

**OCCURRENCE AND SURVIVABILITY
OF YERSINIA IN PORK**

By

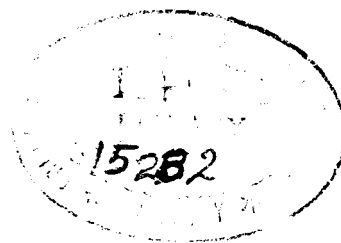
B. SUNIL

THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University



Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy, Thrissur

1994

DECLARATION

I hereby declare that the thesis entitled **"Occurrence and Survivability of Yersinia in Pork"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or any other similar title, of any other University or Society.

Mannuthy,

S .8.1994.



B. SUNIL

CERTIFICATE

Certified that the thesis entitled "Occurrence and Survivability of Yersinia in Pork" is a record of research work done independently by Mr. B. Sunil, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.



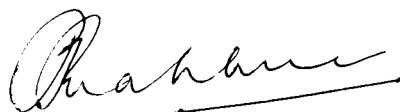
Dr. P. Prabhakaran
Chairman, Advisory Committee
Professor
Department of Veterinary
Public Health,
College of Veterinary &
Animal Sciences,
Mannuthy, Thrissur

Mannuthy,

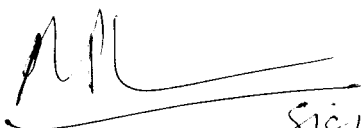
8 .8.1994.

CERTIFICATE

We, the undersigned members of the Advisory Committee of Mr. B. Sunil, a candidate for the degree of Master of Veterinary Science in Veterinary Public Health, agree that the thesis entitled "Occurrence and Survivability of Yersinia in Pork" may be submitted by Mr. B. Sunil, in partial fulfilment of the requirement for the degree.



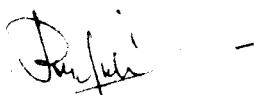
Dr. P. Prabhakaran
Chairman, Advisory Committee
Professor
Department of Veterinary Public Health,
College of Veterinary & Animal Sciences,
Mannuthy



Dr. R. Padmanabha Iyer
Professor and Head
Department of Veterinary
Public Health
(Member)



Dr. P. Kuttinarayanan
Associate Professor
Meat Technology Unit
(Member)



Dr. R. Madhusoodanan Pillai
Associate Professor
Department of Microbiology
(Member)

Metty 21/11/94
External Examiner

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to

Dr. P. Prabhakaran, my major adviser, for his scholastic advice, ardent support and constructive discussions. I am deeply indebted.

Dr. R. Padmanabha Iyer, Professor and Head, Department of Veterinary Public Health, Dr. P. Kuttinarayanan, Associate Professor, Meat Technology Unit and Dr. R. Madhusoodanan Pillai, Associate Professor, Department of Microbiology, members of the advisory committee for their erudite guidance and critical assessment of the work.

Dr. M. Soman, Professor and Dr. E. Nanu, Professor, Department of Veterinary Public Health for their propitious suggestions.

Dr. J. Abraham, Officer-in-charge, Meat Technology Unit, for providing the facilities for the work and unflagging encouragement during the study.

Dean, College of Veterinary and Animal Sciences for providing the facilities for study and research.

Kerala Agricultural University, for providing the financial assistance in the form of Junior fellowship.

Dr. M. Sunil, Dr. K. Rajeev, Dr. C. Latha, Dr. Abdul Kader Kunhy and Dr. Sheela for their keen interest and unstinted help during every phase of the work.

My parents, uncle and brother for their stable support and providing me with the much needed pep to complete this work.

B. SUNIL

To my loving parents

CONTENTS

Chapter No.	Title	Page No.
1.	INTRODUCTION	1
2.	REVIEW OF LITERATURE	4
3.	MATERIALS AND METHODS	28
4.	RESULTS	36
5.	DISCUSSION	44
6.	SUMMARY	49
	REFERENCES	51
	APPENDICES	
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1.	Result of biosamples screened for Yersinia in pigs	38
2.	Primary screening tests of the isolates	39
3.	Presumptive identification tests of the isolates	40
4.	Confirmatory tests of the isolates	41
5.	Biochemical reactions of the standard strains of <u>Yersinia enterocolitica</u> and <u>Yersinia pseudotuberculosis</u> and the isolates	42

Introduction

INTRODUCTION

Food of animal origin, especially meat, forms a major source of food borne diseases. *Yersinia* has attracted attention as an organism causing food borne infections (Gravani, 1986). The presence of *Yersinia* in pigs suggests that it may also be present in meat, especially pork and pork products, and could thus form a major source of infection to human beings.

The genus *Yersinia*, named in honour of French bacteriologist A.J.E. Yersin, is now included as a member of the family Enterobacteriaceae. There has been a steady increase in the recorded incidence of infection and the number of countries which report *Yersinia* infections. The increase could probably be due to the greater awareness about this fleeting organism, or because of improved enrichment and isolation techniques with the advent of newer and more selective media. Data from available literature clearly points to an actual increase in the frequency of infection, causing alarm and anxiety in public health circles.

The organism has been isolated from a wide range of animals including pig, cattle, sheep, cat, dog and hare in European countries and also from monkey, deer and cockatoo in

Japan. In India, *Yersinia* has been isolated from goats (Sulochana and Sudharma, 1985) and pig, man and cat (Pramanik, et al., 1980). Among the meat animals, pigs are considered by many investigators as a potential source of clinically significant types of *Yersinia enterocolitica*. There are reports of isolation of human serotypes from caecal contents, tonsils, mesenteric lymph nodes and throat swabs of pigs. Thus pigs may be a potential reservoir of human infection.

Although the mode of transmission is not fully understood, contact with infected animals, person to person transmission (Sonnenwirth and Weaver, 1970) and consumption of contaminated food and water have been incriminated. The infection in pigs may take place through faecal contamination. The major source of Yersiniosis in man could be contaminated pork, as these pathogenic strains were isolated from both pig carcasses and retail raw pork.

The occurrence of *Yersinia* has been reported from a wide range of materials like soil, water, milk, red meat, vegetables and sea food. It has been recovered from food sources such as beef, pork, lamb and chicken in different parts of the world. *Yersinia* has been increasingly implicated in outbreaks of food and water borne enteritis. The major

source of transmission of the organism to food could be through faeces and urine of infected animals.

Yersinia has the distinction of surviving and multiplying at refrigeration temperature. The psychrotrophic nature of this organism has resulted in added problem in maintaining food safety.

Taking into account the pathogenic nature of *Yersinia* organisms in animals and man, and pigs as a major reservoir, a study was carried out to find out

1. Occurrence of *Yersinia* species in pigs
2. Survivability of *Yersinia* in pork on chilling and freezing.

This would help in assessing the role of pork as a potential source of Yersiniosis in man. The results are expected to provide base line data on the occurrence of *Yersinia* in pigs and the effect of chilling and freezing on its survivability.

Review of Literature

REVIEW OF LITERATURE

Pig

Several workers have reported the isolation of Yersinia from tonsils, mesenteric lymph nodes, caecal contents, faeces and throat swabs of healthy pigs. This indicates that swine may be a potential reservoir of human infection.

In the epidemiological study of Yersinia enterocolitica infections, Esseveld and Goudzwaard (1973) found pigs as a source of infection to man. Yersinia enterocolitica was frequently isolated from pig faeces.

Toma and Deidrick (1975) isolated Yersinia enterocolitica and Yersinia pseudotuberculosis from caecal contents of swine slaughtered at Toronto abattoir. These findings further strengthened the view that pigs act as a reservoir of human infection.

Tsubokura et al. (1975) concluded that isolation of Yersinia enterocolitica from the caecal contents of swine was dependant on season. The organism was isolated more frequently in winter to early spring than in summer.

Tsubokura et al. (1976) isolated Yersinia pseudotuberculosis from caecal contents of apparently healthy pigs which indicated pigs as an important reservoir. Narucka and Westendorp (1977) isolated Yersinia enterocolitica from 22 tonsils of 163 clinically normal pigs and Yersinia pseudotuberculosis from seven pigs; but they were not isolated from mesenteric lymph nodes or muscle tissues. All the 60 samples of minced pork meat were negative.

Szita et al. (1978) isolated Yersinia enterocolitica from pigs. Pigs were considered the main source of human infection, a conclusion supported by higher antibody titre in people in contact with pigs than those in contact with other animals.

Christensen (1981) isolated a predominant human pathogenic serotype of Yersinia from tonsils of 26 per cent of 284 healthy pigs, but none could be isolated from 65 samples of pork sausages or minced or salted pork meat.

Schiemann and Fleming (1981) found the prevalence of Yersinia enterocolitica among slaughter pigs varying from 20 per cent in tonsils to 50 per cent in throat swabs and 55 per cent in tongues.

Weber and Knapp (1981) isolated Yersinia enterocolitica (33) and Yersinia pseudotuberculosis (7) from

1206 faecal samples collected from clinically healthy pigs. Yersinia enterocolitica was isolated during months of late autumn, winter and spring, with higher isolation in January.

Boemi et al. (1982) recovered 25 isolates of Yersinia enterocolitica from 16 of 125 slaughter pigs from two abattoirs.

Weber and Lembke (1982) isolated Yersinia pseudotuberculosis from five of 1098 rectal swabs and from 28 of 480 tonsils taken from clinically healthy slaughter pigs. They were of the opinion that pigs act as carriers and excretors of Yersinia pseudotuberculosis.

Zeng et al. (1982) obtained six isolates (6.8 per cent) from 88 jejunal and ileal contents of swine in China.

Brewer and Corbel (1983) isolated 38 cultures of Yersinia from faeces of apparently healthy pigs in UK.

Cattean et al. (1983) assayed 50 tongues of slaughter pigs originating from France (30), Belgium (15), and Germany (5); thirty-eight yielded Yersinia enterocolitica. Thirteen tongues yielded Yersinia intermedia, Yersinia pseudotuberculosis, Yersinia kristensenii and Yersinia frederiksenii.

Fukushima et al. (1983) concluded that Yersinia enterocolitica was apparently transmitted among pigs from infected faeces or picked up from floor of the contaminated pen. The regular schedule of pig movement among the pens was an important factor in its spread within a piggery.

Harmon et al. (1983) examined 43 samples derived from swine carcass, porcine tongue and trims from an abattoir and obtained eight isolates of Yersinia enterocolitica and related species.

Stengel (1983) examined 103 tonsils from healthy slaughter pigs, of which 14 yielded Yersinia enterocolitica, but failed to isolate from 224 samples of minced pork.

In an outbreak of piglet diarrhoea, Yu et al. (1983) obtained 54 isolates of Yersinia enterocolitica from faecal samples.

From the caecal contents and lingual tonsils of 200 healthy pigs, Delia et al. (1984) isolated Yersinia enterocolitica (41), Yersinia fredericksenii (4) and Yersinia pseudotuberculosis (1).

Fukushima and Naramura (1984) inoculated fattening pigs with human pathogenic strains of Yersinia enterocolitica by

catheter into stomach. The pigs excreted the organism in faeces at 10^2 - 10^6 cells/g for a few weeks.

Popovic and Markovic (1984) isolated Yersinia enterocolitica from swine tonsils.

Verma and Misra (1984) obtained five isolates of Yersinia by direct plating of 127 rectal swabs from healthy pigs in Uttar Pradesh. Four isolates were Yersinia enterocolitica and one isolate was Yersinia fredericksenii.

Fukushima and Tsubokura (1985) examined pigs in one farm for carrier status of Yersinia. The most predominant strain of pathogenic Yersinia enterocolitica isolated from pig faeces was the new biotype 3 B serotype O3 phage type II.

Das (1985) studied the seroprevalence of Yersiniosis and Brucellosis among indigenous pigs and concluded that Yersiniosis was more prevalent than Brucellosis among indigenous boars and less prevalent in sows.

Vasilev (1986) isolated 17 strains of Yersinia enterocolitica from throat of 203 pigs.

Sung and Choi (1987) reported that isolation rate of Yersinia in pigs varied from 5.9 to 8.0 per cent in piggeries and was highest in summer and autumn, and in fattening pigs.

Asplund et al. (1988) isolated Yersinia enterocolitica 0:3 from throat muscles of Finnish pigs.

Okoroafor et al. (1988) obtained four strains of Yersinia enterocolitica from tongue and one each from throat and tonsillar swabs.

Turkson et al. (1988) recovered only one strain of Yersinia enterocolitica from 150 healthy pigs examined, indicating that Yersinia enterocolitica was rare in Kenya.

Asplund et al. (1990) found that prevalence rate of Yersinia enterocolitica in pig tonsil varied from 31.3 to 38.3 per cent. Isolation of the organism could not be made from 104 samples of pork and minced meat collected from retail markets in Helsinki.

Fukushima et al. (1990) investigated the prevalence of Yersinia in 1200 slaughter pigs from 96 pig farms of Shimane prefecture and isolated five serotypes of Yersinia pseudotuberculosis and four serotypes of Yersinia enterocolitica.

Stefanov and Kunev (1990) examined 225 samples of tonsil, 158 of tongue, 152 of faeces and 180 carcass swabs and obtained 109 isolates of Yersinia enterocolitica, 30 of

Yersinia kristensenii, 31 of Yersinia frederiksenii 22 of Yersinia intermedia and four of Yersinia pseudotuberculosis.

Boer and Nouws (1991) isolated pathogenic strains of Yersinia enterocolitica from tonsil, tongue, rectal swab and samples of pork.

Sathyanarayana Rao et al. (1991) isolated Yersinia enterocolitica from twenty one out of 240 pigs (8.75%). Higher percentage of carrier status was observed among graded pigs (10.83%) than desi pigs (6.67%).

Shiozawa et al. (1991) collected tonsils and caecal contents of 140 pigs, oral cavity swabs of 40 pigs from four farms and masseter samples of 25 pigs from five farms. Isolation rate of Yersinia enterocolitica 0:3 from tonsils, caecal contents, mouth swab and masseter muscle was 24.3, 24.3, 8.5 and 36 per cent respectively. It was concluded that Yersinia enterocolitica 0:3 which colonises in tonsils of healthy slaughter pigs is an important source of bacterial contamination of carcass and pork.

Adesiyun et al. (1992) found only two per cent of the 294 piglets to be infected with Yersinia enterocolitica in Trinidad.

Jackova and Urgeova (1992) examined samples of meat and tissue from oral cavity (tonsils, tongue and masticatory muscles) of 920 slaughter pigs. Yersinia enterocolitica 0:3 was isolated, with the highest number of isolation from tonsils.

Occurrence of Yersinia in other species

There has been a steady increase in the recorded incidence of Yersinia infections from a number of countries. The various animal species and environmental specimens from which the bacteria was isolated has increased over the years.

Ruminants (Cattle, Sheep and Goat)

Yersinia enterocolitica was isolated from faecal sample of cattle (Hawari et al., 1981).

Zamora et al. (1981) isolated Yersinia enterocolitica from caecum of cattle.

Jones et al. (1982) isolated Yersinia pseudotuberculosis from milk of aborted female goat.

Kolos et al. (1985) isolated Yersinia from faeces, udder washings and milk of sheep.

Sulochana and Sudharma (1985) isolated Yersinia pseudotuberculosis from cases of abortion in goats, in Kerala.

Bullians (1987) isolated Yersinia pseudotuberculosis and Yersinia intermedia from cattle. Yersinia was also isolated from lambs.

Kwaga et al. (1987) isolated Yersinia enterocolitica from fecal samples of camel in Nigeria.

Pavlov et al. (1988) obtained Yersinia from rectal contents, bulk milk and individual milk samples of cattle.

Non-ruminants

From India, Srivastava et al. (1978) reported an outbreak of Yersinia pseudotuberculosis infection in Guinea pigs.

Fukushima et al. (1984) isolated Yersinia species from apparently healthy dogs in Japan.

Koneuchi et al. (1989) isolated Yersinia species from faecal samples of migratory birds, ducks, seagulls and swallows in Japan.

Nwosuh and Adesiyun (1989) detected Yersinia enterocolitica in the organs of experimentally infected chicken and stressed the public health importance.

Khalafalla (1990) isolated Yersinia enterocolitica

from chicken, ducks and turkey. Isolation rates were higher in chicken than in ducks or turkey.

Fukushima et al. (1990) isolated *Yersinia* species from mice and moles. Wild mice in mountainous areas of Japan were the important reservoirs of *Yersinia pseudotuberculosis*.

Fukushima and Gomyoda (1991) isolated *Yersinia pseudotuberculosis* from deer, hares and birds. The close relationship between the regional distributions of *Yersinia pseudotuberculosis* in wild animals and man suggest that wild animals can act as a source of infection.

Sanekata et al. (1991) reported isolation of *Yersinia pseudotuberculosis* from caecal contents of a pet cockatoo in Japan.

Man

According to Stern and Pierson (1979), *Yersinia* may be transmitted directly or indirectly to food and water through faecal contamination or by urine of a carrier animal. Man could get infected by consumption of the contaminated food or water.

Bottone (1981) described the zoonotic importance of *Yersinia enterocolitica* infection, its host range, clinical

manifestation and mode of transmission between animals and man.

Shayegani et al. (1983) isolated Yersinia enterocolitica from persons at a summer camp in New York. The same serogroup was also isolated from reconstituted dried milk and turkey 'Chow-mein' used at the camp.

Morse et al. (1984) reported an outbreak of gastroenteritis at a summer camp and isolated Yersinia enterocolitica from 54 per cent of 69 persons examined. Epidemiological evidence indicated that illness was associated with consumption of reconstituted dried milk and/or 'Chow-mein'.

Doyle (1985) attributed enterocolitis caused by Yersinia enterocolitica in infants and young children to consumption of contaminated chocolate milk, reconstituted dried milk and pasteurized milk.

Nunes and Ricciardi (1986) isolated Yersinia enterocolitica from faeces of adults, children and dogs in four adjacent households. All were apparently healthy.

Fukushima et al. (1987) concluded that most Yersiniosis was due to Yersinia pseudotuberculosis and

Yersinia enterocolitica. Yersinia pseudotuberculosis was isolated from patients.

Schoilew and Kosarow (1987) detected specific antibodies against Yersinia enterocolitica 0.3 in 28.3 per cent of human beings with rheumatism and from 10.9 per cent of clinically healthy blood donors. The importance of Yersinia enterocolitica infection as a zoonosis was underlined by the occurrence of 0.3 antibody in 51.2 per cent of cows and 9.4 per cent of sows.

Tauxe et al. (1987) isolated Yersinia enterocolitica from 40 human patients with gastroenteritis or liver abscess in Belgium. Twenty-three cases were strongly associated with eating raw pork.

Fukushima et al. (1988) isolated Yersinia enterocolitica from swab of a skinner at a slaughter house.

Fukushima et al. (1989) reported Yersinia pseudotuberculosis infection in boys. They concluded that Yersinia pseudotuberculosis was transmitted through water, sand and soil contaminated by faeces of cats infected with Yersinia pseudotuberculosis.

Mraz et al. (1989) isolated Yersinia enterocolitica from two of the 243 personnel examined.

Greenwood and Hooper (1990) isolated Yersinia enterocolitica from 19 patients. The same serotype was isolated from pasteurized milk supplied to the ward. Epidemiological evidence indicated that contaminated pasteurized milk was the source of Yersinia organism excreted by the patients.

Merilahti-Palo et al. (1991) observed antibodies against Yersinia enterocolitica more often in sera of abattoir workers especially in butchers handling swine (27 per cent), than in sera of healthy blood donors (10 per cent). It was concluded that Yersiniosis was an occupational health risk to abattoir workers.

Nesbakken et al. (1991) detected antibodies to Yersinia enterocolitica in 25 (11.1 per cent) of 225 employees at three pig processing plants and in 14.5 per cent of 91 employees at two poultry processing plants. Prevalence of antibody was higher in those directly involved in slaughter. Antibodies were detected in 17 (9.9 per cent) of 171 veterinarians.

Fukushima et al. (1991) isolated Yersinia pseudotuberculosis from the mesenteric lymph nodes of a girl with acute mesenteric lymph adenitis.

Ichinohe et al. (1991) isolated Yersinia enterocolitica 0.8 from a four year old Japanese boy. This was the first report of this serotype from a human infection outside North America.

Food

Yersinia has been isolated from a variety of food items like beef, pork, milk, cream and vegetables. This highlights the role of food in transmission of infection to man.

Hanna et al. (1976) isolated Yersinia enterocolitica like organisms from vacuum packaged beef and lamb stored at 1-3°C for 21-35 days. A higher percentage of isolates were obtained from cuts packaged under high vacuum conditions.

Yersinia has been isolated from milk and/or milk products (Black et al., 1978; Schiemann and Toma, 1978; Huges, 1979; Aldova et al., 1981; Franzin and Fantino, 1984; Pugina et al., 1984; Umoh et al., 1984; Aiello et al., 1985; Vasavada and Larkin, 1985; Mercado and Ibanez, 1986; Walker and Gilmour, 1986; Zowghi and Ebadi, 1986; McManus and Lanier, 1987 and Rohrbach et al., 1992).

Aldova et al. (1981) isolated Yersinia enterocolitica from meat, pastry and water.

Hurvell et al. (1981) noted that, of the 216 samples of raw minced beef stored at 4-10°C, 15.7 per cent were positive for Yersinia enterocolitica. From 124 other chilled foods mainly chicken products, three isolates were obtained.

Norlyng (1982) examined 1279 samples of food items like milk, pork, beef and meat products and was able to isolate Yersinia enterocolitica from 23 samples.

Marinelli et al. (1985) recovered Yersinia enterocolitica from surface water.

Boer et al. (1986) isolated Yersinia enterocolitica and related species from a number of foods in Netherlands. High isolation rates were reported from meat products, raw vegetables and egg.

Gravani (1986) identified the food involved in cases of Yersiniosis as milk products, meat, sea food and fresh vegetables.

Fukushima et al. (1987) isolated Yersinia enterocolitica from raw beef, pork and chicken.

Rhodehamel et al. (1987) observed that Yersinia enterocolitica grew best in high protein food.

Rindi et al. (1989) isolated six non-pathogenic strains of Yersinia enterocolitica from meat products.

Ibrahim and Macrae (1991) recovered 114 isolates consisting of Yersinia enterocolitica, Yersinia intermedia and Yersinia frederiksenii from beef, lamb, pork and milk.

Isolation technique and media

Different methods of isolation procedures are followed by scientists like cold enrichment at various temperatures. They also compared the effectiveness of direct plating and plating after cold enrichment.

Knapp and Thal (1973) differentiated Yersinia enterocolitica by biochemical reactions and shortlisted the most important reactions of confirmed Yersinia enterocolitica strains, in relation to other species of the genus Yersinia, Pasteurella and Salmonella.

Wauters (1973) suggested improved methods for isolation and identification of Yersinia enterocolitica. The use of Salmonella-Shigella agar with 2 per cent sodium dioxycholate was recommended.

Aulisio et al. (1980) suggested a new culture method employing potassium hydroxide treatment. The new method increased the recovery of Yersinia species four-fold and

sensitivity hundred-fold, shortened the incubation period and appreciably decreased the growth of non-Yersinia bacteria.

Hanna et al. (1980) effected isolation through enrichment in cold mannitol broth or a bile medium at 4°C for 10-60 days. Enrichment in modified Rappaport medium at 25°C for more than two days sharply reduced recovery of Yersinia enterocolitica from tonsil.

Doyle et al. (1981) used cold enrichment in Phosphate buffer saline followed by treatment with dilute potassium hydroxide or subsequent enrichment with modified Rappaport broth.

Weber and Lembke (1981) isolated human pathogenic strains of Yersinia enterocolitica from tonsil of slaughter pigs by cold enrichment in phosphate buffer saline, pH 7.6 at 4°C for three weeks. Thirty-three isolates were made using modified Rappaport medium, plated after incubation for two days at 22°C.

Schiemann (1982) suggested the use of phosphate-buffer saline as a common enrichment medium. Although Cefsulodin-Irgasan-Novobiocin agar (CIN) was considerably more selective than other enteric media used for isolation of Yersinia, it was unable to completely suppress the background flora present after cold enrichment.

Zink et al. (1982) suggested cold enrichment in $\frac{1}{15}$ M disodium phosphate at pH 7.6 for 14-21 days at 4°C. They also suggested CIN agar to be part of every isolation scheme.

Davey et al. (1983) suggested cold enrichment for three weeks at 5°C, followed by plating on CIN agar for better efficiency in isolation of *Yersinia* species.

Harmon et al. (1983) evaluated selective differential plating media for isolation of *Yersinia enterocolitica* and concluded that CIN agar was the most effective medium. However *Yersinia enterocolitica* 0:12, 25 was slightly inhibited on CIN agar.

Feinhaken et al. (1984) compared cold enrichment for three weeks and six weeks in phosphate buffer saline (pH 7.6) at 4°C. All *Yersinia* isolates obtained were after three weeks of cold enrichment.

Yurukov and Slavchev (1984) successfully applied "alkali method" for rapid recovery of *Yersinia enterocolitica* from food as described by Aulisio et al. (1980). Enrichment gave better results at 4°C than 22°C.

Fukushima (1985) recovered *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* by direct treatment with potassium

hydroxide, without enrichment. This formed an important time saving method for isolation of *Yersinia* species from meat.

Marinelli et al. (1985) recovered *Yersinia enterocolitica* from surface water by a simplified method. Samples were given a short pre-incubation warm up in peptone-sorbitol-bile salt broth and quickly passed to an alkali solution and then plated on to MacConkey agar.

Fukushima and Gomyoda (1986b) were of the opinion that CIN media should not be used alone for isolating *Yersinia*.

Guallini et al. (1986) used a method, exploiting the resistance of *Yersinia enterocolitica* to ampicillin, for its isolation from tonsil and lymph nodes of pigs.

Fukushima (1987) described a new selective agar medium containing cefsulodin, irgasan, josamycin and oleandomycin for isolation of virulent *Yersinia enterocolitica* (VYE agar) and found to have high selectivity. The presence of mannitol and esculin in media could differentiate virulent *Yersinia* (red colonies) from environmental *Yersinia* (dark colonies) and other gram negative bacteria.

Okoroafor et al. (1988) cultured tongue, throat and tonsillar swabs from 746 pigs by enrichment in phosphate buffer saline at 4°C for 21 days. Four (0.53 per cent)

isolates of Yersinia enterocolitica were obtained from tongue and one each from throat and tonsillar swabs.

Wauter et al. (1988) developed new enrichment media for recovery of pathogenic Yersinia enterocolitica from naturally infected meat products, based on three selective agents - irgasan, ticarcillin and potassium chlorate (ITC).

Greenwood and Hooper (1989) recovered twice the number of organisms following enrichment of samples in Tris-buffered peptone water (pH 8.0) incubated at 9°C, compared to that from buffered peptone water (pH 7.2) at 4°C. Weekly sub-culture on CIN agar for three weeks was found to achieve maximum recovery of Yersinia.

Aldova et al. (1990) used porcine tongue as models to test culture methods for isolation of Yersinia enterocolitica. They concluded that, two minute treatment of samples with 0.5 per cent potassium hydroxide after cold enrichment in phosphate buffer saline and culturing on CIN agar as the most effective method.

Cox et al. (1990) compared enrichment and plating media for isolation of Yersinia. With Yersinia enterocolitica 0:3, recoveries were more frequently made using phosphate buffer saline, sorbitol bile, followed by plating on pectin,

CIN, bismuth-sulphite or modified Rimler-sholts agar. CIN and pectin agars performed better.

Kwaga et al. (1990) recommended irgasan, ticarcillin and potassium chlorate (ITC) for isolation of virulent strains of Yersinia enterocolitica.

Survivability

Since *Yersinia* can grow at refrigeration temperatures commonly used to store food, investigations has been carried out to test the survivability of the organism in various food at low temperatures.

Leistner et al. (1975) reported good growth of *Yersinia pseudotuberculosis* at 4°C.

Busta (1976) studied the practical implications of injured microorganism in food and suggested that recognition of sub-lethally impaired microorganism was essential for practical interpretation of data in food microbiology. Cellular modifications observed in injured cells included susceptibility to selective agents and anti-microbials and leakage of intracellular material. Injury was characterized by the capability of the microorganism to return to normalcy during a resuscitation process.

Hanna et al. (1977 a) found increase in count of Yersinia enterocolitica on raw beef held over a period 10-days at 1°C. When inoculated on raw, or cooked beef and stored at 7°C (0-10 days) or at 25°C (0-24 h), large increase in Yersinia enterocolitica occurred. At 25°C, the increase in Yersinia enterocolitica was greater on cooked than on raw products.

Hanna et al. (1977 b) observed extensive destruction of Yersinia enterocolitica on beef during frozen storage.

Stern et al. (1980) noted that clinical strains of Yersinia enterocolitica showed significantly greater tolerance to pH (4.6-9.0) level and sodium chloride concentration than did the environmental strain at 25°C. These findings suggested that Yersinia enterocolitica may be resistant to some common methods of food preservation.

EL-Zawahry and Grez (1981) observed that freezing Yersinia enterocolitica at -18°C and -75°C for one hour resulted in cell inactivation and cell injury.

Stanfield et al. (1985) observed that Yersinia enterocolitica survived for more than 21 days at 4°C in milk cartons, which could be a public health hazard.

Ahmed et al. (1986) observed a slow decrease in viable counts of Yersinia enterocolitica in yoghurt during storage.

Fukushima (1986) observed the failure of Yersinia enterocolitica 0:3 to grow in naturally infected raw pork at both refrigeration temperature of 6°C and room temperature of 25°C. The growth was suppressed by other environmental Yersinia organisms and aerobic microbial flora normally seen in pork.

Palumbo (1986) confirmed that Yersinia enterocolitica was capable of growth at 5°C in fresh pork.

Roy (1986) observed that viable counts of Yersinia enterocolitica inoculated into sterile cream increased at 4°C, but decreased at -10°C.

Fukushima and Gomyoda (1986a) found that Yersinia enterocolitica 0:3 did not grow, but survived in inoculated raw ground pork at 6°C and 25°C.

Adesiyum et al. (1989) observed a significantly higher growth of Yersinia enterocolitica at 25°C in colostrum than milk of goats.

Slavchev (1989) concluded that Yersinia enterocolitica multiplied in butter stored at 0-6°C. At -17 to -10°C it survived for 20 days in sweet cream butter and for 44-72 days in sour cream butter.

Corbel (1990) reported Yersinia pseudotuberculosis to survive and multiply at temperatures down to 0°C.

Sorqvist and Danielsson-Tham (1990) concluded that only small numbers of Yersinia could be expected to survive in scalding water at 58-62°C. Hence temperature above 62°C were recommended to minimise the risk of contamination.

Tashiro et al. (1991) observed that Yersinia enterocolitica 0:3 disappeared most rapidly from soil at both temperatures of 4°C and 20°C, and from river water at 20°C. The virulent strains survived longer at 4°C than at 20°C in soil and river water.

Materials and Methods

MATERIALS AND METHODS

Specimen for bacterial isolation

The biomaterials employed for isolation comprised of samples collected from pigs slaughtered at Meat Technology Unit of the College of Veterinary and Animal Sciences, Mannuthy (MTU Samples) and from the Trichur municipal slaughter house (MSH Samples).

Collection of samples

Samples of tonsils, mesenteric lymph nodes, caecal contents, rectal swabs and buccal swabs were collected from 30 healthy pigs slaughtered at MTU and nine pigs slaughtered at MSH. Meat samples (MTU) of approximately 10 g each were collected from four animals slaughtered at random. All the samples were collected immediately after slaughter.

The tonsils and mesenteric lymphnodes were collected into separate sterile beakers containing 30 ml phosphate buffer saline (PBS, pH 7.6).

The surface of the caecum was sterilized with hot spatula, incised and approximately five gram of caecal contents were taken in a sterile test tube containing 30 ml PBS. The contents were mixed thoroughly.

The rectal and buccal swabs were taken in sterile test tubes containing 30 ml PBS and the contents mixed thoroughly. The samples were transported to the laboratory and processed within two hours of collection.

Processing of tonsils, mesenteric lymph nodes and meat

The tonsils, mesenteric lymph nodes and meat were processed by triturating each of them separately with 30 ml PBS in a sterile mortar and pestle. The triturated suspension of the samples were then taken in sterile test tubes.

Isolation

A loopful of inoculum from the supernatant fluid of each individual sample was streaked on to Yersinia isolation agar containing the antibiotic supplement (YIA) (Appendix-I).

The rest of the suspensions of all the samples were cold enriched for 21 days at 4°C (Weber and Lembke, 1981) and then plated on to YIA in the aforesaid manner.

Incubation of inoculated plates

The inoculated plates were incubated at 25°C for 48 h.

Selection of suspected colonies

Translucent, pin point colonies with or without slight

pink center were picked from the selective plating media. This was done by using a straight needle and touching only the center of the suspected colony to obtain pure cultures (Cruickshank et al., 1975). The suspected colonies were subcultured on to nutrient agar plates. The reserve stock cultures and working stock cultures were made on nutrient agar slants.

Biochemical screening

The biochemical screening was carried out as elucidated by Feeley (1971).

Initial screening tests

This included Gram's reaction, motility, Triple sugar iron (TSI) reaction and urease test.

Gram's reaction

The morphology and staining reaction of the colony was studied by Gram's method of staining (Cruickshank et al., 1975). Gram negative rods were selected.

Motility

The motility was tested by inoculating the culture into two 1 per cent peptone water tubes, and incubated one

tube at 22°C (Schiemann, 1982) overnight and the second at 37°C. Motility was tested by dark field microscopy.

Triple sugar iron

The selected colonies were stabbed into the butt using a straight wire and streaked on the slope of TSI agar. The tubes were incubated at 25°C for 24 h. Isolates giving acid slant and acid butt or acid butt and alkaline slant, without production of gas or hydrogen sulphide were selected.

Urease test

The selected colonies were inoculated on to Christensen's urea agar slant and incubated at 37°C for four days. Positive reaction was indicated by bright pink colour on the slant. Tubes were declared negative only after four days of incubation.

Presumptive identification tests

Only those colonies found positive on initial screening for *Yersinia* were subjected to the following tests.

Oxidase test

Dry filter paper method of Kovacs was used. The strip was laid on a microslide and moistened with distilled water. The colony was picked up with a platinum loop and smeared over

the moist area. The development of a deep purple colour within 30 seconds was taken as positive reaction. The presence or absence of colour after 30 seconds was considered as negative reaction.

Decarboxylase tests

Tubes of decarboxylase media containing one per cent L-lysine hydrochloride and another tube containing one per cent L-ornithine hydrochloride were inoculated with a straight wire through the paraffin layer. A control tube without the amino acid was also kept. Incubated at 37°C and read daily for four days. A violet colour indicated the presence of the specific decarboxylase and negative reaction was indicated by yellow colour in both the test and control tubes.

Arginine dihydrolase test

One tube of the amino acid medium containing 0.5 per cent of L-arginine monohydrochloride and one tube of control medium (without the addition of the amino acid) were inoculated with the test culture. The inoculated tubes were incubated at 37°C and examined daily until the test became positive or four days, whichever was earlier. Positive reactions were indicated by violet or reddish violet colour and negative by yellow colour in both the test and control tubes.

Phenylalanine deaminase test

The phenylalanine agar slope was inoculated and incubated for 24 h at 37°C. A few drops of 10 per cent ferric chloride solution was allowed to run down the growth on the slope. A positive test was indicated by development of a green colour in the fluid and the slope.

Indole

Inoculated the medium and incubated for 48 h at 37°C. Added 0.5 ml Kovac's reagent and shook gently. A red colour in the alcohol layer indicated a positive reaction, and negative by brownish colour.

Confirmatory tests

Tests for fermentation of sugars

Fermentation of sugars such as lactose, maltose, sucrose, melibiose, raffinose and rhamnose were carried out following the procedure described by Cowans (1974). One per cent solution of the above sugars in peptone water were used. After inoculation, the sugar solutions were incubated at 37°C and examined daily for seven days. A positive reaction was given by a change in colour of the medium to yellow.

The details of media used for biochemical tests are given in Appendix-I.

Survivability studies

The isolate obtained in the present study was used for the survivability studies. Fresh pork was ground in a blender aseptically, and 18 g of it was placed in eight sterile plastic bags each (Fukushima and Gomyoda, 1986a).

Inoculation of the organism into pork

A fixed dilution of the organism (10^4 org/ml) was prepared using McFarland nephelometer standards (Appendix-II). Two millilitre of this suspension was inoculated into each of the eight bags and thoroughly mixed. The bags were placed at two temperatures - three bags at 4°C and the remaining five bags at -15°C. Duplicate samples were maintained at both temperatures. The study was repeated four times.

Examination of the bags

The bags kept at 4°C were examined on days 3, 5 and 7 after inoculation. The bags kept at -15°C were examined on days 7, 15, 30, 45 and 60.

Testing the survivability

A representative sample (2g) was made into a suspension using 10 ml of peptone water. A loopful from this was plated on to YIA.

For bags kept at -15°C , in addition to direct plating, the suspension was further incubated at 25°C for 48 h and then plated on to YIA (Busta, 1976). The isolates were identified by standard procedures (Feeley, 1971).

Results

RESULTS

Yersinia pseudotuberculosis was isolated from three of the 171 samples screened from 39 animals (Table 1). Two of the isolates were obtained from the buccal cavity and tonsil of the same animal and the third isolate was from the tonsil of another animal. The isolates were numbered as Isolate 1, 2 and 3. Isolate 1 and 2 were from tonsils and Isolate 3 was from the buccal cavity. Isolates 2 and 3 were from the same animal. *Yersinia* could not be isolated from the mesenteric lymph nodes, caecal contents, rectal swabs and meat tested. The organism could not be isolated by direct plating, but cold enrichment was found to be effective in isolating the organism from the same sample.

The results of various biosamples screened are given (Table 1). The organisms were identified based on morphological, cultural and biochemical characters as described by Feeley (1981) and Bisset (1981). The results of primary screening, presumptive identification tests and confirmatory tests are given in Tables 2, 3 and 4 respectively.

All the identification tests carried out are summarised in Table 5. The biochemical reactions of the

standard strains of Yersinia enterocolitica and Yersinia pseudotuberculosis furnished therein are as described by Brenner (1981).

Survivability of the isolates at 4°C

The organism could be recovered at 4°C on days three, five and seven from inoculated meat sample in all four trials. The survivability studies were discontinued beyond 7 days as spoilage of meat had set in.

Survivability of the isolates at -15°C

Yersinia pseudotuberculosis could be recovered on seven, 15 and 30 days of storage by direct plating. On day 45, the organisms could not be recovered by direct plating, while the organisms were recovered after resuscitation. But the organism could not be recovered either by direct plating or by resuscitation on the 60th day of storage. The above results were obtained in all the four trials. The results are presented in Table 6.

Table 1. Result of biosamples screened for Yersinia in pigs

Location	No. of animals screened	Buccal cavity	Tonsils	Caecal	Rectal	MLN	Meat	No. of animals positive
		SCR + -	SCR + -	SCR + -	SCR + -	SCR + -	SCR + -	
MTU samples	30	14 1 13	60 2 58	12 - 12	30 - 30	30 - 30	4 - 4	2
MSH samples	9	3 - 3	18 - 18	- - -	- - -	- - -	- - -	-
Total	39	17 1 16	78 2 76	12 - 12	30 - 30	30 - 30	4 - 4	2

SCR - Number of samples screened

MLN - Mesenteric lymph nodes

(+) Positive

MTU - Meat Technology Unit

MSH - Municipal Slaughter House

(-) Negative

Table 2. Primary screening tests of the isolates

Sl. No.	Test	Isolates		
		Isolate 1	Isolate 2	Isolate 3
1.	Grams reaction	Gram negative	Gram negative	Gram negative
2.	Cell morphology	Rods	Rods	Rods
3.	Motility			
	22°C	+	+	+
	37°C	-	-	-
4.	Urease	+	+	+
5.	TSI	Alk slant Acid butt	Alk slant Acid butt	Alk slant Acid butt

Alk - Alkaline (Red) Acid - Yellow

Table 3. Presumptive identification tests of the isolates

Test	Isolates		
	Isolate 1	Isolate 2	Isolate 3
Oxidase	-	-	-
Lysine decarboxylase	-	-	-
Ornithine decarboxylase	-	-	-
Arginine dihydrolase	-	-	-
Phenylalanine deaminase	-	-	-
Indole	-	-	-

Table 4. Confirmatory tests of the isolates

Test	Isolates		
	Isolate 1	Isolate 2	Isolate 3
Lactose	-	-	-
Maltose	+	d (+)	+
Sucrose	-	-	-
Melibiose	+	+	+
Raffinose	+	-	-
Rhamnose	+	+	+

d - doubtful

Table 5. Biochemical reactions of the standard strains of Yersinia enterocolitica and Yersinia pseudotuberculosis and the isolates

Test	<u>Yersinia</u> <u>enterocolitica</u>	<u>Yersinia</u> <u>pseudotuberculosis</u>	Isolate 1	Isolate 2	Isolate 3
Grams reaction	-	-	-	-	-
Motility					
22	+	+	+	+	+
37	-	-	-	-	-
TSI (H ₂ S)	-	-	-	-	-
	* **				
	Acid/acid	Alk/acid	Alk/acid	Alk/acid	Alk/acid
Urease	+	+	+	+	+
Oxidase	-	-	-	-	-
Lysine decarboxylase	-	-	-	-	-
Ornithine decarboxylase	+	-	-	-	-
Arginine	-	-	-	-	-
Phenylalanine deaminase	-	-	-	-	-

Table 5 (Contd.)

Test	Yersinia enterocolitica	Yersinia pseudotuberculosis	Isolate 1	Isolate 2	Isolate 3
Indole	+	-	-	-	-
Lactose	-	-	-	-	-
Maltose	+	+	d(+)	+	+
Sucrose	+	-	-	-	-
Melibiose	-	+	+	+	+
Raffinose	-	v	+	-	-
Rhamnose	-	+	+	+	+

** - Butt

* - Slant

d - doubtful

v - variable

Alk - Alkaline

Discussion

DISCUSSION

Pig rearing among small farmers in Kerala has now come to occupy an important place in the rural economy as a supplementary occupation. Pigs are also known to be carriers of various human diseases and thus their role in transmission of zoonotic diseases is important. Infection with Yersinia pseudotuberculosis has been receiving wide attention as an emerging zoonotic diseases (Fukushima et al., 1987; Fukushima et al., 1989; Fukushima et al., 1991).

Pigs form the reservoir host for Yersinia, as these organisms are carried even by healthy animals (Toma and Deidrick, 1975; Tsubokura et al., 1976; Szita et al., 1978; Weber and Lembke, 1982). From Kerala, Sulochana and Sudharma (1985) were able to isolate Yersinia pseudotuberculosis from a case of abortion in goat.

During the present study, 39 pigs (30 pigs from the University Pig breeding farm slaughtered at the Meat technology unit and nine pigs from the sub-urban area, slaughtered at the Municipal slaughter house) were screened for Yersinia. One hundred and seventy one samples obtained from various sites were examined for this purpose. Yersinia pseudotuberculosis was isolated from two animals (Table 1).

One isolate each was obtained from the oral swab and the tonsil of the same animal. The third isolate was from the tonsil of another animal. Overlapping of the organism in more than one organ of the same animal was also reported by Sathyanarayana Rao (1991). Christensen (1981) and Stengel (1983) were able to isolate *Yersinia* from the tonsil of pigs. Cattean et al. (1983); Delia et al. (1984) and Weber and Lembke (1982) had reported isolation of *Yersinia pseudotuberculosis* from the buccal cavity of pigs. Shiozawa et al. (1991) had concluded that *Yersinia* colonises in the tonsils of healthy pigs and forms an important source of bacterial contamination of pork. Christensen (1981) and Stengel (loc. cit.) isolated *Yersinia* from samples of pork sausages or minced or salted pork meat. Anderson (1988), Norlyng (1982) and Fukushima (1985) could isolate the organism from pork. They attributed this to the use of head meat and tonsillar tissue in meat, and recommended the practice of removal of head carefully and avoid including the head meat along with other meat to prevent contamination with *Yersinia*. This will help in preventing the organism from gaining access to the food chain, thus reducing the consumer risk.

During the present study, the organism could not be isolated from samples collected from sites other than buccal cavity and tonsil. Boer and Nouws (1991) were also unable to

isolate *Yersinia* from pig carcasses and head meat. The organism could not be isolated from the mesenteric lymph nodes, a finding also reported by Fukushima et al. (1989).

Competitive inhibition of *Yersinia* by other bacteria could make its detection by direct plating difficult. Fukushima and Gomyoda (1986a) found potent antagonistic effect of aerobic microbial flora on growth of *Yersinia* in raw pork. The growth inhibition on raw pork may depend on the metabolic crowding by competing microflora. Hence enrichment procedure is recommended for specific isolation of the organism. In the present investigation the organism could be isolated only after cold enrichment, followed by the use of selective media (*Yersinia* isolation agar).

All the isolates gave identical biochemical reaction, except for isolate 1, which gave a positive reaction for raffinose. Variable raffinose reaction for *Yersinia pseudotuberculosis* has been reported by Brenner (1981).

The organism could enter the domestic animals like pigs through mice and moles, which act as wild-reservoirs for this organism (Fukushima et al., 1990). The presence of *Yersinia pseudotuberculosis* in the buccal cavity and tonsils in the present study may be due to the exposure of pigs to rodents. It is suggested that screening of rodents may help

in identifying the source of infection. Elimination of rodent population in and around the piggery and slaughter house could prevent infection in pigs.

Survivability of Yersinia pseudotuberculosis in pork during chilled/frozen storage

The organism could be recovered from experimentally inoculated pork, stored at 4°C, on days three, five and seven by direct plating on selective media. Yersinia pseudotuberculosis is reported to have survived and multiplied at 10°C (Corbel, 1990). The organism could survive 4°C during the period of refrigerated storage. Thus contamination during processing may result in potential health hazard to consumers.

From frozen meat, the organism could be recovered upto 30 days by direct plating as above. Though it was not possible to recover the organism by direct plating, resuscitation helped to recover the organism from meat frozen for 45 days.

The importance of resuscitation in recovery of the organism under frozen storage was reported by Busta (1976). Freezing causes cell injury to microorganisms. Isolation of injured microorganism on selective media was difficult without resuscitation. The injured microorganism becomes sensitive to antibiotics or other chemicals in selective media to which

they were previously tolerant, due to cell membrane or cell envelope modification (Busta, 1976).

When frozen, depending on duration and the temperature at which it is held, the intensity of freeze-injury could vary. Asakawa et al. (1979) reported that *Yersinia* count of sliced ham stored at -10°C decreased from 3000 to less than 50 per gram within seven days. El-Zawahry and Grez (1981) showed that freezing caused substantially great injury. This could have been the reason for non-recovery of the organism after 60 days.

Contamination of foods or food environments with injured cells that are capable of repairing should be anticipated during production process. It may not be possible to isolate these injured cells, thus masking their presence in food, which could lead to an increased consumer risk and a false sense of safety in consumption of inspected food. But freezing meat beyond 60 days may cause extensive freeze-injury, resulting in destruction of the pathogen as evidenced by this study. This could help to reduce the hazard of infection to consumers. Further studies on these lines could throw more light on the use of freezing as a method for reducing the risk of human infection with *Yersinia pseudotuberculosis*.

Summary

SUMMARY

A study was carried out to find out the occurrence of *Yersinia* species in pigs and also its survivability in pork during chilled and frozen storage.

Biomaterials collected from pigs slaughtered at Meat technology unit (30) and Municipal slaughter house (9) were used for isolation of *Yersinia*.

Samples of tonsils, mesenteric lymph nodes, caecal contents, rectal swabs and oral swabs were collected in Phosphate-buffer saline. Meat samples were collected from four animals slaughtered at Meat technology unit.

The processed samples were used for isolation by direct plating on to *Yersinia* isolation agar (Himedia). The remaining suspensions were cold enriched for 21 days at 4°C and then plated on to *Yersinia* isolation agar.

The initial screening tests included Grams reaction, motility, TSI and urease test. The presumptive identification tests included oxidase, lysine and ornithine decarboxylase tests, arginine dihydrolase test, phenylalanine deaminase test and indole test. The confirmatory tests included the test for lactose, maltose, sucrose, melibiose, raffinose and rhamnose.

Yersinia pseudotuberculosis could be isolated from three of 171 samples screened from 39 animals. Two isolates were obtained from the buccal cavity and tonsil of the same animal and the third from the tonsil of another animal.

Even when it was not possible to isolate the organism by direct plating, cold enrichment was found to be effective in isolating the organism from the same sample. The present study indicated that healthy pigs harboured Yersinia pseudotuberculosis.

The survivability study was conducted using the isolate obtained in the present study. Survivability was tested at 4°C and -15°C. The organism survived upto 7 days in pork at 4°C, beyond which the experiment was discontinued as the meat was spoiled. At -15°C, the organism could be recovered upto 30 days after inoculation, by direct plating. On 45th day it was recovered only after resuscitation. The organism could not be recovered on 60th day even after resuscitation. The study highlighted the importance of resuscitation for isolation of Yersinia from frozen meat to assess consumer risk during quality control. Further studies on role of rodents in transmission of the infection among pigs and the effect and duration of freezing as a measure for preventing infection to human beings will be useful.

References

REFERENCES

- *Adesiyum, A.A. and Lombin, L.H. (1989). Multiplication of Yersinia enterocolitica, Staphylococcus aureus, Escherichia coli in Caprine colostrum and experimental mastitis in does. Israel. J. Vet. Med. 45 (1): 1-11. Dairy Sci. Abstr. 52 (5): 3559.
- Adesiyum, A.A., Kaminjolo, J.S., Loregnard, R. and Kitson-Piggot, W. (1992). Frequency of isolation of Yersinia enterocolitica from livestock in Trinidad. Vet. Rec. 131: 516.
- *Ahmed, A.A.H., Moustafa, M.K. and El-Bassway, T.A. (1986). Growth and survival of Yersinia enterocolitica in yoghurt. J. Fd. Prot. 49 (12): 983-985. Dairy Sci. Abstr. 49 (4): 2339.
- *Aiello, P., Noto, A.M.D. and Guerico, V. (1985). Isolation of Yersinia enterocolitica from Cow's raw milk. Atti della Societe Italiana de Buiatria. 17: 589-592. Dairy Sci. Abstr. 49 (5): 2894.
- *Aldova, E., Sobotkova, J., Brezinova, A., Cerna, J., Janeckova, M., Pegrimkova, J. and Pokorna, V. (1981). Yersinia enterocolitica in water and foods. Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene. 173 (6): 464-470. Dairy Sci. Abstr. 44 (6): 3804.

- *Aldova, E., Svandova, E., Votypka, J. and Sourek, J. (1990). Comparative study of culture methods to detect Yersinia enterocolitica serogroup 0:3 on swine tongues. Zentralblatt fur Bakteriologie. 272 (3): 306-312. Vet. Bull. 60 (7): 4453.
- *Anderson, J.K. (1988). Contamination of freshly slaughtered pig carcasses with human pathogenic Yersinia enterocolitica. Int. J. Fd. Microbiol 7 (3): 193-202. Vet. Bull. 59 (12): 6879.
- Asakawa, Y., Akahane, S., Shiozawa, K. and Homa, T. (1979). Investigations of source and route of Yersinia enterocolitica infection. Contr. Microbiol. Immunol. 3. Cited by Stern, N.J. and Pierson, M.D. (1979). Yersinia enterocolitica: A review of the psychrotrophic water and food borne pathogen. J. Fd. Sci. 44: 1736-1742.
- *Asplund, K., Siitonen, A. and Hirn, J. (1988). Prevalence of Yersinia enterocolitica serobiotpe 0:3,4 in Finnish pigs. Suomen Elainleakarilehti. 94 (4): 177-180. Vet Bull. 58 (8): 4678.
- *Asplund, K., Tuovinen, V., Veijalainen, P. and Hirn, J. (1990). Prevalence of Yersinia enterocolitica 0:3 in Finnish pig and pork. Acta Veterinaria Scandinavica 31 (1): 39-43. Vet. Bull. 61 (1): 45.
- Aulisio, C.C.G., Mehlman, I.J. and Sanders, A.C. (1980). Alkali method for rapid recovery of Yersinia enterocolitica and Yersinia pseudotuberculosis from foods. Appl. Environ. Microbiol. 39 (1): 135-140.

- Bisset, M.J. (1981). Microbiological aspects of Yersinia pseudotuberculosis. In: Yersinia enterocolitica, (Ed.) Bottone, E.J., CRC Press, Inc., Florida. pp. 31-40.
- Black, R.E., Jackson, R.J., Tsai, T., Medvesky, M., Shayegani, M., Feeley, J.C., Macleod, K.I.E. and Wakelee, A.M. (1978). Epidemic Yersinia enterocolitica infection due to contaminated chocolate milk. N. Engl. J. Med. **298**: 76-79.
- *Boemi, G., Guerra, C., Levi Della Vida, A., Levi Della Vida, M.V. and Pinzi, M. (1982). Isolation of Yersinia enterocolitica from soft palate of fattening pigs. Annali Scalvo. **24** (1): 25-30. Vet. Bull. **53** (6): 3783.
- *Boer, E.dE. and Nouws, J.F.M. (1991). Slaughter pigs and pork as a source of human pathogenic Yersinia enterocolitica. Int. J. Fd. Microbiol. **12**: 379-382.
- *Boer, D.dE., Seldam, W.M. and Oosterom, J. (1986). Characterization of Yersinia enterocolitica and related species isolated from foods and porcine tonsils in Netherlands. Int. J. Fd. Microbiol. **3** (4): 217-224. Dairy Sci. Sci. **48** (10): 5702.
- Bottone, E.J. (1981). Yersinia enterocolitica CRC press, Inc. Florida. pp.4, 9-14, 31-39, 161-169.
- Brenner, D.J. (1981). Classification of Yersinia enterocolitica In: Yersinia enterocolitica. (Ed.) Bottone, E.J., CRC Press, Inc., Florida. pp. 1-8.

- *Brewer, R.A. and Corbel, M.J. (1983). Characterization of Yersinia enterocolitica strains isolated from cattle, sheep and pigs in UK. J. Hyg. 90 (3): 425-433. Vet. Bull. 53 (10): 6207.
- Bullians, J.A. (1987). Yersinia species infection of lamb and cull cows at an abattoir. N.Z. Vet. J. 35 (5): 65-67.
- Busta, F.F. (1976). Practical implications of injured micro-organisms in food. J. Milk Fd. Technol. 39 (2): 138-145.
- *Cattean, M., Krembel, C. and Wauters, G. (1983). Isolation of Yersinia enterocolitica from tongues of swine. Recueil de Medicine Veterinaire. 159 (2): 89-94. Vet. Bull. 53 (7): 4410.
- *Christensen, S.G. (1981). The Yersinia enterocolitica prevalence in slaughter animals, water and raw milk in Denmark. Psychrotrophic microorganisms in spoilage and pathogenicity. London Academic Press. pp. 439-445. Dairy Sci. Abstr. 45 (2): 1133.
- Christensen, S.G. (1987). The Yersinia enterocolitica situation in Denmark. Contr. Microbiol. Immunol. 9: 93-97. Cited in Fukushima et al. (1989). Role of contaminated skin of pigs in fecal Yersinia contamination of pig carcasses at slaughter. Fleischwirtsch. 69 (3): 369-372.
- Corbel, M.J. (1990). Yersinia. In: Topley and Wilson's Principles of Bacteriology, Virology and Immunity. Vol.2. (Eds.) Parker, M.T. and Duerden, B.I. 8th Edn. p.449.

- *Cox, N.A., Bailey, J.S., Correl, D.F. and Shotts, E.B. (1990). Comparison of enrichment and plating media for isolation of *Yersinia*. Poult. Sci. 69 (4): 686-693. Fd. Technol. Abstr. 26 (9): 1991.
- Cowan, S.T. (1974). Cowan and Steele's Manual for Identification of Medical Bacteria. 2nd Edn. Cambridge University Press. pp. 140-180.
- Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. (1975). Medical Microbiology. Vol.II. 12th Edn. pp. 34-35.
- Das, A.M. (1985). Seroprevalence of Yersiniosis and Brucellosis among indigenous pigs. Ind. J. Anim. Sci. 55 (9): 753-758.
- Davey, G.M., Bruce, J. and Drysdale, E.M. (1983). Isolation of *Yersinia enterocolitica* and related species from faeces of cow. J. Appl. Bacteriol. 55 (3): 439-443.
- *Delia, S., Mauro, A. and Donia, D., Zaccona, R. and Fauci, V. (1984). *Yersinia enterocolitica* in Italian slaughter pigs. Igiene Moderna. 81 (2): 298-302. Vet Bull. 55 (2): 630.
- *Doyle, M.P. (1985). Food borne pathogens of recent concern. Annual Review of Nutrition. 5: 25-41. Dairy. Sci. Abstr. 49 (7): 4424.
- Doyle, M.P., Hugdahl, M.B. and Taylor, S.L. (1981). Isolation of Virulent *Yersinia enterocolitica* from porcine tongues. Appl. Environ. Microbiol. 42 (4): 661-666.

- EL-Zawahry, A.Y. and Grez, N. (1981). Inactivation and injury of Yersinia enterocolitica by radiation and freezing. Appl. Environ. Microbiol. **41**: 464-468.
- Esseveld, H. and Goudzwaard, C. (1973). On the epidemiology of Yersinia enterocolitica infections: Pigs as a source of infections in man. Contrib. Microbiol. Immunol. **2**: 99-101.
- Feeley, J.C. (1971). Isolation techniques for Yersinia enterocolitica. In: Yersinia enterocolitica, Ed. Bottone, E.J. CRC press, Inc. Florida. pp. 9-14.
- *Feinhaken, D., Sechter, I., Press, L. and Elad, D. (1984). Yersinia enterocolitica in pigs and cattle, Israel, 1982/83. Israel J. Med. Sci. **20** (5): 475. Vet. Bull. **54** (12): 7672.
- *Franzin, L. and Fantino, P. (1984). Isolation of Yersinia enterocolitica from raw milk. Igiene Moderna. **81** (3): 459-482. Vet. Bull. **55** (2): 627.
- Fukushima, H. (1985). Direct isolation of Yersinia enterocolitica and Yersinia pseudotuberculosis from meat. Appl. Environ. Microbiol. **50** (3): 710-712.
- Fukushima, H. (1986). Yersinia enterocolitica serotype O3 in naturally infected raw pork. Jpn. J. Vet. Sci. **48** (1): 183-187.
- *Fukushima, H. (1987). New selective agar medium for isolation of virulent Yersinia enterocolitica. J. Clin. Microbiol. **25** (6): 1068-1073. Vet. Bull. **57** (10): 6217.

Fukushima, H. and Gomyoda, M. (1986a). Inhibition of Yersinia enterocolitica serotype O3 by natural microflora of pork. Appl. Environ. Microbiol. 51 (5): 990-994.

*Fukushima, H. and Gomyoda, M. (1986b). Growth of Yersinia pseudotuberculosis and Yersinia enterocolitica biotype 3B serotype O3 inhibited on cefsulodin-Irgasan. Novobiocin agar. J. Clin. Microbiol. 24 (1): 116-120. Vet. Bull. 56 (10): 6658.

Fukushima, H. and Gomyoda, M. (1991). Intestinal carriage of Yersinia pseudotuberculosis by wild birds and mammals in Japan. Appl. Environ. Microbiol. 57 (4): 1152-1155.

Fukushima, H., Gomyoda, M., Ishikura, S., Nishio, T., Morki, S., Endo, J., Kaneko, S. and Tsubokura, M. (1989). Contaminated environmental substances lead to Yersinia pseudotuberculosis infection in children. J. Clin. Microbiol. 27 (12): 2706-2709.

Fukushima, H., Gomyoda, M. and Kaneko, S. (1990). Mice and moles inhabiting mountainous areas of Shimane peninsula as sources of infection with Yersinia pseudotuberculosis. J. Clin. Microbiol. 28 (11): 2448-2455.

Fukushima, H., Hoshina, K., Nakamura, R. and Ito, Y. (1987). Occurrence of Yersinia spp. in raw beef, pork and chicken. Zbl. Bakt. Hyg. B 184: 50-59.

- Fukushima, H., Hoshina, K., Nakamura, R., Ito, Y. and Gomyoda, M. (1987). Epidemiological study of Yersinia enterocolitica and Yersinia pseudotuberculosis in Shimane prefecture, Japan. Contr. Microbiol. Immunol. 9: 103-110.
- Fukushima, H., Maruyama, K., Omori, I., Ito, K. and Iorihara, M. (1989). Role of the contaminated skin of pigs in faecal Yersinia contamination of pig carcasses at slaughter. Fleischwirtschaft. 69 (3): 369-372.
- Fukushima, H., Maruyama, K., Omori, I., Ito, K. and Iorihara, M. (1990). Contamination of pigs with Yersinia at the slaughter house. Fleischwirtschaft. 70 (11): 1330-1335.
- Fukushima, H., Maruyama, K., Omori, I., Ito, K. and Kaneko, S. (1988). Isolation of sucrose-negative Yersinia enterocolitica biotype 3 serotype O3 strains and their pathogenicity. Curr. Microbiol. 17: 199-202.
- Fukushima, H., Nakamura, R., Iitsuka, S., Tsubokura, M., Otsuki, K., Kawaoka, Y. (1984). Prospective systematic study of Yersinia spp. in dogs. J. Clin. Microbiol. 19 (5): 616-622.
- Fukushima, H., Nakamura, R., Ito, Y. and Saito, K. (1983). Ecological studies on Yersinia enterocolitica. I. Dissemination of Yersinia enterocolitica in pigs. Vet. Microbiol. 8 (5): 469-483.

- Fukushima, H. and Nakamura, R. (1984). Ecological studies of Yersinia enterocolitica. II. Experimental infection with Yersinia enterocolitica in pigs. III. Cross protection against faecal excretion between Yersinia enterocolitica serovar 3 and 5.27 in pigs. Vet. Microbiol. 9 (4): 375-389.
- Fukushima, H., Sato, T., Nagasako, R. and Takeda, I. (1991). Acute mesenteric lymphadenitis due to Yersinia pseudotuberculosis lacking a virulence plasmid. J. Clin. Microbiol. 29 (6): 1271-1275.
- Fukushima, H. and Tsubokura, M. (1985). Yersinia enterocolitica biotype 3B serotype O3 phage type II infection in pigs. Jpn. J. Vet. Sci. 47 (6): 1011-1015.
- *Gravani, R.B. (1986). Yersinia enterocolitica. Dairy and food sanitation. 6 (10): 443-444. Dairy Sci. Abstr. 49 (3): 1608.
- *Greenwood, M.H. and Hooper, W.L. (1989). Improved methods for isolation of Yersinia species from milk and foods. Fd. Microbiol. 6 (2): 95-104. Fd. Technol. Abstr. 1990. 25 (1): 143.
- *Greenwood, M.H. and Hooper, W.L. (1990). Excretion of Yersinia species associated with consumption of pasteurized milk. Epidemiology and Infection. 104 (3): 345-350. Dairy Sci. Abstr. 52 (9): 6541.

- *Guallini, L., Malaspina, C. and Pastoni, F. (1986). Variant of traditional method of isolating Yersinia enterocolitica from samples of tonsils and intestinal lymphnodes of swine. Archivlo Veterinario Italiana. 37 (3/6): 163-169. Vet. Bull. 57 (5): 2489.
- *Hanna, M.O., Stewart, J.C., Carpenter, Z.L. and Vanderzant, C. (1977a). Heat resistance of Yersinia enterocolitica in skin milk. J. Fd. Sci. 42 (4): 1134. Dairy Sci. Abstr. 1978 (40): 1521.
- Hanna, M.O., Stewart, J.C., Zink, D.L., Carpenter, Z.L. and Vanderzant, C. (1977b). Development of Yersinia enterocolitica on raw and cooked beef and pork at different temperatures. J. Fd. Sci. 42 (5): 1180-1181.
- Hanna, M.O., Smith, G.C., Hall, L.C., Vanderzant, C. and Childres, A.B. (1980). Isolation of Yersinia enterocolitica from pig tonsils. J. Fd. Prot. 43 (1): 23-25.
- Hanna, M.O., Zink, D.L., Carpenter, Z.L. and Vanderzant, C. (1976). Yersinia enterocolitica like organisms from vacuum packaged beef and lamb. J. Fd. Sci. 41 (5): 1254-1256.
- Harmon, M.C., Yu, C.L. and Swaminathan, B. (1983). An evaluation of selective differential plating media for isolation of Yersinia enterocolitica from experimentally inoculated fresh ground pork homogenate. J. Fd. Sci. 48 (1): 6-9.

- *Hawari, A.D., Amtsberg, G. and Kripal, G. (1981). Cultural and serological investigation into the frequency of Yersinia enterocolitica infection in pigs and cattle. Berliner and Munchener Tierarztliche Wochenschrift. 94 (20): 404-409. Vet. Bull. 52 (7): 3705.
- *Huges, D. (1979). Isolation of Yersinia enterocolitica from milk and a dairy farm in Australia. J. Appl. Bacteriol. 46 (1): 125-130. Vet. Bull. 48 (8): 4323.
- *Hurvell, B., Danielsson-Tham, M.L. and Olsson, E. (1981). Zoonotic aspects of Yersinia enterocolitica with special reference to its ability to grow at low temperature. Psychrotrophic microorganism in spoilage and pathogenicity - London, UK. Academic Press. pp. 393-399. Dairy Sci. Abstr. 45 (2): 986.
- Ibrahim, A. and Macrae, I.C. (1991). Isolation of Yersinia enterocolitica and related species from red meat and milk. J. Fd. Sci. 56 (6): 1524-1526.
- Ichinohe, H., Yoshioka, M., Fukushima, H., Kaneko, S. and Maruyama, T. (1991). First isolation of Yersinia enterocolitica serotype 0:8 in Japan. J. Clinical Microbiol. 29 (4): 846-847.
- *Jackova, S. and Urgeova, E. (1992). Yersinia enterocolitica 03 in the oral cavity of slaughter pigs, and in the meat and meat products. Biologia (Bratislava), 47 (9): 737-743. Vet Bull. 63 (10): 6395.
- Jones, T.O., Mair, N.S. and Fox, E. (1982). Caprine mastitis associated with Yersinia pseudotuberculosis. Vet. Rec. 110 (10): 231.

- *Khalafalla, F.A. (1990). Yersinia enterocolitica in processed poultry. Fleischwirtschaft. 70 (3): 305-306. Vet Bull. 61 (6): 3924.
- Knapp, W. and Thal, E. (1973). Differentiation of Yersinia enterocolitica by biochemical reactions. Contr. Microbiol. Immunol. 2: 10-16.
- *Kolos, E.N., Gnutov, I.N., Yushchenko, G.V. and Gunaev, V.I. (1985). Agricultural animals as a source of Yersiniosis. Zhurnal Mikrobiologii Epidemiologii, Immunobiologii 4: 77-80, 128. Dairy. Sci. Abstr. 49 (9): 5725.
- Koneuchi, C., Shibata, M., Kawasaki, T., Karia, T., Kanzaki, M. and Maruyama, T. (1989). Occurrence of Yersinia species in migratory birds, ducks, sea gulls and swallows in Japan. Jpn. J. Vet. Sci. 51 (4): 805-807.
- Kotula, A.W. and Sharar, A.K. (1993). Presence of Yersinia enterocolitica serotype 0:5, 27 in slaughter pigs. J. Fd. Prot. 56 (3): 215-218.
- Kwaga, J.P., Adesiyun, A.A., Lombin, L.H., Mijinyawa, S.M. and Agbonlahor, D.E. (1987). Cultural and serological studies on camels in Nigeria. Can. Vet. J. 28 (1/2): 42-44.
- Kwaga, J.P., Iversen, J.C. and Saunders, J.R. (1990). Comparison of two enrichment protocols for detection of Yersinia in slaughtered pigs and pork products. J. Fd. Prot. 53 (12): 1047-1049.

- Leistner, L., Hechelmann, H., Kashiwazaki, M. and Albertz, R. (1975). Nachweis Von Yersinia enterocolitica in Faeces and Fleisch Von Schweinen, Rindern und Geflugel, Fleischwirtschaft, 55, 1599. Cited In: Bottone, E.J. (1981). Yersinia enterocolitica, p. 167. CRC Press, Inc., Florida.
- Marinelli, G., D'innocenzo, C., Fabiani, L. and Leoni, V. (1985). Application of a simplified method for recovery of Yersinia enterocolitica from surface water. Appl. Environ. Microbiol. 49 (5): 1348-1349.
- McManus, C. and Lanier, J.M. (1987). Salmonella, Campylobacter jejuni and Yersinia enterocolitica in raw milk. J. Fd. Prot. 50 (1): 51-55.
- *Mercado, E.C. and Ibanez, S.B. (1986). Isolation of Yersinia enterocolitica from raw cow milk in Argentina. J. Food. Microbiol. 3 (4): 237-242. Dairy Sci. Abstr. 48 (10): 5703.
- *Merilahti-Palo, R., Lehesmaa, R., Granfors, K., Gripenberg-Lerche, C. and Toivanen, P. (1991). Risk of Yersinia infection among butchers. Scandinavian. J. Infectious Diseases. 23 (1): 55-61. Vet. Bull. 61 (5): 3214.
- *Morse, D.L., Shayegani, M. and Gallo, R.J. (1984). Epidemiological investigation of a Yersinia camp outbreak linked to a food handler. Amer. J. Public Hlth. Dairy Sci. Abstr. 47 (5): 2695.



- *Mraz, O., Cizek, A. and Hejlicek, K. (1989). Concurrent surveys for Yersinia in slaughter pigs, synanthropic animals and livestock personnel in a limited area. Veterinari Medicina. 34 (10): 613-622. Vet. Bull. 60 (9): 6046.
- *Narucka, U. and Westendorp, J.F. (1977). Occurrence of Yersinia enterocolitica and Yersinia pseudotuberculosis in clinically normal pigs. Tijdschrift Voor Diergeneseskunde. 102 (5): 299-303. Vet Bull. 47 (8): 4181.
- *Nesbakken, T., Kapperud, G., Lassen, J. and Skjerve, E. (1991). Yersinia enterocolitica 0:3 antibodies in slaughter house employees, Veterinarians and military recruits. Occupational exposure to pigs as a risk factor for Yersiniosis. Contr. Microbiol. Immunol. 12: 32-39. Vet. Bull. 62 (4): 1685.
- *Norlyng, P. (1982). Occurrence of Yersinia enterocolitica in raw meat, meat products and raw and heat treated milk. Dansk Veterinaertidsskrift. 65 (9): 421-424. Vet. Bull. 52 (8): 4484.
- *Nunes, M.P. and Ricciardi, I.D. (1986). Yersinia enterocolitica: Concomitant isolation from faeces of man and dog. Revista de Microbiologia, Brazil. 17 (3): 220-224. Vet. Bull. 57 (3): 1176.
- Nwosuh, E.N. and Adesiyun, A.A (1989). Experimental studies on Yersinia enterocolitica infection in chicken exposed at 1 day old. British Poult. Sci. 30 (1): 91-99. Vet. Bull. 60 (3): 1396.

- Okoroafor, E., Adesiyu, A.A. and Agbonlahor, D.E. (1988). Prevalence and characteristics of Yersinia enterocolitica strains isolated from pigs in Jos, Nigeria. British Vet. J. **144** (2): 131-138.
- *Palumbo, S.A. (1986). Is refrigeration enough to restrain food borne pathogens? J. Fd. Prot. **49** (12): 1003-1009. Dairy Sci. Abstr. **49** (4): 2168.
- *Pavlov, A., Filipov, V., Khvurchilkov, V. and Danev, I. (1988). Occurrence of Yersinia enterocolitica in lactating cows. Veterinarna Sbirka. **86** (2): 47-49. Vet. Bull. **58** (9): 5180.
- *Popovic, M. and Markovic, B.S. (1984). Isolation and identification of Yersinia enterocolitica. Veterinarski Glasnik. **38** (3): 227-231. Vet. Bull. **55** (9): 5442.
- Pramanik, A.K., Bhattacharyya, H.M., Chatterjee, A. and Sengupta, D.N. (1980). Isolation of Yersinia enterocolitica from human and animal sources in the same family. Ind. J. Anim. Hlth. **19** (1): 79-81.
- *Pugina, P., Benzi, G., Giralaldi, V., Mingrane, M.G. and Fantasia-Mazzotti, M. (1984). Yersinia species strains isolated from raw milk samples and bovine and human stools in Rovigo Province. Bollettino dell Institute Sieroterapico Milanex. Dairy Sci. Abstr. **47** (1): 228.

- *Rhodehamel, E.J., Reddy, N.R. and Pierson, M.D. (1987). Growth of Staphylococcus aureus S-6 and Yersinia enterocolitica CDCA 2635 in cow, soy and winged bean milk. J. Fd. Qly. 9 (6): 387-398. Dairy Sci. Abstr. 49 (9): 5724.
- *Rindi, S., Cerri, D. and Farina, R. (1989). Yersinia enterocolitica in some foods of animal origin. Industrie Alimentari. 28 (273): 713-715. Dairy Sci. Abstr. 52 (2): 911.
- Rohrbach, B.W., Draughow, F.A., Davidson, M.P. and Oliver, S.P. (1992). Prevalence of Listeria monocytogenes, Campylobacter jejuni, Yersinia enterocolitica and Salmonella in tank milk: Risk factors and risk of human exposure. J. Fd. Prot. 55 (2): 93-97.
- Roy, R.N. (1986). The lipolytic activity of extracellular enzymes of Yersinia enterocolitica inoculated into chilled and frozen cream. J. Dairy Sci. 69 (71): 236.
- Sanekata, T., Yoshikawa, N., Otsuki, K. and Tsubokura, M. (1991). Yersinia pseudotuberculosis isolation from Cockatoo. J. Vet. Med. Sci. 53 (1): 121-122.
- Sathyanarayana Rao, M., Upadhye, A.S. and Raghavan, R. (1991). Isolation and characterization of Yersinia enterocolitica from apparently healthy pigs. Mysore J. Agric. Sci. 25: 370-375.
- Schiemann, D.A. (1982). Development of a two step enrichment procedure for recovery of Yersinia enterocolitica from food. Appl. Environ. Microbiol. 43 (1): 14-27.

- Schiemann, D.A. and Fleming, C.A. (1981). Yersinia enterocolitica isolated from throat of swine in Eastern and Western Canada. Canadian J. Microbiol. 27 (12): 1326-1333. Vet. Bull. 52 (6): 2884.
- Schiemann, D.A. and Toma, S. (1978). Isolation of Yersinia enterocolitica from raw milk. Appl. Environ. Microbiol. 35 (1): 54-58.
- *Schoilew, C. and Kosarow, A. (1987). Yersinia enterocolitica infections in man and in some species of farm animals in Bulgaria. Monatshefte fur Veterinarmedizin. 42 (5): 164-165. Vet. Bull. 57 (8): 4776.
- *Shayegani, M., Morse, D., DeForge, I., Root, T., Parsons, L.M. and Maupin, P.S. (1983). Microbiology of a major food borne outbreak of gastroenteritis caused by Yersinia enterocolitica Serogroup 0:8. J. Clin. Microbiol. 17 (1): 35-40. Dairy Sci. Abstr. 45 (7): 497.
- *Shiozawa, K, Nishina, T., Miwa, Y., Mori, T., Akahena, S. and Ito, K. (1991). Colonization in tonsils of swine by Yersinia enterocolitica. Contr. Microbiol. Immunol. 12: 63-67. Vet. Bull. 62 (4): 1686.
- *Slavchev, G. (1989). Growth and survival of Yersinia enterocolitica in butter. Veterinarna Sbirka. 87 (3): 5052. Dairy Sci. Abstr. 52 (2): 1068.

- Sonnenwirth, A.C. (1990). Gram negative bacilli, Vibrios and Spirilla. In: Gradwohl's Clinical Laboratory Methods and Diagnosis. Vol.II. Ed. by Sonnenwirth, A.C. and Jarett, L. pp. 1731-1784. B.I. Publications Ltd., New Delhi.
- *Sonnenwirth, A.C. and Weaver, R.E. (1970). Yersinia enterocolitica. N. Engl. J. Med. 283: 1464. Cited by Hanna et al. (1977) in Effect of treating, freezing and pH on Yersinia enterocolitica - like organism from meat.
- Sorqvist, S. and Danielsson-Tham, M.L. (1990). Survival of Campylobacter, Salmonella and Yersinia species in scalding water used at pig slaughter. Fleischwirtschaft. 70 (12): 1451-1454. Vet. Bull. 61 (6): 3903.
- Srivastava, C.P., Iyer, P.K.R., Gupta, S.C. and Mataney, C.F. (1978). A note on psudotuberculosis in Guinea pigs. Ind. J. Anim. Sci. 48 (7): 552-555.
- *Stanfield, J.T., Jackson, G.J. and Aulisio, C.C.G. (1985). Yersinia enterocolitica: Survival of a pathogenic strain on milk containers. J. Fd. Prot. 48 (11): 947-948. Dairy Sci. Abstr. 49 (2): 951.
- Stern, N.J. and Pierson, M.D. (1979). Yersinia enterocolitica: A review of the psychrotrophic water and food borne pathogen. J. Fd. Sci. 44: 1736-1741.

- Stern, N.J., Pierson, M.D. and Kotula, A.W. (1980). Effects of pH and sodium chloride on Yersinia enterocolitica growth at room and refrigeration temperatures. J. Fd. Sci. 45: 64-67.
- *Stefanov, I. and Kunev, Z. (1990). Isolation of Yersinia enterocolitica and related species from slaughtered pigs. Veterinarna sbirka. 88 (5/6): 43-46. Vet. Bull. 61 (2): 991.
- *Stengel, G. (1983). Occurrence of Yersinia enterocolitica. Inaugural-Dissertation, Fachbereich Veterinarmedizin, Freie Universitat, Berlin. pp. 150. Vet Bull. 54 (2-3): 460.
- *Sung, K.C. and Choi, W.P. (1987). Characterization of Yersinia species isolated from animals in Korea. Korean J. Fd. Res. 27 (2): 235-243. Vet. Bull. 56 (6): 3243.
- Sulochana, S. and Sudharma, D. (1985). Isolation of Yersinia pseudotuberculosis from cases of abortion in goats. Kerala J. Vet. Sci. 16 (2): 139-142.
- *Szita, J., Svidro, A., Kubinyi, J., Nyomarkay, I. and Mihalyfi, I. (1978). Yersinia enterocolitica infection of animals and persons in contact with them. Magyar Allatorvosok Lapja. 33 (6): 398-402. Vet. Bull. 48 (12): 7039.
- Tashiro, K., Kubokura, Y., Kato, Y., Kaneko, K. and Ogawa, M. (1991). Survival of Yersinia enterocolitica in soil and water. J. Vet. Med. Sci. 53 (1): 23-27.

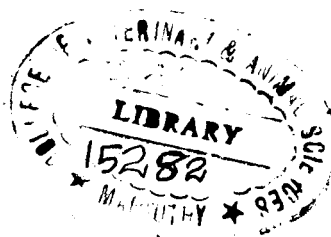
- Tauxe, R.V., Vandepitte, J., Wanters, G., Martin, S.M., goossens, V., Mol, P.DE., Noyen, R.V. and Their, G. (1987). Yersinia enterocolitica infection and pork: the missing link. Lancet. 1 (8542): 1129-1132.
- Toma, S. and Deidrick, V.R. (1975). Isolation of Yersinia enterocolitica from swine. J. Clin. Microbiol. 2 (6): 478-481.
- Tsubokura, M., Fukuda, T., Otsuki, K., Kubota, M., Itagaki, K., Yamaoka, K. and Wakatsuki, M. (1975). Studies on Yersinia enterocolitica. II. Relationship between detection from swine and seasonal incidence and regional distribution of the organism. Jap. J. Vet. Sci. 38 (1): 1-6.
- Tsubokura, M., Otsuki, K., Fukuda, T., Kubota, M., Inamura, M., Itagaki, K., Yamaoka, K. and Wakatsuki, M. (1976). Studies on Yersinia pseudotuberculosis IV. Isolation of Yersinia pseudotuberculosis from healthy swine. Jap. J. Vet. Sci. 38 (6): 549-552.
- *Turkson, P.K., Lindqvist, K.J. and Kapperud, G. (1988). Isolation of Campylobacter spp. and Yersinia enterocolitica from domestic animals and human patients in Kenya. Acta Pathologica, Microbiologica et Immunologica scandinavica. 96 (2): 141-146. Vet. Bull. 56 (6): 3218.
- *Umoh, V., Dangana, A. and Umoh, J.U. (1984). Isolation of Yersinia enterocolitica from milk and milk products in Zaria, Nigeria. Int. J. Zoonoses. 11 (2): 223-228. Vet. Bull. 55 (8): 4795.

- *Vasavada, P.C. and Larkin, L. (1985). Incidence of Yersinia enterocolitica in relation to quality of milk. J. Dairy Sci. 68 (1): 82. Dairy Sci. Abstr. 47 (11): 6920.
- *Vasilev, P. (1986). Yersinia enterocolitica in throat of pigs. Veterinarna sbirka. 84 (2): 30-31. Vet. Bull. 56 (7): 4220.
- Verma, N.K. and Misra, D.S. (1984). Isolation of enterotoxigenic Yersinia enterocolitica from pigs in UP. Ind. J. Anim. Sci. 54 (7): 659-662.
- *Walker, S.J. and Gilmour, A. (1986). Incidence of Yersinia enterocolitica and Yersinia enterocolitica like bacteria in goat milk in Northern Ireland. Letters in Applied Microbiology. 3 (2): 49-52. Vet. Bull. 57 (1): 41.
- Wauters, G. (1973). Improved methods for the isolation and recognition of Yersinia enterocolitica. Contr. Microbiol. Immunol. 2: 68-70.
- Wauters, G., Goossens, V., Janssens, M. and Vandepitte, J. (1988). New enrichment for isolation of pathogenic Yersinia enterocolitica serogroup 0:3 from pork. Appl. Environ. Microbiol. 54 (4): 851-854.
- *Weber, A. and Knapp, W. (1981). Detection of Yersinia enterocolitica and Yersinia pseudotuberculosis in faecal samples of healthy slaughter pigs in relation to season. Zentralblatt fur Veterinarmedizin B. 28 (5): 407-413. Vet. Bull. 52 (7): 3704.

- *Weber, A. and Lembke, C. (1981). Comparison of two selective methods and five selection media for isolation of Yersinia enterocolitica from tonsil of slaughter pigs. Zentralblatt fur Bakteriologie Mikrobiologic und Hygiene. 250 (4): 72-77. Vet. Bull. 52 (2): 543.
- *Weber, A. and Lembke, C. (1982). Occurrence of Yersinia pseudotuberculosis in slaughter pigs. Fortschritte der Veterinarmedizin. 35: 356-358. Vet. Bull. 52 (11): 6695.
- *Yu, E.S., She, J.H., Cai, Z.Q., Chen, E., Zhang, B.Y. and Zhu, H.F. (1983). Outbreak of Yersinia enterocolitica infection in pigs. Chinese J. Vet. Med. 9 (5): 5-6. Vet. Bull. 54 (9): 5466.
- *Yurukov, M. and Slavchev, G. (1984). Improved method for isolating Yersinia enterocolitica. Veterinarnomeditsinski Nanki 21 (6): 75-83. Vet. Bull. 54 (12): 7670.
- *Zamora, J., Munoz, A. and Alonso, O. (1981). Isolation of Yersinia enterocolitica from caecal contents of cattle in South Chile. Zentralblatt fur Veterinarmedizin B. 28 (6): 503-505. Vet. Bull. 52 (5): 2173.
- *Zeng, G.J., Chen, M.G. and Sun, Y.Q. (1982). Isolation and identification of Yersinia enterocolitica in swine for first time in China. Chinese J. Vet. Med. 8 (2): 2-5. Vet. Bull. 53 (2): 942.

- Zink, D.L., Victorlachica, R. and Dubel, J.R. (1982). Yersinia enterocolitica and Yersinia enterocolitica like species: Their pathogenicity and significance in food. J. Fd. Safety. 4: 223-241.
- Zowghi, E. and Ebadi, A. (1986). Isolation and identification of Yersinia enterocolitica serotype 0:9 in cattle in Iran. Archives de L'Institut Razi. 36/37: 79-83. Vet. Bull. 58 (2): 655.

* Originals not seen



Appendices

Appendix-I

1. *Yersinia* isolation agar (Himedia)

	Grams/litre
Peptone special	20.0
Yeast extract	2.0
Mannitol	20.0
Sodium pyruvate	2.0
Sodium chloride	1.0
Magnesium sulphate	0.01
Sodium deoxycholate	0.50
Neutral red	0.03
Crystal violet	0.001
Agar	12.5

Final pH 7.4 ± 0.2

Yersinia selective supplement FD 034

Formula

Per vial (Sufficient for 500 ml medium)

Cefsulodin	7.5 mg
Irgasan	2.0 mg
Novobiocin	1.25 mg

Add 2 ml of sterile distilled water and 1 ml of ethyl alcohol to 1 vial. Shake gently to dissolve and add to 500 ml of Yersinia isolation agar.

2. Urea medium (Cowan, 1974)

Peptone	1.00 g
Sodium chloride	5.00 g
Secondary potassium phosphate (KH_2PO_4)	2.00 g
Agar	20.00 g
Distilled water	1000 ml

Dissolved the ingredients by heating, adjusted pH to 6.8, sterilized at 115°C for 20 minutes.

Added

Glucose	1.00 g
Phenolred 0.2% sol	6 ml

to molten base, steamed for one hour and cooled to 50-55°C

Added urea 20 per cent aqueous solution, sterilized by filtration, 100 ml aseptically to the base. Distributed aseptically into sterile test tubes and allowed to cool as slopes.

3. Oxidase strips (Cruickshank et al., 1975)

Dry filter paper method

Strips of Whatman's No.1 filter paper are soaked in a freshly prepared one per cent solution of tetramethyl p-phenylene-dimamine dihydrochloride. The strips are dried and stored in dark bottle, tightly sealed. The papers have a light purple tint.

4. Decarboxylase media (Cowan, 1974)

Peptone	1 g
Yeast extract	3 g
Glucose	1 g
Distilled water	1000 ml
Brom Cresol purple 0.2 per cent solution	10 ml

5. Phenylalanine deaminase medium

Yeast extract	3 g
L-phenylalanine	1 g
Disodium hydrogen phosphate (Na_2HPO_4)	1 g
Sodium chloride	5 g
Agar	12 g
Distilled water	1000 ml
Adjust pH to 7.4	

6. Indole Medium (Cruickshank, et al., 1975)

Peptone	20 g
Sodium chloride	5 g
Distilled water	1000 ml

Adjust pH to 7.4. Dispensed and sterilized by autoclaving at 121°C for 15 minutes.

Kovac's reagent

Iso-amyl alcohol	150 ml
p-dimethyl-aminobenzaldehyde	10 g
Concentrated hydrochloric acid	50 ml

Dissolved the aldehyde in alcohol and slowly added acid.
Stored in refrigerator shaken gently before use.

7. Sugar fermentation

Basal media (Cowan, 1974)

Peptone	10 g
Sodium chloride	5 g
Distilled water	1000 ml

Dissolved the ingredients in warm water, adjusted pH 8.0 to 8.4. Boiled for 10 minutes filtered, adjusted the pH to 7.2 to 7.4 and sterilized at 115°C for 20 minutes.

Sterilization of sugars

Dissolved the sugar in distilled water and sterilized by filtration using seitz filter.

Preparation of sugar media (Cowan, 1974)

To 900 ml of basal media already prepare, added 10 ml of 0.2 per cent Bromthymol blue indicator and sterilized at 115°C for 20 minutes. Added approximately 90 ml of the appropriate sugar solution which was previously prepared, sterilized and mixed. About 5 ml of the mixture was distributed into sterile test tubes.

Appendix-II

McFarland Nephelometer standards (Sonnenwirth and Jarett, 1990)

Tube	Sulphuric acid one per cent aq. solution (ml)	BaCl ₂ one per cent (ml)	Corresponding density (million/ml)	I.U. of opacity
1.	9.9	0.1	300	3
2.	9.8	0.2	600	7
3.	9.7	0.3	900	10
4.	9.6	0.4	1200	12
5.	9.5	0.5	1500	15
6.	9.4	0.6	1800	--
7.	9.3	0.7	2100	20
8.	9.2	0.8	2400	--
9.	9.1	0.9	2700	--
10.	9.0	1.0	3000	30

Standardization of bacterial suspensions

1. Make sets of nephelometric tubes by mixing one per cent sulphuric acid with one per cent barrium chloride.
2. Tubes must be of uniform size.
3. Plug tubes with rubber stoppers and carefully seal with paraffin. Set tubes in rack.
4. For estimation, compare bacterial suspension with standards.
5. This serves to determine appoximately the number of bacteria present in saline suspension.

OCCURRENCE AND SURVIVABILITY OF YERSINIA IN PORK

By

B. SUNIL

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Veterinary Public Health
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy, Thrissur

1994

ABSTRACT

Investigation was carried out to find out the the presence and survivability of Yersinia in pork. One hundred and seventy one samples were collected from 39 animals. Three isolates of Yersinia pseudotuberculosis were obtained using Yersinia isolation agar (Himedia). Two of the isolates were from the buccal cavity and tonsil of the same animal and the third from the tonsil of another animal. Even when the organism could not be isolated by direct plating, cold enrichment enabled isolation of the organism from the same sample.

The organism could be recovered from inoculated and stored (4°C) pork samples upto seven days. At -15°C storage, the organism could be recovered upto 30 days by direct plating. Direct plating failed to recover the organism on 45th day, but resuscitation technique was effective, which underlined the importance of resuscitation for isolation of the organism from frozen samples. On 60th day resuscitation also failed to recover the organism.

15282