

OCCURRENCE OF YERSINIA ENTEROCOLITICA IN PORK

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ABSTRACT

In the present study, 69 pork samples including 23 each of pig tongue, lymphoid tonsils and ground pork were procured from local market and processed for isolation of *Yersinia enterocolitica* using both direct broth enrichment and cold broth enrichment techniques. In both enrichment procedures, before inoculation on selective agar medium, inoculum was subjected to alkali treatment with 0.5% KOH in saline to inhibit the non-yersinia organisms. Of the 69 samples, twelve (17.69%) were positive by the culture methods including three (13.0%) of pig tongue, eight (34.78%) of lymphoid tonsils and one (4.35%) of ground pork. The positive samples included six (8.69%) by direct broth enrichment and one (1.44%) by cold broth enrichment method alone and five (7.25%) were positive by both the methods. The number of pork samples positive for *Y. enterocolitica* by direct broth enrichment was more (11; 15.94%) as compared to cold broth enrichment (6; 8.69%). The present investigation, thus, revealed that isolation rate of *Y. enterocolitica* from pork samples was better with direct broth enrichment than with cold broth enrichment.

Key words: *Y. enterocolitica*, ground pork, isolation, tonsils, tongue

Yersinia enterocolitica is a zoonotic pathogen widely distributed in nature that can cause acute gastroenteritis, mesenteric lymphadenitis, appendicitis and has emerged as an important food borne pathogen causing infection in human and animals (Bottone, 1999). Pigs are reported to harbour this organism in their throat and tonsils (Funk *et al.*, 2000) and shed the organisms in faeces (Rasmussen *et al.*, 1995). The organism has also been isolated from a variety of foods such as raw and pasteurized milk (Moustafa *et al.*, 1983), beef (Inoue and Kurose, 1975), oysters (Toma and Lafleur, 1974), pork (Fredriksson-Ahomaa *et al.*, 2007) and masala item of panipuri (Sathis Babu and Rati, 2003). Further, *Y. enterocolitica* is capable of multiplying at refrigeration temperature and in vacuum packed meats, milk and other food products which support its greater public health significance.

Current methods for detection of *Y. enterocolitica* infection include conventional method of culture and modern molecular techniques viz. polymerase chain reaction (PCR). However, molecular techniques like PCR are quite expensive and cumbersome. On the other hand, culture method is economical, easy to perform and gives confirmatory diagnosis. Conventionally, the isolation method usually involves selective enrichment of the food sample followed by plating on to selective media. The present paper describes the isolation of *Y. enterocolitica*

from pork samples including pig tongue, lymphoid tonsils and ground pork by adopting both direct broth enrichment and cold broth enrichment treatments before inoculation on selective agar medium.

MATERIALS AND METHODS

A total of 69 pork samples comprising 23 each from pig tongue, lymphoid tonsils and ground pork were collected aseptically from the local market and transported to the laboratory under cold conditions. For cold broth enrichment of *Y. enterocolitica*, the method of Doyle *et al.* (1981) was used. For this process, 25 g of sample was blended aseptically with 225 ml of 0.067 M di-sodium-PBS (pH 7.6) for 2 min in a homogenizer and the homogenate was incubated at 4°C for 21 days. Subsequently, 1 ml of the inoculum was transferred into 10 ml modified Rappaport broth (MRB) and incubated at 25°C for 48 h. Then, alkali treatment was given for 30 seconds by mixing 0.5 ml of enriched broth culture with 4.5 ml of 0.5% KOH in saline. The treated enriched broth culture was streaked within 60 seconds on both Cefsulodin Irganon Novobiocin Agar (CIN, Hi-media) and MacConkey Lactose Agar (MLA, Hi-media) plates and incubated at 25°C for 24-48 h.

Isolation of *Y. enterocolitica* by direct broth enrichment was done as per method described by Bhaduri *et al.* (1997) with slight modifications wherein a total of 25 g sample was blended in PBS aseptically as done in case of cold broth enrichment and incubated at 25°C for

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24 h. After transferring 1 ml inoculum into 10 ml of MRB, the enriched broth was further incubated at 25°C for 4 days followed by alkali treatment and streaking on selective medium plates as in cold broth enrichment. The plates were examined for the appearance of characteristic red ‘bull eye’ colonies of *Y. enterocolitica* on CIN and colourless colonies on MLA.

These presumptive *Y. enterocolitica* isolates were purified on Tryptone Soya Agar (TSA) and examined for their morphology and Gram’s staining reaction. Biochemical identification of *Y. enterocolitica* was performed as per the Bergey’s Manual of Determinative Bacteriology (Holt *et al.*, 1994). However, standard methods of biochemical examination of *Y. enterocolitica* were used as described by Mac-Faddin (1976) and Cowan (1974).

RESULTS AND DISCUSSION

It is well known that *Y. enterocolitica* obligately feeds on lymphoid tissue, so it can be easily parasitic on pig throat tonsils, which are rich in lymphoid tissue (Horter *et al.*, 2003). Pigs (mainly their tonsils) are assumed to be the main source for pathogenic *Y. enterocolitica* because the pig is so far the only animal species from which pathogenic strains have frequently been isolated (Lindblad *et al.*, 2007). In the present study, the bacteriological examination of 69 pork samples resulted in isolation of *Y. enterocolitica* organisms from 12 (17.39%) samples (Table 1). All the isolates of *Y. enterocolitica* on CIN agar showed characteristic red “bull eye” appearance with light periphery. Similar appearance of red “bull eye” colonies of *Y. enterocolitica* on CIN agar showing deep red centre and transparent margins have been reported by Van Damme *et al.* (2010). Likewise the above protocol for isolation of *Y. enterocolitica* has also been used by other workers and CIN agar was reported to be inhibitory to *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* (Walker

and Gilmour, 1986).

Of the 69 pork samples examined, six (8.69%) were positive only by direct broth enrichment, only one (1.44%) by cold broth enrichment and five (7.25%) by both the methods. The samples positive for *Y. enterocolitica* by direct broth enrichment method were mainly from lymphoid tonsil (4) and tongue (2) of pig but none of the samples of ground pork was found positive for *Y. enterocolitica*. However, the only sample positive by cold broth enrichment was of lymphoid tonsils (Table 1). Thus the isolation of *Y. enterocolitica* was maximum from pig lymphoid tonsil (8, 34.78%) followed by tongue (3, 13.0%) and ground pork (1, 4.34%). Similarly Mundi *et al.* (1999) has reported the prevalence rate of *Y. enterocolitica* up to 11.76% (4/34) and 8.96% (6/67) in pig lymphoid tonsils and tongue, respectively. Earlier Fredriksson-Ahomaa *et al.* (2007) has also reported highest isolation rate of this organism from pig tonsil samples indicating tonsils as a significant source for carcass and offal contamination than faeces in slaughter houses. Likewise, *Y. enterocolitica* was isolated from 19.53% (878/4,495) of the tonsil samples, 7.51% (93/1,239) of intestinal contents, and 5.30% (161/3,039) of faeces (Liang *et al.*, 2012) which also supports the findings of the present study.

Detection rate of *Y. enterocolitica* in pork samples was found to be higher by direct broth enrichment (15.94%) as compared to cold broth enrichment (8.69%) suggesting use of cold broth enrichment may underestimate the prevalence of this pathogen in pork. Besides, the cold broth enrichment is also considered a time consuming protocol. Wauters *et al.* (1988) reported that direct Irganon ticarcillin chlorate (ITC) enrichment broth derived from modified Rappaport to be a better method for detection of pathogenic *Yersinia* strains than cold enrichment in PBS broth. The present investigation, thus, revealed that isolation rate of *Y. enterocolitica* from

Table 1
Detection of *Y. enterocolitica* from pork by culture method

Type of sample (n= 69)	No. positive only by direct broth enrichment (%)	No. positive only by cold enrichment (%)	No. positive by both methods (%)	Total (%)
Pig tongue (23)	2	-	1	3 (13.0)
Lymphoid tonsils (23)	4	1	3	8 (34.78)
Ground pork (23)	-	-	1	1 (4.34)
Total	6 (8.69)	1 (1.44)	5 (7.25)	12 (17.39)

pork samples was better with direct broth enrichment than cold broth enrichment.

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