# ASSESSMENT OF THE SENSITIVITY OF CURRENT STANDARD PROCEDURES FOR THE ISOLATION OF *YERSINIA ENTEROCOLITICA* FROM PORK MINCE

A DISSERTATION PRESENTED IN PARTIAL FULFILMENT (25%) OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF VETERINARY STUDIES IN VETERINARY PUBLIC HEALTH AT MASSEY UNIVERSITY

DURAI SUBRAMANIAM

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# ABSTRACT

*Y. enterocolitica* and related species have been isolated from many types of food. The majority of isolates differ in biochemical and serological characteristics from typical pathogenic strains and are termed non-pathogenic or environmental strains. Usually the number of *Y. enterocolitica* organisms present in food products is low compared with the dominant background flora. The ability of current enrichment procedures to recover pathogenic strains of *Y. enterocolitica* from different foods is often inadequate probably because different strains require different conditions for optimum growth (De Boer 1992). An efficient enrichment procedure should confer some selective advantage to the desired type of microorganism by promoting its growth relative to the competing microflora. At present, there is no single ideal isolation procedure available for the recovery of pathogenic strains of *Y. enterocolitica* from foods.

The aim of this study was to determine the recovery rate of *Y. enterocolitica* biotype 4/serotype O:3 from samples of pork mince inoculated with known numbers of the microorganism using different enrichment parameters (Time, temperature and pH) and Cefsulodin-Irgasan-Novobiocin (CIN) agar as the selective medium. The experiment was conducted in two trials using different bacterial dilutions. Three pork mince samples in duplicate were inoculated with known quantities of *Y. enterocolitica* biotype 4/serotype O:3 organisms and subjected to cold enrichment in phosphate buffered saline (PBS) with a pH of 7.6, 6.6 and 5.5 at 25°C for 2 days, 10°C for 7 days and 4°C for 21 days. CIN agar was used as the selective medium. Pre-inoculation control samples were selected and plated in CIN on day 0 and on day 21 after PBS enrichment at 4°C.

In Trial one *Y. enterocolitica* organisms were recovered from all 3 samples incubated at 25°C for 2 days and from 1 out of 3 inoculated samples incubated at 4°C for 21 days. There were no organisms recovered from other inoculated samples. The control sample did not show any environmental contamination with *Yersinia* species. In Trial two, *Y. enterocolitica* was recovered from 1 out of 3 duplicate samples enriched in PBS with pH 6.6 and incubated at 25°C for two days. *Y. enterocolitica* was not recovered from other inoculated samples. *Y. intermedia* was isolated from all pH, temperature and time combinations and also from control samples.

The following conclusions can be drawn from this experiment. Incubation at high temperature (25°C) and short duration (48 hours) can be used as an efficient method for isolating *Y. enterocolitica* from pork samples. The standard incubation period of 21 days required for cold enrichment at 4°C is too long for the isolation of pathogenic strains, because of possible growth of environmental microorganisms. A pH of 6.6 is less efficient than 7.6 for enrichment although occasional isolation can be made using this pH. Enrichment in PBS with a pH of 5.5 with any time as well as temperature combinations and incubation at 10°C for 7 days are not ideal for isolation of pathogenic *Yersinia enterocolitica* strains. Of the three enrichments (PBS 7.6, 6.6, 5.5) used in this experiment, PBS with pH 7.6 was found to be most efficient to others.

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# LIST OF TABLES