Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

,

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

٠...

i

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 JULY 1999

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.

•....

ACKNOWLEDGEMENTS

I am thankful to Professor Colin R. Wilks for giving me the opportunity to study for a Masters degree in Veterinary Public Health at the Faculty of Veterinary Science in Massey University.

I am very grateful to my supervisor Mr Per Madie for his constant encouragement and endless enthusiasm in guiding me throughout these years to reach my goal.

I am also thankful to Dr Stan Fenwick for his timely advice and help during experiments and thesis writing.

In addition I would like to acknowledge with respect the following: Mr. Peter Wildbore, Ms. Jan Schrama, Ms. Magda Gwodz, Ms. Kylie Walker, Ms. Pam Slack, Ms Pat Davey and Mr Faris Sharpe for their for their help during my work.

The other members of my masters programme Alice Alma Bungay and Kyung Ho Shin for their help especially during the experiments.

I would like to thank staff members of the former Department of Pathology and Public health: Professors Bill Mantkelow and Bob Jolly, Associate professors Tony Charleston, Maurice Alley and Keith Thomson, Drs Mark Collet, John Lumsden, Bill Pomroy, Jane Hunter, Joanne Meers and Mr Neil Christenson for teaching me the techniques in Diagnostic pathology, Public health and Meat hygiene.

Mrs Allain Scott, Mrs Sheryll Crawford, Mrs Wendy Graham and Ms. Nicola Purdue-Smith are gratefully recognised for their administrative assistance.

I am also thankful Dr N.S. Bolan, and Mr M.J Birttles for their help and encouragement during my study.

I wish to say my special thanks to my wife Chitra for her love, patience and encouragement and also to my two sons Arjun and Adithya and to my parents and in-laws, brother and sister and in-laws and friends in India for their support for my study and wellbeing. Finally, thanks to Mohan my brother-in-law, without his help I would not have achieved my trip to New Zealand.

CHAPTER I	1
REVIEW OF LITERATURE	1
SECTION-A	1
THE GENUS YERSINIA	1
INTRODUCTION	1
YERSINIA PSEUDOTUBERCULOSIS	2
YERSINIA ENTEROCOLITICA	3
GENERAL CHARACTERISTICS OF THE YERSINIA SPECIES	4
THE BACTERIUM	4
GROWTH REQUIREMENTS	5
ISOLATION	7
IDENTIFICATION	9
BIOTYPES AND SEROTYPES	10
EPIDEMIOLOGY	13
ENVIRONMENT	13
HUMANS	13
ANIMALS	14
FOOD	15
SEASONAL PREVALENCE	16

vii

TRANSMISSION	16
YERSINIOSIS IN HUMANS	17
YERSINIA PSEUDOTUBERCULOSIS	17
YERSINIA ENTEROCOLITICA	
TERSINIA ENTEROCOLITICA	18
YERSINIOSIS IN ANIMALS	20
YERSINIA PSEUDOTUBERCULOSIS	20
YERSINIA ENTEROCOLITICA	20
YERSINIOSIS IN NEW ZEALAND	21
HUMAN INFECTION	21
ANIMAL INFECTION	22
DIAGNOSIS	23
	43
TREATMENT	25
PREVENTION AND CONTROL	25
	25
SECTION-B	28
YERSINIOSIS AND FOOD HYGIENE	28
TEROINIODIO AND FOOD IITOIMAE	20
INTRODUCTION	28
MEAT	28
PORK AND PORK PRODUCTS	28 28
BEEF AND CHICKEN	30

viii

SEAFOODS	30
OTHER FOODS	30
MILK AND MILK PRODUCTS	30
WIER AND WIER PRODUCTS	50
VEGETABLES	31
FOODBORNE OUTBREAKS	31
PUBLIC HEALTH SIGNIFICANCE OF FOODBORNE YERSINA	32
ISOLATION AND IDENTIFICATION OF YERSINIA SPECIES	
FROM FOOD	33
ENRICHMENT	34
TEMPERATURE AND TIME	35
EFFECT OF pH	36
	36
PLATING MEDIA	37
IDENTIFICATION	39
COMPARISON OF DIFFERENT ISOLATION TECHNIQUES	
YERSINIA SPECIES FROM FOOD SAMPLES	40
IDENTIFICATION OF PATHOGENIC YERSINIA SPECIES FROM	
FOOD SAMPLES	42
YERSINIA OUTER MEMBRANE PROTEINS (YOPS)	42
IN VITRO TESTS	43
IN VIVO TESTS	45

~

CHAPTER II	46
EXPERIMENT	46
SECTION-A	46
MATERIALS AND METHODS	46
INTRODUCTION	46
INOCULATION AND SUBSEQUENT RECOVERY OF Y. ENTEROCOLITICA FROM PORK PRODUCTS	47
OUTLINE OF METHODS USED	48
PREPARATION OF BACTERIAL CULTURE	48
PROCESSING THE SAMPLES FOR ENRICHMENT	50
TRIAL ONE	50
TRIAL TWO	51
PLATING THE SAMPLES	52
PRESUMPTIVE SCREENING FOR YERSINIA SPECIES	53
BIOTYPING AND SEROTYPING OF YERSINIA ISOLATES	53
SECTION B	54
RESULTS	54
SUMMARY OF RESULTS	64
DISCUSSION	65
CONCLUSIONS	67
APPENDIX	69
REFERENCES	76

х

LIST OF TABLES

Table	Contents	Page
1	Biochemical differentiation of Y. enterocolitica	11
2	Biochemical differentiation of species in the genus Yersinia	12
3	Flow diagram used for isolation of Yersinia species	49
4	Classification of samples in trial one	51
5	Classification of samples in trial two	52
6	Trial one, results of the pH 7.6 group	57
7	Trial one, results of the pH 6.6 group	58
8	Trial one, results of the pH 5.5 group	59
9	Trial two, results of the pH 7.6 group	60
10	Trial two results of the pH 6.6 group	61
11	Trial two results of the pH 5.5 group	62
12	Control samples for trial one and trial two	63