THE TONSILLAR CARRIAGE OF *YERSINIA* SPECIES BY PIGS

A THESIS PRESENTED IN PARTIAL FULFILMENT (40%)
OF THE REQUIREMENTS FOR THE DEGREE OF MASTER
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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.