica* is positive.

Biotypes and Serotypes

Determination of the antigenic structure will confirm the identity of the organism and indicate the serogroup to which it belongs. Serogrouping is performed by slide-agglutination using single-factor O-agglutinating antisera prepared from rabbits hyperimmunized with autoclaved strains of different serogroups and subgroups (Mair *et.al.*, 1960). *Yersinia pseudotuberculosis* has been separated into six serogroups, Types I to VI (Thal and Knapp, 1971; Tsubokura *et.al.*, 1971). Subtypes labelled A and B have been determined for Types I, II, IV and V.

Unlike *Y. pseudotuberculosis*, *Y. enterocolitica* and its related species represent a complex and heterogeneous group from the point of view of their biochemical and antigenic patterns. *Yersinia enterocolitica* O-antigen representing factor 1 to 57 (Wauters, 1981) are recognised. Slide-agglutination with absorbed, specific O-antisera prepared against serovars 0:3, 0:5,27, 0:8 and 0:9 should be sufficient to identify most strains responsible for human yersiniosis (Mair and Fox, 1986).

Initially, *Y. enterocolitica* was grouped into 5 biotypes by Nilehn (1969). The biotyping scheme proposed by Wauters *et.al.* (1987) involved the addition of new biochemical tests: Pyrazinamidase reaction, 5-D-Glucosidase and proline peptidase. Biotype 1 was divided by these authors to differentiate the non-pathogenic environmental strains (1A) from the North American human pathogenic strains (1B). These authors also proposed the creation

of biotype 6 to accommodate the non-pathogenic *Y. enterocolitica* strains within biotype 3, biotypes 3A and 3B. Due to further biochemical characterisation, biotype 3A and 3B is now called *Yersinia mollaretii* and *Yersinia bercovieri* respectively (Wauters *et al*, 1988). Table 1.3 shows the differentiation of species within the genus *Yersinia* (Bercovier *et.al.*, 1984; Wauters *et.al.*,1988).

Biochemical differentiation of the 6 biogroups of *Y. enterocolitica* is shown in Table 1.4 (Wauters *et.al.*,1987).

Table 1.3: Biochemical differentiation of species within the genus *Yersinia*.

Biochemical Test	*Y. pestis*	*Y. pseudotuberculosis*	*Y. enterocolitica*	*Y. frederiksenii*	*Y. intermedia*	*Y. kristensenii*	*Y. aldovae*	*Y. bercovieri*	*Y. mollaretii*	*Y. rohdei*
Indole	-	-	V	+	+	V	-	-	-	-
Ornithine	-	-	+	+	+	+	+	+	+	+
Motility 25 °C to 28 °C	-	+	+	+	+	+	+	+	+	+
Fermentation of sucrose	-	-	+	+	+	-	-	+	+	+
Rhamnose	-	+	-	+	+	-	+	-	-	-
Cellobiose	-	-	+	+	+	+	-	+	+	+
Sorbitol	-	-	+	+	+	+	+	+	+	+
Melibiose	V	+	-	-	+	-	-	-	-	V

+ = 90% or more strains are positive; - = 90% or more strains are negative; V = 11%-89% of strains are positive. Adapted from: Bercovier *et.al.*, 1984; Wauters *et.al.*, 1988. All tests were performed at 25 °C to 28 °C.

Table 1.4: Biochemical differentiation of *Y. enterocolitica* biogroups.

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

EPIDEMIOLOGY

Reservoirs

Yersinia enterocolitica and related species are widely distributed in the terrestrial environment and in lakes, well and stream waters, which are sources of the organism for warm-blooded animals. *Yersinia enterocolitica* is more animal adapted and is found more often among human isolates than the other species. Of 149 strains of human origin in the U.S.A., 81, 12, 5.4 and 2% were respectively *Y. enterocolitica*, *Yersinia intermedia*, *Yersinia frederiksenii* and *Yersinia kristensenii* (Shayegani *et.al.*,1981). *Yersinia intermedia* and *Y. frederiksenii* are found mainly in fresh water, fish and food and are only occasionally isolated from humans. *Yersinia kristensenii* is found mainly in soils and other environmental samples as well as in foods but is rarely isolated from humans (Kreig, 1984).

Yersinia enterocolitica serotypes 0:3 and 0:9 are rarely isolated from the environment. Pigs are the principal animal species which have been identified as carriers, and they are most probably the reservoir of these strains (Carniel and Mollaret, 1990) as well as serotype 0:5,27 (Hanna *et.al.*,1980). Pigs carry pathogenic yersiniae (0:3, 0:9, 0:5,27) in their throat and on their tonsils and shed them in faeces for up to 30 weeks (Fukushima *et.al.*,1979). In contrast, serotype 0:8, the predominant human pathogen in the USA, appears to be rare in pigs (Schiemann, 1989). Sources of infection with serotype 0:8 are

much wider than with the above serotypes. They involve mainly ingestion of water (Aulisio *et.al.*,1983; Keet, 1974) particularly drinking water from streams and wells, food washed with contaminated water (bean sprouts, tofu) and milk products (Black *et.al.*, 1978).

Dogs, cats and rats may occasionally be faecal carriers of 0:3 and 0:9, the relative intimate contact between man and pets suggests a potential reciprocal transmission, although such an epidemiological link has not been confirmed (Carniel and Mollaret, 1990). Pathogenic *Y. enterocolitica* was recovered from crabs in Alaska (Fughri, 1984) and *Y. enterocolitica* biotype 2, serotype 0:5,27 demonstrating virulence characteristics was recovered from a common garter snake in Canada (Kwaga and Iversen, 1993).

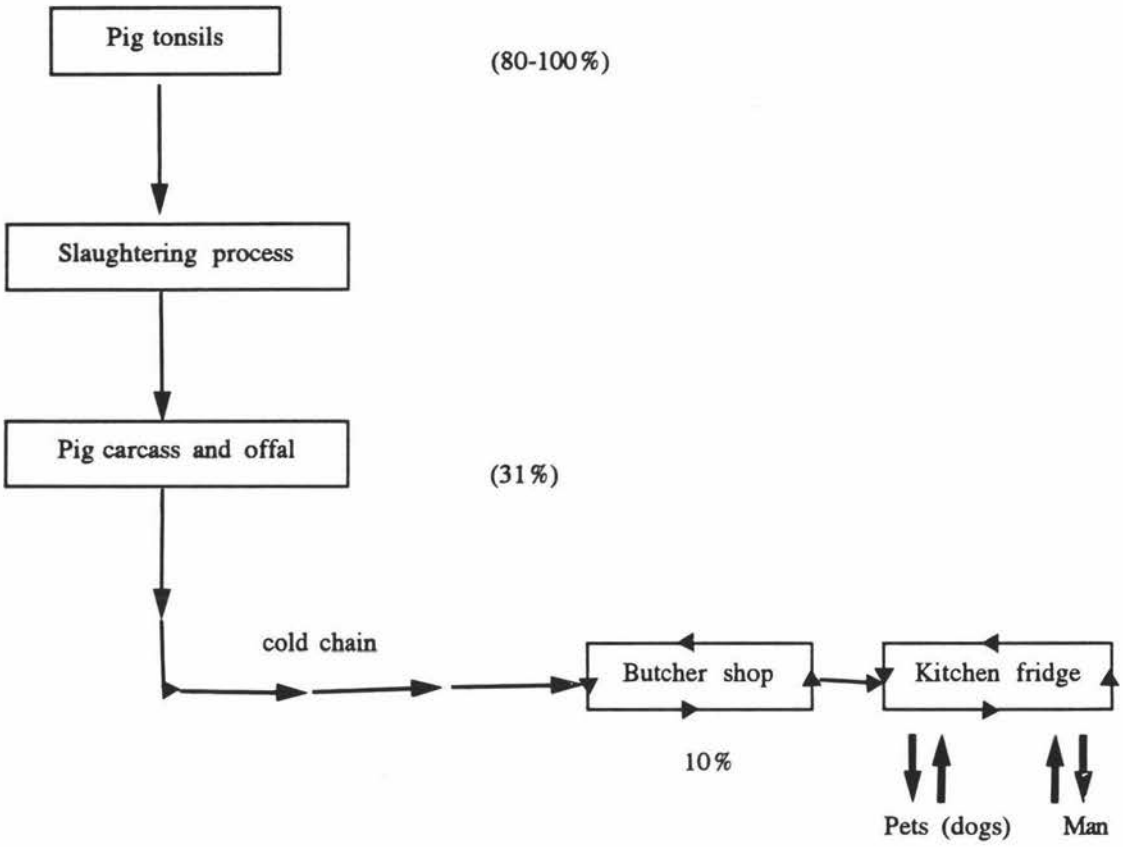
Yersinia pseudotuberculosis is widely spread in the environment, the environment itself is contaminated by the faeces of infected animals, mainly rodents and birds (Carniel and Mollaret, 1990).

Transmission

Clinical and epidemiological data indicate that the most likely portal of entry of *Y. enterocolitica* infection in both animals and man would be the digestive tract. Since *Y. enterocolitica* has been isolated from the intestinal contents of a large number of animals and occurs abundantly in different environments, it has been presumed that this organism would follow the same epidemiological pattern as other enterobacteria, e.g. various *Salmonella* and *Escherichia* species (Hurvell, 1981).

The most frequently documented mode of transmission has been by foods, milk, milk drinks and water (Cover and Aber, 1989). A case control study in Belgium implicated the consumption of raw pork (Tauxe *et.al.*, 1987). The route of infection of *Y. enterocolitica* 0:3 in Denmark seems to be closely related to the major reservoir in pigs (Fig. 1.1), with modern slaughtering technology contributing to the spread of the organism on the carcass. Pigs are healthy carriers and detection at routine, organoleptic meat inspection is consequently not possible (Christensen, 1987).

Figure 1.1: The proposed route of infection with *Y. enterocolitica* 0:3.



Cross-contamination in the kitchen from raw pork chitterlings to prepared infant foods appeared to be the mode of transmission to infants in an outbreak in the U.S.A. (Lee *et.al.*, 1990). Faecal-oral spread by handling infected pigs, dogs and cats has been reported (Carniel and Mollaret, 1990). Serotypes 0:3 and 0:9 may also spread from person-to-person by the faecal-oral route, and this transmission from asymptomatic "carriers" could be a possible explanation of some sporadic cases.

Although spread of *Y. enterocolitica* within families and institutions is well documented, direct person-to-person transmission is uncommon. A possible explanation for this may be that *Y. enterocolitica* is especially likely to initiate infection when grown at temperatures below 30 °C. The observation in many temperate countries that sporadic cases of yersiniosis occur chiefly during late autumn and early winter supports experimental evidence that environmental growth temperature influences bacterial pathogenicity (Robins-Browne, 1992).

Serotype 0:8 appears to be different to the other pathogenic serotypes, there is no evidence of person-to-person transmission or of carriers, and the origin of this serotype is most likely to be contaminated water or food (Carniel and Mollaret, 1990). Infection caused by contamination of food by human excreters of the organism has not been described.

Transmission by blood transfusion has been reported (Cover and Aber, 1989). The few *Y. enterocolitica* present during transient bacteraemia may multiply in blood products stored at 4°C, without manifestly altering their appearance. Accordingly, large numbers of bacteria may be infused directly into patients receiving a blood transfusion. Administered in this manner, even environmental strains of *Y. enterocolitica* could be as lethal as enteropathogenic strains (Robins-Browne, 1992). A septic shock syndrome produced upon transfusion is fatal in 70% of the cases (Jacobs *et.al.*, 1989).

Transmission of *Y. pseudotuberculosis* occurs by consumption of contaminated food (oral contamination). There is an increased incidence of clinical infections due to *Y. pseudotuberculosis* during the cold season for at least two reasons: first, the bacteria survive and multiply better in the environment at low temperatures; second, animals which are healthy carriers of the organism excrete larger numbers in their faeces after a stress like cold weather or starvation (Carniel and Mollaret, 1990). The cycle is very simple: infected stools from contaminated animals are spread in the environment, new animals become infected by ingestion of the contaminated water or grass and in turn spread the organisms throughout the environment. Most often, human cases occur by consuming raw contaminated vegetables. From time to time, *Y. pseudotuberculosis* is also transmitted directly from animals (pets) to humans or from humans to humans via dirty hands (Carniel and Mollaret, 1990).

Susceptible Species

With *Y. enterocolitica*, serotypes 0:3, 0:8 and 0:9, the main susceptible species is man. Experimental infection of rodents with serotype 0:8 leads to rapid death whereas serotypes 0:3 and 0:9 only cause very mild diarrhoea or no symptoms at all. A few animal species are sensitive to strains which are not common human pathogens: hares

and goats (biotype 5, serotype 0:2,3), chinchillas (biotype 3, serotype 0:1, 0:5b). The majority of the other animal species are resistant to *Y. enterocolitica* infections (Carniel and Mollaret, 1990) although some reports exist of infection in sheep, cattle and dogs (Fantasia *et al*, 1993; Slee and Button, 1990b; Slee and Skilbeck, 1992)

Almost all animal species are potential carriers of *Y. pseudotuberculosis*. However, as mentioned earlier, the disease often only appears after stress. The organism has a worldwide distribution, but most cases occur in the northern hemisphere with numerous reports of outbreaks in zoological collections and aviaries (Barton, 1992).

PATHOGENESIS AND CLINICAL PRESENTATION

Yersinia pseudotuberculosis infection

Infection with *Y. pseudotuberculosis* may cause severe disease in animals. After oral contamination the bacteria pass through the stomach and the small intestines and invade the mesenteric lymph nodes at the end of the ileum. They spread via the lymphatic vessels to the distal lymph nodes and invade the spleen and liver where abscesses are formed. Rapid invasion of blood vessels leads to septicaemia and death of the animal (Carniel and Mollaret, 1990). *Yersinia pseudotuberculosis* serotypes I, II and III are now recognised as the cause of enteric or septicaemic disease in a wide range of species including man and other primates, rodents, birds and domestic ungulates (cattle, deer, goats, sheep) (Comba *et.al.*,1993; Henderson, 1983; Jerrett *et.al.*,1990; Mackintosh and Henderson, 1984; Obwolo, 1976; Slee and Button, 1990 a,b; Slee *et.al.*,1988; Slee and Skilbeck, 1992). *Yersinia. pseudotuberculosis* is considered as a possible cause of abortion in sheep in Germany where the organism was detected in addition to chlamydiae in a flock of aborting sheep (Mirle *et.al.* 1993).

The disease in man is usually less severe than in animals. The bacterium migrates to the ileum and mesenteric lymph nodes, but it neither colonizes the spleen and liver nor invades the blood stream (Carniel and Mollaret, 1990). There is frequent fever but this is usually low grade (38-39 °C). The most constant symptom is abdominal pain in the right lower quadrant. This pain is due to a mesenteric lymphadenitis which mimics

appendicitis and often leads to surgery (Mair and Fox, 1986). After laparotomy, the appendix is found to be normal but is surrounded by enlarged, inflamed and infected mesenteric lymph nodes. Progressively, the infection ascends along the lymphatic route, the distal lymph nodes are infected while the proximal become sterile. Usually the disease is self-limiting and the clinical symptoms disappear even without treatment. Rarely, the infection leads to intestinal necrosis (Ahvonen, 1972). Diarrhoea accompanying the abdominal pain is mild and not constantly found. The most common secondary complications are erythema nodosum and reactive polyarthritis; this occur a few weeks after the acute phase. Arthritis is frequently associated with the presence of HLA-B27 antigen (Aho *et.al.*, 1973). Far-eastern scarlatiniform fever or epidemic pseudotuberculosis, a severe form of pseudotuberculosis which appears to be confined to the far-eastern territories of the former Soviet Union, is characterized by fever, a scarlatiniform rash, arthralgia and acute polyarthritis. Symptoms indicative of fever and gastro-intestinal lesions have also been reported (Mair and Fox, 1986). This "Far East Scarlet-fever-like disease" occurred in epidemics and some 5,000persons in the Far East (Somov and Martinevsky, 1973) are known to have contracted this disease. Similar clinical findings in children due to *Y. pseudotuberculosis* infection have been reported in Japan, but they are somewhat different from those of European reports (Bottone, 1977).

Yersinia pseudotuberculosis was the most common cause of human yersiniosis a few years ago in France (Carniel and Mollaret, 1990). Gradually, the importance of this species in human infections has decreased and it has been replaced by the prominence of *Y. enterocolitica* enterocolitis.

Yersinia enterocolitica infections

Yersinia enterocolitica is an invasive enteric pathogen. The bacteria gain access to the small intestine (terminal end of ileum), penetrate the lamina propria by passing through epithelial cells, multiply in Peyer's patches and lymph follicles and causes typical *Y. enterocolitica*, terminal ileitis (Carniel and Mollaret, 1990, Robins-Browne, 1992). Usually the infection is limited to this area of the intestines, but sometimes the micro-organisms invade the mesenteric lymph nodes and give rise to a systemic infection.

Yersinia enterocolitica has been recognised as a cause of terminal ileitis and mesenteric adenitis in human beings since the 1930's, and a number of domestic animals including dogs, cats, cattle, sheep and pigs have been suggested as possible sources for these infections (Robins-Browne, 1989). There are few reports linking *Y. enterocolitica* with naturally occurring disease in animals other than man. However, the characteristic lesions of yersiniosis have been produced by dosing mice, caesarean-derived or new-born piglets and rabbits with *Y. enterocolitica* (Carter, 1975; Pai *et.al.*, 1980; Robins-Browne *et.al.*, 1985, Schiemann, 1988). Corbel *et.al.* (1992) produced an experimental placentitis with abortion or delivery of infected lambs 50 days after intravenous inoculation. Corbel *et.al.* (1990) characterized *Y. enterocolitica* strains associated with ovine abortion, the majority of which were identified as *Y. enterocolitica* biotype 1 or 2 or *Y. intermedia*.

The clinical presentation of acute yersiniosis in man varies according to age (Robins-Browne, 1992). Most symptomatic infections occur in children, especially in those under five years and are associated with serotype 0:3. In these patients yersiniosis presents as diarrhoea, often accompanied by fever. The diarrhoea is characterized by frequent loose or watery stools which are sometimes bloody. Diarrhoea is the most important and frequent symptom caused by serotype 0:3, 0:9 and 0:5,27 (Carniel and Mollaret, 1990). Children with *Yersinia*-induced diarrhoea often complain of abdominal pain and headache. Sore throat is a frequent accompaniment. In adults, acute pharyngitis, accompanied by tonsillitis, may dominate the clinical picture (Robins-Browne, 1992).

In children more than five years of age, acute yersiniosis commonly presents as abdominal pain and fever, with little or no diarrhoea. It may resemble the pseudo-appendicular syndrome found with *Y. pseudotuberculosis* and is most often associated with serotype 0:8 infection (Carniel and Mollaret, 1990). Of patients with this syndrome who undergo laparotomy up to 80% have ileitis, while the remainder show evidence of mesenteric adenitis. *Yersinia enterocolitica* rarely causes true appendicitis.

Although *Y. enterocolitica* is seldom isolated from extra-intestinal sites, there appears to be no tissue in which it cannot persist (Robins-Browne, 1992). Cases of *Yersinia*-induced meningitis, endocarditis, mycotic aneurysm (see Table 1.5, cardiovascular infections with

Y. enterocolitica), sinusitis, empyema, septic arthritis, osteomyelitis and pyomyositis are well documented.

Yersinia enterocolitica may also produce abscesses in the liver, spleen and kidneys. Rabson *et.al.* (1972) reported a case in which a 57-year-old African labourer on a pig farm complained of mild cough, chest pain and bloody diarrhoea, and later died. At necropsy there was evidence of a terminal lobar pneumonia, but the striking feature was the presence of numerous small yellow, necrotic foci in the liver and spleen. Histological examination of the liver showed a fine monolobular cirrhosis and numerous pyaemic abscesses. In addition there were fairly prominent haemosiderin deposits. The spleen similarly showed haemosiderosis and pyaemic abscesses. The bacteria isolated from blood, hepatic and splenic abscesses showed typical features of *Y. enterocolitica* serogroup 0:3, phagetype IXa. *Yersinia enterocolitica* serogroup 0:3 was isolated from rectal swabs and faeces of six out of 12 pigs with which the patient was closely associated. Five of these isolates belonged to phagetype IXa and one to phage type X^3.

Septicaemia is a rare complication of infection with *Y. enterocolitica* (Mair and Fox, 1986; Robins-Browne, 1992). Some strains, notably the comparatively invasive, biotype 1B yersiniae are more likely to cause septicaemia than others (Robins-Browne, 1992). Nonetheless, almost all patients who develop systemic yersiniosis have an underlying immune or metabolic disorder. Predisposing conditions include lymphoma, hepatic cirrhosis, uraemia, diabetes and iron overload (Gallant *et.al.*, 1986; Melby *et.al.*, 1982; Rabson *et.al.*, 1975) and the mortality associated with septicaemia is high (45-50%) (Rabson *et.al.*, 1975).

Of the immunological sequelae of yersiniosis, reactive arthritis is the best known (Robins-Browne, 1992). This manifestation is infrequent before the age of 10 years. Most cases occur in Scandinavian countries where serogroup 0:3 strains and the HLA-B27 histocompatibility antigen are prevalent. Men and women are equally affected. Arthritis typically follows the onset of diarrhoea or the pseudo-appendicular-like syndrome by two weeks, with a range of from one to 30 days. The joints most commonly involved are the knees, ankles, toes, tarsal joints, fingers, wrists and elbows. Usually the arthritis is migratory with the joints being affected one after another. The

duration of arthritis is generally less than three months, and the long term prognosis in patients followed for up to 16 years is excellent. Many patients with arthritis have extra-articular symptoms, including urethritis, eye inflammation and erythema nodosum. The latter occurs most frequently in women, in whom the lesions are seen predominantly on the shins (Robins-Browne, 1992). Other autoimmune complications of yersiniosis include Reiter's syndrome, iridocyclitis, acute proliferative glomerulonephritis and rheumatic-like carditis. These have been reported almost exclusively from Scandinavian countries.

Studies carried out in various countries indicate that *Y. enterocolitica* is the causative agent of 2.8% of the enteritis in Montreal (Marks *et.al.*,1980), 2.9% of the enteritis in The Netherlands (HoogKamp-Korstanje *et.al.*,1986) and 1.4% in Italy (Mingrone *et.al.*, 1987). In Norway, Kapperud (1986) reported that *Y. enterocolitica* is the fourth most common causative agent of acute enteritis (1%) after *Salmonella* (5%), *Campylobacter* (3%) and **Shigella** (3%).

Table 1.5: Cardiovascular infections with *Y. enterocolitica*.

Case No.	Reference	Sex and age (years)	Localisation, Pathology, Predisposition	Country	Serotype (Biogroup)	Treatment	Outcome
1	Plotkin and O'Rourke (1981)	M 53	Mycotic aneurysm of the left internal carotid artery, diabetes	USA	? (4)	Gentamicin + excision	Recovery
2	Quisant *et.al.* (cited by Van Noyen *et.al.*, 1986)	M 72	Aortic valve endocarditis, liver and splenic abscesses, rectal carcinoma	France	0:8 (1)	Doxyceline + gentamicin + co-trimoxazole	Recovery
3	Urbano-Marquez (1983)	M 72	Mitral valve endocarditis, rheumatic heart disease	Spain	0:3 (4)	Ampicillin + gentamicin	Died
4	Appelbaum *et.al.* (1983)	F 73	Aortic valve endocarditis + bullous skin lesion, hypertensive-nephrosclerosis	USA	?	Cefamandole + tobramycin	Recovery
5	Verhaegen *et.al.* (1985)	M 79	Infection of aortic bifurcation prosthesis	Belgium	0:3 (4)	Ampicillin + gentamicin + excision	Died
6	Van Noyen *et.al.* (1986)	M 76	Mycotic aneurysm of the abdominal aorta	Belgium	0:9 (2)	Cefazoline, gentamicin + co-trimoxazole + excision	Recovery

N.B. The serotypes and biotypes listed have been isolated from pigs.

YERSINIOSIS IN PIGS

Infections with *Y. enterocolitica, Y. pseudotuberculosis and Y. pestis*

Yersinia enterocolitica is found throughout the world and has been recorded from pigs in many countries (De Barcellos and De Castro, 1981; Bockemuhl *et.al.*, 1979; Cantoni *et.al.*, 1979; Doyle *et.al.*, 1981; Hunter *et.al.*, 1983; Schiemann and Fleming, 1981). Table 1.6 shows the prevalence of *Y. enterocolitica* based on the findings of various investigators. Infections may not be general, since not all herds are infected (Christensen, 1980). The organism is shed in the faeces of infected pigs for up to 30 weeks and has been shown to be transmitted to human food and elsewhere on farms by flies (Fukushima *et.al.*, 1979). Feed for pigs has been found to be infected, and studies of the dissemination of infection in pig facilities (Fukushima *et.al.* 1983) indicate that infection is transmitted from contaminated pens in which infection can persist for three weeks. Other studies suggest that faeces can remain infected for up to 12 weeks and that, in suitable substrates, the organism can multiply at 20-22°C. It appears that transmission from pig to pig is via faecal contamination of accommodation, water and feed (Fukushima *et.al.*, 1983).

The ability of pigs to carry and shed *Y. enterocolitica* is very important in the epidemiology of the infection. The organism is able to colonise the lymphoid tissue and gastro-intestinal tract without the animals producing significant levels of antibodies against them (Fukushima *et.al.*, 1984, Schiemann, 1988). This results in the establishment of carrier animals which have been shown to shed the organism in their faeces (Fukushima *et.al.*, 1983).

It appears that *Y. enterocolitica* serotype 0:3 has a herd or farm-wide distribution. During a one-year survey of *Y. enterocolitica* in Danish pigs, serotype 0:3 was constantly isolated from the tonsils of pigs in six farms but not from those in another four farms, indicating a herd-wise distribution (Christensen, 1980). Furthermore, this farm survey revealed that the *Y. enterocolitica* 0:3-positive farms were all of the "open management type", where 6-8 weeks-old pigs were purchased from various pig markets or pig producers, indicating that this type of management was an important factor in the spread of *Y. enterocolitica* 0:3 within pig herds. Zheng (1987), in a study of *Y. enterocolitica* isolated from faeces of diarrhoeic pigs from four farms in China, reported all four farms were of the "open

management type". He also suggested that the difference in incidence of *Y. enterocolitica* in the four pig farms was probably related to the extent of pre-sampling treatment given to the pigs. Farms that were partially treated for diarrhoea with *Coptis* rhizomes were all positive for *Y. enterocolitica* and in one farm where complete treatment was carried out no *Y. enterocolitica* was isolated.

Yersinia pseudotuberculosis is less commonly demonstrated in America than in Europe or Japan and is less commonly isolated from pigs than *Y. enterocolitica* (Taylor, 1992). It is commonly found in rodents, which probably represents the main source of infection for pigs. *Yersinia pestis* may infect wild pigs in California, presumably from infection present in rodents (Clark *et.al.*,1983).

Yersinia enterocolitica has been shown to infect pigs orally, to multiply and be shed in the faeces within 2-3 weeks of infection, and to disappear from the faeces within 30 weeks (Fukushima *et.al.*,1984). In Canada studies conducted in suckling mice have shown that isolates from pigs were able to produce enterotoxin. However in the same study, the Sereny test for invasiveness was negative for similar pig strains (Mosimbale and Gyles, 1982). A study by Erwerth and Natterman (1987) suggested that oral infection is followed by establishment of infection in the tonsils and the development of enteritis in the ileum and large intestines.

Although pigs are regarded as asymptomatic carriers of yersiniae, a few reports exist on it's association with clinical disease. Disease has been associated only with *Y. enterocolitica* and *Y. pseudotuberculosis*. *Yersinia pestis* is clearly capable of producing serologic reactions (Clark *et.al.*,1983) but no clinical disease has been described. *Yersinia enterocolitica* was isolated in profuse culture from outbreaks of diarrhoea in weaned pigs from which no other infectious agent could be recovered. Mild fever (39.4 °C) was present and the diarrhoea contained no blood or mucous and was blackish in colour (Taylor, 1992), but similar clinical signs have been seen in animals receiving tiamulin or lincomycin. Blood-stained mucous may also be found in some diarrhoea and on solid faeces passed by pen mates. The organism has been isolated from the rectal mucosa in cases of rectal stricture (Taylor, 1992).

Zheng (1987) also suggested that *Y. enterocolitica* may be capable of producing illness in pigs. He reported a prevalence of 49.4% (60 out of 124 samples) from the faeces of diarrhoeic pigs. Fifty eight of the isolates (96.6%) were shown to belong to serotype 0:3 or 0:9.

A few reports have associated *Y. pseudotuberculosis* infection with clinical signs in pigs. The organism has been recovered from a clinical case in Canada (Langford, 1972), from diarrhoeic pigs in Brazil (De Barcellos and De Castro, 1981), from sick and dead pigs in New Zealand (Hodges *et.al.*,1984), from pigs with chronic diarrhoea in Argentina (Fain Binda *et.al.*,1992), and in Japan from pigs with diarrhoea and oedema of the eyelids, lower face, and dependent parts of the abdomen (Morita *et.al.*,1968). *Yersinia pseudotuberculosis* serotype III was the most frequently isolated strain from the reports described above.

Erwerth and Natterman (1987), described in detail the lesions caused by *Y. enterocolitica* which consisted of catarrhal enteritis in both small and large intestines. Microcolonies of the organism were seen in the disrupted intestinal epithelium, and in pigs with rectal lesions, bacterial penetration and inflammation reached the muscularis mucosa. Tzipori *et.al.*(1987) studied the characteristics of intestinal mucosal lesions experimentally induced by *Y. enterocolitica* serotype 0:3 in gnotobiotic piglets. The histopathological changes, in particular the diffuse ileitis accompanied by mucosal ulceration, with sparing of the crypts, resembled the findings described in man (Bradford *et.al.*,1974; Vantrappen *et.al.*,1982).

Lesions due to generalised infection with *Y. pseudotuberculosis* in pigs have been described (Fain Binda *et.al.*,1992; Morita *et.al.*,1968). They resembled those of *Y. pseudotuberculosis* in other species, with miliary grey-white spots in the liver and spleen and swollen, grey-white mesenteric lymph nodes. Microscopic lesions in the large intestines showed central caseous necrotic foci, surrounded by lymphocytes, plasmacytes and macrophages in the submucosa, with oedema and chronic inflammatory infiltrates in the mucosa.

The clinical signs are not distinctive, but the occurrence of mild fever with blood and mucus on solid faeces can indicate yersiniosis in the absence of swine dysentery (Taylor, 1992), where rectal stricture is common, the organism may be responsible for diarrhoea in younger age groups (Taylor, 1992).

Diagnosis of *Yersinia* infections in pigs depends upon isolation of the organism and its identification. Serology has been used to identify *Y. pestis* (Clark *et.al.*, 1982), but most accounts of yersiniosis suggest that, although production of antibodies may result from infection, isolation methods are required for diagnosis (Taylor, 1992). Yersiniosis in pigs, particularly when *Y. enterocolitica* 0:9 is involved is difficult to diagnose by serological methods because of its cross reactivity with *Brucella abortus* (Mittal and Tizard, 1979). For this same reason, it would be unfair to declare the prevalence of brucellosis in a pig farm without a comprehensive study ruling out the prevalence of *Y. enterocolitica* 0:9 infection.

There is at present no general indication for the treatment of yersiniosis in pigs, since clinical signs are very rare. However, some *in vitro* studies indicate that isolates are often sensitive to oxytetracycline, furazolidone, neomycin, sulphonamides and streptomycin. Tetracyclines have been used in feed to eliminate infection and clinical signs (Taylor, 1992).

Table 1.6: The prevalence of *Yersinia enterocolitica* in pigs based on the findings of various investigators.

Country	Investigator	Year Studied	Prevalence (%) in Pigs	Specimens
Australia	Blackall (1977)	1977	0	intestinal/faecal
Belgium	Wauters & Janssens (1976)	1976	53.0	tongues
Canada	Toma & Deidrick (1975)	1975	3.8	caecal
China	Zheng (1987)	1987	48.4	faeces
Denmark	Pedersen (1976)	1976	4.0	colon
Denmark	Pedersen & Petersen (1977)	1977	24.0	tonsils
Denmark	Pedersen & Winblad (1979)	1979	4.5	colon
Denmark	Christensen (1980)	1979	26.05	tonsils
Denmark	Andersen (1988)	1988	24.7	rectal swab
Denmark	Andersen *et.al.* (1991)	1982-83	25.0	tonsillar swab
England	Hunter & Hughes (1983)	1983	5.8	colon/caecal faeces, tonsils
Finland	Aspuland *et.al.* (1990)	1990	38.3	faeces
Finland	Merilahti-Palo *et.al.* (1991)	1991	45.8	tonsils
Holland	Esseveld & Goudzwaard (1973)	1968-70	0.5	caecal
Holland	Esseveld & Goudzwaard (1973)	1970	8.2	faecal
Holland	Narucka & Westendoorp (1977)	1977	73.5	tonsils
Holland	de Boer *et.al.* (1986)	1986	72.5	tonsils
Holland	de Boer & Nouws (1991)	1991	42.0	tonsils
Italy	Fantasia *et.al.* (1993)	1986	4.0	faeces
Italy	Mazzeo *et.al.* (1992)	1992	4.4	faeces/tonsils
Japan	Tsubokura *et.al.* (1973)	1973	4.3	caecal
Japan	Zen-Yoji *et.al.* (1974)	1974	15.3	caecal
Japan	Tsubokura *et.al.* (1976a,b)	1972	4.3	caecal
Japan	Maruyama (1987)	1983-84	11.8	caecal
Japan	Schiozawa *et.al.* (1991)	1991	24.3	tonsils
	" " "	"	24.3	caecal
	" " "	"	85.0	oral cavity swab
	" " "	"	36.0	massiter muscles
Nigeria	Lombin *et.al.* (1985)	1985	1.6	tongue/rectal swab
Nigeria	Okoroafor *et.al.* (1988)	1988	0.53	tongue/throat/tonsil swab
Norway	Nesbakken & Kapperud (1985)	1984	44.4	tonsils
Singapore	Ho & Koh (1981)	1981	4.87	rectal swab
Slovak Rep.	Jackova & Urgeova (1993)	1987-90	43.63	tonsils
Sweden	Hurvell (1978)	1970	3.3	caecal
Trinidad	Adesiyun *et.al.* (1992)	1991	2.0	rectal swab
U.S.A.	Doyle *et.al.* (1981)	1981	51.6	tongues
U.S.A.	Harmon *et.al.* (1984)	1983	18.6	tongue/carcass swab
U.S.A.	Kotula & Sharar (1993)	1992	4.6	caecal/faecal
W. Germany	Leistner *et.al.* (1975)	1974	39.0	faecal
W. Germany	Weber & Lembke (1980)	1979	3.8	rectal

Anatomical distribution

It is well documented that *Y. enterocolitica* 0:3 can be isolated from approximately 25-50% of tonsils and tongues of healthy pigs (Andersen *et.al.*,1991; Christensen, 1980; Doyle *et.al.*, 1981; Nesbakken and Kapperud, 1985; Pedersen, 1979; Schiemann and Fleming, 1981;

Wauters, 1979). Nesbakken (1985) was able to isolate *Y. enterocolitica* 0:3 from 68% of 47 pigs by enrichment of tongues and tonsillar tissue.

Isolation from faeces and intestinal contents has been reported to be of much lower frequency, approximately one-tenth of that found in tongues and tonsils (Aldova *et.al.*, 1980; Esseveld and Goudzwaard, 1973; Pedersen and Winbald, 1979; Szita *et.al.*, 1980; Toma and Deidrick, 1975; Tsubokura *et.al.*,1973; Weber and Lembke, 1981; Zen-Yoji *et.al.*, 1974). Higher frequencies of isolation from faeces have, however, also been reported. Thus Fukushima *et.al.* (1983) found 7.1% of faecal samples from five pig farms infected, with as much as 14.6% infected in one of the farms. Maruyama (1987) examined caecal contents at 41 abattoirs and found a constant high percentage of pigs infected; out of 9423 pigs examined, 1114 (11.8%), were found to be infected with the human pathogenic serotypes 0:3, 0:9 or 0:5,27. Of a total of 1458 faecal samples, Andersen (1988) reported 24.7% positive for *Y. enterocolitica* 4/0:3. Zheng (1987) and Bockemuhl *et.al.* (1979) reported findings of 48.4% and 27.3% respectively.

Only a few reports on the occurrence of human pathogenic strains of *Y. enterocolitica* on the surface of pig carcasses have been published. Nesbakken (1988) isolated *Y. enterocolitica* 0:3 from the surface of 19 of 30 pig carcasses with a higher frequency rate from the anterior part of the carcass, and Christensen (1987) isolated *Y. enterocolitica* 0:3 from 31 of 100 pigs examined with an even distribution on the carcass. However, de Boer and Nouws 1991 did not isolate pathogenic yersiniae from samples of 210 pig carcasses or from 20 samples of porcine headmeat, suggesting that contamination of carcasses during the slaughtering process with yersiniae from either faecal material or from the tonsillary region does not seem to occur frequently and this may explain the reported low contamination rate of pathogenic *Y. enterocolitica* found for pork (deBoer and Nouws (1991)). Differences in sampling techniques, enrichment and/or slaughtering practices may explain these results. In contrast, Andersen (1988) isolated pathogenic *Y. enterocolitica* from carcass surfaces with varying frequencies depending on the evisceration technique used. Manual evisceration was found to correspond with high frequencies of contamination: 26.3% on the medial aspect of the hind limb and 12.9% on the split sternum. The use of a mechanized bung cutter was found to reduce the rate of contamination, especially when the use of the bung cutter was supplemented by enclosing

the anus and rectum in a plastic bag to minimise faecal contamination. When carcasses were eviscerated in this way, it was possible to reduce contamination to 1.9% on the medial hind limb, 1.0% in the pelvic duct and 2.2% on the split sternum.

Seasonal occurrence

The isolation of *Y. enterocolitica* from the caecal contents of pigs was dependent on season (Tsubokura *et.al.*, 1976). The organism was isolated more frequently in winter to early spring than in summer. No relationship could be established between rate of isolation and serogroup distribution (Tsubokura *et.al.*, 1976).

The incidence of asymptomatic infection with *Y. enterocolitica* is pigs was markedly related to season (Bockemuhl *et.al.*, 1979). The lowest incidence in faecal samples was observed during the summer months (August, 1976, 0%) but increased steadily to a maximum in spring (April 1977, 71.2%). With one exception, serotype 0:3 and 0:9 were only isolated during October to December (Bockemuhl *et.al.*, 1979).

Weber and Knapp (1981) studied the variation of infection in pig faeces, from month to month and found a peak in January (winter) with 14% (13 of 95) infected, with a mean of 2.7% (33 of 1206) infected throughout the year.

Y. pseudotuberculosis infections and isolation from mammals and pork were found in the colder months in Japan (Fukushima *et.al.*, 1987). The authors also reported isolation of *Y. enterocolitica* from breeding pigs throughout the year and stated that seasonal changes in the prevalence of pathogenic *Y. enterocolitica* serotypes in slaughtered pigs matched the winter peaks found in human isolations in Germany and Japan. Rakovsky *et.al.* (1973) proposed that one cause of the increased frequency of yersiniosis in man in Czechoslovakia over the period December to March could be that it coincides with the period of domestic pig slaughter.

YERSINIA ENTEROCOLITICA IN FOOD HYGIENE

Occurrence in pork, related products and other foodstuffs

The occurrence of *Y. enterocolitica* in foods, including pork, has been amply documented (Kapperud, 1991; Lee, 1977; Mollaret *et.al.*,1979; Morris and Feeley, 1976) indicating that this microbe is a potential foodborne pathogen.

Unlike most enteric pathogens *Y. enterocolitica* is a psychrotroph which is able to multiply at temperatures approaching 0 °C, a circumstance which means that it can grow in refrigerated foods. Several investigators have documented the growth of *Y. enterocolitica* on raw and cooked meat and in milk during extended refrigerated storage (Hanna *et.al.*, 1977; Lee *et.al.*, 1981). In one study (Hanna *et.al.*, 1977) a few hundred cells of *Y. enterocolitica* in raw pork held at 7 °C grew to more than 10^9 cells/g. within 10 days. The bacteria is also able to multiply in vacuum-packed meat during refrigerated storage (Hanna *et.al.*, 1976). As refrigeration is now being increasingly applied to the preservation of perishable foods, the ability to propagate at 4 °C is of considerable significance to food hygiene. *Yersinia enterocolitica* can survive in frozen foods for long periods (Schiemann, 1989).

Some results indicate that the ability of *Y. enterocolitica* to compete with other psychrotrophic organisms normally present in foods may be poor (Schiemann, 1989; Stern *et.al.*,1980). It has been suggested that "Dark, Firm and Dry" (DFD) meat may favour the growth of *Y. enterocolitica*, not only because of the elevated pH level, but primarily due to the low glycogen content, a factor which may result in reduced competition with the lactobacillary flora (Skjelkvale, 1981, cited by Andersen *et.al.*,1991).

Raw pork products have often been found to be contaminated with human pathogenic *Y. enterocolitica* and the bacteria have been found to be part of the environment of butchers' shops (Christensen, 1987; Wauters and Janssens, 1976). Investigations conducted by Nesbakken *et.al.*(1985) indicate that *Y. enterocolitica* and *Y. enterocolitica*-like bacteria are common in Norwegian pork products, having been isolated from 27.0% of samples investigated. The frequency of isolation reported from other countries varies considerably.

Schiemann (1980) recovered *Y. enterocolitica* from 40.0% of raw and 7.2% of processed pork products in Canada. A relatively high isolation rate (34.5%) was also reported from the Federal Republic of Germany (Leistner *et.al*; 1975). In contrast only 4 (1.3%) of 300 pork samples and 6 (4.0%) of 150 samples of sliced ham from Japan contained *Y. enterocolitica* (Asakawa et.al; 1979). Yields as high as 53% have been reported from pork tongues retailed in Belgium (Wauters and Janssens, 1976). Twenty one strains of *Y. enterocolitica* strains were isolated from 31 tongues from freshly slaughtered pigs in the U.S.A. representing six serotypes, with 0:8 the most common and 0:6,30 the second most commonly isolated (Doyle and Hudgahl, 1981). The other serotypes recovered were 0:3, 0:13,7, 0:18 and 0:46.

A detailed survey of a variety of foods in Holland, found the highest isolation rates on raw pork (73%), unpasteurized egg (43%), raw vegetables (43%) and raw beef (42%), with a surprisingly low recovery (10%) of *Y. enterocolitica* from raw milk, which is often implicated in outbreaks of yersiniosis (deBoer *et.al*; 1986). These authors stressed that only 4% of the isolates from foods were recognised pathogenic strains, all being obtained from pork products.

Of 100 milk samples examined in the U.S.A., 23 raw and 1 pasteurized yielded *Y. enterocolitica* (Moustafa *et.al.*, 1983). In Eastern France, 81% of 75 samples of raw milk contained *Y. enterocolitica* following enrichment, with serotype 0:5 being the most predominant (Vidon *et.al.*, 1981). In Australia 35 isolates were recovered from raw goats' milk, with 71% being rhamnose positive (Hughes and Jensen, 1981). In Brazil, 16.8% of 219 samples of raw milk and 13.7% of 280 pasteurized milk contained yersiniae, with *Y. enterocolitica*, *Y. intermedia* and *Y. frederksenii* constituting 34, 65 and 2.7% respectively (Tibana *et.al.*, 1987). From samples of raw beef and chicken in Brazil, 80% contained yersiniae, 60% of ground beef and liver, and 20% of pork were also positive (Warnken, 1987). Five strains of *Y. enterocolitica* were isolated from 450 samples of cold foods (cooked ham, salami) in Argentina (Velazquez *et.al*; 1993). These included 4 strains of serotype 0:9 and 1 strain of serotype 0:5, however these strains lacked pathogenic activity. According to Kleemann and Scheibner (1993) dry sausage should be considered in epidemiological studies of human infection caused by *Y. enterocolitica*. They recovered

pathogenic strains (0:3; 0:8; 0:9) from experimentally infected sausages stored for more than 50 days at 3 ˚C to 18 ˚C.

In New Zealand, investigations conducted in environmental samples from a supermarket delicatessen revealed one strain of *Y. enterocolitica* (Hudson and Motti, 1993). This strain reacted with antisera raised against a pathogenic serotype (0:8) but did not fall into any pathogenic biotype. This low prevalence of *Y. enterocolitica* correlates with previous observations (Hudson *et.al.*,1992) that this species is rarely isolated from retail flesh foods, and that a majority of the strains which are isolated do not fall into a pathogenic serogroup.

Foodborne outbreaks

Although it is theoretically possible for virulent strains of *Y. enterocolitica* to contaminate and then grow in many types of refrigerated foods, actual foodborne outbreaks caused by *Y. enterocolitica* are comparatively rare (WHO, 1987). In some countries, however, extensive outbreaks have occurred (U.S.A., Japan) while in other countries only sporadic cases have been detected (Cover and Aber, 1989; Schiemann, 1989). In Japan, several large outbreaks have been reported. In all but one case, *Y. enterocolitica* serotype 0:3 was the causative agent but the source of the infection was not detected in any of these cases. The endemic level of yersiniosis in the U.S.A. is lower than in Europe and many other countries, however, six major foodborne outbreaks have been documented in the U.S.A., the food vehicles were identified as chocolate milk, powdered milk, chow mein probably contaminated by a food handler, "tofu" (soyabean curd) manufactured with contaminated water, pasteurized milk indirectly involving pigs, bean sprouts immersed in well water and chitterlings (a dish made from pig intestines). Four outbreaks involved serotype 0:8, one involved serotype 0:13a, 13b, whereas the fifth outbreak, which implicated chitterlings, was caused by serotype 0:3, a serotype which has been rarely isolated in the U.S.A. (Lee *et.al.*, 1990). Table 1.7 summarises some documented outbreaks of yersiniosis.

Table 1.7: Summary of some documented outbreaks of yersiniosis.

Year	Location	Vehicle	Characteristics of Outbreaks	Serotype	Reference
1972	Japan	Unknown	47% of 182 school children and 1 teacher infected.	0:3	Asakawa *et.al.* (1973)
1972	Japan	Unknown	53% of 993 children and adults at a primary school affected.	0:3	Asakawa *et.al.* (1973)
1972	Japan	Unknown	198 of 1,086 junior high school pupils infected.	-	Zen-Yoji *et.al.* (1973)
1972	North Carolina (U.S.A.)	Dog/puppies	16 of 21 persons in 4 families infected.	-	Gutman *et.al.* (1973)
1975	North Carolina (U.S.A.)	Food handler	Two common-source outbreaks occurred in nursery schools. The children also ate snow covered with maple syrup.	-	Olsovsky *et.al.* (1975)
1975	Montreal (Canada)	Raw milk	57 elementary school children and 1 adult infected, serovar 0:5,27 was recovered from milk; 0:6,30 from victims.	-	CDWR, 1976 (cited by Jay, 1992)
1976	New York State (U.S.A.)	Chocolate milk	Some 218 school children were infected. Chocolate syrup added to pasteurized milk in open vat was the apparent vehicle.	0:8	Black *et.al.* (1978)
1981	New York State (U.S.A.)	Powdered milk and chow mein	About 35% of 455 teenage campers and staff were infected from dissolved powdered milk and/or chow mein. Five had appendectomies.	0:8	Shayegani *et.al.* (1982)
1982	Washington State (U.S.A.)	Commercial tofu	Of 87 victims, 56 had positive stools. Water used in processing was the apparent source of the organism.	0:8 0:Tacoma	Aulisio *et.al.* (1983) Tacket *et.al.* (1985)
1982	Connecticut (U.S.A.)	Pasteurized milk	53 of 300 became victims with attack rate being greatest among the 6-13 year olds. 20 of 52 stools were positive for the serovar noted.	0:8	Amsterdam *et.al.* (1984, cited by Jay 1992)
1982	Tennessee, Arkansas, Mississippi (U.S.A.)	Pasteurized milk	More than 172 cases resulted from ingestion of farm milk pasteurized in Tennessee. Seventeen patients underwent appendectomies, and 41% of cases were under age 5.	0:13a, 13b, 0:18	CDC (1982, cited by Jay 1992)
1988-89	Georgia (U.S.A.)	Raw chitterlings	15 black children were affected including 14 infants. The persons cleaning the pig intestines were also caring for the children.	0:3	Lee *et.al.* (1990)

YERSINIOSIS IN NEW ZEALAND

Yersiniosis in New Zealand has followed the worldwide trend, with the role of yersiniae as human and animal pathogens being recognised only in the last three decades. However, reports of disease and of isolation from healthy subjects have been sporadic, and an active search for possible hosts of yersiniae has only begun in earnest in recent years (Lanada, 1990).

Human infections

A few reports exist of human infections with both *Y. pseudotuberculosis* and *Y. enterocolitica*. Henshall (1963), reported the first human case of mesenteric lymphadenitis due to *Y. pseudotuberculosis* in this country, which incidentally was the first reported isolation of *Y. pseudotuberculosis* as a human pathogen outside of Europe. Later, *Y. pseudotuberculosis* was associated with a case of reactive arthritis in New Zealand, although this was only shown serologically (Rose, 1976). Malpass (1981) reported another case of mesenteric lymphadenitis involving *Y. pseudotuberculosis* in a 19 year old soldier.

However, infections with *Y. enterocolitica* in humans are becoming more commonly diagnosed in New Zealand (McCarthy and Fenwick, 1990). Clinical features of infection include diarrhoea, and reactive arthritis following enteritis (Ameratunga *et.al.*,1987; Jones and Burns, 1987). Lello and Lennon (1992) described a syndrome in a young boy in which a *Y. enterocolitica* infection was initially diagnosed as acute rheumatic fever. The patient had complained of a sore throat a few days prior to admission. *Yersinia enterocolitica* serotypes 0:3, 0:5, 27, 0:8 and 0:9 have been detected in New Zealand (Beeching *et.al.*, 1985). In contrast, an earlier survey in Palmerston North (Watson *et.al*; 1979), failed to isolate any *Y. enterocolitica*, leading them to conclude that the organism is an infrequent cause of enteritis in this country.

Animal infections

Animal infections with yersiniae were, again, only reported occasionally, until the late 1970's when the expanding deer industry brought along some new diseases, one of the most prominent of which was yersiniosis (Lanada, 1990).

Yersiniosis, caused by *Y. pseudotuberculosis*, commonly affects young farmed deer (*Cervus elaphus*) in New Zealand (Mackintosh, 1992). The first recorded outbreak of yersiniosis in farmed red deer occurred in 1978 (Beatson and Hutton, 1981). Since then yersiniosis due to *Y. pseudotuberculosis* serotypes I, II and III has become one of the most common causes of death in this species in New Zealand (Beatson, 1984; Mackintosh, 1990; Mackintosh and Henderson, 1984a, b; Wilson, 1984). *Yersinia enterocolitica* may occasionally cause this disease, but it is usually part of the normal intestinal flora (Henderson, 1983). Outbreaks of the disease can affect up to 40% in a group although usually 5% to 20% are affected (Mackintosh, 1992). While it has caused tremendous problems for the deer industry, *Y. pseudotuberculosis* has also been shown to cause disease in other domestic animals in New Zealand. Cases of yersiniosis have been reported in cattle (Hodges *et.al.*,1984) and in sheep, where the organism has been reported in cases of abortion (Hartley and Kater, 1964).

Yersinia enterocolitica has also been involved in a variety of clinical syndromes in domestic animals in this country. 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). A strain of *Y. enterocolitica* serotype 0:5,27 has been isolated from a slaughtered deer in New Zealand (Bosi, 1992), but no history or clinical signs were reported.

An interesting feature of *Y. enterocolitica* infection in New Zealand is the isolation of biotype 5 from domestic animals. *Yersinia enterocolitica* biotype 5 has been previously isolated only in Europe from hares and rabbits (Bercovier *et.al.*,1978; Nilehn, 1969) and goats (Krogstad *et.al.*,1972). Isolation of this biotype in New Zealand has been reported from deer (Henderson, 1984), sheep (Bullians, 1987), and goats (Buddle *et.al.*, 1988; Lanada, 1990).

Pigs appear to be reservoirs of pathogenic yersiniae in New Zealand. Hodges *et.al.*(1984) isolated 7 strains of *Y. pseudotuberculosis* from dead or sick pigs. Fenwick (unpublished data 1989) isolated several strains of *Y. pseudotuberculosis* and pathogenic *Y. enterocolitica* from the tonsils of slaughter pigs.

VIRULENCE ACTIVITY

Some, but not all, strains of *Y. enterocolitica* can cause human disease. This fact is well accepted, but the difficulty has been in the differentiation of the virulent from the non-virulent strains. Strains known to be pathogenic have not always been positive in all virulence assays. Virulent strains of *Y. enterocolitica* contain a 70-kilobase virulence plasmid (45 megadaltons) and the loss of this plasmid is accompanied by a loss in the ability to invade tissue and cause disease (Farmer *et.al.*,1987). Table 1.8 lists many of the methods which have been used to measure virulence. The "simple" methods listed in the table have application in routine clinical microbiology because they are easy to perform. The other methods, particularly those that use animal models are more difficult and are usually carried out only in reference laboratories or in clinical laboratories where there is a special interest in yersiniae. Some simple methods are described below.

Table 1.8: Methods used to define virulence in cultures of *Yersinia enterocolitica*.

Method	Dependence of Virulence Plasmid
Simple Laboratory Methods	
Pyrazinamidase test	-
Biotyping	-
Calcium-dependent growth at 37 °C on congo red agar	+
Calcium-dependent growth at 37 °C on magnesium oxylate agar	+
Congo red uptake	+
O antigen group (such as 0:3, 0:8 or 0:9)	-
Temperature-dependent colonial morphology on trypticase soy agar	+
Temperature-dependent autoagglutination in tissue culture or other broth media	+
Other Laboratory Methods	
Plasmid profile analysis	+
Serotyping, complete	-
Tissue culture, adherence to human epithelial cells such as Hep-2	?
Tissue culture, invasion of human cell lines such as HeLa, Hep-2 and Hence	-
Tissue culture, cytotoxicity to human epithelial cells such as Hep-2	?
Serum resistance (to normal human serum)	?
Agglutination in "virulence specific" antiserum	?
Electron microscopy - detection of fibril structures	+
DNA probes	?
Other membrane protein profiles	?
Haemagglutination (mannose-resistant) of guinea pig erythrocytes	?
Phagocytosis of *Y. enterocolitica* cells	+
Growth within macrophages	+
Animal Models	
Sereny test (guinea pig eye)	+
Mouse lethality	+
Infant mouse intestine - accumulation of fluid (enterotoxin test)	+

Adapted from Farmer *et.al.* (1987).

Pyrazinamidase Test: This test measures the enzyme pyrazine-carboxylamidase (pyrazinamidase), and was originally used to characterize the mycobacteria and corynebacteria (Farmer *et.al.*,1987). Kandolo and Wauters (1985) used it to characterize 381 strains of yersiniae. They found an excellent correlation between virulence and a negative test for pyrazinamidase, which is indicated by non-coloration of the medium. *Yersinia pestis* and *Y. pseudotuberculosis* were pyrazinamidase-negative, as were strains of *Y. enterocolitica* which belonged to bio-serogroups usually associated with human disease. One interesting observation was that the pyrazinamidase test does not depend on the actual presence of the 70-kilobase virulence plasmid. Strains cured of this plasmid remained pyrazinamidase-negative. The advantage of the pyrazinamidase test is that it can detect

strains that were originally virulent, but which have lost their virulence plasmid through laboratory manipulations such as picking off a single colony and/or growth at 37 °C.

Calcium dependent growth at 37 °C: The requirement of calcium for the proper growth of some *Yersinia* strains has been shown to be associated with the virulence plasmid (Gemeski *et al*; 1980). The demonstration of this characteristic is, however, dependant on temperature and is usually only shown at 37 °C. A culture of *Y. enterocolitica* is grown on a medium such as magnesium oxalate agar (MOX, tryptic soy agar with added magnesium chloride and sodium oxalate). Strains that contain the virulence plasmid are calcium dependent at 37 °C and thus fail to grow or produce small colonies (Gemski *et.al.*, 1980; Noble *et.al.*, 1987).

Congo Red Uptake: This test demonstrates the ability of plasmid bearing yersiniae to bind congo red. In cultures of *Y. enterocolitica* grown at 37 °C on tryptic soy agar with added congo red dye (Prpic *et.al.*, 1983), strains which contain the virulence plasmid take up the dye and appear as red colonies. Congo red is now added to magnesium oxalate agar (MOX). This combination is referred to as congo red-magnesium oxalate agar (CRMOX). Calcium-dependent growth at 37 °C and congo red uptake can now be determined in one assay (Riley and Toma, 1989).

Autoagglutination: This method was developed by Laird and Cavanaugh (1980), to detect the production of outer membrane proteins (YOPS) and expression of surface fibrillae. A culture of *Y. enterocolitica* is inoculated into two tubes of tissue culture media (such as Eagle's minimum essential medium plus Earle salts and L-glutamine) one tube is incubated at 37 °C and the other at 22 °C. Autoagglutination at 37 °C but not at 22 °C, is usually associated with the secretion of YOPS and surface fibrillae found in pathogenic yersiniae. Other broth media can also be used, e.g. MR-VP broth (Farmer *et.al.*, 1992).

Pathogenic strains of *Y. enterocolitica* produce a heat-stable enterotoxin (YST), however it appears that this agent is not critical for virulence. Some evidence for the lack of importance of YST were provided by Schiemann (1981) who demonstrated positive Hela-cell and Sereny-test responses, with a 0:3 strain that did not produce enterotoxin. On the other hand, each of 49 isolates belonging to serovar 0:3 and the other four virulent serovars (0:5,27; 0:8; 0:9; 0:1,2,3) produced YST (Mors and Pai, 1980).

All three pathogenic yersiniae (*Y. pestis, Y. pseudotuberculosis, Y. enterocolitica*) harbour a similar species of plasmid (PYV) which is required for the full expression of virulence (Cornelis *et.al.*, 1987; Portnoy and Martinez, 1985). This plasmid which is about 70Kb governs growth restrictions of yersiniae at 37 °C in the absence of calcium ions and correlates with the massive release of a set of proteins called Yops (Cornelis *et.al.*,1989b; Gemski *et.al.*, 1980; Wolf-Watz, 1987). It has become clear that PYV and Yops are essential for the pathogenicity and virulence of yersiniae including *Y. enterocolitica* (Bolin *et.al.*, 1988; Brubaker, 1991; Portnoy and Martinez, 1985; Portnoy *et.al.*, 1984). In *Y. enterocolitica* the virulence plasmid has been found only in strains of serogroups that are traditionally considered to be pathogenic (Sory *et.al.*,1990).

The finding that Yops are essential for *Yersinia* virulence (Brubaker, 1991) and are conserved among human and pig strains (Kapperud *et.al.*,1985; Kwaga and Iversen, 1993) is further evidence for the role of pigs as reservoirs of human pathogenic strains. Kwaga and Iversen (1993) using a large collection of yersiniae of pig origin, have found that *Y. enterocolitica* serogroups 0:3; 0:1,2,3; 0:5,27 and 0:9 harbour Yops that are indistinguishable from human strains belonging to the same serogroups. These findings complement those of Kapperud and Colleagues (1985, 1990) and Kwaga and Iversen (1992) that pig constitute an important reservoir of pathogenic *Y. enterocolitica*. Further studies are required to confirm that Yops of pig and human strains are specifically and similarly recognized by either monospecific or monoclonal antibodies (Kwaga and Iversen, 1993).

DIAGNOSTIC METHODS

As with most bacterial diseases, definitive diagnosis is based on the isolation and identification of the organism from appropriate clinical specimens taken when symptoms are present. Some aspects of the diagnosis (isolation, identification, pathology) of infections with *Yersinia* have already been mentioned elsewhere in this review.

Yersiniosis is sometimes considered in the differential diagnosis of a patient's illness in the absence of positive culture results (Bottone, 1981; Fowler and Brubaker, 1985). Pathologically, *Y. enterocolitica* enteritis may resemble a number of inflammatory lesions of the ileocecal region caused by other bacteria such as *Salmonella typhi*, *Brucella* spp, *Francisella tularensis* and *Y. pseudotuberculosis* (Bradford *et.al.*,1974). Similarly, enteritis due to yersiniae may superficially resemble the acute stage of regional enteritis (Crohn's

disease) and ulcerative colitis. Differentiation from the lesions of lymphogranuloma venereum, cat scratch disease, and bovine tuberculosis is facilitated by anatomic location, and microscopic appearance (Bradford *et.al.*, 1974). Antibody levels for *Y. pseudotuberculosis* and *Y. enterocolitica* may be used as a diagnostic aid.

Serodiagnosis of *Y. enterocolitica* infection is a complex subject. A wide range of serological techniques are available to measure antibodies to *Y. enterocolitica*. These include enzyme-linked immunosorbent assay (ELISA), radio-immunoassay and complement fixation tests (Robins-Browne, 1992), but most experience has been gained with whole-cell agglutination (Widal) tests. Interpretation of all these assays is fraught with difficulty. Sera from healthy individuals commonly contain low levels of antibodies to yersiniae, while sera from patients with yersiniosis may exhibit a prozone phenomenon (Robins-Browne, 1992). Furthermore, *Y. enterocolitica* serogroup 0:9 shares antigens with *Brucella abortus*, *Escherichia coli*, *Morganella (Proteus) morganii* and *Salmonella* spp. Consequently, false-positive reactions are common in tests that use 0:9 as the antigen.

The reliability of serological diagnosis is considerably improved by demonstrating a four-fold or greater difference in titre between acute and convalescent sera (Robins-Browne, 1992), but in patients who present late in the course of the illness, this is seldom possible. Patients and animals convalescing from yersiniosis also develop antibodies to plasmid-encoded Yops. Tests which detect antibodies to these proteins are more sensitive than those using bacterial cell-wall components as antigens. Robins-Browne *et.al.* (1993) confirmed the value of an enzyme immunoassay (EIA) using Yops as antigen in the sero-diagnosis of infection with pathogenic strains of *Y. enterocolitica* and *Y. pseudotuberculosis* in lambs. All lambs infected with PYV^+ strains of yersiniae seroconverted to Yops whereas uninfected lambs did not. The presence of anti-Yops antibodies in animals with subclinical infections indicates that EIA may be useful in sero-epidemiological surveys of yersiniosis in various animal species (Robins-Browne *et.al.*, 1993). An EIA could also be used to screen food animals, such as sheep and pigs, for yersiniae, and probably even as a component in addition to the widely used passive haemaggulation test (HA) for plague surveillance in rats (WHO, 1970).

A few rapid methods have recently been developed for detection of pathogenic species of *Yersinia* from clinical samples and food. Using a synthetically produced oligonucleotide probe, it was found that the prevalence of pathogenic yersiniae in Norwegian pork products

was substantially higher than previously demonstrated using conventional methods (Kapperud *et.al.*, 1990b). A simple colony immunoblotting method using monoclonal antibodies (MABS) was developed to detect *Y. enterocolitica* serotype 0:3 in the faeces of pigs (Li *et.al*; 1992). Several reports have described the use of the polymerase chain reaction (PCR) for detection of pathogenic *Y. enterocolitica* species from various sources including human, animals, food and water (Fang *et.al.*, 1992; Fenwick and Murray, 1991; Ibrahim *et.al.*, 1992; Kapperud *et.al.*, 1993; Kwaga *et.al.*, 1992; Nakajima *et.al.*, 1992; Wren and Tabaqchali, 1990). The indirect immunofluorescence (IF) assay has been used successfully to detect *Y. enterocolitica* 0:3 cells in the tonsils of pigs (Shiozawa *et.al.*, 1991) and *Y. enterocolitica* 0:3, 0:5, 0:8 and 0:9 from histological preparations of human clinical specimens (HoogKamp-Korstanje *et.al.*, 1986). Use of contaminated blood has led to 10 cases of bacteriaemia due to *Y. enterocolitica* with seven deaths in the U.S.A., since 1987, four of the deaths could be traced to *Y. enterocolitica* serotype 0:3 (Li *et.al.*, 1993). These observations point to the need for rapid and specific methods to identify *Y. enterocolitica* 0:3 in clinical samples from domestic animals and humans (Li *et.al.*, 1993).

TREATMENT, PREVENTION AND CONTROL

Treatment

As yersiniosis is for the most part self-limiting, specific treatment of infection is seldom required. Most strains produce B-lactamases, which render them resistant to "older" penicillins and first-generation cephalosporins, including ampicillin and cephalothin (Robins-Browne, 1992).

When the disease is mild (enterocolitis, uncomplicated pseudoappendicular syndrome), antimicrobial chemotherapy is not useful. However, systemic infection or extra-intestinal localizations should be treated. The antibiotic of choice will be based on the results of the sensitivity test. Before the results of antimicrobial-susceptibility tests, a combination of doxycycline and an aminoglycoside can be administered to patients with septicaemia (Horstein *et.al.*, 1985).

Although very few formal trials of antimicrobial therapy have been reported, anecdotal evidence suggests that patients with prolonged diarrhoea or generalized infection will respond to treatment with trimethoprim-sulphamethoxazole, tetracycline, chloramphenicol, ciprofloxacin or an appropriate B-lactam antibiotic. Isolates of *Y. enterocolitica* are highly

susceptible to aminoglycosides *in vitro*, but patients treated with these agents may respond poorly, because of the large proportion of bacteria located intracellularly (Robins-Browne, 1992).

Prevention and control

At present, prevention of yersiniosis relies chiefly on good hygienic practices, especially with regard to food preparation. In endemic regions, where pigs are the major reservoir of infection, measures could be taken to reduce the bacterial load in slaughtered animals.

Preventative and control measures should not be different, in general, from those which are intended to prevent and control zoonotic salmonellosis (WHO, 1987). These include three main lines of defence:

(i) the pathogen-free breeding and rearing of slaughter animals;

(ii) the improvement of hygiene during transportation, slaughtering and processing including different means of decontamination; and

(iii) education of all categories of people involved in production, processing and final preparation of food products.

Strict hygiene is particularly necessary during food processing and preparation, because *Y. enterocolitica* is able to propagate at temperatures approaching 0 °C, therefore chilling of food products should not be considered as an effective control measure for this microbe (Kapperud, 1991).

At the farm level, newborn piglets are easily colonized and become long-term pharyngeal and intestinal carriers, without any signs of illness (Schiemann, 1989). This observation, as well as the widespread occurrence of pathogenic *Y. enterocolitica* in herds of pigs, indicates that the organism may be difficult to control efficiently at this stage (Andersen, 1984, cited by Kapperud, 1991).

During commercial slaughtering and processing, bacteria from the oral cavity or intestinal contents may easily contaminate the carcasses and the environment in the slaughter house.

Although some contamination is unavoidable on a highly mechanized slaughter line application of the HACCP concept and improved hygiene at critical control points should be attempted. Special attention should be paid during:

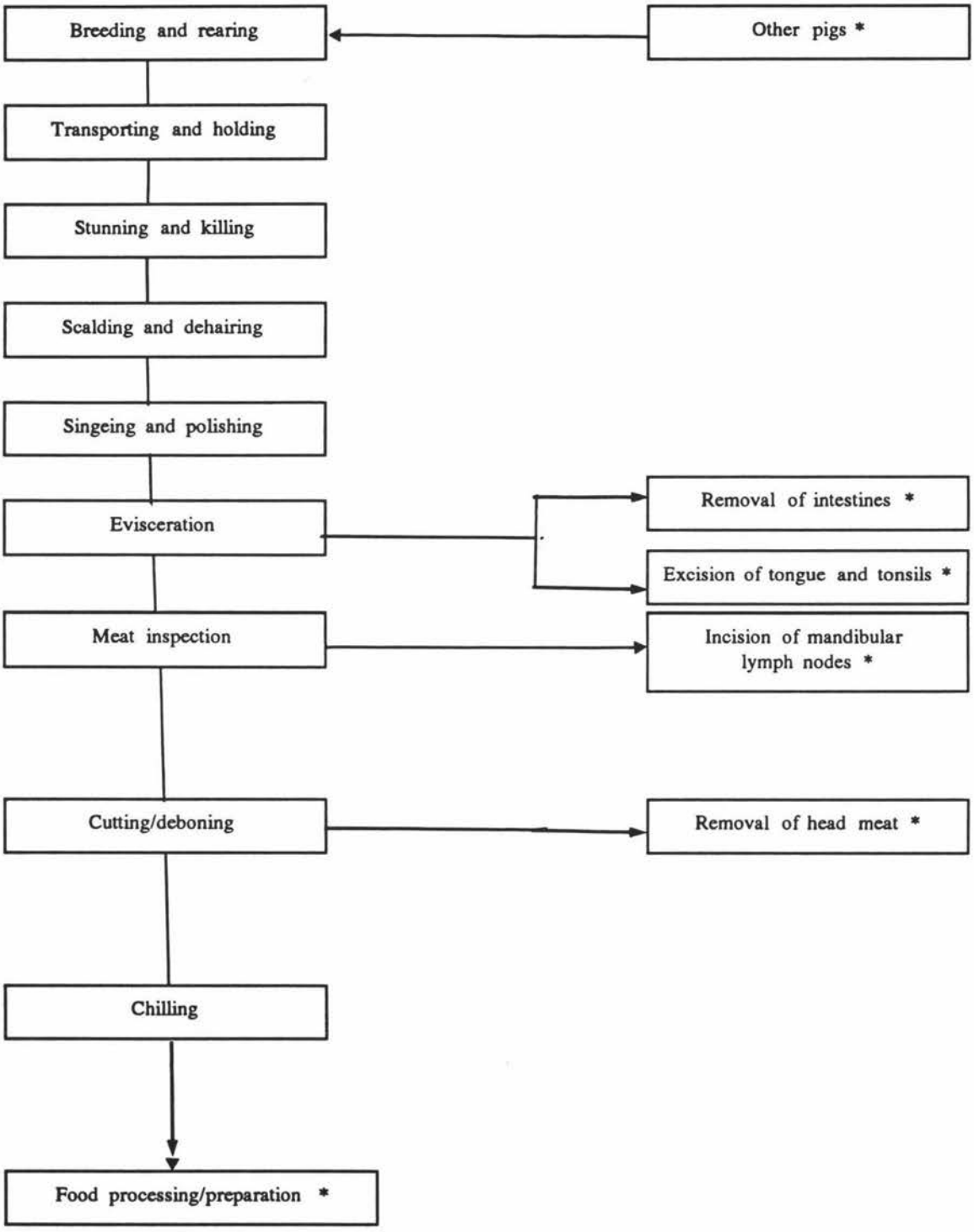
(i) circumanal incision and removal of the intestines;

(ii) excision of the tongue, pharynx, and particularly the tonsils;

(iii) post-mortem meat inspection procedures which involve incision of the mandibular lymph nodes; and

(iv) deboning of head meat. See Fig. 1.2 (Kapperud, 1991).

Figure 1.2: **Flow diagram for production and processing of pork products. Sites where major contamination with *Y. enterocolitica* 0:3 is expected to occur are indicated (*). Adapted from Kapperud (1991).**



A critical point on the slaughter line is the meat inspection because incision of the mandibular lymph nodes is compulsory according to the meat inspection procedures in most countries and thus represents a cross-contamination risk (Nesbakken, 1988), also after inspection, any remaining tonsillary tissue is removed in connection with dressing of the head. *Yersinia enterocolitica* may thus be transmitted from the tonsillary region to other parts of the carcass by the knives and hands of the personnel involved (Nesbakken, 1988).

Likewise, the knife of the slaughterman and the carcass may be contaminated during circumanal incision and removal of the intestines. Andersen (1988) found that faecal contamination was of major importance in the spread of *Y. enterocolitica* 0:3 to the carcass. He reported that the frequency of isolation from the surface of pig carcasses vary greatly with the evisceration technique use. Manual evisceration produced the greatest incidence of *Y. enterocolitica* 0:3 on the carcass surface whereas the use of a bung cutter reduced this markedly.

In man, efforts should be made to reduce the incidence of systemic yersiniosis in patients with iron overload. This may involve temporary cessation of treatment with desferrioxamine B, and the prompt administration of appropriate antimicrobials to patients at risk (Robins-Browne, 1992).

PUBLIC HEALTH IMPLICATIONS

Yersiniosis is now considered to be a foodborne disease responsible for high morbidity and occasional fatality in man. As emphasized throughout this review, pigs appear to be major reservoirs of pathogenic species of yersiniae worldwide. The organism has also been isolated from many foodstuffs including pork and pork products, milk and dairy products. Isolates from pigs are indistinguishable, by currently available methods, from similar human isolates. The histories of many patients with yersiniosis contain data on pork ingestion (Bottone, 1977) or direct contact with pigs or pig keepers (Rabson *et.al.*,1972; Radovsky *et.al.*,1973; Szita *et.al.*,1973). In the United States, one outbreak of human yersiniosis was associated with raw chitterlings, a dish prepared from pig intestines. In Scandinavian countries, where the incidence of human infections due to *Y. enterocolitica* serotype 0:3 is highest, the consumption of raw pork has been implicated as the source of the infection.

The highest isolation rates in pigs have been from the tonsils and oral cavity. During the modern slaughtering technique with excessive use of water, the organism may be readily spread from the throat and faeces to the rest of the carcass. It can be assumed that persons involved in the slaughtering process of pigs may be especially exposed to the risk of infection. A report by Merilahti-Palo *et.al.* (1991) concluded that infections with yersiniae are an occupational health risk to workers slaughtering pigs in Finland. A similar situation could exist among pig abattoir workers in New Zealand since similar slaughter procedures and techniques are employed.

Yersiniae can propagate in the cold chain from slaughter house to butcher shops, on meat counters and in home refrigerators. For this reason an action plan consisting of strict hygienic measures and public health education should be implemented at major sites where contamination with the organism is expected to occur.

Section B: The Porcine Palatine Tonsils

INTRODUCTION

In the porcine species, tonsils are concentrated in symmetrical plates in the posterior soft palate. In the dog and cat the tonsils are usually prominent and protrude slightly from the tonsillar fossa. In other species, the tonsils are diffuse; they are subject to the usual condition involving lymphoid tissue and undergo progressive atrophy with age (Jubb, Kennedy and Palmer, 1985; Taylor, 1992).

By virtue of their function in immune surveillance in the oropharynx, the tonsils are constantly exposed to antigenic stimuli and may serve as an entrance for infectious agents. Some bacteria native to the oropharynx may inhabit the tonsillar crypts, resulting in sub-clinical carriers. A significant percentage of pigs may carry *Erysipelothrix rhusiopathiae* and *Salmonella* spp. in their tonsils. Consequently, the tonsils may serve as portals of entry for a variety of bacterial agents including *Streptococcus suis*, and intra-cellular organisms such as *Yersinia* spp. Primary multiplication of viruses may also occur in the tonsils and this frequently leads to necrosis and lymphoid depletion. For diagnostic purposes, tonsils are collected for bacteriology for isolation of bacterial pathogens, e.g. *E. coli*, *Salmonella* and *Yersinia*, and for immunohistochemistry to detect viral antigens in such infections as pseudorabies (DNA virus, *Herpes suis*) and hog cholera (RNA virus, *Pestivirus suis*) (Jubb, Kennedy and Palmer, 1985; Taylor, 1992).

LOCATION AND ONTOGENESIS

Frandson (1981), describes the tonsils as more or less circumscribed masses of lymphoid tissue, named according to their location. They are surrounded by a connective tissue capsule of variable shape and size typical of the different species (Sisson, Grossman and Gethy, 1975).

In the pig, the palatine tonsils are paired oval lympho-epithelial organs (Plate I) located in the substance of the soft palate on either side of the median furrow, marked by numerous crypts (Frendson, 1981; Sisson *et.al.*,1975). This location is peculiar to the pig; the palatine or (faucial) tonsils in other domestic animals and in man are found in the lateral walls of the *isthmus faucium*, often within tonsillar fossae between the pharyngopalatine and

glossopalatine arches (Bloom and Fawcett, 1975; Frandson, 1981; Ham, 1969; Sisson, 1953; Tortora and Anagnostakes, 1984).

The palatine tonsils develop from a two-chambered tonsillar sinus which incorporates the remnants of the second gill pouch. The entodermal epithelium lining the sinus drives solid buds into the mesenchyme, and these buds later become canalized, forming the tonsillar crypts, into which the tonsillar glands empty (Nickel *et.al.*, 1982).

CLASSIFICATION OF TONSILS

Although all tonsils are similar, two distinguishable groups are defined on the basis of the relationship of the tonsillar tissue with the surface epithelium, i.e. tonsils with crypts and tonsils without crypts (Banks, 1986; Dellmann, 1971).

Tonsils with crypts (follicular tonsils) are characterized by deep invaginations of the surface epithelium called crypts (Bacha and Wood, 1990). A crypt with its associated lymphatic tissue constitutes a tonsillar follicle (Plate II), and several follicles form the tonsils. Examples of tonsils with crypts include the following: lingual tonsils of the horse, pig and cow; tubal tonsils of the pig; palatine tonsils of the horse, pig and ruminants. Tonsils without crypts have a smooth somewhat folded or bulging surface but lack deep invaginations of the epithelium. Tubal tonsils of ruminants, the paraepiglottic tonsil of the cat and palatine tonsils of carnivores are examples (Dellmann, 1971).

MICROSCOPIC ANATOMY

Although structural differences exist (Chukalorjkaya, 1956, cited by Williams and Rowland, 1972), histologically the porcine tonsils (Anderson, 1974) present essentially the same features as those of other species (Bacha and Wood, 1990) including man (Bloom and Fawcett, 1975).

The epithelium covering the tonsils is of the non-keratinized stratified squamous type, supported by dense connective tissue (Anderson, 1974; Curran and Jones, 1977; Ham, 1969; Howie, 1980; Nickel *et.al.*, 1981). The surface epithelium invaginates to form crypts (Plate III) (Bacha and Wood, 1990; Banks, 1986). The continuity of each crypt with the surface is not always evident (Bacha and Wood, 1990).

The follicles with their prominent germinal centres are embedded in a diffuse mass of lymphoid tissue under the epithelium (Banks 1986; Bloom and Fawcett, 1975). The epithelial crypts with their surrounding sheets of lymphoid tissue (Plate IV) are partially separated from one another by thin partitions of loose connective tissue which invaginate from the capsule. In this connective tissue there are always numerous lymphocytes of various sizes, mast cells and plasma cells (Anderson, 1974). The presence of a large number of polymorphonuclear leukocytes is indicative of inflammation, which is very common in tonsils (Bloom and Fawcett, 1975).

The lumen of the crypts may contain large accumulations of living and degenerated lymphocytes mixed with desquamated squamous epithelial cells, granular detritus and micro-organisms (Bloom and Fawcett, 1975). Glands are associated with the palatine tonsils, but their ducts open beside it and not into the crypts (Ham, 1969), hence the crypts are not flushed out as they are in the lingual tonsils.

Afferent lymph vessels are lacking in the tonsils but aggregated follicles of the tonsils are drained by efferent lymph vessels (Banks, 1986; Dellmann, 1971).

In calves and pigs the tonsils are one of the portals of entry into the body for bacteria and inert particles (Payne and Derbyshire, 1963) because of their exposed position in the oropharynx.

In a detailed study, Williams and Rowland (1972) demonstrated, using india ink particles, an afferent route leading from the oral cavity of the pig through the tonsillar crypt epithelium to the sub-epithelial lymphoid parenchyma. Although these authors were not able to demonstrate an efferent pathway from the tonsils to the associated lymph nodes, they postulated that the significance of the tonsils to the general body defences lay in their contribution of lymphoid cells stimulated by bacterial antigens in the oral cavity.

PATHOLOGY

The tonsils are a common site of functional lymphoid hyperplasia and physiological inflammation due to their constant exposure to antigenic stimuli. Desquamated epithelium, bacteria, necrotic debris and neutrophils are normally present in moderate amounts in tonsillar crypts (Jubb, Kennedy and Palmer, 1985). In certain bacterial infections, the

reaction is exaggerated and may be associated with ulceration of the crypts and suppuration of involuted tonsillar lymphoid tissue causing the formation of macroscopic yellowish nodules. Such bacterial tonsillitis may occur in pasteurellosis of pigs and sheep, and necrobacillosis in all species. Haemorrhagic necrotizing tonsillitis has been reported in porcine anthrax (Jubb, Kennedy and Palmer, 1985).

Yersinia enterocolitica, serotype 0:3 has been implicated in the aetiology of a mild chronic tonsillitis in pigs. Shiozawa *et.al.* (1991), found no apparent macroscopic differences between those tonsils from which *Y. enterocolitica* could be isolated and those from which it could not. However, histological examinations revealed mild inflammation with mononuclear infiltration and epithelial cell destruction around the crypts of *Y. enterocolitica*-positive tonsils. *Yersinia enterocolitica* 0:3 cells were observed mainly in the epithelium of tonsillar crypts by immunofluorescence (IF) assay. *Yersinia enterocolitica* 0:3 was also occasionally detected in the tonsillar lymphoid centres by IF assay. However, it was not detected in any sections of tonsils from which *Y. enterocolitica* 0:3 could not be isolated.

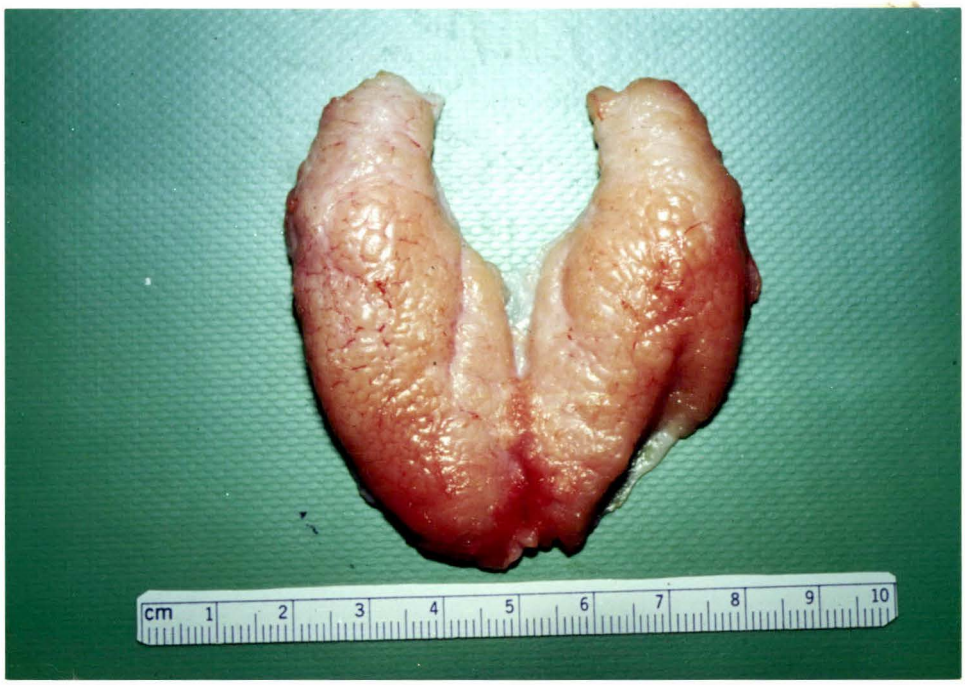
During the early phase of a number of lymphotrophic diseases such as feline panleukopenia, canine parvovirus infection, canine distemper, bovine viral diarrhoea, rinderpest and swine vesicular disease, involution of B-dependent tonsillar lymphoid follicles due to viral lymphocytolysis may occur (Jubb, Kennedy and Palmer, 1985). Numerous karyorrhexic nuclei, lymphocyte depletion and prominent histocytes appear in such conditions. In distemper, involuted tonsils are susceptible to secondary bacterial invasion and suppuration. Compensatory lymphoid hyperplasia may occur during the post-viraemic phase of parvoviral infection and distemper (Jubb, Kennedy and Palmer, 1985).

Plant fibres or other foreign bodies that lodge in the tonsillar fossa may produce a localized unilateral inflammation or a peritonsillar abscess (Frazer, 1986).

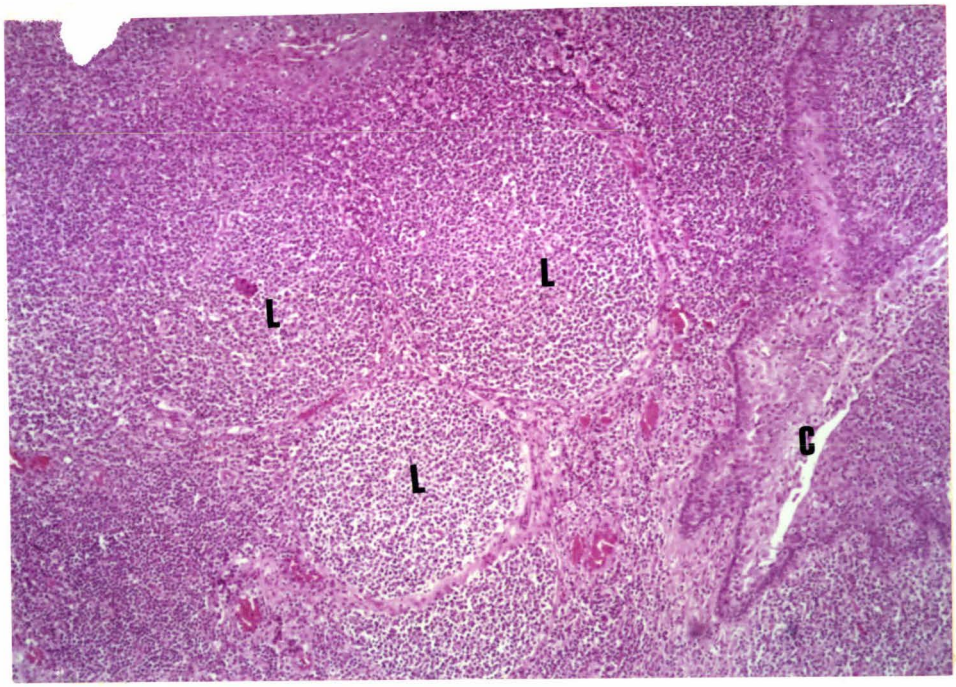
The most frequent neoplasms of the tonsils of animals are squamous cell carcinomas and malignant lymphomas (Smith, Jones and Hunt, 1972). In humans, malignant tumours of the tonsils are often mistaken for inflammatory lesions until incontrovertible proof of neoplasia appears. Both the anterior and posterior faucial pillars may be involved, and the tumour may spread into the soft palate and the uvula. Inferiorly, the tumour may extend into the glossopharyngeal sulcus and into the base of the tongue (Galante *et.al.*, 1985).

Histologically, malignant tumours of the tonsil may be poorly differentiated epidermoid carcinomas (75%), lymphosarcoma (15%) or lymphoepitheliomas (10%). Distant metastasis to bone, lungs and liver occurs frequently, particularly with lymphoepitheliomas.

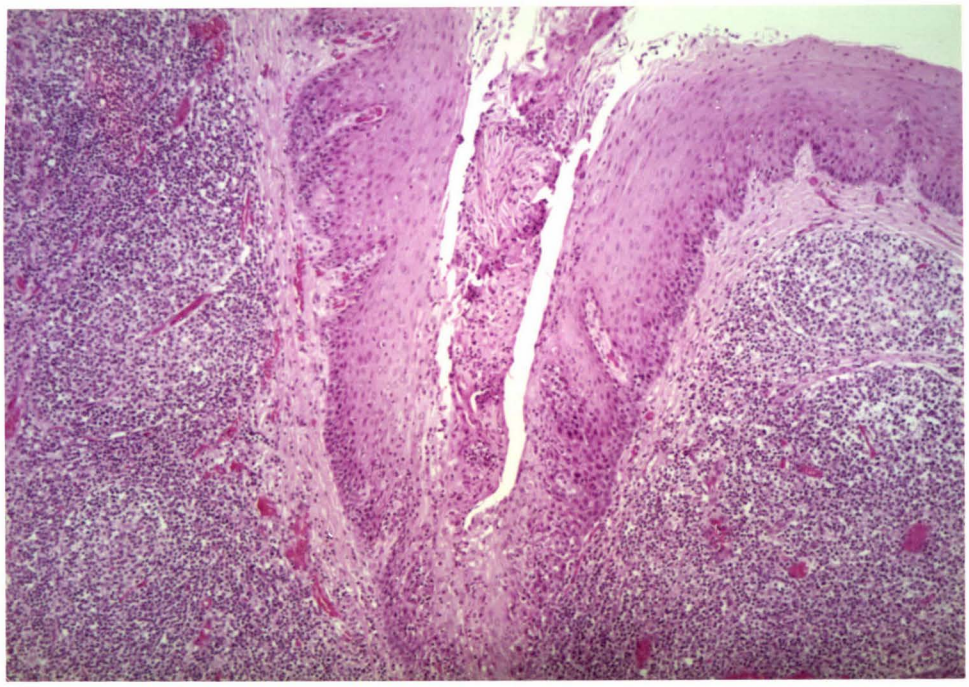
PLATES I - IV



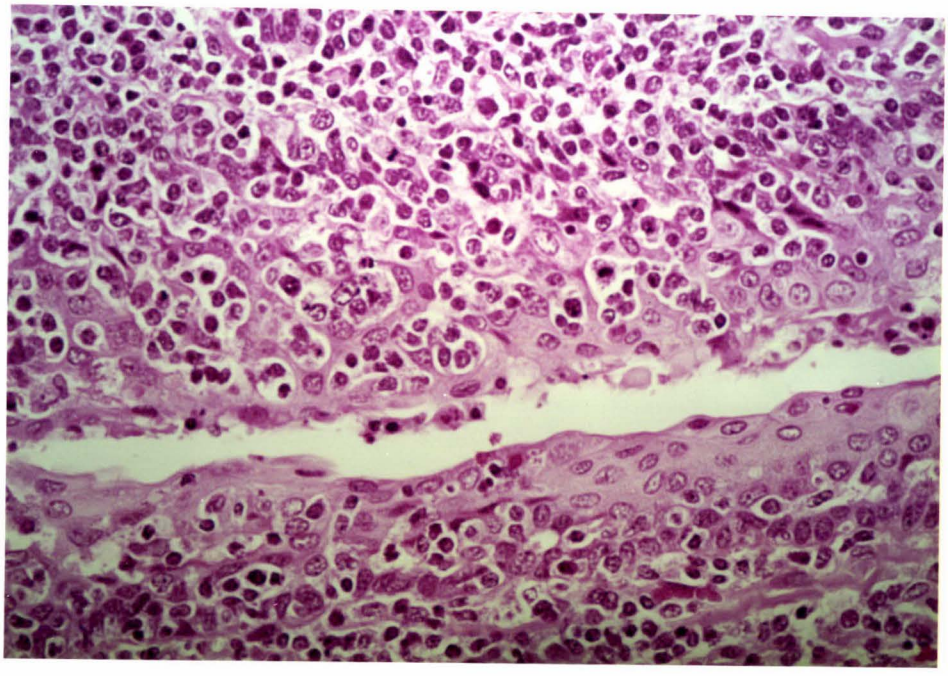
I. The palatine tonsils of the pig; paired lympho-epithelial organs.



II. Histologic section of pig's tonsil, showing a tonsillar follicle, consisting of a crypt (C) with its associated lymphatic centres (L) H & E stain (x 70).



III. Histologic section of pig's tonsil, showing invagination of surface epithelium to form a crypt. H & E stain (x 100).



IV. Histologic section of pig's tonsil, showing active lymphoid epithelium of a crypt. H & E stain (x 350).

CHAPTER TWO

CROSS-SECTIONAL STUDY OF THE OCCURRENCE OF YERSINIA SPECIES IN THE TONSILS OF SLAUGHTER PIGS IN THE MANAWATU

INTRODUCTION

During the past few decades a number of species of *Yersinia* have been isolated from both meat and dairy products (Lee *et.al.*, 1981). This has attracted considerable attention in connection with food hygiene, even though attempts to isolate the bacterium from foods incriminated as sources of foodborne disease outbreaks have seldom proved successful (Schiemann, 1989; WHO, 1987). However, the reported incidence of *Yersinia enterocolitica* in man has increased dramatically in the last ten years (Gilmour and Walker, 1988).

Among meat animals, pigs are regarded as a major reservoir of pathogenic *Yersinia enterocolitica* since the serovars most commonly involved in human infections (i.e. 0:3; 0:5,27;0:9) have been often isolated from the oral cavity or intestinal tract of healthy pigs (Doyle, 1985; Schiemann, 1989).

Several investigators in Europe (de Boer *et.al.*, 1986, 1991; Nesbakken and Kapperud, 1985), America (Doyle and Hugdahl, 1983; Doyle *et.al.*, 1989; Schiemann and Flemings, 1981) and Japan (Fukushima *et.al.*, 1989; Shiozawa *et.al.*, 1987, 1988) have reported that pathogenic strains of *Yersinia* are carried on the tongue and in the throat of healthy pigs, and suggest that those strains may be disseminated from the tonsils to other pharyngeal regions, such as tongue and throat leading to carcass contamination during slaughter.

Results of many other surveys (Table 1.6) suggest that infection is distributed worldwide in pigs, that strains considered pathogenic to man are normal residents in the oral cavity and that pigs play a major role in the epidemiology of human infection.

This study was undertaken to determine the presence of species of *Yersinia*, particularly pathogenic strains, in the tonsils of slaughter pigs and their distribution among farms supplying pigs for slaughter within the Manawatu.

MATERIALS AND METHODS

Determination of Sample Sizes

In this study the actual population for pig farms submitting animals for slaughter was unknown. However, varying numbers of animals from each farm were submitted weekly for slaughter.

It was assumed that a 30% prevalence of *Yersinia* infection was present in these herds. This estimation of prevalence was based on internationally published studies and data from an unpublished study conducted in New Zealand (Fenwick, *pers.comm.* 1989). A table for the determination of sample sizes at the 95% level of confidence was consulted (Cannon and Roe, 1982). This table showed that for an infinite population size at a 30% level of infection a total of 9 animals per herd were required for detecting at least one infected animal from a herd. The number of tonsillar samples collected (pigs sampled) ranged from 10-30 per herd (average: 16).

Slaughter of Pigs and Sample Collection

The pigs were subjected to the standard slaughtering procedures (electrical stunning, exsanguination, scalding, dehairing, evisceration), applied in New Zealand pig abattoirs at a Ministry of Agriculture and Fisheries (MAF) inspected slaughter house and pork processing plant at Longburn, Palmerston North, New Zealand. Animals for slaughter were obtained from the various pig farms within the Manawatu and Wairarapa regions and the age of the animals at slaughter ranged from 20-24 weeks. Sampling was carried out during

the period August to September 1992. The tongue, tonsils and pharynx were removed as one block following evisceration (Plate V) and placed into a sterile tray corresponding to the animal undergoing evisceration.

Pairs of palatine tonsils were then removed using sterile scissors, placed into separate sterile containers and transported to the laboratory at Massey University, Department of Veterinary Pathology and Public Health. In some instances tonsils were stored at 4 °C for 1-3 hours before processing. An average of 30 pairs of tonsils per abattoir visit and a total of 124 during the sampling period were collected. Samples were identified using a code, representing the farm's name, sex of the pig and sample number, e.g. WF10, farm W, female, sample No. 10. Pigs from eight herds were examined for the presence of yersiniae.

Processing of Samples for Cold Enrichment

Samples were processed individually and aseptically. Special care was taken to avoid cross contamination and samples from the various herds were processed separately.

Tonsils were trimmed to remove excess non-tonsillar tissue. On each occasion the instruments used (scissors, forceps), were disinfected with 95% alcohol and flamed in a Bunsen burner.

From each sample a cross-section (0.5 x 1 cm) was fixed in 10% neutral buffered formalin for 18-24 hours, embedded in paraffin wax and stored for future immuno-cytochemical study (not part of this thesis). The remaining portion was sliced several times (using a sterile surgical blade on each occasion) and placed in a stomacher bag, to which was added 25-30 ml of phosphate buffered saline (PBS), pH 7.6 (Appendix I). This was then homogenized in a stomacher "400"apparatus (Colworth 400, London), for 15 minutes. The suspensions were then transferred to sterile screw-capped universal bottles, labelled and stored at 4 °C for 21 days.

Cultivation of Cold-enriched Samples

After 21 days of cold enrichment a loopful ($0.1cm^3$) of suspension from each sample was plated onto Cefsulodin-Irgasan-Novobiocin agar (CIN, DIFCO, U.S.A.)(Schiemann, 1979). See Appendix I. Plates were incubated at 29 °C and examined frequently after 18-48 hours.

Colonies resembling yersiniae (pink to dark red "bulls-eye" colonies surrounded by a transparent border, varying in size from pin-point within 24 hours to approximately 2.5 mm in diameter at 48 hours) were picked off and sub-cultured on to blood agar plates (Appendix I). Cultures that were haemolytic on blood agar were rejected. From blood agar, cultures were transferred to bijoux bottles containing 3 ml of tryptone water (TW). This was incubated for 24 hours and used as the inoculum for further characterisation.

Screening for *Yersinia* Species

Triple-sugar-iron agar (TSI), lysine-arginine-iron agar (LAIA) (Weagant, 1981) and urea slopes were inoculated and incubated for 24-48 hours at 29 °C. At this point the mortality test was performed.

Cultures which gave at least one of the following reactions were rejected: H_2S production (blackening on TSI), excessive gas production or non-fermentation (TSI), non-fermentation (LAIA) and urea negative.

Cultures which gave either 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 LAIA, plus urea positive (pink), with motility at 29 °C were considered as presumptive yersiniae. At this point one drop of the inoculum was transferred to bijoux bottles each containing 4ml of tryptone water (TW) and further incubated for 24-48 hours. This was used as the inoculum for biochemical characterization and subsequent identification of the isolate (see Table 2.1).

Identification and Serotyping of *Yersinia* Isolates

Species of *Yersinia* were identified on the basis of morphological, biochemical and cultural parameters as described elsewhere (Bercovier *et.al.*, 1980).

Each isolate was subjected to the following biochemical tests at 29 °C: ornithine decarboxylase, methyl red, voges-proskauer (MRVP) (also at 37 °C), indole production, acid production from the following carbohydrates: sucrose, L-rhamnose, D-melibiose, D-trehalose, D-xylose, salicin and aesculin hydrolysis. All tests were read daily for 2 days.

Serotyping was carried out using commercially available typing sera (Eco-Bio, Belgium) representing O-antigen factors available for *Y. enterocolitica* (0:3, 0:5,27, 0:6,38, 0:8, 0:9) and factors I to III of *Y. pseudotuberculosis*. The slide agglutination technique was used in this assay (Wauters, 1981).

All isolates identified as species of yersiniae from this study were stored at -20 °C and -70 °C in 15% glycerol broth.

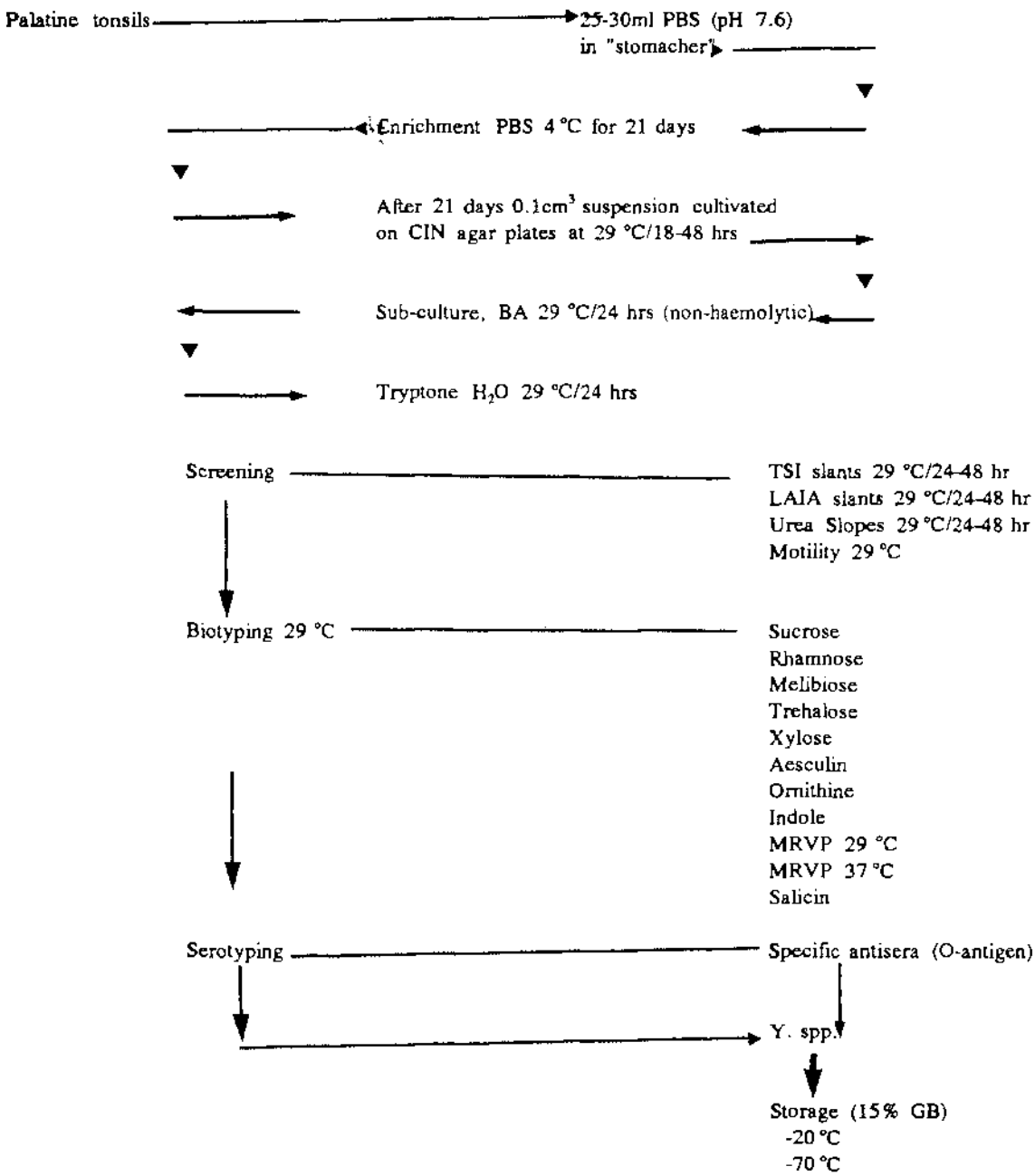
Virulence Assay

All *Y. enterocolitica* and related species isolated in this survey were tested for potential virulence by the pyrazinamidase activity test (PYZ). The test for PYZ activity was performed and interpreted as recommended by Kandolo and Wauters (1985). (Appendix I). Overnight broth cultures of *Y. enterocolitica*, *Y. frederiksenii* and *Y. intermedia* were inoculated over the entire slant of PYZ agar, incubated at 29 °C for 48 hours, and tested with 1ml of a freshly prepared solution of 1% ferrous ammonium sulphate. A positive test was evidenced by pink to brownish discolouration on the slant, while no colour change was associated with a negative reaction.

Biochemical Media Used

Preparation of biochemical media used in this study is described in Appendix I. This was carried out according to labelled instructions by the manufacturers, modifications by investigators and established criteria in microbiology.

Table 2.1: Flow diagram used in the isolation of *Yersinia* species from tonsils.



CIN - (Cefsulodin-Irgasan-Novobiocin agar)
BA - (Blood agar)
TSI - (Triple-sugar-iron agar)
LAIA - (Lysine-arginine-iron agar)
GB - (Glycerol broth)

RESULTS

Occurrence and Distribution of Yersinia Isolates

Table 2.2 shows the occurrence and distribution of species of *Yersinia* isolated from the tonsils of slaughter pigs. Isolates were obtained from the tonsils of 73 (58.9%) of the 124 pigs sampled. Four pigs harboured two distinct strains giving a total of 77 isolates (62.1%). *Yersinia enterocolitica* were present in 42 the (33.9%) of pigs sampled, followed by *Y. pseudotuberculosis* 27 (21.8%), *Y. frederiksenii* 7 (5.6%) and *Y. intermedia* 1 (0.8%). Yersiniae were isolated from all eight farms sampled in this survey with the number of isolates per farm ranging from 3 to 13 (20-100%), while the number of *Yersinia* species isolated per farm ranged from 1 to 3.

Table 2.2: Occurrence and distribution of Yersinia species from porcine tonsils.

Farm	No. of Samples	*Y.ent*(%)	*Y.fred*(%)	*Y.int.*(%)	*Y.ptb.*(%)	No. of isolates	Prev (%)
H	30	-	4 (13.3)	1 (3.3)	1 (3.3)	6	(20)
K	11	1 (9.1)	1 (9.1)	-	7 (63.6)	9	(81.8)
M	12	3 (25)	2 (16.7)	-	2 (16.7)	7	(58.3)
N	15	11 (73.3)	-	-	-	11	(73.3)
P	10	2 (20.0)	-	-	1	3	(30.0)
R	20	-	-	-	15 (75.0)	15	(75.0)
T	13	13 (100.0)	-	-	-	13	(100.0)
W	13	12 (92.3)	-	-	1 (7.7)	13	(100.0)
Total	124	42 (13.9)	7 (5.6)	1 (0.8)	27 (21.8)	77	
Mean (%)		33.9	5.6	0.8	21.8		62.1

NOTE: (%) (Number of isolates of each species/total number of samples)

Biochemical and Serological Characterization of Isolates

Table 2.3 shows the biochemical and serological characterization of the 77 isolates. A total of 65 of the 77 isolates (84.4%) were serotyped. The remainder were untypable by available antisera. *Yersinia enterocolitica* comprised 42 (54.6%) of the isolates, there were 26 (33.8%) isolates of bio-serotype 4/0.3, 12 (15.6%) of bio-serotypes 2 and 3/0:5,27, and 4 (5.2%) of biotype 1A.

Yersinia pseudotuberculosis comprised 27 (3.5%) of the isolates. These were 13 (16.9%) isolates of serotype III, 13 (16.9%) of serotype II, and 1 (1.3%) of serotype I.

Yersinia enterocolitica related species (*Y. intermedia* and *Y. frederiksenii*), comprised 9 (10.4%) of the isolates.

Table 2.3: Biochemical and serological characterization of 77 *Yersinia* isolates.

Bio-serotype	No. of Isolates	% of Total (n = 77)
Y. ent. bio/4 0:3	26	33.8
Y. ent. bio/2/3 0:5,27	12	15.6
Y. ent. bio/1A	4	5.2
Total	42	54.6%
Y. ptb I	1	1.3
II	13	16.9
III	13	16.9
Total	27	35.1%
Y. fred.	7	9.1
Y. int.	1	1.3
TOTAL	8	10.4%

Pathogenic Yersinia Isolates

Table 2.4 shows the prevalence of potential pathogenic species isolated from the tonsils. *Yersinia enterocolitica* 4/0:3 was isolated from 26 (21%) of pigs sampled. *Yersinia enterocolitica* 0:5,27 from 12 (9.8%). *Yersinia pseudotuberculosis* from 27 (21.8%). Therefore 65 (52.6%) of the pigs sampled yielded pathogenic species of yersiniae.

Table 2.4: Prevalence of pathogenic *Yersinia* from the tonsils of pigs

Species/serotype	No. Isolates	Prevalence % (n = 124)
Y. ent. 0:3	26	21
Y. ent. 0:5,27	12	9.8
Y. ptb. (I, II, III)	27	21.8
Total	65	52.6%

Virulence Assay

Pyrazinamidase activity (PYZ) was absent in 36 (72%) of 50 Yersinia strains tested and present in 14 (28%) (Table 2.5). All negative strains belonged to *Y. enterocolitica* bio-serotypes 4/0:3 and bio-serotype 2/3/0:5,27. Two strains of bio-serotype 3/0:5,27 gave weak positive reactions and were considered positives. All isolates of *Y. enterocolitica* 1A, *Y. frederiksenii* and *Y. intermedia* showed positive pyrazinamidase activity.

Table 2.5: Presence of pyrazinamidase activity (PYZ) in the isolates of *Yersinia*.

Species	O-Serogroup	No. of Strains	Pyrazinamidase (PYZ) (-)	(+)
Y. enterocolitica	0:3	26	26	0
Y. enterocolitica	0:5,27	12	10	2
Y. enterocolitica Bio/1A	-	4	0	4
Y. frederiksenii	-	7	0	7
Y. intermedia	-	1	0	1
Total		50	36(72%)	14(28%)

DISCUSSION

The prevalences of *Y. enterocolitica*, based on the findings of various investigators from several countries are given in Table 1.6. Most of the early reports of *Y. enterocolitica* in pigs were based on examination of stools, mesenteric lymph nodes, or caecal contents with relatively low isolation rates between 0% and 16% being reported (Esseveld and Goudzwaard, 1973; Szita *et.al.*, 1980; Toma and Deidrick, 1975; Zen-Yoji *et.al.*, 1974). Later studies demonstrated that the frequency of isolation of these bacteria was approximately ten times greater from the tongues and tonsils than was obtained from faeces

(Pedersen, 1979; Schiemann, 1980; Wauters, 1979). Isolation rates ranging from 25 to 80% were reported (Christensen, 1980; Doyle *et.al.*, 1980; Nesbakken, 1985; Pedersen, 1979; Wauters, 1979) depending on the type of samples examined (tongues, tonsils, throat swabs), geographical origin and efficacy of the isolation method. The results of this study are in agreement with studies performed elsewhere and clearly demonstrate that *Y. enterocolitica* and related species are common in the tonsils of New Zealand slaughter pigs in the region under study. The frequency of isolation of pathogenic species of *Yersinia* for this study is not very different from recently published data from Europe and Japan (Asplund *et.al.*, 1990; Christensen, 1987; de Boer and Mouws, 1991; Nesbakken and Kapperud, 1985; Shiozawa *et.al.*, 1991).

Yersinia enterocolitica bio-serotype 4/0:3, which is the most common human pathogenic strain isolated in New Zealand (McCarthy and Fenwick, 1990), was isolated from 21% of the pigs sampled. According to Lassen 1985 (cited by Nesbakken and Kapperud, 1985), this bio-serotype constitutes about 99% of the human clinical isolates in Norway. Kwaga *et.al.* (1990) isolated *Y. enterocolitica* serotype 0:5,27 from throats and caecal swabs of pigs in Canada. Fukushima *et.al.* (1990) recovered 0:5,27 from pigs grown on farms in Japan. Other studies have confirmed that pigs frequently carry this strain (Fukushima *et.al.*, 1984; Zen-Yoji *et.al.*, 1974). In the present study, serotype 0:5,27 was isolated from 9.8% of pigs sampled and constituted 15.6% of the total number of isolates. This serotype has been reported to cause yersiniosis in humans in California (Bisset, 1979) and New Zealand (McCarthy and Fenwick, 1990). More recently it has been reported to be involved with human infections worldwide (Aleksic *et.al.*, 1988).

Yersinia pseudotuberculosis serotypes I, II and III were isolated in this study, giving a combined isolation rate of 21.8% of pigs sampled or 35.1% of the total number of isolates. *Yersinia pseudotuberculosis* is associated with a variety of human diseases such as mesenteric lymphadenitis, terminal ileitis, erythema nodosum, arthritis and septicaemia (Mollaret, 1965). Studies carried out in Europe, Canada and Japan have shown that pigs are an important reservoir of *Y. pseudotuberculosis* (Narucka and Westendoorp, 1977; Toma and Deidrick, 1975; Tsubokura *et.al.*, 1984; Zen-Yoji *et.al.*, 1974). The presence of the organism in the tonsils of slaughter pigs suggests that pig carcasses and pork products could become contaminated and act as a source of infection to man.

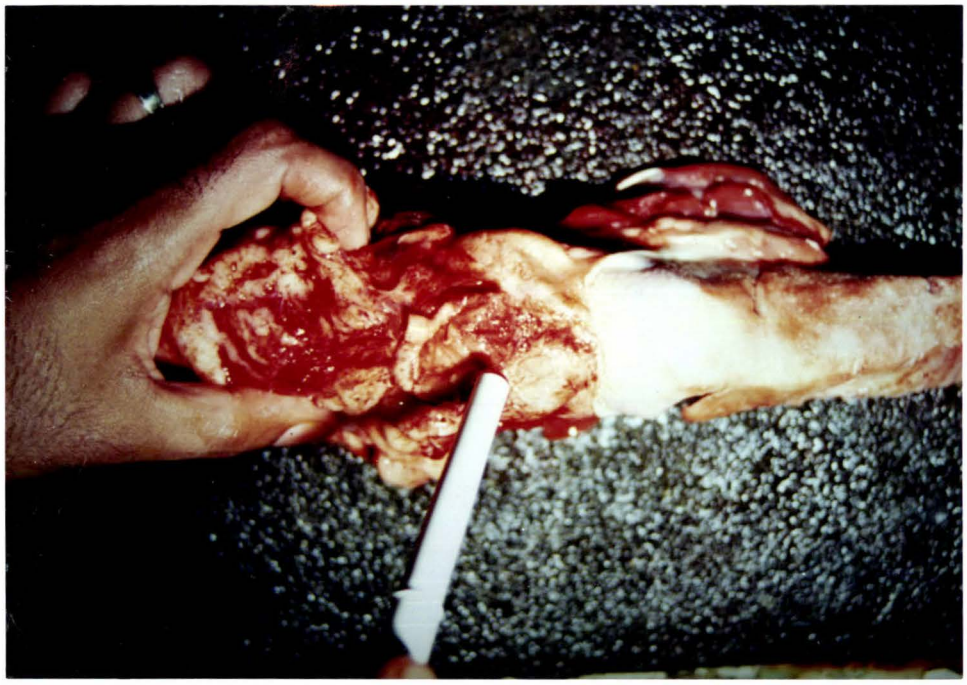
Pyrazinamidase activity (PYZ) has been described as a simple assay that is a reliable indicator of virulence in species of *Yersinia* (Cornelis *et.al.*, 1987; Kandolo and Wauters, 1985; Miller *et.al.*, 1988). An excellent correlation between a negative PYZ test and virulence was demonstrated by Carter *et.al.* (1988). From the findings of this study, this may be true also for *Y. enterocolitica* biotype 4/0:3; biotype 2 or 3/0:5,27,but not for biotype 1A and other *Yersinia* isolates. All 12 of these isolates which are non-pathogens give positive PYZ reactions, an indication that the test is useful in separating pathogenic from non-pathogenic yersiniae. Similar observations were reported by Kwaga and Iversen (1991) who investigated the virulence of strains of *Y. enterocolitica* and related species isolated from pigs and pork products in Canada.

Since this study was conducted during the colder months of August and September, it was thought that this may have been the reason for the relatively high isolation rates encountered. Another observation in this study was the ability of a particular species or serotype to predominate in a herd. *Yersinia enterocolitica* serotype 0:3 was present in two farms with isolation rates of 100% and 73.3% respectively. Serotype 0:5,27 was present in another farm with an isolation rate of 100%. *Yersinia pseudotuberculosis* also predominated in two farms with isolation rates of 75% and 63.3% respectively (Table 2.2). Therefore a longitudinal study was considered necessary to look into these aspects.

CONCLUSION

From this study the following can be concluded. Pigs sent for slaughter within the Manawatu and Wairarapa regions carry species of *Yersinia* in their tonsils. Pathogenic strains of *Y. enterocolitica* and *Y. pseudotuberculosis* comprised a high percentage of the isolates and a particular species or serotype prevailed in a specific herd for the majority of herds investigated. The pyrazinamidase activity (PYZ) test for potential virulence correctly identified 96% of the isolates tested with 72% belonging to pathogenic strains of *Y. enterocolitica*.

PLATE V



V. Tongue, tonsils and pharynx of the pig. Location of tonsils are indicated.

CHAPTER THREE

THE EFFECTS OF SEASON ON PREVALENCE AND SPECIES OF *YERSINIA* ISOLATED FROM THE TONSILS OF SLAUGHTERED PIGS

INTRODUCTION

There are several reports on the seasonal incidence of *Yersinia* infection in slaughtered pigs (Bockemuhl *et.al.*, 1979; Tsubokura *et.al.*, 1973, Zen-Yoji *et.al.*, 1974). The incidence of asymptomatic *Y. enterocolitica* infections detected by isolation from the faecal material of healthy slaughter pigs in Northern Bavaria (Germany) was closely related to season (Bockemuhl *et.al.*, 1979). The lowest incidence was observed during the summer months (August, 0%), but this increased steadily to a maximum in April (71.2%). With one exception, the serotypes 0:3 and 0:9 were only isolated during October to December.

In another report from Germany, on the seasonal isolation of *Y. enterocolitica* and *Y. pseudotuberculosis* from the tonsils of healthy slaughter pigs, *Y. enterocolitica* was isolated throughout the whole year, but especially in the months of November (17.5%) and December (32.5%). *Yersinia pseudotuberculosis* was found during the winter and spring, most frequently in the months of January (30%) and December (17.5%) (Weber and Knapp, 1981).

Most cases of human yersiniosis appear to occur during the colder months of the year (Asakawa *et.al.*, 1973; De Groote *et.al.*, 1982; Mingrone *et.al.*, 1987; Vandepitte and Wauters, 1979). In the United States, a study conducted in human patients (Metchock *et.al.*, 1991) revealed that cases of yersiniosis due to *Y. enterocolitica* occurred throughout the year and increased in the winter months. This contrasts with data from Canada which

indicated highest isolation rates in the summer (Marks *et.al.*,1980).

The cross-sectional study (Chapter Two) was conducted during the colder months of the year and it was believed that this may have been the reason for the high relatively isolation rates encountered. Another observation in this study was the tendency for a particular species or serotype to prevail in a herd, this included *Y. enterocolitica* serotypes 0:3; 0:5,27, and *Y. pseudotuberculosis*. For this reason the under-mentioned hypotheses were set out.

- The same species and serotype of *Yersinia* is maintained in a herd over an extended period of time.
- There is a seasonal effect on the prevalence of isolation and type of *Yersinia* isolated.

The knowledge of the distribution of the various species of *Yersinia* in pigs is vital in understanding some aspects of the epidemiology of the infection. Such information is of tremendous importance in assessing the potential significance of *Yersinia* infections in pigs, and the subsequent relationship to infections in humans and other animal species.

MATERIALS AND METHODS

Farm selection

From the eight farms surveyed during the cross-sectional study (Chapter Two), four were selected for this longitudinal study. This selection was based on the dominant species or serotypes isolated from three farms. The fourth farm was considered a negative farm since only a few atypical strains of *Yersinia* were detected during the cross-sectional study. Hence farm H was selected as negative, while farm K, farm T, and farm W were selected due to the dominance of *Y. pseudotuberculosis*, *Y. enterocolitica* 4/0:3,and *Y. enterocolitica* 3/0:5,27 respectively.

Determination of sample sizes

The method used for determination of sample sizes in the cross-sectional study (Chapter

Two) was applied in this study. The prevalence of species of *Yersinia* in these four farms was already known (Table 2.2). An annual total of 180 samples per farm (15 per month) was to be collected. This number was greater than that established in the table (Cannon and Roe, 1982) "Sample size for the estimation of prevalence" at the 95% level of confidence and a 10% desired accuracy for "infinite" populations.

Animal reference

A similar code to that used in phase one of the study, representing the animal reference was applied in this phase of the study. This comprised the first letter of the farm's name, the sex of the animal, the month in which sampling was carried out, and the sample number in question. Thus, WM1-10 referred to farm W, sex male, January, sample number 10 (Appendix IIB).

Sampling plan

Sampling of tonsils was carried out once per month, usually during the first week of the month, over a period of 12 months from February 1993 to January 1994. A total of 60 samples per month (15 per farm), and 180 samples per farm per year were expected to be collected during the sampling period, however, one farm (farm T) did not supply any pigs for slaughter during the month of January 1994. Therefore an overall number of 705 samples were collected during the twelve month period.

Samples were collected, processed for cold-enrichment, cultured and isolates identified exactly as described in Chapter Two.

RESULTS

Distribution and prevalence of species of *Yersinia*

Table 3.1 shows the farm distribution and prevalence of species of *Yersinia* isolated from the tonsils of slaughtered pigs during the study period. Of a total of 705 pigs sampled from the four selected farms, of 264 strains of *Yersinia* were isolated giving an overall prevalence

of 37.4%. Twelve pigs harboured two distinct strains and one pig harboured three distinct strains (See Appendix IIB). Individual farm prevalences varied as follows: farm H 2.8%, farm K 31.7%, farm T 55.2% and farm W 61.7%. (See Figure 3.1).

***Fig 3.1* Individual Farm Prevalence of Species of *Yersinia* Isolated from the Tonsils of Pigs during the period Feb 1993-Jan 1994**

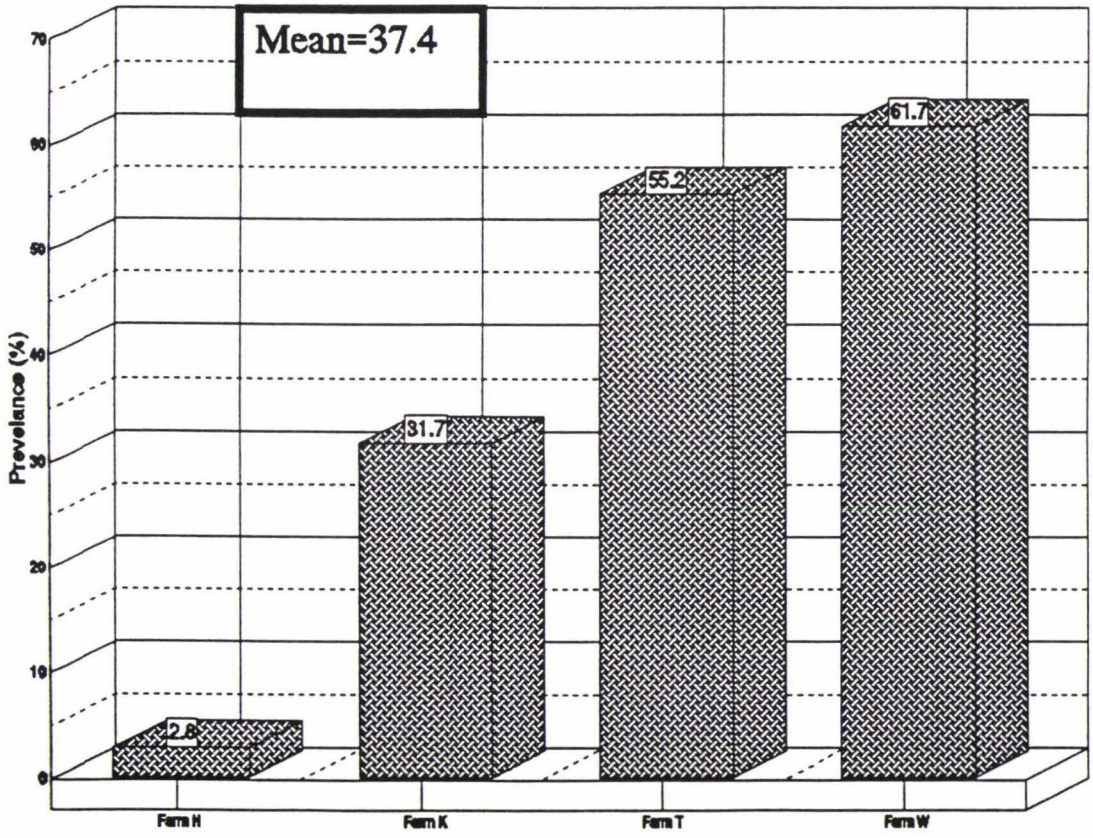


Table 3.1: **Farm distribution and prevalence of species of *Yersinia* isolated from the tonsils of pigs during the period February 1993 to January 1994.**

Farm	No. of samples	No. of isolates of:						
		Y.ent.(%)	*Y.ptb.*(%)	*Y.int.*(%)	*Y.fred.*(%)	*Y.kris.*(%)	Total	Prevalence %
H	180	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	1 (0.6)	5	2.8
K	180	8 (4.4)	46 (25.6)	1 (0.6)	2 (1.1)	-	57	31.7
T	165	80 (48.5)	8 (4.8)	3 (1.8)	-	-	91	55.2
W	180	109 (60.6)	-	-	2 (1.1)	-	111	61.7
TOTAL	**705**	**198 (28.1)**	**55 (7.8)**	**5 (0.7)**	**5 (0.7)**	**1 (0.2)**	**264**	**37.4**

Note: (%) = number of isolates of each species/total number of samples.

Y. enterocolitica was isolated from all four farms as follows: farm H 1 (0.6%), farm K 8 (4.4%), farm T 80 (48.5%) and farm W 109 (60.6%) (n = 180 for H, K and W and 165 for T).

Yersinia pseudotuberculosis was isolated from all but one farm (W) as follows: farm H 1 (0.6%), farm K 46 (25.6%) and farm T 8 (4.8%).

Yersinia frederiksenii was isolated from all but one farm (T) as follows: farm H 1 (0.6%), farm K 2 (1.1%) and farm W 2 (1.1%).

Yersinia intermedia was isolated from all but one farm as follows: farm H 1 (0.6%), farm K 1 (0.6%) and farm T 3 (1.8%).

Yersinia kristensenii was isolated only from one farm, farm H 1 (0.6%).

Altogether, 198 (28.1%) isolates of *Y. enterocolitica* were recovered, 55 (7.8%) of *Y. pseudotuberculosis*, 5 (0.7%) of *Y. intermedia*, 5 (0.7%) of *Y. frederiksenii*, and 1 (0.2%) of *Y. kristensenii* representing 75%, 20.8%, 1.9%, 1.9% and 0.4% of the total number (264) of isolates respectively. Table 3.2.

Table 3.2: Species of *Yersinia* isolated in the study.

Species	No. of isolates	% of total n = 264
Y. enterocolitica	198	75.0%
Y. pseudotuberculosis	55	20.8%
Y. frederiksenii	5	1.9%
Y. intermedia	5	1.9%
Y. kristensenii	1	0.4%
TOTAL	**264**	**100.0%**

Monthly isolation of yersiniae

Table 3.3 shows the monthly isolation of species and serotypes of *Yersinia* during the study period. A total of 60 samples were collected each month except for January where only 45 samples were collected due to the non-supply of pigs from one farm (farm T).

Yersiniae were isolated throughout the entire year with isolation rates of 40% or more during the months of: April, 26 (43.3%); May, 24 (40.9%); June, 31 (51.7%); July, 30 (50.0%); August, 32 (53.3%); September, 24 (40.0%); October, 32 (53.3%); and December, 25 (41.7%).

Yersinia. enterocolitica

Yersinia enterocolitica serotype 0:3 was isolated throughout the year with 5 or more isolates being made during the months of March (8), April (7), May (5), June (12), July (10), August (7), September (8) and December (11). The annual total of 70 isolates, were 29.5% of the total number of isolates.

Yersinia enterocolitica serotype 0:5,27 was isolated throughout the year except in the month of November, with 5 or more isolates recorded during the months of March (9), April (12), May (11), June (12), July (8), August (14), September (8), October (16) and December (7). The annual total of 105 isolates, were 39.8% of the total number of isolates.

Sporadic isolations of *Y. enterocolitica* biotype 1A strains were made, these included serotype 0:5 with 5 isolates recorded in the month of September, and an annual total of 8, constituting 3.0% of the total number of isolates. Serotypes 0:6 and 0:8 and non-typable (NT) strains accounted for 3 (1.1%), 2 (0.8%), and 2 (0.8%) of the total number of isolates respectively.

Other *Yersinia* species

A number of other *Yersinia* species were also sporadically isolated and accounted for 11 (4.2%) of the total number of isolates. These comprised 5 (1.9%) of *Y. intermedia*, 5 (1.9%) of *Y. frederiksenii*, and 1 (0.4%) of *Y. kristensenii* (see Table 3.2).

Table 3.3: Monthly isolation of yersiniae isolated from the tonsils of pigs during the period February 1993 to January 1994.

Year	Month	No. of Monthly Samples	Number Positive												
			Total	%	*Y. ent. Serotypes*						*Y. ptb. Serotypes*			Other Species		
					0:3	0:5,27	0:5	0:6	0:8	NT	I	II	III	*Y. int.*	*Y. fred.*	*Y. krist.*
1993	February	60	9	15.0	1	4	-	-	-	-	-	-	-	4	-	-
	March	60	19	31.7	8	9	-	-	-	-	-	-	2	-	-	-
	April	60	26	43.3	7	12	-	-	-	-	-	-	6	-	-	1
	May	60	24	40.0	5	11	-	-	-	-	-	-	4	1	3	-
	June	60	31	51.7	12	12	1	1	-	-	-	3	2	-	-	-
	July	60	30	50.0	10	8	-	-	1	2	3	2	3	-	-	-
	August	60	32	3.3	7	14	1	-	1	-	1	3	5	-	-	-
	September	60	24	40.0	8	8	5	-	-	-	-	-	2	-	1	-
	October	60	32	53.3	4	14	1	1	-	-	2	1	7	-	-	-
	November	60	5	8.3	3	-	-	-	-	-	-	1	1	-	-	-
	December	60	25	41.7	11	7	-	1	-	-	-	-	6	-	-	-
1994	January	45	7	15.6	2	4	-	-	-	-	-	-	1	-	-	-
Total		**705**	**264**		**78**	**105**	**8**	**3**	**2**	**2**	**6**	**10**	**39**	**5**	**5**	**1**
Mean %				**37.4**	**11.1**	**14.9**	**1.1**	**0.4**	**0.3**	**0.3**	**0.9**	**1.4**	**5.5**	**0.7**	**0.7**	**0.1**

Yersinia pseudotuberculosis

Yersinia pseudotuberculosis serotype I was isolated only in the months of July (3), August (1) and October (1) (Table 3.3). *Yersinia pseudotuberculosis* serotype II was only isolated between the months of June and November, comprising of 3 isolates each in June and August, 2 in July and 1 each in October and November. However, serotype III was isolated throughout the year except for February and isolates of 5 or more were recorded in the months of April (6), August (5), October (7), and December (6).

Altogether a total of 55 (20.9%) of the total number of isolates belonged to *Y. pseudotuberculosis* with serotype I, 6 (2.3%); serotype II, 10 (3.8%); and serotype III, 39 (14.8%) respectively (Table 3.4).

Table 3.4: Serological characterization of 253 strains of *Y. enterocolitica* and *Y. pseudotuberculosis* isolated during the study.

Specie/Serotype	No. of isolates	% of total n = 264
Y. ent./0:3	78	29.5
Y. ent./0:5,27	105	39.8
Y. ent./0:5	8	3.0
Y. ent./0:6	3	1.1
Y. ent./0:8	2	0.8
Y. ent./NT	2	0.8
Total	**198**	**75.0%**
Y. ptb. I	6	2.3
Y. ptb. II	10	3.8
Y. ptb. III	39	14.8
Total	**55**	**20.9**

Relationship between monthly isolation of yersiniae and average daily temperatures

Fig. 3.2 shows the relationship between monthly isolation of yersiniae and the average daily temperatures (Data ex AgResearch, Palmerston North) for each month during the period February 1993 to January 1994. Yersiniae were isolated throughout the year with peaks

occurring in the colder months between June and October. The lowest isolation rates occurred in the months of November, January, and February. A total of 25 isolates were recorded during the month of December.

(See Figure 3.2)

Distribution and seasonal incidence of species of *Yersinia* on individual farms

1. *Farm H*

This farm showed no dominance of a particular species or serotype of *Yersinia*. Isolations were sporadic and in fact only 5 (2.8%) strains of *Yersinia* were isolated (Table 3.1). These comprised one strain each of *Y. enterocolitica* 1A/0:6, *Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii* and *Y. pseudotuberculosis* serotype III. Four strains were isolated between April and June and one strain in December (Table 3.5).

Table 3.5: Monthly isolations of species of *Yersinia* from farm H

Month	Number Positive					
	Total	*Y.ent.* Serotype 0:6	*Y.ptb* Serotype III	Other Species		
				Y.fred.	*Y.int.*	*Y.krist.*
April	1	-	-	-	-	1
May	2	-	-	1	1	-
June	1	1	-	-	-	-
December	1	-	1	-	-	-
TOTAL	**5**	**1**	**1**	**1**	**1**	**1**

2. *Farm K*

The data from this farm shows that *Y. pseudotuberculosis* is the predominant strain (Figs. 3.3, 3.4, 3.5). A total of 46 (80.7%) of the 57 isolates from this farm were *Yersinia pseudotuberculosis*. These consisted of 6 (10.5%) isolates of *Y. pseudotuberculosis* serotype I, 10 (17.5%) isolates of *Y. pseudotuberculosis* II, and 30 (52.6%) isolates of *Y. pseudotuberculosis* III. The remaining 11 (19.3%) of the isolates belonged to other species of *Yersinia* consisting of 8 strains of *Y. enterocolitica*, 1 strain of *Y. intermedia* and 2 strains of *Y. frederiksenii* (Table 3.6).

Yersinia pseudotuberculosis serotype I was isolated only in winter and early spring (Fig. 3.3). *Y. pseudotuberculosis* serotype II was also isolated during the colder months of winter and spring (Fig. 3.4). *Y. pseudotuberculosis* serotype III was isolated throughout the year except in the month of February, with the highest peak in autumn (Fig. 3.5) and lowest in late spring and summer.

***Fig 3.2* Relationship between Monthly Isolation of Species of *Yersinia* and Average Daily Temperature during the period Feb 1993-Jan 1994**

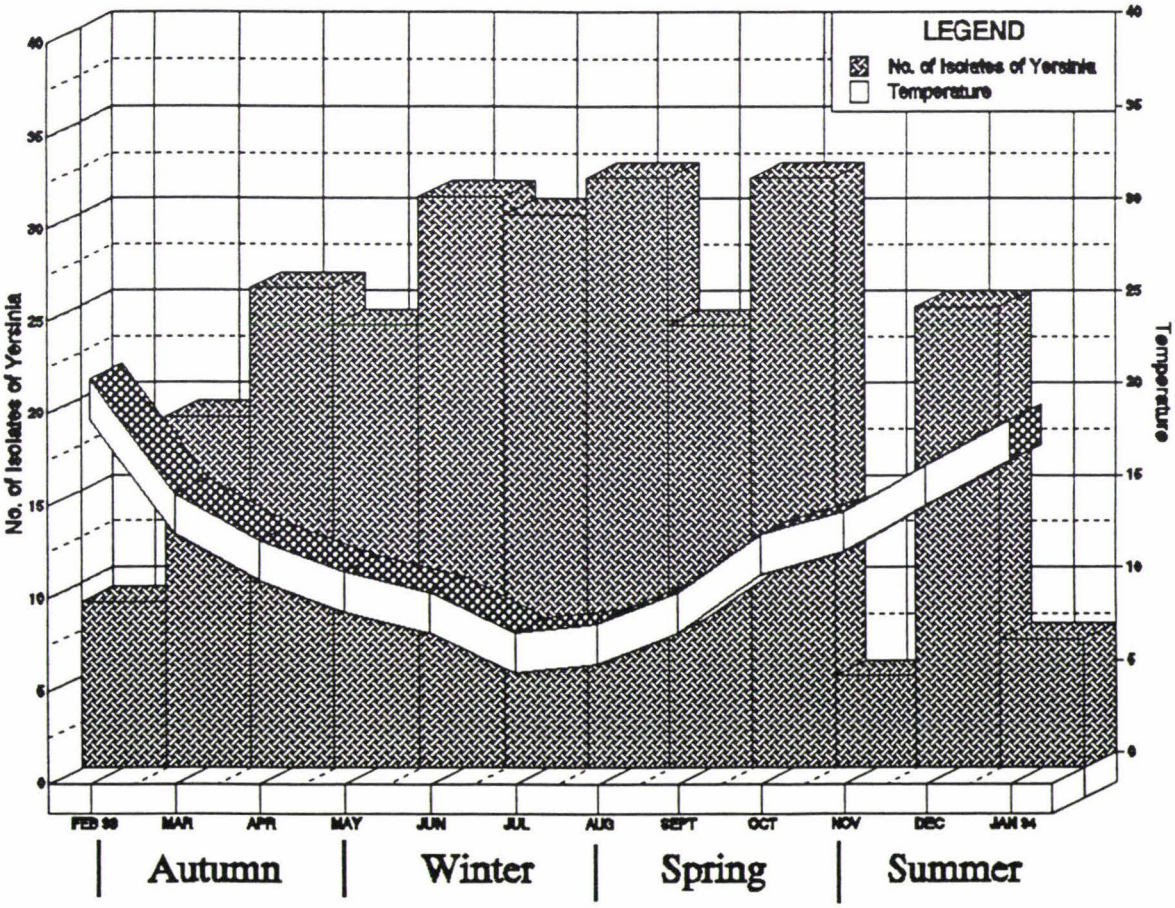


Fig 3.3 **Seasonal Distribution and Prevalence of *Y. pseudotuberculosis* Serotype I from Farm K**

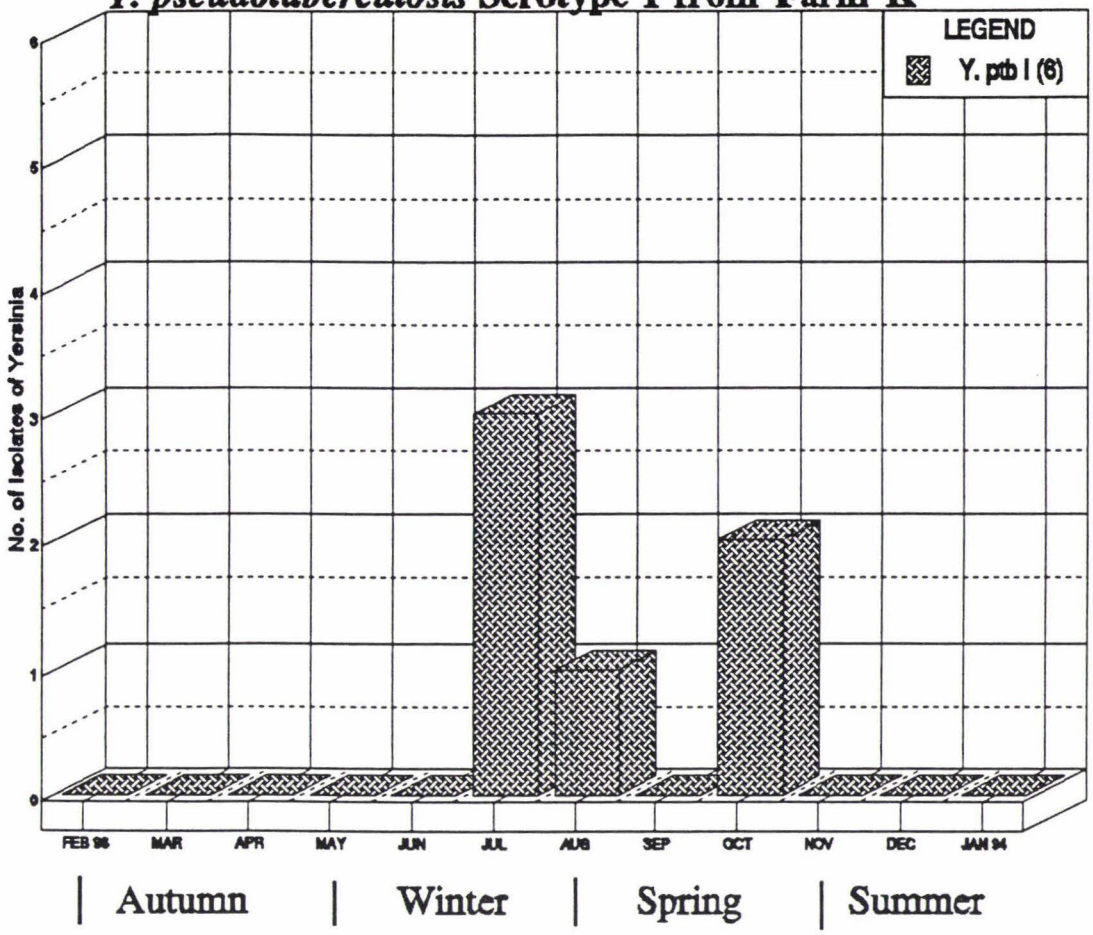


Fig 3.4 **Seasonal Distribution and Prevalence of *Y. pseudotuberculosis* Serotype II from Farm K**

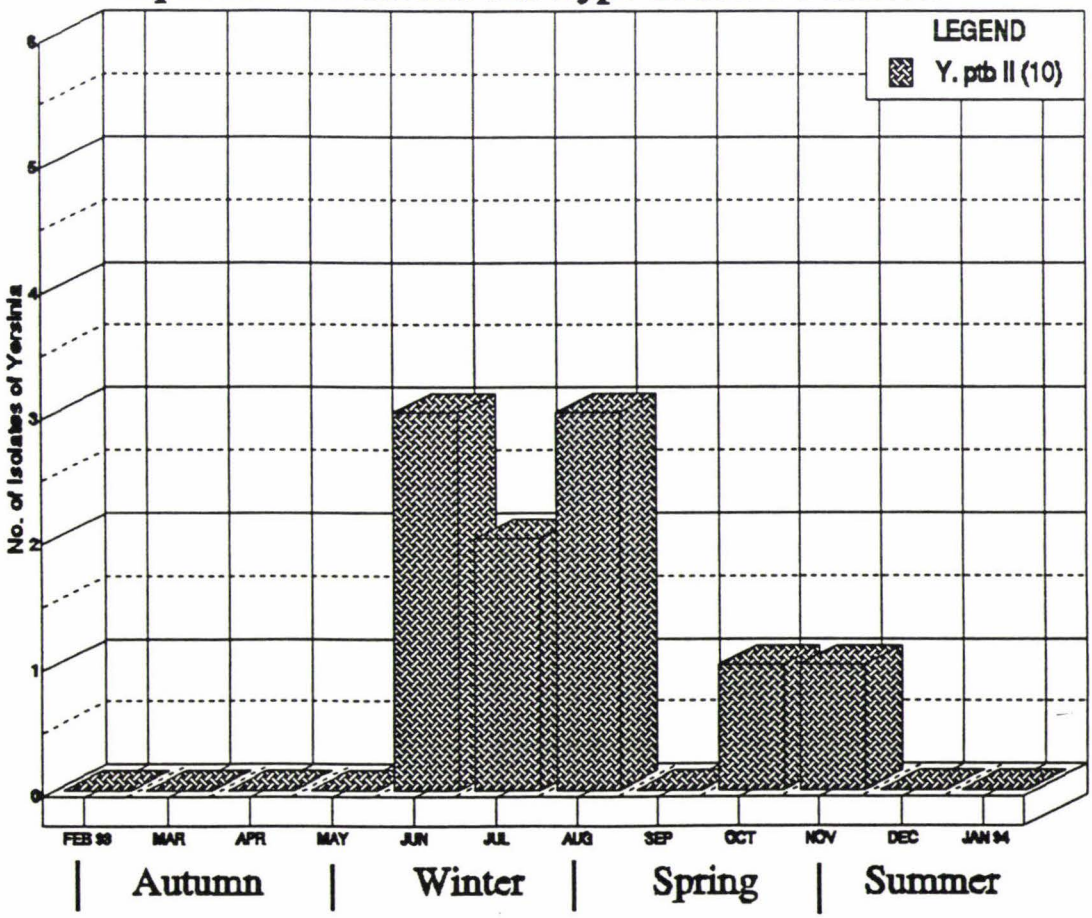


Fig 3.5 **Seasonal Distribution and Prevalence of *Y. pseudotuberculosis* Serotype III from Farm K**

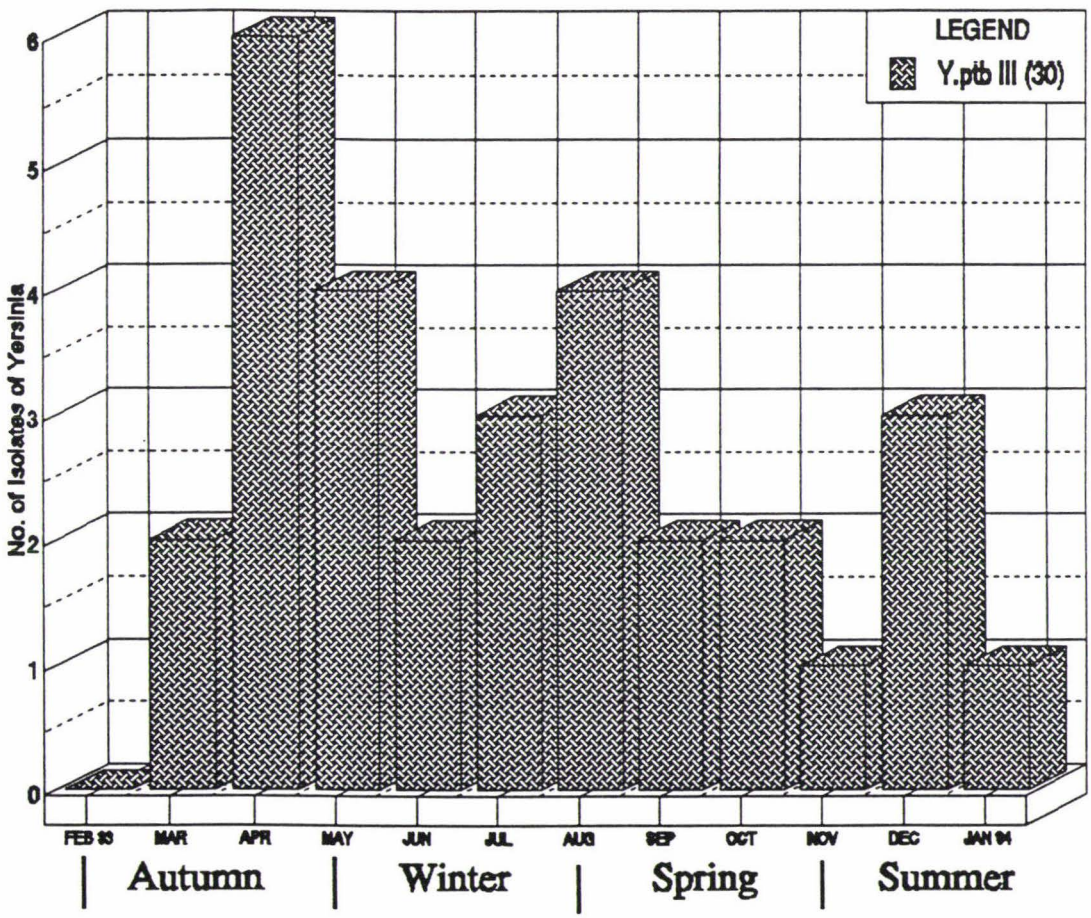


Table 3.6: Monthly isolations of species of *Yersinia* other than *Y. pseudotuberculosis* from farm K

Month	Number Positive									
	Total	*Y.ent.* Serotypes						Other Species		
		0:3	0:5,27	0:5	0:6	0:8	NT	*Y.fred.*	*Y.int.*	*Y.krist.*
Feb	1	-	-	-	-	-	-	-	1	-
May	1	-	1	-	-	-	-	-	-	-
June	3	3	-	-	-	-	-	-	-	-
July	2	-	-	-	-	1	-	1	-	-
August	1	-	-	-	-	1	-	-	-	-
Sept	3	-	1	1	-	-	-	-	1	-
Total	**11**	**3**	**2**	**1**	**-**	**2**	**-**	**1**	**2**	**-**

3. *Farm T*

The data from this farm clearly shows that *Y. enterocolitica* serotype 0:3 is the predominant strain (Fig. 3.6). *Yersinia enterocolitica* serotype 0:3 was isolated throughout the year except in the month of January when samples were not obtained due to non-supply of pigs for slaughter. Peak prevalence occurred during the autumn, winter and early summer months with the lowest prevalence in November and February. The overall prevalence of species of *Yersinia* in this farm was highest in the months of December, July and October. Of the 91 isolates, 70 (76.9%) were of *Y. enterocolitica* serotype 0:3, the remaining 21 isolates (23.1%) comprised *Y. enterocolitica* 0:5,27 (3), 0:5 (3), 0:6 (2), NT (2), *Y. intermedia* (3) and *Y. pseudotuberculosis* serotype III (8) (Table 3.7).

Fig 3.6 **Seasonal Distribution and Prevalence of *Y. enterocolitica* Serotype 0:3 from Farm T**

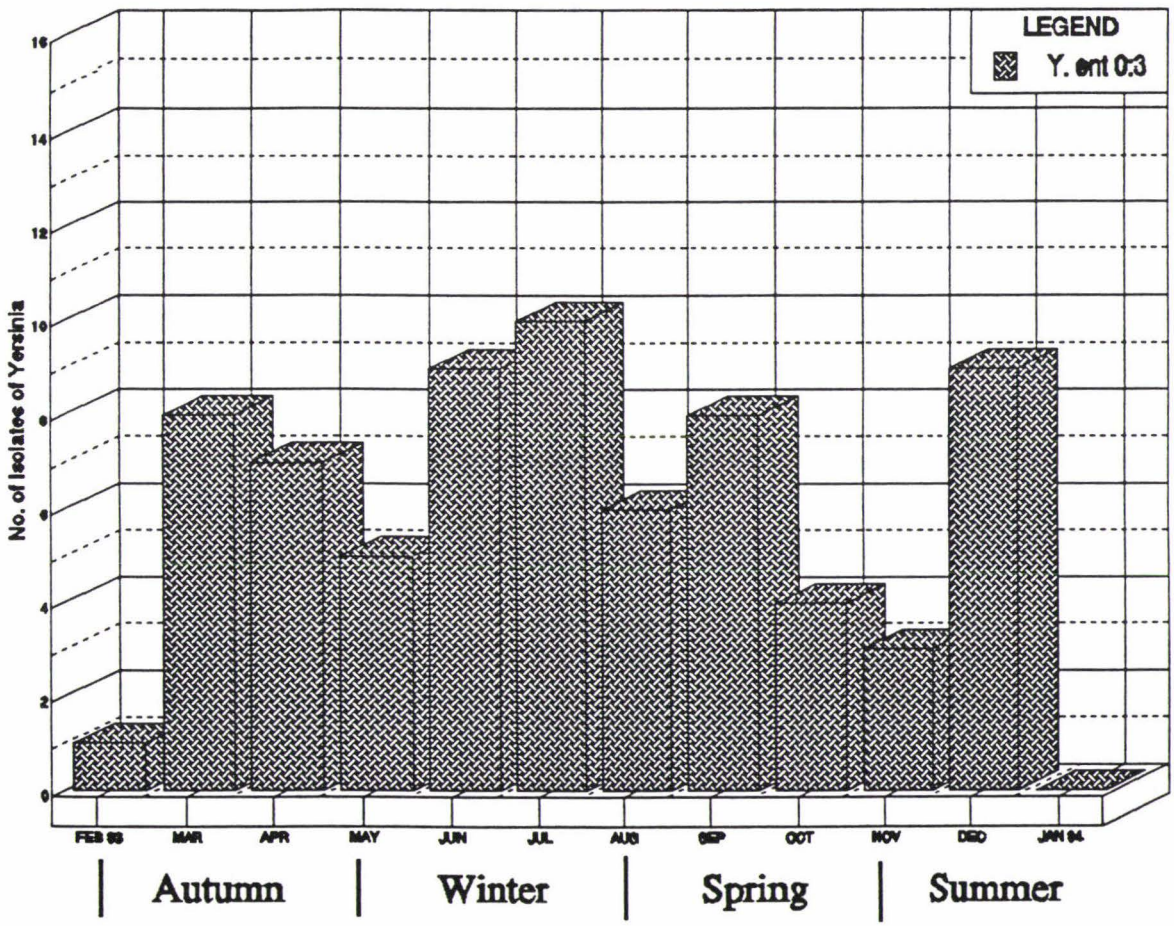


Table 3.7: Monthly incidence of species of *Yersinia* other than *Y. enterocolitica* serotype 0:3 from farm T.

Month	Number Positive										
	Total	*Y. ent.* Serotype					*Y. ptb.* Serotype			Other Species		
		0:5,27	0:5	0:6	0:8	NT	I	II	III	*Y.fred.*	Y.int.	Y.krist.
Feb	3	-	-	-	-	-	-	-	-	-	3	-
July	2	-	-	-	-	2	-	-	-	-	-	-
Aug	3	1	1	-	-	-	-	-	1	-	-	-
Sept	1	-	1	-	-	-	-	-	-	-	-	-
Oct	8	1	1	1	-	-	-	-	5	-	-	-
Dec	4	1	-	1	-	-	-	-	2	-	-	-
Total	**21**	**3**	**3**	**2**	**-**	**2**	**-**	**-**	**8**	**-**	**3**	**-**

4. *Farm W*

Yersinia enterocolitica serotype 0:5,27 was the predominant serotype in this farm (Fig. 3.7). This serotype was isolated throughout the year except in the month of November. The peak prevalence occurred in the month of October with high isolation rates in the autumn and winter months. The lowest prevalence occurred during the summer months. Of the 111 isolates, 100 (90.0%) were *Y. enterocolitica* serotype 0:5,27,the remaining 11 (10%) of the isolates were *Y. enterocolitica* biotype 4/0:3 (5), *Y. enterocolitica* biotype IA/0:5 (4) and *Y. frederiksenii* (2) (Table 3.8). *Yersinia pseudotuberculosis* was never isolated from this farm.

Fig 3.7 **Seasonal Distribution and Prevalence of *Y. enterocolitica* Serotype 0:5,27 from Farm W**

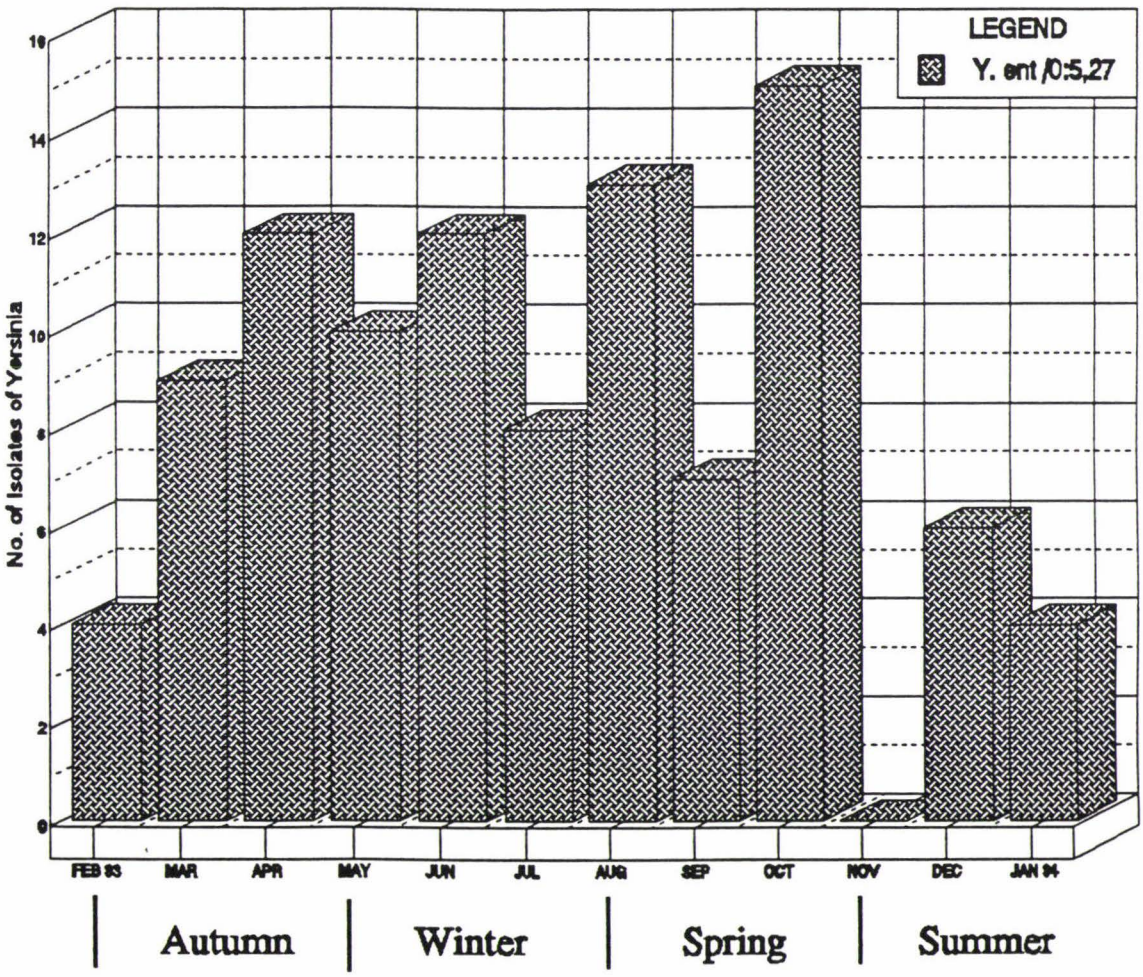


Table 3.8: Monthly isolations of species of *Yersinia* other than *Y. enterocolitica* serotype 0:5,27 from farm W.

Month	Number Positive								
	Total	*Y.ent.* Serotype					Other Species		
		0:3	0:5	0:6	0:8	NT	*Y.fred.*	*Y.int.*	*Y.krist.*
May	2	-	-	-	-	-	2	-	-
June	1	-	1	-	-	-	-	-	-
Aug	1	1	-	-	-	-	-	-	-
Sept	3	-	3	-	-	-	-	-	-
Dec	2	2	-	-	-	-	-	-	-
Jan	2	2	-	-	-	-	-	-	-
Total	**11**	**5**	**4**	**-**	**-**	**-**	**-**	**2**	**-**

DISCUSSION

This study has confirmed that pigs sent for slaughter in the lower North Island of New Zealand are frequent carriers of species of *Yersinia* in their tonsils, including those that may be pathogenic for humans.

The overall prevalence of 37.4% ranks New Zealand among countries with reported high isolation rates of species of *Yersinia* from the tonsils of pigs. These include Scandinavian countries, other parts of Europe, and Japan (Table 1.6). It is important to note that the prevalence reported in this study could have been substantially higher if other farms were selected. For this study only four farms were considered which included one (farm H) with a relatively low isolation rate. During the cross-sectional study several other farms had higher isolation rates (see Table 2.2) but were not selected due to the objectives of the current study.

The individual farm prevalence in this study followed a similar pattern to that detected in the earlier study. Farm H continued to show sporadic isolations and the lowest prevalence, followed by farm K, farm T and farm W which maintained their particular dominant species or serotypes throughout the study period. However, the number of samples tested in the

case of farm T (165) was less than that for the other farms (180 each).

This study has also confirmed that *Yersinia enterocolitica* is the most predominant species of *Yersinia* harboured in the tonsils of slaughtered pigs in this region of New Zealand's North Island. Of the 264 isolates, 198 (75%) were *Y. enterocolitica* followed by 55 (20.8%) strains of *Y. pseudotuberculosis* (Table 3.2). Other *Yersinia* species considered to be environmental in origin were only isolated sporadically during the study (11 isolates of 4.2% of the total).

Yersinia enterocolitica serotype 0:5,27 was the most common serotype isolated during the study representing 105 (39.8%) of the total number of isolates, followed by *Y. enterocolitica* serotype 0:3 representing 78 (29.5%) (Table 3.4). This situation appears to be unique to New Zealand. Several studies from other countries (Christensen, 1980; Jackova and Uregeova 1993; Kotula and Sharar, 1993; Maruyama, 1987; Shiozawa *et.al.*, 1991) have reported *Y. enterocolitica* serotype 0:3 to be the most common isolate from the tonsils of pigs, with serotype 0:5,27 being isolated in insignificant numbers or totally absent. However, the figures from this study may have been biased by the deliberate selection of the four farms. The study was not designed to give an estimate of the relative prevalence of different serotypes in the pig population of New Zealand.

There are numerous reports on the seasonal incidence of *Yersinia* infections in slaughtered pigs (Bockemuhl *et.al.*, 1979; Tsubokura *et.al.*, 1973, 1976; Weber and Knapp, 1981; Zen-Yoji *et.al.*, 1974). It is well known that *Y. pseudotuberculosis* infections occur more frequently in winter (Tsubokura *et.al.*, 1976b). As for *Y. enterocolitica*, no seasonal variation has been reported in any early publications (Nilehn, 1969). Later, many workers, however, reported that *Y. enterocolitica* infections were more prevalent in autumn and winter (Ahvonen, 1973; Arvastson, 1971; Winblad, 1973) or winter or early spring (Rakovky, 1973; Vandepitte *et.al.*, 1973). On the other hand, Rabson and Koornhof (1973) reported that these infections appeared most frequently in late summer and early autumn in South Africa. Delorme *et.al.* (1974) pointed out that the monthly distribution of the organism showed no such seasonal patterns as described by previous workers, but that a peak of infection occurred in Canada in mid-summer. In Japan, *Y. enterocolitica* was mainly isolated in winter (Tsubokura *et.al.*, 1976a).

In this study, yersiniae were isolated throughout the entire year from the tonsils of slaughtered pigs with peak prevalences in the colder months between June and October (Fig. 3.2). The isolation rate in the month of November was relatively low compared with that for December. One reason for this may have been an over growth of competitive microflora in the PBS cold-enrichment causing an inhibition of the growth of yersiniae. Numerous colonies of unidentified organisms were observed on the CIN plates during culture especially from farm W where, surprisingly, no yersiniae were isolated during that month. From Fig. 3.2, one can also suggest that the isolation rate for January is substantially lower than that for the month of December, but it must be remembered that only 45 of the expected 60 samples were collected during January.

Yersinia enterocolitica serotype 0:3 was found throughout the year except in January when samples were not available from the farm in which this serotype predominated, however, the low isolation rate in February suggests that there was a real decrease in summer. The peak prevalence was observed in winter with high isolation rates in early summer, autumn and spring. *Yersinia enterocolitica* serotype 0:5,27 was also found throughout the year on another farm except in November (a possible explanation for this is given above). The peak prevalence was observed in mid-spring with high isolation rates during the autumn and winter.

The reason for the ecological pattern of *Y. enterocolitica* in this study is unknown. Carter and Collins (1974) and Nilehn (1973) reported that this organism is more pathogenic when grown at 25 °C than at 37 °C. It has been suggested that the organism might remain as a source of infection in the winter months because *Y. enterocolitica* can replicate at temperatures as low as 4 °C (Gutman *et.al.*, 1973).

This study has shown a seasonal variation among serotypes of *Y. pseudotuberculosis* isolated from the tonsils of pigs. *Yersinia pseudotuberculosis* serotype I was isolated only in mid-winter and early spring. *Yersinia pseudotuberculosis* serotype II was isolated throughout the winter and spring. However, *Y. pseudotuberculosis* serotype III was isolated throughout the year in a similar manner to *Y. enterocolitica* 0:3 and 0:5,27. The lowest prevalence occurred in late summer, with a peak in autumn and constant isolation throughout winter and spring. This finding could suggest that serotypes I and II are more cold-tolerant and hence may

be more virulent than serotype III.

This study has also demonstrated the ability of a particular species or serotype to be maintained on a farm over an extended period. The pattern of occurrence in the individual farms observed during the cross-sectional study was maintained during the twelve months of the current study. However, sporadic isolation of other species or serotypes did occur in one or more of the farms under study (Table 3.5-3.8).

Several factors, including sources of infection, type of management and husbandry practices or establishment of cross-protection between serotypes may be responsible for the individual farm situations observed in this study. This could be an area for further carefully planned ecological and epidemiological investigations of yersiniae under the circumstances where pigs are raised. This is of considerable potential importance to public health because there are farms where pathogenic strains are maintained and farms where they are absent. If the determining factors could be identified then it may be possible by changing some management factors, for example, to influence the species of yersiniae which predominate.

CONCLUSIONS

Yersinia enterocolitica is the most common species of *Yersinia* isolated from the tonsils of pigs sent for slaughter in the lower North Island of New Zealand. *Yersinia enterocolitica* biotype 4 serotype 0:3 and *Y. enterocolitica* biotype 3 serotype 0:5,27 are the two most common pathogenic variants of *Y. enterocolitica* harboured in the tonsils of these animals. *Yersinia pseudotuberculosis* is also a frequent inhabitant in the tonsils. Serotypes I, II and III were detected with serotype III being the most consistent isolate.

Yersiniae were isolated throughout the year with a peak prevalence in the colder months. Seasonal differences in the occurrence of serotypes 0:3 and 0:5,27 however, are not really significant, both species being found throughout the year with lowest isolation rates in summer.

Seasonal variation in the isolation of serotypes of *Y. pseudotuberculosis* was evident. Serotypes I and II were only detected in winter and spring. Serotype III was detected

throughout the year with a peak in autumn and a low in summer.

The ability of a particular species and/or serotype to be maintained in a specific herd over an extended period was established. The findings from this study further reinforce the assumption that pigs constitute an important reservoir for pathogenic yersiniae in many countries, including New Zealand.

CHAPTER FOUR

IN VITRO ASSESSMENT OF VIRULENCE AMONG STRAINS OF *YERSINIA ENTEROCOLITICA* AND *YERSINIA PSEUDO-TUBERCULOSIS* ISOLATED FROM THE TONSILS OF SLAUGHTERED PIGS. PUBLIC HEALTH SIGNIFICANCE OF POTENTIAL PATHOGENS

INTRODUCTION

Yersinia enterocolitica has become well established as an enteric pathogen since it was first described in 1939 (Bottone, 1987; Cornelis *et.al.*,1987). However, considerable confusion exists in the literature because not all strains of this species can cause intestinal infection. Unlike intrinsic pathogens such as *Salmonella* and *Shigella* species, there is a strain-to-strain variation in the pathogenicity of *Y. enterocolitica* (Bottone, 1981; Cornelis *et.al.*,1987; Kay *et.al.*,1983; Miller *et.al.*,1989; Wachsmuth *et.al.*,1984). Several studies have shown an excellent correlation between the serotype and biotype of *Y. enterocolitica* and its ability to cause infections of the intestinal tract and to invade tissue (Bottone, 1981; Cornelis *et.al.*, 1987; Kay *et.al.*,1983; Miller *et.al.*,1989; Zink *et.al.*,1980).

Different bio-serotypes of *Y. enterocolitica* are associated with human infections in different regions of the world (Doyle, 1990). Of the many bio-serotypes of *Y. enterocolitica*, only a few regularly cause disease in man (Robins-Browne *et.al.*,1989). Because non-pathogens may readily contaminate food, water, or even clinical specimens, it is essential to be able to distinguish true pathogens from their similar, but comparatively benign, relatives (Lee *et.al.*, 1990; Robins-Browne *et.al.*, 1989), and this requires information on markers of virulence.

Epidemiological evidence has shown that human infections with *Y. enterocolitica* arise from the ingestion of raw pork (Tauxe *et.al.*,1987). Molecular genetic studies have also failed

to differentiate among isolates recovered from humans with disease, carrier pigs, and pork, which supports the hypothesis that pigs constitute an important source of human infection (Caugant *et.al.*,1989; Kapperud *et.al.*,1990).

Yersinia pseudotuberculosis has consistently been recovered from the intestinal contents and tonsils of healthy slaughtered pigs in various parts of the world (Toma and Deidrick, 1975; Tsubokura *et.al.* 1976; Weber and Knapp, 1981; Zen-Yoji *et.al.*,1974). However, only a few reports are available on the virulence and the presence of plasmids in strains of *Y. pseudotuberculosis* isolated from pigs or pork (Shiozawa *et.al.*,1988; Tsubokura *et.al.*,1984).

Several different tests and methods have been used over the years to determine the pathogenic potential of *Y. enterocolitica* (Table 1.8), and also of *Y. pseudotuberculosis*, but it has only recently been recognised that an important component of virulence in both these species is determined by a plasmid that is easily lost (Kandolo and Wauters, 1985; Kay *et.al.*,1982; Wachsmuth *et.al.*,1980). For this reason there is confusion on how well the various methods identify pathogenic serotypes and determine pathogenic potentials (Farmer *et.al.*,1992).

The present study was conducted with the following objectives:

(i) To investigate the *in vitro* virulence-associated characteristics of strains of *Yersinia* isolated from the tonsils of pigs, with the aim of determining the potential role of pigs as reservoirs of pathogenic strains.

(ii) To assess the potential public health implications of pathogenic strains of *Yersinia* isolated from the tonsils of slaughtered pigs.

MATERIALS AND METHODS

Bacterial strains

A total of 150 isolates of *Yersinia* recovered from the tonsils of slaughtered pigs during the period June to November 1993 were tested for virulence-associated characteristics. These comprised 114 isolates of *Yersinia enterocolitica* including 57 isolates of *Y. enterocolitica* 3/0:5,27,43 isolates of *Y. enterocolitica* 4/0:3; 8 isolates of *Y. enterocolitica* 1A/0:5, and 2 isolates each of *Y. enterocolitica* 1A/0:6, 1A/0:8 and 1A/NT (non-typable).

Yersinia pseudotuberculosis accounted for 36 of the isolates tested for virulence-associated characteristics. These comprised 6, 10 and 20 isolates of *Y. pseudotuberculosis* serotypes I, II and III respectively.

Growth, isolation and identification procedures

All aspects relating to cultivation, isolation, screening, identification and subsequent serotyping of yersiniae presented in this study are described in Chapter Two.

Evaluation of simple tests used to define pathogenic serotypes of *Y. enterocolitica*

Since antisera for typing clinical isolates of *Y. enterocolitica* are not widely available, several simple tests that can be used to identify pathogenic serotypes have been proposed (Farmer *et.al.*,1992). Table 4.1 summarises the expected results for pathogenic and non-pathogenic serotypes in these tests.

Table 4.1: Properties of *Y. enterocolitica* strains.

Property	Pathogenic serotypes with virulence plasmid:		Non-pathogenic serotypes
	Present	Absent[a]	
Small red colonies on CR-MOX agar	+	-	-
Temperature-dependent autoagglutination	+	-	-
Invasiveness (cell culture)	+	+	-
Pyrazinamidase production	-	-	+
Salicin fermentation	-	-	+
Aesculin hydrolysis	-	-	+
Belonging to serotypes 0:1,2,3;0:2,3;0:3; 0:4,32; 0:5,27;0:8; 0:9; 0:13a,13b; 0:18; 0:20; or 0:21	+	+	-
Belonging to other serotypes	-	-	+

[a] Presumably, the virulence plasmid was originally present but it was lost during sub-culture or storage. Adapted from Farmer *et.al.* (1992).

From the table (4.1) shown above, tissue culture invasion was not tested for strains of *Y. enterocolitica* in the present study. In addition tissue culture invasion, with salicin fermentation and aesculin hydrolysis were not considered for strains of *Y. pseudotuberculosis*. The various simple *in vitro* tests used in this study are described below.

Temperature-dependent autoagglutination

This test was conducted as described by Farmer *et.al.* (1992). Cultures of all 150 isolates of yersiniae undergoing investigation were inoculated into two tubes of Methyl-red, Voges-Proskauer broth (MR-VP, Difco, U.S.A.) during the routine biochemical characterisation of the isolates. One tube was incubated at 37 °C and the other at 29 °C. After 18-24 hours, the tubes were observed for agglutination, with care taken not to shake or disturb the sediment at the bottom and along the side of the tubes. Pathogenic yersiniae that contain the virulence plasmid agglutinate at 37 °C but not at 29 °C. Strains that agglutinated at both temperatures were considered "rough".

Congo red, magnesium oxalate agar (CR-MOX)

The use of congo red as an indicator of the potential virulence of isolates of *Yersinia* was proposed by Prpic *et.al.* (1983). These authors were able to show that plasmid-bearing strains of *Y. enterocolitica* had the ability to bind congo red. Mair and Fox (1986) used magnesium oxalate agar (MOX) to demonstrate calcium dependency at 37 °C, for some strains of *Y. enterocolitica*. Riley and Toma (1989) modified magnesium oxalate agar (MOX) to include congo red (CR) which allows visualization of calcium-dependent growth and uptake of congo red dye on the same plate.

Isolates were first grown on 5% sheep blood agar for 24 hours. Individual colonies were then picked off and sub-cultured onto CR-MOX agar plates. These plates were incubated at 37 °C, and observed for the presence of small red colonies at 24 and 48 hours. Freshly isolated strains of pathogenic yersiniae contain the virulence plasmid and are CR-MOX positive. When the plates are kept at 37 °C for several additional days it is often possible to observe the development of a large colourless colony from a small red colony due to loss of plasmid in some cells. Non-pathogenic strains are CR-MOX negative producing only large, colourless colonies.

Pyrazinamidase test

The pyrazinamidase test was performed as described by Kandolo and Wauters (1985). The method and interpretation of this virulence assay are described in Chapter Two.

Salicin fermentation - aesculin hydrolysis

Salicin and aesculin are both B-glucosides that vary in the chemical structure of the compound attached to D-glucose by a beta-linkage. Both compounds are hydrolysed by the enzyme B-glucosidase.

Both tests are routinely carried out during biochemical characterization of isolates of yersiniae. Salicin fermentation was determined by inoculating 2-3 drops of a test strain into bijoux containing 3ml of the preparation (Appendix I) which was then incubated at 29 °C and read after 24 and 48 hours. A negative reaction is indicated by no colour change while a positive reaction is indicated by a pink to red colouration of the preparation. Aesculin

hydrolysis was determined by similarly inoculating 2-3 drops of a test strain onto aesculin agar slopes (Appendix I), these were also incubated at 29 °C and read after 24 to 48 hours. A positive reaction is indicated by blackening of the medium while no change in the medium indicates a negative reaction. Pathogenic strains of *Y. enterocolitica* are salicin-aesculin negative while non-pathogenic strains are positive in both tests.

RESULTS

Virulence-associated characteristics in strains of *Y. enterocolitica*

The results of assays used for the determination of virulence-associated characteristics of *Y. enterocolitica* are presented in Table 4.2. Based on these results the isolates can be divided into two groups. The first group consists of *Y. enterocolitica* strains belonging to biotypes 3 and 4 which are generally recognised as pathogenic biotypes based on their ecological behaviour (Cornelis *et.al.*, 1987). The other group consists of *Y. enterocolitica* biotype 1A, that is believed to constitute non-pathogenic strains.

Table 4.2: Occurrence of virulence-associated characteristics among strains of *Yersinia enterocolitica* (Ye) isolated from the tonsils of pigs.

Species/ bioserotype	No. of isolates tested	Salicin fermentation	Aesculin hydrolysis	No. of isolates giving positive virulence assay in:		
				Auto-agglutination	CR-MOX agar	Pyrazinam-idase[a]
Ye 3/0:5,27	57	0	0	57	56	57
Ye 4/0:3	43	0	0	43	40	43
Ye 1A/0:5	8	8	8	0	0	0
Ye 1A/0:6	2	2	2	0	0	0
Ye 1A/0:8	2	2	2	0	0	0
Ye 1A/NT*	2	2	2	0	0	0
TOTAL	114	14	14	100	96	100

[a] A negative reaction for pyrazinamidase correlates to correlate with virulence potential (Kandolo and Wauters, 1985).

NT* Non-typable

As can be observed from Table 4.2, all strains of *Y. enterocolitica* biotypes 3 and 4 tested positive virulence assay results in both autoagglutination and pyrazinamidase tests (100%

sensitivity). However CR-MOX agar identified only 98.2% (56 out of 57) strains of biotype 3, and 93% (40 out of 43) strains of biotype 4. All of these strains were salicin-aesculin negative. All strains of biotype 1A, regardless of serotype, gave negative results in the autoagglutination, CR-MOX agar and pyrazinamidase tests, and all were salicin-aesculin positive.

Virulence-associated characteristics in strains of *Y. pseudotuberculosis*

The results obtained in the virulence assays for 36 strains of *Y. pseudotuberculosis* are given in Table 4.3.

Table 4.3: Occurrence of virulence-associated characteristics among strains of *Yersinia pseudotuberculosis* isolated from the tonsils of pigs.

Serotype	No. of strains tested	No. of strains giving positive virulence assay in:		
		Autoagglutination	CR-MOX agar	Pyrazinamidase[a]
I	6	6	6	6
II	10	10	10	9
III	20	20	18	19
TOTAL	36	36	34	34

[a] A negative reaction for pyrazinamidase correlates with virulence potential (Kandolo and Wauters, 1985).

As can be observed from Table 4.3, all these serotypes (I, II, III) demonstrated at least one virulence-associated characteristic. All 36 test strains of *Y. pseudotuberculosis* were positive in the autoagglutination test (100% sensitivity), while CR-MOX agar and the pyrazinamidase test identified 34 of 36 strains respectively (94.4% sensitivity).

DISCUSSION

This study has confirmed that bio-serotypes of *Y. enterocolitica* isolated from the tonsils of slaughtered pigs in this locality include those that are potentially pathogenic for humans as assessed by their *in vitro* virulence-associated characteristics. Cornelis *et.al.* (1987) suggested that bio-serotyping provides a useful means for predicting virulence in *Y. enterocolitica,* as is evident in the present study.

This study has also shown that serotypes of *Y. pseudotuberculosis* isolated from the tonsils of slaughtered pigs in the same locality may be potentially pathogenic for humans as is evident by the high number of strains giving positive virulence assay results in autoagglutination, calcium dependency and congo red uptake (CR-MOX agar) and pyrazinamidase tests. Several studies (Toma and Deidrick, 1975; Tsubokura *et.al.*, 1976; Weber and Knapp, 1981; Zen-Yoji *et.al.*, 1974) have shown consistent recovery of *Y. pseudotuberculosis* from the tonsils and intestinal contents of healthy slaughtered pigs; moreover, a large number of strains isolated from these animals belonged to serotype III. This finding is confirmed in the present study, since serotype III accounted for 55.6% (20 out of 36) of the isolates of *Y. pseudotuberculosis*. Kanazawa *et.al.* (1974) isolated a serotype III strain from a housewife in Japan and they suspected contaminated pork to be the mode of transmission since she often ate this meat. This, according to the authors, was the only case of isolation of a melibiose-nonfermenting strain of *Y. pseudotuberculosis* from a host (human) other than healthy pigs. It is important to note that all serotype III strains tested for virulence-associated characteristics in the present study were melibiose-nonfermenting strains.

Only a few reports are available (Shiozawa *et.al.*, 1988; Tsubokura *et.al.*, 1984) on the virulence and presence of plasmids in *Y. pseudotuberculosis* strains isolated from pigs or pork. In Japan, Fukushima (1985) and Shiozawa *et.al.* (1987) reported that retail pork was contaminated with *Y. pseudotuberculosis* serotype IVB. The results of that study showed that such isolates possess a 42 Mda plasmid which is associated with virulence factors, such as autoagglutination and calcium dependency. There were also no differences in these virulence factors found among pig, pork, and human clinical isolates of *Y. pseudotuberculosis* in Japan. In the present study the majority of strains of *Y. pseudotuberculosis* serotypes I, II and III were positive in the autoagglutination and CR-MOX assays (Table 4.3) which suggests that these strains possess a similar size plasmid. Thus, these strains should be considered to pose a potential risk for people in direct contact with infected pigs, such as veterinarians, pig farmers and farm workers, and abattoir workers. In Finland, studies carried out to evaluate the risk of infection among butchers (Merilahti-Palo *et.al.*, 1991; Seuri and Granfors, 1992) concluded that infections with *Y. pseudotuberculosis* are an occupational health risk to workers slaughtering pigs in the abattoirs. In addition, if pork is contaminated with virulent strains of *Y. pseudotuberculosis* and improperly cooked or allowed to cross-contaminate other foods, its consumption may

result in the development of a *Y. pseudotuberculosis* infection. Therefore pigs and pork may play an important role in the epidemiology of human infections caused by *Y. pseudotuberculosis*.

An interesting finding in this study was the isolation of two strains of *Y. enterocolitica* serotype 0:8 from the tonsils of slaughtered pigs. This serotype which belongs to the pathogenic biotype 1B has so far dominated in human infections in the U.S.A. (Mollaret *et.al.*,1979). However, in this study both strains of serotype 0:8 were associated with the non-pathogenic biotype 1A of *Y. enterocolitica*, which from a clinical and public health standpoint, is not considered an enteric pathogen. This finding has provided further evidence that serological typing alone is not sufficient for classification of strains of *Yersinia* and may not always predict virulence (Chiesa *et.al.*,1991). In these strains, the 0:8 factor could have been associated with biotype 1A, serotype 0:7,8, a non-pathogenic strain of *Y. enterocolitica*, however, this was not verified (Wauters, 1981).

Farmer *et.al.* (1992) have stated that pyrazinamidase, salicin-aesculin and CR-MOX agar can be used to screen for pathogenic serotypes of *Y. enterocolitica* since 0:antisera are not generally available. A study by Chiesa *et.al.* (1993) reaffirms that the use of 0:antisera without prior complete biochemical characterisation of the *Y. enterocolitica* isolates could lead to considerable overestimation of pathogenic serotypes (Chiesa *et.al.*,1987; Chiesa *et.al.*,1991; Wauters, 1981; Wauters *et.al.*,1991). The authors agreed that simple tests, such as those mentioned above, are a useful primary tool to identify *Y. enterocolitica* isolates as belonging to pathogenic or non-pathogenic phenotypes, while 0:typing is important in the understanding of the epidemiology and ecology of this species. Further evidence confirming this finding has been demonstrated in the present study (Table 4.2).

The autoagglutination assay in MR-VP broth was easy to perform and was easily adaptable to the daily routine since MR-VP broth is readily available in most laboratories (Farmer *et.al.*,1992). This virulence assay provides a rapid presumptive indication of the pathogenic potential of *Yersinia* isolates (Laird and Cavanaugh, 1980; Stern and Damare 1982). However, negative results should be interpreted with caution since this plasmid-associated property may easily be lost on sub-culture. In this study the autoagglutination assay was highly effective in detecting pathogenic species. This is probably due to the fact that all

strains tested were fresh isolates that were not stored for any extended period of time, thus reducing the likelihood that they had lost their virulence plasmids.

The CR-MOX test provides a simple method to screen for plasmid-containing pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* and yields results in 24 hours. The advantage over other virulence tests is that each colony on CR-MOX is tested for both calcium dependency and congo red absorption (Riley and Toma, 1989). A strain which has lost the plasmid will be CR-MOX negative but, if it is a potential pathogen, it will be salicin-aesculin negative.

The pyrazinamidase assay has been shown to play a significant role in the characterisation of biotypes of *Y. enterocolitica*. This test has also been correlated with the ability of the strain to harbour the virulence plasmid and not to the presence of the virulence plasmid itself (Kandolo and Wauters, 1985). In fact, there are potentially pathogenic strains of *Y. enterocolitica* without the plasmid and they are usually pyrazinamidase, salicin and aesculin negative (Chiesa *et.al.*,1991). In this regard the assay has been established as a valuable indicator in the separation of non-pathogenic from potentially pathogenic strains of yersiniae.

The potential virulence of strains of *Y. enterocolitica* and *Y. pseudotuberculosis* isolated from the tonsils of slaughtered pigs in the present locality during this study, further supports the hypothesis that pigs may constitute an important source of human infection (Caugant *et.al.*, 1989; Kapperud *et.al.*, 1990). There is no evidence to suggest that the organisms are pathogenic for pigs themselves. On the other hand, yersiniae may easily be disseminated from the tonsils during slaughtering and processing and may thus represent a hazard to consumers (Nesbakken, 1992).

Reports of human infection due to yersiniae have only been sporadic in New Zealand, but infections with *Y. enterocolitica* are becoming more commonly diagnosed (McCarthy and Fenwick, 1990). However, no reports to date have implicated pigs or pork products in the epidemiology of human infection with this organism in New Zealand. It is therefore recommended that further studies be carried out on potential pathogenic isolates from this study and similar isolates from humans in an attempt to evaluate the relationship among strains from both sources.

CONCLUSION

The virulence assays of autoagglutination, CR-MOX agar, and pyrazinamidase, combined with salicin fermentation and aesculin hydrolysis can be used successfully to identify isolates of *Y. enterocolitica* as pathogenic or non-pathogenic strains. Similarly, these assays are also useful in determining the pathogenic potential of serotypes of *Y. pseudotuberculosis*.

Yersinia enterocolitica bioserotypes 4/0:3 and 3/0:5,27 and *Y. pseudotuberculosis* serotypes I, II and III isolated from the tonsils of slaughtered pigs in this locality may be potentially pathogenic for humans as assessed by their virulence-associated characteristics. Such strains could pose a risk of infection to persons in direct contact with infected pigs. During slaughtering and processing these strains may easily be disseminated from the tonsils, contaminate carcasses and products and thus may represent a hazard to consumers.

CHAPTER FIVE

GENERAL DISCUSSION

The need to investigate the role of pigs as reservoirs of pathogenic yersiniae implicated in human infections came about as a result of the increasing frequency of isolation of *Yersinia enterocolitica* from humans in New Zealand. Results of similar studies carried out at the Department of Veterinary Pathology and Public Health in deer and goats have not implicated either of these animal species as important sources of human pathogenic strains of *Y. enterocolitica*. Results of the present study have clearly indicated that pigs are major reservoirs not only for human pathogenic *Y. enterocolitica* but also for *Y. pseudotuberculosis*.

During this study a total of 341 strains of *Yersinia* were isolated from the tonsils of 829 pigs. This constitutes an overall prevalence of 41.1% ranking New Zealand among countries with high rates of isolation of this organism. Of the isolates, 104 (30.5%) were *Y. enterocolitica* serotype 0:3, 117 (34.3%) were *Y. enterocolitica* serotype 0:5,27, 82 (24.6%) were *Y. pseudotuberculosis* and 38 (11.1%) were environmental strains. These results, coupled with the fact that several pigs were concurrently-infected with distinct species or serotypes of the organism, clearly indicates the widespread distribution of species of *Yersinia* in the tonsils of pigs sent for slaughter.

One reason for the high isolation rate encountered in this study may have been the efficacy of the isolation method used. The "stomacher" method combined with cold-enrichment in PBS at pH 7.6, for 21 days at 4 °C proved highly efficient for the isolation of species of *Yersinia* from the tonsils of pigs. Nesbakken and Kapperud (1985) claimed that PSB (PBS + Sorbitol and bile) cold-enrichment should be included in any combination as it was the single most efficient method for recovery of *Y. enterocolitica* biotype 4/0:3. However the high isolation rate in this present study indicated that their method need not be used.

Pigs are regarded as natural carriers of pathogenic species of *Y. enterocolitica*, but the source of infection for pigs themselves remains unclear. However, it is commonly believed

that wild animals, birds and rodents are the primary reservoirs for *Y. pseudotuberculosis*. There appears to be a variation in the geographical distribution of *Y. pseudotuberculosis* in New Zealand. The widespread distribution of *Y. pseudotuberculosis* serotype III among domestic animals in the North Island (Hodges *et.al.*,1984) may explain why the majority of the *Y. pseudotuberculosis* strains isolated from pigs in the study belonged to serotype III, which was rarely detected in domestic animals in the South Island (MacKintosh and Henderson, 1984).

The frequency of isolation of *Y. pseudotuberculosis* serotype III varies between countries (Tsubokura *et.al.*,1984). In the United States (Dickinson and Mocquot, 1961) and Canada (Toma and Deidrick, 1975) all *Y. pseudotuberculosis* isolates from faeces or the alimentary tracts of healthy pigs belonged to serotype III. In Germany, however, serotype III strains were not frequently detected from the faeces of healthy pigs (Weber and Knapp, 1981); moreover, strains isolated from the tonsils of healthy slaughter pigs belonged to serotypes I and II and serotype III strains were not detected. In Japan, many of the *Y. pseudotuberculosis* isolates from healthy pigs belonged to serotype III (Tsubokura *et.al.*,1970, 1976, 1984; Zen Yoji *et.al.*,1974). In the present study, 52 (63.4%) of the isolates of *Y. pseudotuberculosis* were serotype III.

Epidemiological investigations of *Y. enterocolitica* have focused mainly on pigs as a source of infection with serotype 0:3. Serotype 0:5,27 has been isolated from pigs all over the world (Fukushima and Tsubokura, 1985; Hurvell, 1981) including New Zealand (this thesis) and Asakawa *et.al.* (1979) isolated 0:5,27 from raw pork in Japan. A study by Fukushima *et.al.* (1993) suggested the possibility that pigs and dogs are the sources of serotype 0:5,27 infection for humans. Restriction endonuclease analyses of virulence plasmid DNA (REAP) of isolates from these animals produced patterns similar to those produced by human isolates. Similar studies need to be conducted on isolates of serotype 0:5,27 from this study in an attempt to elucidate the role of pigs as a possible reservoir for human infection with this serotype. Whether or not there is a causal relationship between human infection and that in animals such as pigs and dogs should be clarified in the future, with carefully planned epidemiological studies.

Several other serotypes of *Y. enterocolitica* were sporadically isolated during this study. These included serotypes 0:5, 0:6, 0:6,30, 0:8 and strains which did not react to any of the

typing sera available and were termed NT (non-typable). *Yersinia* species, namely *Y. frederiksenii*, *Y. intermedia* and *Y. kristensenii* were also detected. These serotypes and species are regarded as environmental strains and are not usually associated with disease in people. The isolation of two strains of serotype 0:8 was interesting. This serotype belongs to the pathogenic biotype 1B, but the biochemical profile of these two strains indicated that they belonged to the non-pathogenic biotype 1A. A similar strain of *Y. enterocolitica* serotype 0:8 was detected in an environmental sample taken from a supermarket delicatessen (Hudson and Motti, 1993). These authors indicated that the prevalence of *Y. enterocolitica* in retail flesh foods, including pork, in New Zealand is low and that the majority of strains isolated did not fall into a pathogenic serogroup. These observations correlate well with previous observations by Hudson *et.al.*,(1992).

There was no evidence during this study to suggest that the pathogenic strains of *Yersinia* isolated from the tonsils of pigs are themselves pathogenic for pigs, however, some reports have implicated both *Y. enterocolitica* and *Y. pseudotuberculosis* in clinical disease in pigs (see Yersiniosis in Pigs, Chapter One). Non-specific colitis is an infection seen especially in fattening pigs. It causes diarrhoea, often benign, whose economic consequences can however be important due to its effects on animal production (Neef, 1993). Its aetiology is poorly understood but several infectious agents including *Y. enterocolitica* and *Y. pseudotuberculosis* have been implicated. The widespread distribution of these species of *Yersinia* in the tonsils of pigs in New Zealand may suggest a possible role in the aetiology of this infection and similar infection of the intestinal tract of pigs in this country. Studies should be conducted in an attempt to clarify the role of species of *Yersinia* in enteric infection of pigs in this country.

In the first phase of this study, pigs from eight farms were investigated for the presence of species of *Yersinia*. Four of these farms were then selected for the second phase based on the particular species and/or serotype which they were carrying. In general, *Y. enterocolitica* serotype 0:3 was detected from five of the eight farms at varying prevalence, but was absent from another three farms suggesting a herd or farm-wide distribution. Christensen (1980) found a similar situation in Danish pigs. The farms positive for *Y. enterocolitica* serotype 0:3 in Denmark were all of the "open management type", where 6-8 weeks-old pigs were purchased from various pig markets. This suggested that the "open management type" may be an important factor in the spread of *Y. enterocolitica* serotype 0:3 within pig herds.

Zheng (1987) in China found a similar situation as regards to the "open management type" of farm. A similar situation could exist in New Zealand with regards to the dissemination of *Y. enterocolitica* serotype 0:3 within pig herds and consideration should be given to this aspect in future epidemiological studies.

A correlation between temperature and the isolation of species of *Yersinia* was noted. In general, yersiniae were isolated throughout the year with a peak in the colder months. 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). The variation in monthly temperatures between May and November (winter and spring) is minimal and this could suggest why *Yersinia* are more frequently isolated during these months.

The individual farm data suggests that some farms are heavily contaminated with yersiniae especially *Y. enterocolitica*. The isolation of the organism throughout the year suggests that variations in temperature did not influence the spread of the organism in a highly contaminated environment. These results would also be consistent with colonisation of the tonsils of pigs and later shedding of yersiniae in the faeces, allowing pig-to-pig transmission via faecal contamination of accommodation, water and feed.

This study has provided some insights into "the tonsillar carriage of *Yersinia* species by pigs in New Zealand". The study cannot give complete information about the true prevalence of species of *Yersinia* in the whole of New Zealand but it can be considered to represent prevalence in the locality from which pigs were taken for slaughter in the North Island of New Zealand. The majority of yersiniae isolated in this study belonged to pathogenic bio-serotypes of *Yersinia*, and pigs can be considered to be reservoirs of pathogenic *Yersinia* in New Zealand. Assessment of virulence-associated characteristics showed that most strains of *Y. enterocolitica* serotype 0:3, 0:5,27 and *Y. pseudotuberculosis* had *in vitro* properties indicating virulence. The ability of a particular species and/or serotype to be maintained in a particular herd and the virtual exclusion of other species over a period of time was demonstrated.

This study was conducted on slaughtered pigs, however, it has been suggested that investigations on slaughtered pigs do not reflect the true ecological pattern in breeding pigs

(Fukushima *et.al.*,1987). Future studies therefore should be conducted in breeding pigs on particular farms in an attempt to gain further insights on the dissemination of species of *Yersinia* in pigs and its their relevance to human infections in New Zealand.

RECOMMENDATIONS

According to Schiemann (1989), new-born piglets are easily colonized and become long-term healthy carriers of *Y. enterocolitica* and *Y. pseudotuberculosis* in the tonsils and intestines. This phenomenon, together with the widespread occurrence of the bacterium at the herd level, as demonstrated in this study, suggests that its control at this stage is hardly possible unless one could define and eliminate the source of infection for the new-born piglets. Pathogenic organisms from the tonsils and intestines are likely to contaminate carcasses and the slaughter house environment. Such carcasses are not detected by routine post-mortem meat inspection procedures. Moreover, there is the risk of cross-contamination from crude meat products to heat-treated products occurring at a later stage, for example in a processing department or in a kitchen (Nesbakken, 1992).

It is therefore recommended that emphasis be placed on the following critical control points during slaughter and dressing:

(i) scalding,
(ii) singeing,
(iii) circumanal incision and removal of the intestines,
(iv) excision of the tongue, pharynx and particularly the tonsils,
(v) post-mortem meat inspection procedures which involve incision of the mandibular lymph nodes,
(vi) deboning of the head (see Plates VI to IX).

Personal conversation with workers at the slaughter house where this study was conducted revealed limited knowledge of the role they are likely to play in the spread of bacterial food-borne infections such as yersiniosis and the danger of *Yersinia* infection as an occupational health risk. It is recommended that these workers be constantly reminded

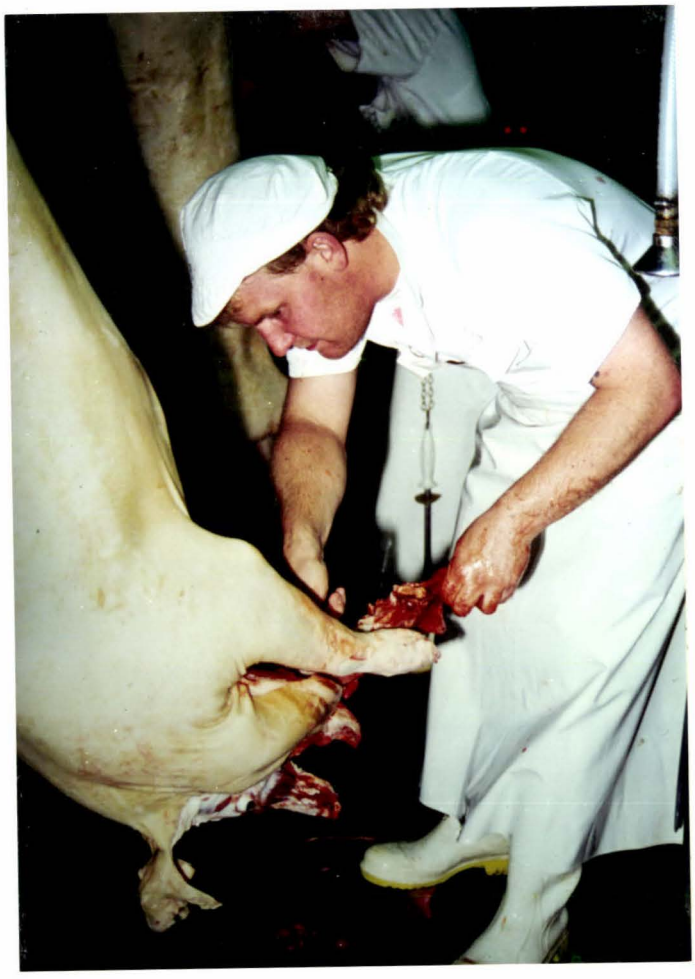
of their hygienic duties during slaughtering and processing with the aim of preventing contamination of carcasses and products, becoming self-infected and avoiding person-to-person transmission of *Yersinia* infections. Information to the public on the consequences of changed dietary habits, and of proper kitchen hygiene, will thus be one of the most important prophylactic measures in the control of yersiniosis.

PLATES VI - IX

Hazard analysis critical control points (HACCP) on the slaughter line.

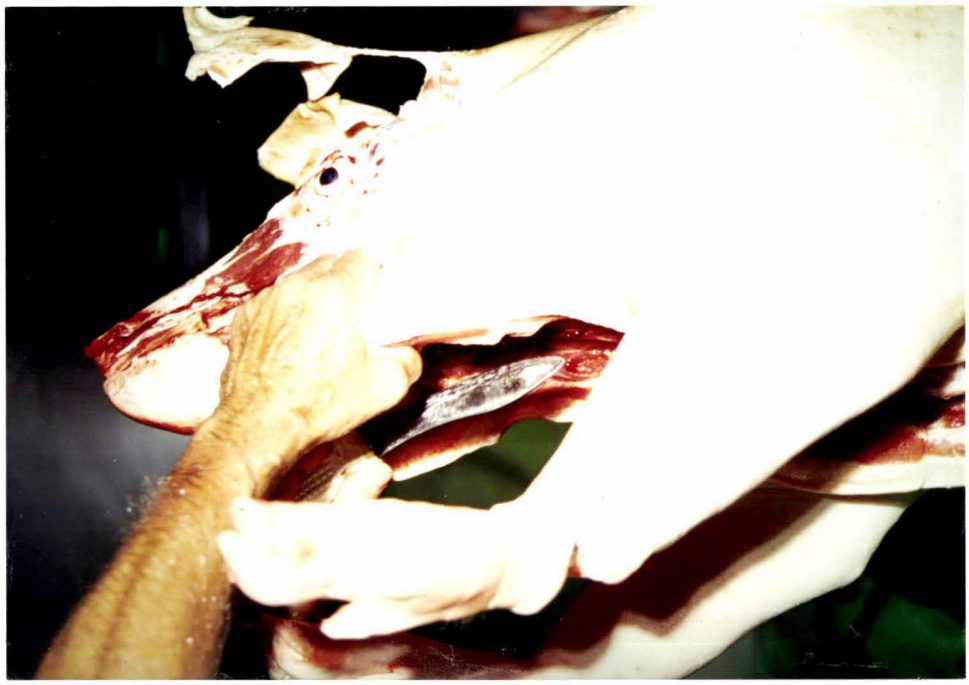
VI. Circumanal incision and removal of the intestines.

VII. Excision of tongue, pharynx and particularly the tonsils.



VIII. Postmortem meat inspection involving incision of the mandibular lymph nodes.

IX. Deboning of head meat.



APPENDICES

APPENDIX I — Preparation of media used for the isolation, identification, virulence tests, and storage of *Yersinia* isolates.

APPENDIX II(A) — Catalogue of strains of *Yersinia* isolated from the cross-sectional study (Chapter Two).

APPENDIX II(B) — Catalogue of strains of *Yersinia* isolated from the longitudinal study (Chapter Three).

APPENDIX III(A) — Reactions of 144 strains of *Y. enterocolitica* tested for virulence-associated characteristics (Chapter Four).

APPENDIX III(B) — Reactions of 36 strains of *Y. pseudotuberculosis* tested for virulence-associated characteristics (Chapter Four).

APPENDIX I

Preparation of media used for the isolation, identification, virulence tests, and storage of *Yersinia* isolates.

Aesculin Hydrolysis

1. To 1 litre of distilled water, add the following materials:

   1 gm Aesculin (DIFCO)
   0.5 gm Ferric Iron Citrate
   25 gm Heart Infusion Broth (DIFCO)
   15 gm Bacto Agar (DIFCO)

2. Dissolve by gentle heating.

3. Mix thoroughly and dispense 3 ml amounts into bijoux bottles.

4. Sterilise by autoclaving for 15 minutes at 121 °C.

5. Cool in a slanting position and store at 4 °C.

Autoagglutination Test

This test was performed in Methyl Red - Voges Proskauer medium (MR-VP) as described by Aleksic *et.al.* (1988) and Farmer *et.al.* (1992).

1. To 1 litre of distilled water, add 17 gm of MR-VP medium (MERCK) and dissolve by shaking.

2. Dispense 3 ml amounts into bijoux bottles.

3. Sterilise by autoclaving for 15 minutes at 121 °C.

4. Cool and store at 4 °C.

Blood Agar

This medium comprises a salt base and a blood base.

Salt Base

1. To 1 litre of distilled water add 15.0 gm Bacto Agar (DIFCO) and 5.0 gm Sodium Chloride (NaCL), and dissolve by heating.

2. Autoclave for 15 minutes at 121 °C.

3. Cool to 45-50 °C.

4. Dispense 10 ml amounts into each petri dish and allow to cool and dry.

Blood Base

1. To 1 litre of distilled water, add 44.0 gm of Columbia Blood Agar Base (DIFCO) and dissolve by heating.

2. Autoclave for 15 minutes at 121 °C.

3. Cool to 45-50 °C.

4. Add 5% (W/V) of sheep blood.

5. Dispense 10-15 ml amounts over the already prepared salt base.

6. Allow to cool and dry, and store at 4 °C.

Carbohydrates

1. To 1 litre of distilled water, add the following materials:

   10 gm peptone (DIFCO)
   3 gm Meat Extract (GIBCO, BRL, SCOTLAND)
   5 gm Sodium Chloride (NaCL)
   10 ml Andrades Solution

2. Dissolve by stirring and adjust pH to 7.2.

3. Dispense into 200 ml bottles.

4. Autoclave for 15 minutes at 121 °C.

5. Add 20 ml (10%) Seitz filtered carbohydrate*

6. Dispense aseptically in 3 ml amounts into bijoux bottles. Store at 4 °C.

* The following were used: Comprises of sucrose, trehalose, rhamnose, melibiose, raffinose, alpha-methyl-D-glucoside, sorbose, sorbitol, cellobiose, maltose, xylose, adonitol, arabinose, salicin and lactose.

Cefsulodin-Irgasan-Novobiocin Agar (CIN)

(Schiemann, 1979)

The preparation of this medium is achieved by the combination of the *Yersinia* selective agar base and the *Yersinia* antimicrobic supplement Cefsulodin Novobiocin (CN).

1. To 1 litre of distilled water, add 59.5 gm of *Yersinia* Selective Agar Base (DIFCO) and dissolve completely by boiling.

2. Sterilise by autoclaving for 15 minutes at 121 °C.

3. Cool to 45-50 °C.

4. Aseptically add 10 ml rehydrated *Yersinia* Antimicrobic Supplement CN (DIFCO USA).

5. Mix thoroughly avoiding the formation of air bubbles, and dispense 15-20 ml amounts into sterile petri dishes.

6. Allow to cool and dry, store at 4 °C.

Congo Red - Magnesium Oxalate Agar (CR-MOX)

(Riley and Toma, 1989)

1. To 825 ml of distilled water, add 40 gm of Tryptic Soy Agar (DIFCO) and dissolve by boiling.

2. Autoclave for 15 minutes at 121 °C.

3. Cool the molten medium to 55 °C, and add the following solutions:

   80 ml of 0.25 M Magnesium chloride (2.38 gm/100 ml)
   80 ml of 0.25 M Sodium oxalate (3.55 gm/100 ml)
   10 ml of 20% D-galactose
   5 ml of 1% Congo red

4. All solutions are sterilised by autoclaving for 15 minutes at 121 °C, except for D-galactose solution which is filter-sterilised.

5. After thorough mixing, dispense 15-20 ml amounts into sterile petri dishes.

6. Allow to cool and dry. Store at 4 °C.

Decarboxylase Test Broth

1. To 1 litre of distilled water add 10.5 gm Moeller's decarboxylase base medium and dissolve by gentle heating.

2. Dispense into 200 ml bottles.

3. To each 200 ml amount, add 2 gm L-Arginine (SIGMA), or 2 gm L-lysine (SIGMA), or 2 gm L-ornithine (SIGMA), whichever is required.

4. Adjust pH to 6.0.

5. Dispense 3 ml amounts into bijoux bottles.

6. Autoclave for 15 minutes at 121 °C. Store at 4 °C.

Glycerol Broth (15%)

1. To 300 ml of distilled water add the following materials:

   2.4 gm Nutrient Broth
   45 ml Glycerol

2. Mix together (don't need to heat).

3. Dispense 3 ml amounts into bijoux bottles.

4. Autoclave for 15 minutes at 121 °C. Store at 4 °C.

Lysine Arginine Iron Agar (LAIA)

(Weagant, 1983)

1. To 1 litre of distilled water, add the following materials:

   34.5 gm Lysine Iron Agar (DIFCO)
   10.0 gm L-Arginine (SIGMA)

2. Dissolve by heating.

3. Dispense in 6 ml amounts into screw-capped polycarbonate tubes.

4. Autoclave for 15 minutes at 121 °C.

5. Cool in a slanting position. Store at 4 °C.

M/15 Phosphate-Buffered Saline pH 7.6 (PBS)

1. Prepare solution A by dissolving 9.07 gm of Potassium Di-hydrogen Phosphate ($KH_2\ PO_4$) in 1 litre of distilled water.

2. Prepare solution B by dissolving 11.87 gm of Disodium Hydrogen Phosphate ($Na_2\ HP0_4\ 2H_2O$) 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 (NaCL).

4. Autoclave for 15 minutes at 121 °C. Store at 4 °C.

Pyrazinamidase Slopes

(Kandolo and Wauters, 1985)

1. Prepare buffer solution by dissolving 2.37 gm of Tris-Maleate in 500 ml of distilled water.

2. Add the following materials:

   15 gm Tryptic Soy Agar (DIFCO)
   1.5 gm Yeast Extract (DIFCO)
   0.5 gm Pyrazinecarboxamide (MERCK)

3. Dissolve by boiling in buffer.

4. Dispense 5 ml amounts into screw-capped polycarbonate tubes.

5. Sterilise by autoclaving for 15 minutes at 121 °C.

6. Cool in a slanting position, and store at 4 °C.

Triple Sugar Iron Agar (TSIA)

1. To 1 litre of distilled water add 65.0 gm TSIA (DIFCO) and dissolve by heating.

2. Dispense 6 ml amounts into screw-capped polycarbonate tubes.

3. Autoclave for 15 minutes at 121 °C.

4. Cool in a slanting position, and store at 4 °C.

Tryptone Water

1. To 1 litre of distilled water, add 20 gm Tryptone (DIFCO) and dissolve by gentle heating.

2. Dispense in 3 ml amounts into bijoux bottles.

3. Autoclave for 15 minutes at 121 °C, and store at 4 °C.

Urea Agar

This medium comprises an agar base and a urea base.

Agar Base

1. To 1 litre of distilled water, add 15.0 gm Bacto Agar (DIFCO), and dissolve by heating.

2. Dispense into 200 ml bottles.

3. Autoclave for 15 minutes at 121 °C.

4. Cool to 45-50 °C.

Urea Base

1. To 100ml distilled water, add 29.0gm Bacto Urea Agar Base (DIFCO) and mix to dissolve.

2. Filter-sterilise and store at 4 °C.

To prepare the medium, add aseptically 20 ml of Bacto Urea Agar Base to 200 ml Agar Base. Mix thoroughly and dispense 3 ml amount into each sterile bijoux bottle. Cool in a slanting position. Store at 4 °C.

APPENDIX II(A)

Catalogue of strains of *Yersinia* isolated from the cross-sectional study (Chapter Two).

No.	Animal Ref.	Species/Biotype	Serotype
1	HF3	*Y. frederiksenii*	
2	HFIII	"	
3	HM14	*Y. pseudotuberculosis*	II
4	HF15	*Y. frederiksenii*	
5	HM16	*Y. intermedia*	
6	HM18	*Y. frederiksenii*	
7	KF1	*Y. pseudotuberculosis*	II
8	KM2	"	III
9	KM3	"	III
10	KF5	"	II
11	KF6	"	II
12	KFB	*Y. frederiksenii*	
13	* KF10	*Y. enterocolitica*	NT
14	* KF10	*Y. pseudotuberculosis*	II
15	KF11	"	III
16	MM1	*Y. frederiksenii*	
17	* MF2	*Y. pseudotuberculosis*	III
18	* MF2	*Y. enterocolitica* 1A	NT
19	MF4	"	0:6,30
20	MM8	"	0:6,30
21	MM10	*Y. frederiksenii*	
22	MM12	*Y. pseudotuberculosis*	III
23	NF1	*Y. enterocolitica* 4	0:3
24	NF3	"	0:3
25	NF4	"	0:3
26	NF5	"	0:3
27	NF6	*Y. enterocolitica* 4	0:3

28	NF7	"	0:3
29	NF11	"	0:3
30	NM12	"	0:3
31	NF13	"	0:3
32	NF14	"	0:3
33	NF15	"	0:3
34	PF2	"	0:3
35	PF7	"	0:3
36	PM8	*Y. pseudotuberculosis*	III
37	RF3	"	III
38	RF5	"	III
39	RF6	"	II
40	RF7	"	II
41	RF8	"	III
42	RF9	"	II
43	RF11	"	II
44	RF12	"	II
45	RM13	"	III
46	RM14	"	III
47	RM15	"	II
48	RM16	"	II
49	RM17	"	III
50	RF18	"	I
51	RF20	"	II
52	TF1	*Y. enterocolitica* 4	0:3
53	TM2	"	0:3
54	TF3	"	0:3
55	TM4	"	0:3
56	TM5	"	0:3
57	TF6	"	0:3
58	TF7	"	0:3
59	TM8	"	0:3
60	TM9	*Y. enterocolitica* 4	0:3
61	TM10	"	0:3
62	TM11	"	0:3

63		TM12	"	0:3
64		TM13	"	0:3
65	*	WF1	*Y. enterocolitica* 2/3^{+}	0:5,27
66	*	WF1	*Y. pseudotuberculosis*	III
67		WM2	*Y. enterocolitica* 3	0:5,27
68		WF3	*Y. enterocolitica* 2/3^{+}	0:5,27
69		WF4	*Y. enterocolitica* 3	0:5,27
70		WM5	*Y. enterocolitica* 2	0:5,27
71		WM6	*Y. enterocolitica* 3	0:5,27
72		WM7	"	0:5,27
73		WF8	"	0:5,27
74		WF9	"	0:5,27
75		WF10	*Y. enterocolitica* 2/3^{+}	0:5,27
76		WM11	"	0:5,27
77		WF13	*Y. enterocolitica* 3	0:5,27

* Indicates strains isolated from the same animal.

NT Non-typable

$^{+}$ Biotype not possible to determine, both are pathogenic.

APPENDIX II(B)

Catalogue of strains of *Yersinia* isolated from the longitudinal study (Chapter Three).

No.		Animal Ref.	Species/biotype	Serotype
1		HM4-6	*Y. kristensenii*	
2		HM5-12	*Y. intermedia*	
3		HM5-14	*Y. frederiksenii*	
4		HM6-11	*Y. enterocolitica* 1A	NT
5		HM12-15	*Y. pseudotuberculosis*	III
6		KF2-11	*Y. intermedia*	
7		KM3-5	*Y. pseudotuberculosis*	III
8		KF3-6	"	III
9		KF4-3	"	III
10		KF4-4	"	III
11		KF4-5	"	III
12		KM4-8	"	III
13		KF4-9	"	III
14		KF4-11	"	III
15		KM5-1	"	III
16		KM5-2	*Y. enterocolitica* 3	0:5,27
17		KM5-10	*Y. pseudotuberculosis*	III
18		KM5-11	"	III
19		KM5-15	"	III
20		KM6-1	"	II
21		KM6-2	*Y. enterocolitica* 4	0:3
22	*	KF6-6	" 4	0:3
23	*	KF6-6	*Y. pseudotuberculosis*	III
24		KF6-11	"	III
25		KM6-12	*Y. enterocolitica* 4	0:3
26		KF6-13	*Y. pseudotuberculosis*	II
27		KF6-15	*Y. pseudotuberculosis*	II
28		KM7-1	*Y. frederiksenii*	

29		KF7-3	*Y. enterocolitica* IA	0:8
30		KM7-5	*Y. pseudotuberculosis*	~~III~~ I
31	*	KF7-6	"	III
32	*	KF7-6	"	II
33	*	KM7-7	"	I
34	*	KM7-7	"	III
35		KF7-12	"	I
36		KM7-13	"	II
37		KM7-14	"	I
38	*	KM8-1	"	II
39	*	KM8-1	"	I
40		KF8-2	"	III
41		KM8-5	"	II
42		KM8-6	"	III
43		KM8-10	"	II
44		KF8-12	"	III
45		KF8-13	*Y. enterocolitica* 1A	0:8
46		KM8-15	*Y. pseudotuberculosis*	III
47		KF9-3	*Y. enterocolitica* 3	0:5,27
48		KF9-5	*Y. frederiksenii*	
49		KM9-10	*Y. pseudotuberculosis*	III
50		KM9-12	*Y. enterocolitica* 1A	0:5
51		KF9-15	*Y. pseudotuberculosis*	III
52		KM10-1	*Y. pseudotuberculosis*	I
53		KM10-2	"	I
54		KM10-5	"	III
55		KF10-9	"	III
56		KF10-15	"	II
57		KF11-3	"	II
58		KM11-4	"	III
59		KM12-7	"	III
60		KF12-10	*Y. pseudotuberculosis*	III
61		KM12-15	"	III
62		KF1-1	"	III
63		TF2-5	*Y. intermedia*	

64	TF2-7	*Y. enterocolitica* 4	0:3
65	TM2-8	*Y. intermedia*	
66	TM2-13	"	
67	TF3-6	*Y. enterocolitica* 4	0:3
68	TF3-8	"	0:3
69	TM3-10	"	0:3
70	TF3-11	"	0:3
71	TF3-12	"	0:3
72	TF3-13	"	0:3
73	TM3-14	"	0:3
74	TF3-15	"	0:3
75	TF4-1	"	0:3
76	TF4-3	"	0:3
77	TF4-4	"	0:3
78	TF4-5	"	0:3
79	TF4-6	"	0:3
80	TF4-11	"	0:3
81	TM4-15	"	0:3
82	TM5-3	"	0:3
83	TF5-4	"	0:3
84	TF5-5	"	0:3
85	TF5-10	"	0:3
86	TM5-14	"	0:3
87	TF6-1	"	0:3
88	TM6-2	"	0:3
89	TM6-3	"	0:3
90	TF6-4	"	0:3
91	TF6-6	"	0:3
92	TF6-7	"	0:3
93	TM6-9	*Y. enterocolitica* 4	0:3
94	TF6-14	"	0:3
95	TM6-15	"	0:3
96	TM7-1	*Y. enterocolitica* 1A	NT
97	TM7-3	*Y. enterocolitica* 4	0:3
98	TM7-5	"	0:3

99		TM7-6	"	0:3
100		TM7-7	"	0:3
101		TF7-8	"	0:3
102		TF7-9	"	0:3
103	*	TF7-10	"	0:3
104	*	TF7-10	*Y. enterocolitica* 1A	NT
105		TM7-11	*Y. enterocolitica* 4	0:3
106		TM7-13	"	0:3
107		TM7-15	"	0:3
108		TF8-2	"	0:3
109		TF8-6	"	0:3
110	*	TF8-8	"	0:3
111	*	TF8-8	*Y. enterocolitica* 2	0:5,27
112	*	TF8-8	*Y. pseudotuberculosis*	III
113		TF8-9	*Y. enterocolitica* 4	0:3
114		TM8-10	"	0:3
115		TF8-14	"	0:3
116		TF8-15	*Y. enterocolitica* 1A	0:5
117		TF9-2	*Y. enterocolitica* 4	0:3
118		TF9-3	"	0:3
119		TF9-4	"	0:3
120		TF9-5	"	0:3
121		TF9-6	"	0:3
122		TF9-7	"	0:3
123	*	TM9-8	*Y. enterocolitica* 1A	0:3
124	*	TM9-8	*Y. enterocolitica* 4	0:3
125		TM9-11	"	0:3
126		TM10-1	*Y. enterocolitica* 4	0:3
127	*	TF10-2	"	0:3
128	*	TF10-2	*Y. pseudotuberculosis*	III
129		TM10-3	"	III
130		TM10-4	"	III
131	*	TF10-5	*Y. enterocolitica* 3	0:5,27
132	*	TF10-5	*Y. pseudotuberculosis*	III
133		TM10-6	*Y. enterocolitica* 4	0:3

134		TF10-10	*Y. pseudotuberculosis*	III
135		TF10-13	*Y. enterocolitica* 1A	0:6
136		TM10-14	*Y. enterocolitica* 4	0:3
137		TM10-15	*Y. enterocolitica* 1A	0:5
138		TF11-4	*Y. enterocolitica* 4	0:3
139		TF11-8	"	0:3
140		TF11-13	"	0:3
141	*	TM12-1	*Y. pseudotuberculosis*	III
142	*	TM12-1	*Y. enterocolitica* 4	0:3
143		TM12-2	"	0:3
144		TM12-3	"	0:3
145		TF12-5	*Y. enterocolitica* 3	0:5,27
146		TF12-6	*Y. enterocolitica* 4	0:3
147		TF12-7	"	0:3
148		TF12-8	"	0:3
149		TM12-11	"	0:3
150		TF12-13	"	0:3
151		TF12-14	"	0:3
152	*	TF12-15	*Y. enterocolitica* 1A	0:6
153	*	TF12-15	*Y. pseudotuberculosis*	III
154		WM2-7	*Y. enterocolitica* 3	0:5,27
155		WM2-10	"	0:5,27
156		WF2-12	"	0:5,27
157		WM2-13	"	0:5,27
158		WM3-1	"	0:5,27
159		WF3-2	*Y. enterocolitica* 3	0:5,27
160		WM3-3	"	0:5,27
161		WM3-4	"	0:5,27
162		WF3-7	"	0:5,27
163		WM3-8	"	0:5,27
164		WF3-11	"	0:5,27
165		WF3-14	"	0:5,27
166		WF3-15	"	0:5,27
167		WF4-1	"	0:5,27
168		WF4-2	"	0:5,27

169		WF4-3	"	0:5,27
170		WM4-6	"	0:5,27
171		WF4-7	"	0:5,27
172		WM4-8	"	0:5,27
173		WM4-9	"	0:5,27
174		WM4-11	"	0:5,27
175		WF4-12	"	0:5,27
176		WF4-13	"	0:5,27
177		WF4-14	"	0:5,27
178		WF4-15	"	0:5,27
179		WM5-1	"	0:5,27
180		WM5-2	"	0:5,27
181		WF5-3	"	0:5,27
182		WF5-7	"	0:5,27
183		WF5-8	"	0:5,27
184		WF5-9	"	0:5,27
185		WF5-10	*Y. frederiksenii*	
186		WF5-11	*Y. enterocolitica* 3	0:5,27
187		WF5-12	"	0:5,27
188		WF5-13	*Y. frederiksenii*	
189		WF5-14	*Y. enterocolitica* 3	0:5,27
190		WF5-15	"	0:5,27
191	*	WM6-1	*Y. enterocolitica* 1A	0:5
192	*	WM6-1	*Y. enterocolitica* 3	0:5,27
193		WM6-2	"	0:5,27
194		WM6-3	"	0:5,27
195		WM6-5	"	0:5,27
196		WF6-6	"	0:5,27
197		WF6-7	"	0:5,27
198		WF6-8	"	0:5,27
199		WF6-9	"	0:5,27
200		WM6-10	"	0:5,27
201		WF6-12	"	0:5,27
202		WM6-13	"	0:5,27
203		WM6-14	"	0:5,27

204		WF7-1	"	0:5,27
205		WF7-2	"	0:5,27
206		WF7-4	"	0:5,27
207		WF7-14	"	0:5,27
208		WF7-5	"	0:5,27
209		WM7-6	"	0:5,27
210		WM7-10	"	0:5,27
211		WF7-12	"	0:5,27
212		WF8-1	"	0:5,27
213		WM8-2	"	0:5,27
214		WM8-3	"	0:5,27
215		WM8-4	"	0:5,27
216		WM8-5	"	0:5,27
217		WM8-6	"	0:5,27
218		WM8-7	"	0:5,27
219	*	WM8-8	"	0:5,27
220	*	WM8-8	*Y. enterocolitica* 4	0:3
221		WM8-9	*Y. enterocolitica* 3	0:5,27
222		WM8-10	"	0:5,27
223		WF8-11	"	0:5,27
224		WM8-13	"	0:5,27
225		WF8-15	*Y. enterocolitica* 3	0:5,27
226		WM9-1	"	0:5,27
227		WM9-2	*Y. enterocolitica* 1A	0:5
228		WM9-4	"	0:5
229		WM9-7	*Y. enterocolitica* 3	0:5,27
230		WM9-8	"	0:5,27
231		WF9-10	"	0:5,27
232		WF9-12	"	0:5,27
233		WM9-13	"	0:5,27
234		WF9-14	*Y. enterocolitica* 1A	0:5
235		WM9-15	*Y. enterocolitica* 3	0:5,27
236		WM10-1	"	0:5,27
237		WF10-2	"	0:5,27
238		WF10-3	"	0:5,27

239	WF10-4	"	0:5,27
240	WF10-5	"	0:5,27
241	WM10-6	"	0:5,27
242	WM10-7	"	0:5,27
243	WF10-8	"	0:5,27
244	WM10-9	"	0:5,27
245	WM10-10	"	0:5,27
246	WF10-11	"	0:5,27
247	WM10-12	"	0:5,27
248	WF10-13	"	0:5,27
249	WF10-14	"	0:5,27
250	WM10-15	"	0:5,27
251	WM12-2	"	0:5,27
252	WF12-3	*Y. enterocolitica* 4	0:3
253	WF12-4	*Y. enterocolitica* 3	0:5,27
254	WF12-5	"	0:5,27
255	WF12-11	"	0:5,27
256	WF12-12	"	0:5,27
257	WM12-13	*Y. enterocolitica* 4	0:3
258	WM12-14	*Y. enterocolitica* 3	0:5,27
259	WM1-2	"	0:5,27
260	WF1-3	"	0:5,27
261	WF1-6	*Y. enterocolitica* 4	0:3
262	WF1-7	*Y. enterocolitica* 3	0:5,27
263	WF1-10	*Y. enterocolitica* 4	0:3
264	WF1-12	*Y. enterocolitica* 3	0:5,27

* Indicates strains isolated from the same animal.

NT Non-typable.

APPENDIX III (A)

Reactions of 114 strains of *Y. enterocolitica* tested for virulence-associated characteristics (Chapter Four).

NO.	STRAIN REF.	BIO-/SEROTYPE	AA[1]	CRMOX[2]	PYZ[3]	AES/SAL[4]
1	HM6-11	1A/0:6	-	-	+	+
2	KM6-2	4/0:3	+	+	-	-
3	KM6-12	4/0:3	+	-	-	-
4	KF7-3	1A/0:8	-	-	+	+
5	KF8-13	1A/0:8	-	-	+	+
6	KF9-3	3/0:5,27	+	+	-	-
7	KM9-12	1A/0:5	-	-	+	+
8	TF6-1	4/0:3	+	-	-	-
9	TM6-2	4/0:3	+	+	-	-
10	TM6-3	4/0:3	+	+	-	-
11	TF6-4	4/0:3	+	+	-	-
12	TF6-6	4/0:3	+	+	-	-
13	TF6-7	4/0:3	+	+	-	-
14	TM6-9	4/0:3	+	+	-	-
15	TF6-14	4/0:3	+	+	-	-
16	TM6-14	4/0:3	+	+	-	-
16	TM6-15	4/0:3	+	+	-	-
17	TM7-1	1A/NT	-	-	+	+
18	TM7-3	4/0:3	+	+	-	-
19	TM7-5	4/0:3	+	+	-	-
20	TM7-6	4/0:3	+	+	-	-
21	TM7-7	4/0:3	+	+	-	-
22	TM7-8	4/0:3	+	+	-	-
23	TF7-9	4/0:3	+	+	-	-
24	TF7-10	4/0:3	+	+	-	-
25	TF7-10	1A/NT	-	-	+	+

26	TM7-11	4/0:3	+	-	-	-
27	TM7-13	4/0:3	+	+	-	-
28	TM7-15	4/0:3	+	+	-	-
29	TF8-2	4/0:3	+	+	-	-
30	TF8-6	4/0:3	+	+	-	-
31	TF8-8	4/0:3	+	+	-	-
32	TF8-9	4/0:3	+	+	-	-
33	TM8-10	4/0:3	+	-	-	-
34	TF8-14	4/0:3	+	+	-	-
35	TF8-15	1A/0:5	-	-	+	+
36	TF9-2	4/0:3	+	+	-	-
37	TF9-3	4/0:3	+	+	-	-
38	TF9-4	4/0:3	+	+	-	-
39	TF9-5	4/0:3	+	+	-	-
40	TF9-6	4/0:3	+	+	-	-
41	TM9-7	4/0:3	+	+	-	-
42	TM9-8	1A/0:5	-	-	+	+
43	TM9-8	4/0:3	+	+	-	-
44	TM9-11	4/0:3	+	+	-	-
45	TM10-1	4/0:3	+	+	-	-
46	TF10-2	4/0:3	+	+	-	-
47	TF10-5	3/0:5,27	+	+	-	-
48	TM10-6	4/0:3	+	+	-	-
49	TF10-13	1A/0:6	-	-	+	+
50	TF10-14	4/0:3	+	+	-	-
51	TM10-15	1A/0:5	-	-	+	+
52	TF11-4	4/0:3	+	+	-	-
53	TF11-8	4/0:3	+	+	-	-
54	TF11-13	4/0:3	+	+	-	-
55	WM6-1	1A/0:5	-	-	+	+
56	WM6-1	3/0:5,27	+	+	-	-
57	WM6-2	3/0:5,27	+	+	-	-
58	WM6-3	3/0:5,27	+	+	-	-
59	WM6-5	3/0:5,27	+	+	-	-
60	WF6-6	3/0:5,27	+	+	-	-

61	WF6-7	3/0:5,27	+	+	-	-
62	WF6-9	3/0:5,27	+	+	-	-
63	WM6-10	3/0:4,27	+	+	-	-
64	WF6-12	3/0:5,27	+	+	-	-
65	WM6-13	3/0:5,27	+	+	-	-
66	WM6-14	3/0:5,27	+	+	-	-
67	WF6-8	3/0:5,27	+	+	-	-
68	WF7-1	3/0:5,27	+	+	-	-
69	WF7-2	3/0:5,27	+	+	-	-
70	WF7-3	3/0:5,27	+	+	-	-
71	WF7-4	3/0:5,27	+	+	-	-
72	WF7-5	3/0:5,27	+	+	-	-
73	WM7-6	3/0:5,27	+	+	-	-
74	WM7-10	3/0:5,27	+	+	-	-
75	WF7-12	3/0:5,27	+	+	-	-
76	WF8-1	3/0:5,27	+	+	-	-
77	WM8-2	3/0:5,27	+	+	-	-
78	WM8-3	3/0:5,27	+	+	-	-
79	WF8-4	3/0:5,27	+	+	-	-
80	WM8-5	3/0:5,27	+	+	-	-
81	WM8-6	3/0:5,27	+	+	-	-
82	WM8-7	3/0:5,27	+	+	-	-
83	WM8-8	3/0:5,27	+	+	-	-
84	WM8-8	4/0:3	+	+	-	-
85	WM8-9	3/0:5,27	+	+	-	-
86	WM8-10	3/0:5,27	+	+	-	-
87	WF8-11	3/0:5,27	+	+	-	-
88	WM8-13	3/0:5,27	+	+	-	-
89	WF8-15	3/0:5,27	+	+	-	-
90	WM9-1	3/0:5,27	+	+	-	-
91	WM9-2	1A/0:5	-	-	+	+
92	WM9-4	1A/0:5	-	-	+	+
93	WF9-7	3/0:5,27	+	+	-	-
94	WF9-8	3/0:5,27	+	+	-	-
95	WF9-10	3/0:5,27	+	+	-	-

96	WF9-12	3/0:5,27	+	+	-	-
97	WM9-13	3/0:5,27	+	+	-	-
98	WF9-14	1A/0:5	-	-	+	+
99	WM9-15	3/0:5,27	+	+	-	-
100	WM10-1	3/0:5,27	+	+	-	-
101	WF10-2	3/0:5,27	+	+	-	-
102	WF10-3	3/0:5,27	+	+	-	-
103	WF10-4	3/0:5,27	+	+	-	-
104	WM10-5	3/0:5,27	+	+	-	-
105	WM10-6	3/0:5,27	+	+	-	-
106	WM10-7	3/0:5,27	+	+	-	-
107	WF10-8	3/0:5,27	+	+	-	-
108	WM10-9	3/0:5,27	+	+	-	-
109	WM10-10	3/0:5,27	+	+	-	-
110	WF10-11	3/0:5,27	+	+	-	-
111	WM10-12	3/0:5,27	+	+	-	-
112	WF10-13	3/0:4,27	+	+	-	-
113	WF10-14	3/0:5,27	+	+	-	-
114	WM10-15	3/0:5,27	+	+	-	-

1 - Autoagglutination Test

2 - Congo Red - Magnesium Oxalate Agar

3 - Pyrazinamidase Test

4 - Aesculin/salicin

APPENDIX III (B)

Reactions of 36 strains of *Y. pseudotuberculosis* tested for virulence-associated characteristics (Chapter Four).

NO.	STRAIN REF.	SEROTYPE	AA[1]	CRMOX[2]	PYZ[3]
1	KM6-1	II	+	+	-
2	KF6-6	III	+	+	-
3	KF6-11	III	+	+	-
4	KM6-13	II	+	+	-
5	KF6-15	II	+	+	-
6	KM7-5	III	+	+	-
7	KF7-6	III	+	+	-
8	KF7-6	II	+	+	-
9	KM7-7	I	+	+	-
10	KM7-7	III	+	+	-
11	KF7-12	I	+	+	-
12	KM7-13	II	+	+	-
13	KM7-14	I	+	+	-
14	KM8-1	II	+	+	-
15	KM8-1	I	+	+	-
16	KF8-2	III	+	+	-
17	KM8-5	II	+	+	-
18	KM8-6	III	+	+	-
19	KM8-10	II	+	+	-
20	KF8-12	III	+	+	-
21	KM8-15	III	+	+	-
22	KM9-10	III	+	+	-
23	KF9-15	III	+	+	-
24	KM10-1	I	+	+	-
25	KM10-2	I	+	+	-
26	KF10-5	III	+	+	-
27	KF10-9	III	+	+	-

28	KF10-15	II	+	+	-
29	KF11-3	II	+	+	+
30	KM11-4	III	+	+	+
31	TF8-8	III	+	+	-
32	TF10-2	III	+	+	-
33	TM10-3	III	+	+	-
34	TM10-4	III	+	+	-
35	TF10-5	III	+	+	-
36	TF10-10	III	+	-	-

1 - Autoagglutination Test

2 - Congo Red - Magnesium Oxalate Agar

3 - Pyrazinamidase Test

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