CONTAMINATION OF VARIOUS FOODS OF ANIMAL ORIGIN WITH *YERSINIA ENTEROCOLITICA* AND ITS RELATED SPECIES

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Summary

A total of 332 raw foods samples of animal origin, consisting of 219 (66%) raw meat, 93 (28%) meat products and 20 (6%) chicken carcass rinses were examined for the presence of *Yersinia*. This study was conducted at a local Regional Veterinary Diagnostics Laboratory in Petaling Jaya over eight months period of study. The frequencies of the positive samples were 1/94 of raw beef (1.1%), 16/47 of beef burgers (34%), 6/114 of raw chicken meat (5.3%), 1/30 of chicken burger (3.3%) and 1/20 of chicken carcass rinses (5%). The 53 isolates were identified as four different species of *Yersinia* namely *Y. enterocolitica* (29), *Y. frederiksenii* (18), *Y. kristensenii* (3) and *Y. intermedia* (3). All of the *Y. enterocolitica* belonged to biotype 1A as indicated by the positive results for salicin-esculin, xylose, nitrate, pyrazinamidase and therefore regarded as presumptively avirulent food isolates.

Keywords : *Yersinia enterocolitica*, related species, foods of animal origin, biotype 1A, avirulent

INTRODUCTION

Many researchers have investigated the presence of pathogens in food of animal origin in Malaysia. The pathogens included *Listeria monocytogenes*, *Salmonella* serotypes, *Escherichia coli* O157:H7, vancomycin-resistant *Enterococcus faecium*, *Campylobacter jejuni* and *Vibrio parahaemolyticus* (Arumugaswamy *et al.*, 1994; Rusul *et al.*, 1996; Son *et al.*, 1996; Son *et al.*, 1998a; Son *et al.*, 1998b; Son *et al.*, 1999). Though the actual incidence of infection caused by these pathogens is not known, their presence in foods is self-evident and the weight of publications reporting the occurrence of these pathogens in Malaysian foods implies a high contamination risk for human. While there are documentations on many pathogens, there is no report concerning the presence of *Yersinia* in foods of animal origin in Malaysia. *Y. enterocolitica* is recognized as a significant foodborne pathogen overseas especially in temperate countries (Schiemann,1989). However, we cannot assume that this bacteria is absent in Malaysia. Thus, the objectives of this study are to isolate and identify *Y. enterocolitica* and other species from various foods of animal origin. The characterization of *Y. enterocolitica* isolates using simple tests in order to presumptively check their pathogenicity was also carried out.

MATERIALS AND METHODS

Collection of samples

Raw meat, meat products and chicken carcass rinses originating from different locations such as Selangor (150), Negeri Sembilan (42), Melaka (32), Federal Territory (35), Perak (2) and also imported meat (71) were collected and cultured at the Regional Veterinary Diagnostic Laboratory, Petaling Jaya, Malaysia for the presence of *Yersinia*. A total of 332 samples were examined over an eight months study period, from June 1999 to August 1999, October 1999 to December 1999 and November 2000 to December 2000. Raw meats consisted of beef (94), chicken meat (114) and pork (11). Raw meat products consisted of beef burger (47), chicken burger (30), chicken frankfurter (10), pork frankfurter (5) and chicken nugget (1). Chicken carcass rinses (20) were also examined for *Yersinia* and were taken from a poultry processing plant located in Selangor. The carcass rinses were brought in icebox to the laboratory and examined immediately upon arrival.

Enrichment method

Procedures for sampling and enrichment were conducted according to the method recommended by The Meat Industry Research Institute of New Zealand or MIRINZ, (1991). Phosphate-buffered saline (PBS) solution (KH_2PO_4-Na_2HPO_4, 0.067M and NaCl, 0.85%), pH 7.6 was used as an enrichment broth. Each raw food samples (25g) was placed in a sterile stomacher bag and homogenized in 225ml of the enrichment broth for 30 seconds using a stomacher. For chicken carcass rinses sample, the whole chicken was washed in 250ml of the enrichment broth. The enrichment broth was then incubated at 25°C for 2 days.

Isolation and identification

The selective media used for the isolation of *Yersinia*

was cefsulodin-irgasan-novobiocin (CIN) agar (Merck, Darmstadt, Germany). Following enrichment using PBS, 0.5ml of enrichment broth was diluted in 4.5ml of KOH in 0.5% NaCl with a brief gentle mixing (Aulusio *et al.*, 1980) and the diluted broth was then plated directly on CIN plate. Then, the agar plates were incubated at 25°C for 48 hr. *Y. enterocolitica* ATCC 27729, obtained from American Type Culture Collection, was used as internal control strain to validate media and biochemical tests. Typical colonies with a deep red center and well-defined translucent margins (bulls eye appearance) were subcultured onto McConkey agar (Oxoid, Ltd.). Non-lactose fermenters generally produced colorless colonies whereas lactose fermenters exhibited red colonies. Presumptive colorless colonies were confirmed as members of the genus *Yersinia* by heavy inoculation of slopes of Kligler iron agar (KIA), Christensen's urea agar, Simmon's citrate agar, phenylalanine deaminase slant, and a tube of motility agar incubated at 25°C for 48h, and to another tube of motility agar incubated at 37°C for 48h (Reid, 1991). Cultures were identified to the species level by both rapid method using Microbact 24E (Medvet, Australia) and also a cassette of conventional biochemical tests on the basis of their reactions to the following reactions, incubated at 25°C for 48h: nitrate, hidrogen sulfide, indole, MR-VP, lysine, ornithine, raffinose, mellibiose, cellobiose, sucrose, lactose, sorbitol and mannitol (Reid, 1991).

Biochemical tests to screen for

Since antisera for typing isolates of *Y. enterocolitica* are generally not available in the laboratory, several biochemical tests that can be used to identify pathogenic serotypes were performed (Farmer *et al.*, 1992). The tests are pyrazinamidase, salicin-esculin and D-xylose fermentation. The results obtained could be used to presumptively identified pathogenic serotypes of *Y. enterocolitica* thus may indicate the pathogenic potential of individual strains.

RESULTS

The results of the isolation of *Yersinia* strains are presented in Table 1. A total of 332 samples of raw meats and meat products were investigated for the occurrence of *Yersinia*. *Yersinia* spp. were prevalent in all types of samples except chicken frankfurter, raw pork, pork frankfurter and chicken nugget. The frequencies of positive samples were variable with different samples examined. The highest percentage of *Yersinia* was detected in beef burger (34%), followed by raw chicken meat (5.3%), chicken carcass rinses (5%), chicken burger (3.3%) and raw beef (1.1%). Four species of *Yersinia* were identified in the study. *Y. enterocolitica* was the predominant species and was isolated from beef burger (29.4%) and chicken burger (3.3%), followed by *Y. frederiksenii* isolated from raw beef (1.1%), beef burger (4.3%) and raw chicken meat (4.4%). *Y. intermedia* was recovered from beef burger (4.3%), raw chicken meat (0.9%) and chicken carcass rinses (5%). *Y. kristensenii* was the least, recovered from beef burger (2.1%) and raw chicken meat (0.9%).

This study includes local and imported samples revealed Selangor as the main contributor to the contamination. Of 150 samples examined, 40 *Yersinia* isolates were recovered with *Y. enterocolitica* (28) being the predominant species followed by *Y. frederiksenii* (6), *Y. intermedia* (3) and *Y. kristensenii* (3) (Table 2). Negeri Sembilan, Federal Territory and imported samples showed low contamination rate. Samples from Melaka and Perak were found negative for the presence of *Yersinia*. Attempt to screen the *Y. enterocolitica* for pathogenicity by using simple tests revealed all of them were positive for pyrazinamidase, salicin fermentation-esculin hydrolisis and D-xylose fermentation.

DISCUSSION

There is no published report on the detection of *Y. enterocolitica* and its related species in foods of animal origin in Malaysia.

This study presented the occurrence of *Yersinia* spp. contamination in ten types of foods samples that have been examined over 8-month period. They were selected on the basis of being popular choice of convenience food among the public and may act as a source of foodborne infection to the consumer. As example, beef burger, chicken burger and chicken frankfurter are easily available through street vendors and handling them during the raw state favor cross-contamination from the operator to the other foods that are also prepared raw. Furthermore, to cook them without proper heating or half-cooked will provide opportunity for pathogen to survive. Raw beef and raw chicken meat may cross-contaminate the kitchen environment and to other ready-to-eat food or direct hand-to-mouth exposure while handling in domestic kitchen.

Table 1. Occurrence of *Yersinia* in meats and meat products

Sample type	Number of Samples	Number of positive samples (%)	*Y. enterocolitica* (%)	*Y. frederiksenii* (%)	*Y. kristensenii* (%)	*Y. intermedia* (%)
Raw beef	94	1 (1.1)	0 (0)	1 (1.1)	0 (0)	0 (0)
Beef burger	47	16 (34.0)	14 (29.8)	2 (4.3)	1 (2.1)	2 (4.3)
Raw chicken meat	114	6 (5.3)	0 (0)	5 (4.4)	1 (0.9)	1 (0.9)
Chicken burger	30	1 (3.3)	1 (3.3)	0 (0)	0 (0)	0 (0)
Chicken frankfurter	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chicken carcass rinses	20	1 (5)	0 (0)	1 (5)	0 (0)	0 (0)
Raw pork	11	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pork frankfurter	5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chicken nugget	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	332	25 (7.7)	15 (4.5)	9 (2.7)	2 (0.6)	3 (0.9)

Table 2. Distribution of *Yersinia* isolated according to origin of samples

Species	Origin of samples[a]						
	Selangor (n-150)	Negeri Sembilan (n=42)	Melaka (n=32)	Federal Territory (n=35)	Perak (n=2)	Imported (n=71)	Total (%) (n=332)
Y. enterocolitica	28	-	-	1	-	-	29 (54.7)
Y. frederiksenii	6	4	-	-	-	8	18 (34.0)
Y. kristensenii	3	-	-	-	-	-	3 (5.7)
Y. intermedia	3	-	-	-	-	-	3 (5.7)
Total	40 (75.5)	4 (7.5)	-	1 (3.4)	-	8 (15.1)	53

[a] n, number of samples investigated from that particular location.

The result of this study indicates highest occurrence of *Yersinia* in beef burger (34%). The microbiology of a food is usually dependent on the conditions under which it is processed. Beef burger is prepared from ground meats and mixed with animal fats to hold the meat pieces together. If the meat source were highly contaminated, this would contribute to high incidence of microbes in the end products. With the beef contamination rate being very low at 1.1% - it is surprising that the incidence of *Yersinia* in beef burger is very high. It can be suggested that the source of contamination may come from the workers handling the foods, the machines that were not sanitized properly, the already contaminated animal fats or water supply. Further, the presence of *Y. enterocolitica* in beef burgers (29.8%) but not in raw beef, give an indication that contamination may occur during processing. Every slice of the burger was wrapped with two sheets of small plastics and ten pieces were packed together in one plastic bag. Thus, the several steps during the processing and packaging may allow great opportunity for the contamination of the food with *Yersinia*. To date, published report is lacking concerning the isolation rate of *Yersinia* from beef burger from other countries to which we could compare our results.

The detection of the *Yersinia* in other samples was very low. The contamination rate in raw beef, raw chicken meat, chicken burger and chicken carcass rinses were 1.1%, 5.3%, 3.3% and 5% respectively. Ibrahim and MacRae (1991) reported the isolation of *Y. enterocolitica* from beef was 20% in which 18% were identified as *Y. enterocolitica* and 4% as *Y. frederiksenii*. Norberg (1981) recorded the isolation of *Yersinia* from chicken was 24.5%. Floccari *et al.*, (2000) detected *Y. enterocolitica* or related species in 7 out of 70 (10%) samples of chicken carcasses from Argentina. From them, 4.3% were identified as *Y. enterocolitica*, 1.4% as *Y. intermedia*, and 4.3% as *Y. frederiksenii*. De Boer *et al.*, (1982) documented the occurrence of *Y. enterocolitica* in poultry products as high as 68%. However, this study showed that none of *Yersinia* was found in any of the chicken frankfurter or chicken nugget. Pigs are reservoir for *Y. enterocolitica* (Kapperud, 1991). Ramirez *et al.*, (2000) detected 53% of raw pork were positive for the presence of *Yersinia* in which 48.8% were identified as *Y. enterocolitica*, 25% as *Y. kristensenii*, 15% as *Y. intermedia*, 9.1% as Y. *frederiksenii* and 0.9% as *Y. aldovae*. However, the result of this study found negative for any presence of the bacteria in raw pork. In addition, *Yersinia* was not isolated from pork frankfurter.

This is in agreement with the work done by Velazquez *et al.*, (1993) where they detected very low occurrence of *Y. enterocolitica* in pork subproducts, only five out of 450 samples (1.1%) were positive. This could be possibly due to very low contamination during processing or in the case of pork frankfurter, the frequency of contamination is expected since it is a cooked and vacuum-packed product.

Samples from Selangor possessed major contamination risk of *Yersinia* spp. compared among samples in other states where 75.5% of *Yersinia* isolates were detected in samples from Selangor. In contrast, low frequency of *Yersinia* isolates were found in Negeri Sembilan (6.9%), Federal Territory (3.4%) and among imported samples (3.4%). In addition, *Yersinia* was not detected in samples from Melaka and Perak. This could be due to rapid growth of food industry in Selangor as a need to fulfill the market in and around the state. Population's increase in the mid-valley area also demand fast production and may result in ignorance of strict hygiene practice and regulation during processing and packaging of the foods.

The differences in biochemical and virulence behavior of *Y. enterocolitica* strains have led to the division of the species into six biogroups. Each biogroup comprises several serotypes based on lipopolysaccharide O antigens and their virulence potential varies from very high to avirulent. Biogroup 1B, 2, 3, 4 and 5 have been established and associated as pathogen, whereas the status of biogroup 1A is still obscure and usually described as environmental or not virulent (Bottone *et al.*, 1997). The result of this study indicated that all of the *Y. enterocolitica* isolates belonged to biogroup 1A. This was in agreement with Bercovier *et al.*, (1980) who found there was correlation between biogroup and their ecology. Usually biotype 1A does not cause any disease and mainly isolated from food and environment. However, Ratnam *et al.*, (1982) summarized that although biotype 1A may not be associated with human infections, however, there is opportunity to infect patient with underlying disorders. This is due to the detection of this biogroup in feces of six of nine patients. Serotyping of *Yersinia* was not done in this study.

In conclusion, despite low percentage of *Yersinia* contamination, this report establishes the presence of *Yersinia* in foods of animal origin in Malaysia (Table 1). Though there was little presence of *Yersinia* in raw beef, raw chicken meat, chicken carcass rinses and chicken burger, the high incidence detected in beef burger is of great concern. The preparation method of the beef burger would cause the bacteria to be embedded in the food and the psycrophilic nature of *Yersinia* allows its survival even when stored at freezing temperature. Delayed or improper storage of such contaminated food would cause further proliferation. Realizing that environmental *Yersinia* may survive in Malaysian foods, we can assume pathogenic *Yersinia* may survive as well should the foods were contaminated with the organisms. Continuous monitoring and surveillance has to be carried out by diagnostic laboratories to detect presence of pathogenic *Y. enterocolitica* and prevent cross-contamination by rejecting the infected source in an early stage.

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RINGKASAN

KONTAMINASI SAMPEL MAKANAN BERASALKAN HAIWAN OLEH YERSINIA ENTEROCOLITICA *DAN SPESIS YANG BERKAITAN.*

Sejumlah 332 sampel makanan mentah yang berasalkan haiwan, terdiri dari 219 (66%) daging, 93 (28%) hasil daging dan 20 (6%) karkas ayam telah dikaji untuk mengesan kehadiran Yersinia. Kajian ini telah dijalankan di Makmal Kesihatan Awam Veterinar di Petaling Jaya untuk tempoh selama lapan bulan. Frekuensi bagi sampel yang positif adalah seperti berikut, 1/94 bagi daging lembu (1.1%), 16/47 bagi burger daging lembu (34%), 6/114 bagi daging ayam (5.3%), 1/30 bagi burger daging ayam (3.3%) dan 1/20 bagi karkas ayam (5%). 53 isolat telah dipencil dan empat spesis Yersinia telah dikenal pasti iaini sebagai *Y. enterocolitica* (29), *Y. frederiksenii* (18), *Y. kristensenii* (3) dan *Y. intermedia* (3). Kesemua isolat *Y. enterocolitica* telah dikelaskan dalam biotaip 1A sebagaimana yang telah ditunjukkan oleh keputusan positif ujian-ujian biokimia seperti salicin-esculin, xylose, nitrate dan pyrazinamidase, yang mana disifatkan sebagai isolat bukan virulen.