Isolation of *Yersinia enterocolitica* from cases of acute appendicitis and ice-cream

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عزل اليرسنية المعوية القولونية من حالات الالتهاب الحاد بالزائدة وعينات من المثلجات

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خلاصة: تم تحليل سبعين عينة من الزوائد الدودية وثمانين عينة من المثلجات، بحثاً عن اليرسنية المعوية القولونية باستعمال ثلاثة أوساط مختلفة. ولقد اكتشفت اليرسنية المعوية القولونية مع الليمونية الفروبندية في عينات الزائدة الدودية (17.1% و8.6% على التوالي) وفي عينات المثلجات (26.25% و18.75% على التوالي). وكان وسط الثيوغليكولات أكثر انتقائية وإنتاجية في عزل اليرسنية. وظهر أن اليرسنية المعوية القولونية هي أهم مسببات الالتهاب الحاد بالزائدة (11 من أصل 25 عينة أي 44%). ووجد أنها حساسة للكلورامفنيكول والجنتاميسين والتتراسيكلين والترايمثوبريم - سلفاميثوكسازول.

ABSTRACT Seventy (70) appendiceal specimens and 80 ice-cream samples were analysed to detect *Yersinia enterocolitica* using three different media. Both *Y. enterocolitica* and *Citrobacter freundii* were recovered in appendiceal specimens (17.1% and 8.6%) and ice-cream (26.25% and 18.75%) respectively. Thioglycollate medium was more selective and productive in isolating *Yersinia*. *Y. enterocolitica* was the major causative agent of acute appendicitis (11/25, 44%). It was sensitive to chloramphenicol, gentamicin, tetracycline and trimethoprim-sulfamethoxazole.

Isolement de Yersinia enterocolitica chez des cas d'appendicite aiguë et dans les glaces
RESUME Soixante-dix (70) spécimens d'appendice et 80 échantillons de glaces ont été analysés pour la recherche de *Yersinia enterocolitica* en utilisant trois milieux différents. Les deux germes *Y. enterocolitica* et *Citrobacter freundii* ont été retrouvés dans les spécimens d'appendice (17,1% et 8,6%) et les échantillons de glaces (26,25% et 18,75%) respectivement. Le milieu au thioglycolate était plus sélectif et productif dans l'isolement de *Yersinia*. *Y. enterocolitica* était le principal agent étiologique de l'appendicite aiguë 11/25 (44%). Il était sensible au chloramphénicol, à la gentamicine, à la tétracycline et au sulfaméthoxazole-triméthoprime.

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Introduction

Yersinia enterocolitica is an important pathogen worldwide. In the past two decades, there have been many reports of outbreaks of yersiniosis with concomitant right lower quadrant abdominal pain [*1–5*]. *Y. entercolitica* not only causes fulminate infections that exactly mimic acute appendicitis [*6*], which could affect the ability of doctors to make the correct diagnosis, but it also gives rise to chronic colitis and abdominal pain in patients up to 5 years after appendectomy [*1*].

It has been reported that *Y. enterocolitica* invades the epithelium cells of the gastrointestinal tract to produce intestinal disease in animals and humans. It also produces a heat-stable enterotoxin that contributes to the symptoms of gastroenteritis [*7*].

Recently, there has been interest in the recovery of *Y. enterocolitica* from foods as a result of yersinial food poisoning in New York and Tennessee [*8, 9*]. Furthermore, infection of the appendices by enteroinvasive *Y. enterocolitica* is primarily as a result of ingestion of contaminated foods, including milk [*3,4*]. Unlike other enteropathogenic bacteria, it has a wide range of pH (4.4–9) and is psychrophilic in nature (grows at –1 °C).

This study aimed to evaluate the liquid media used to recover *Y. enterocolitica* from appendiceal specimens and also ice-cream as there have been few reports on the occurrence of this organism in such food.

Materials and methods

The patients included in this study lived in the same area where the ice-cream was obtained. Only appendiceal specimens and ice-cream samples were used in the study protocol.

Appendiceal specimens

Appendiceal specimens were taken from 70 patients who had been diagnosed with acute appendicitis and had undergone appendectomies at Tripoli Medical Centre between October and December 1997. Their ages ranged from 7 years to 44 years with an average age of 18.5 years; 16 children were under 16 years of age. Two appendiceal specimens were collected immediately from each operation; one was immersed in a disposable sterile container containing peptone physiological saline solution for bacteriological isolation, and the second was kept in formalin for histopathological examination. Both were then kept in an ice-box (±5 °C) while being transferred to the laboratory. About 5 g of the first specimen were ground aseptically in a disposable plastic tissue grinder in a measured volume (45 ml) of tryptone soya broth (TSB) and incubated for 24 hours at 25 °C. Then, 1 ml of the culture was inoculated into 9 ml each of phosphate buffered saline, and TSB plus polymyxine (20000 U/l) and Irgasan (10 mg/l) (TSB plus supplement). In addition, 3 ml of culture were transferred to 15 ml of thioglycollate medium. All the cultivated broths were incubated at 25 °C for 48 hours. A loopful of the culture was streaked onto a Yersinia-selective medium, Cefsulodin Irgasan Novobiocin (CIN) agar. The plates were incubated at 25 °C for 24–48 hours. MacConkey's medium was not used because of its poor selectivity. Serology was not carried out because of a lack of specific antisera.

Ice-cream samples

Eighty (80) samples of ice-cream were obtained from different groceries and super-

markets. The samples were transferred in a sample case to the laboratory without delay and were prepared as described by Al-Ashmawy [*10*]. The sample was softened by standing in a water-bath kept at 40 °C for 10 minutes; 25 ml of the prepared sample were diluted with 225 ml of TSB and incubated at 25 °C for 24 hours. The inoculation of the culture into the different selective broths and the streaking onto CIN agar were carried out as described before.

Suspected yersinial colonies (deep red centre with a clear halo) were purified on a tryptone soya agar (TSA) slope, incubated at 25 °C for 24 hours and then identified as described by Seeliger and Jones [*11*]. The biochemical tests used were Gram stain, motility test (motile at 25 °C, non-motile at 37 °C), oxidase test, catalase test, Kligler iron agar (KIA) test, followed by API-20E. Specimens and samples were also cultured for other pathogenic microorganisms using standard microbiological methods. The clinical isolates of *Y. enterocolitica* were subjected to an antibiotics sensitivity assay.

Results and discussion

Evaluation of the selective broths

The conventional techniques used to isolate *Y. enterocolitica* from operative specimens as well as food of animal origin are slow and arduous. The problems include the growth of psychrophylic competitors and the length of time required to obtain results, which may be as long as 1–2 months at 4 °C. Thus, there is a need for selective broths which can give reliable results quickly (2 days) so that doctors can arrive at a correct diagnosis. Quick results are also necessary for food safety microbiologist and public health officials, who are responsible for assuring food is free of *Yersinia* contamination.

Table 1 shows the results of a comparison between the three selective broths used for the isolation of *Y. enterocolitica* from the appendix specimens. *Y. enterocolitica* was detected in 12 cases from the CIN agar inoculated with the three selective broths. Seven (7) positive cases were identified using the thioglycollate broth alone and 1 positive case with TSB plus supplement. Two (2) positive cases were identified using TSB plus supplement alone and 1 case with phosphate buffered saline. Phosphate buffered saline alone gave 1 positive case.

The detection of *Y. enterocolitica* in the ice-cream using the three different liquid media is illustrated in Table 2. Using the thioglycollate medium, *Y. enterocolitica* was detected in 22.5% (18/80) of the samples examined, followed by TSB with supplements and phosphate buffered saline.

The thioglycollate medium contained sodium thioglycollate and resazurine, which acts as an oxidation/reduction indicator, which has replaced the more toxic methylene blue in the original formula.

Table 1 **Evaluation of three selective broths for detecting *Y. enterocolitica* in 70 appendiceal specimens**

Selective media	Positive cases	
	No.	%
CIN	12	17.1
Thioglycollate medium	7	10.0
Thioglycollate medium and TSB with supplement	1	1.4
TSB with supplement	2	2.9
TSB with supplement and phosphate buffer saline	1	1.4
Phosphate buffer saline	1	1.4

CIN = Cefsulodin Irgasan Novobiocin
TSB = tryptone soy broth

Table 2 **Evaluation of three liquid media used for detecting *Y. enterocolitica* in 80 ice-cream samples**

Selective media	Positive samples No.	%
Thioglycollate media	10	12.50
Thioglycollate medium and TSB with supplements and phosphate buffered saline	8	10.00
TSB with supplement	3	3.75
Total positive streaked onto CIN agar	21	26.25

Y. enterocolitica *was detected in 40% (20/50) of the samples of home-produced ice-cream and in 3.3% (1/30) of the samples of commercially produced ice-cream.*
TSB = tryptone soy broth
CIN = Cefsulodin Irgasan Novobiocin

Table 3 **Frequency of isolation of *Y. enterocolitica* and other bacteria from appendiceal specimens**

Isolated organism	Positive cases (n = 27) No.	%
Y. enterocolitica	7	25.9
Y. enterocolitica and others	5	18.5
Total isolates of *Y. enterocolitica*	12	44.4
Shigella spp.	2	7.4
Klebsiella pneumoniae	4	14.8
Salmonella spp.	2	7.4
Citrobacter freundii	5	18.5
Pseudomonas aeruginosa	2	7.4
Total isolates other than *Y. enterocolitica*	15	55.6
Y. enterocolitica in acute appendicitis	6/25	24
Y. enterocolitica and others in acute appendicitis	5/25	20
Other bacteria in acute appendicitis	14/25	56
Y. enterocolitica in chronic appendicitis	1/2	50
Other bacteria in chronic appendicitis	1/2	50

Phosphate buffered saline is sensitive to some strains of *Y. enterocolitica* but is deficient in nutrients compared with the other media. Despite the superiority of thioglycollate medium, some positive cultures were not detected and were recovered using other selective broths.

Frequency of bacteria isolated

Y. enterocolitica was the of bacteria most often isolated (44.4%) from the specimens obtained from appendectomy, followed by *Citrobacter freundii* (18.5%) and *Klebsiella pneumoniae* (14.8%); *Salmonella* sp., *Shigella* and *Pseudomonas aeruginosa* each constituted 7.4% (Table 3). Mixed infection was detected in 18.5% of positive cultures. *Y. enterocolitica* was associated with *C. freundii, Salmonella* sp. and *P. aeruginosa* in one specimen for each case, while *Y. enterocolitica* and *Shigella* were recovered in two cases (Table 3). Our finding of mixed infection is higher than that of Stolk-Engelaar et al. who isolated *Y. enterocolitica* together with *Sal. typhimurium* in 0.3% of specimens [*12*].

Of the 70 patients with appendicitis, bacteria were isolated from 25 cases with acute appendicitis; *Y. enterocolitica* was detected in 11 (44%) cases, either alone or with other bacteria, and 14 (56%) cases were infected with other pathogenic bacteria. *Y. enterocolitica* was detected in one case of chronic appendicitis while other

pathogens were detected in another case (Table 3).

Some investigators found 30%–40% of operative specimens with acute appendicitis were infected with Y. *enterocolitica* [*1,5*]. Attwood et al. found *Y. enterocolitica* to be the cause of chronic yersiniosis [*1*].

Ice-cream samples

Of a total of 80 samples of ice-cream, 21 (26.25%) were contaminated with *Y. enterocolitica* (Table 2), while *C. freundii* was found in 18.75% of the samples examined. The majority of the positive yersinial samples were obtained from home-produced ice-cream. This was probably due to poor sanitation; the product was not subjected to heat treatment during processing, which would allow bacteria to enter the product from different environmental sources. Our figures are higher than those reported by El-Gmiey [*13*] who found 9% of the samples analysed contained *Y. enterocolitica* His samples were collected from supermarkets and cafeterias in Mansoura City, Egypt. Walker and Brooks [*14*] recovered *Yersinia* from 4.2% of food samples examined, including milk and dairy products.

The large number of ice-cream samples contaminated by *Y. enterocolitica* increases the possibility that the product can transmit the infectious agent to human appendices, primarily by ingestion of a contaminated product. It suggests an epidemiological link between food-borne transmission and human yersiniosis, which increases the risk of postoperative infection. Some investigators have observed that many patients complain of abdominal pain and chronic colitis for 1–5 years after appendectomy in cases of *Y. enterocolitica* infection [*1,12*].

Treatment

The clinical isolates were resistant to ampicillin and sensitive to chloramphenicol, gentamicin, tetracycline and trimethoprim-sulfamethoxazole. These findings are in agreement with those reported by Stoddard et al. [*4*] and Hoogkaamp et al. [*5*].

Treatment with a suitable drug should be given preoperatively, intraoperatively and postoperatively.

Conclusion

We found a pronounced link between the occurrence of *Y. enterocolitica* in ice-cream (26.25%) and appendiceal infection (17.1%), which indicates that contaminated dairy products are a major cause of human yersiniosis. Techniques for the rapid isolation of *Y. enterocolitica* from foods and operative specimens are necessary to enable doctors to make correct diagnoses. In addition, food safety microbiologists need to be able to monitor food for *Yersinia* contamination. Educational programmes should be designed for dairy-product producers and handlers, and consumers in order to ensure the production of food free of *Yersinia*. The drugs recommended for patients diagnosed with yersiniosis are chloramphenicol, gentamicin, tetracycline and trimethoprim-sulfamethoxazole.

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References

1. Attwood SEA et al. *Yersinia* infections and acute abdominal pain. *Lancet*, 1987 1(8532):529–33.
2. Puylaert JBCM et al. Incidence and sonographic diagnosis of bacterial ileocaecitis masquerading as appendicitis. *Lancet*, 1989, 2(8654):84–6.
3. Bennion RS et al. The role of *Yersinia enterocolitica* in appendicitis in the south-western United States. *American surgeon*, 1991, 57:766–8.
4. Stoddard JJ et al. *Yersinia enterocolitica* infection in a patient with sickle-cell disease after exposure to chitterlings. *American journal of pediatric hematology and oncology*, 1994,16(2):153–5.
5. Hoogkamp-Korstanje JAA, Stolk-Engelaar VMM. *Yersinia enterocolitica* infection in children. *Pediatric infectious diseases journal*, 1995, 14:771–5.
6. Vantrappen G et al. *Yersinia enterocolitica*. *Medical clinics of North America*, 1982, 66:639–53.
7. Coghlan JD. *Yersina, Pasteurella, Francisella*. In: Collee JG et al., eds. *Mackie and McCartney. Practical medical microbiology*, 14th ed. New York, Churchill Livingstone, 1996.
8. Black RE et al. Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. *New England journal of medicine*, 1978, 289:76–9.
9. Multistate outbreak of yersiniosis. *Morbidity and mortality weekly report*, 1982 31:505–6.
10. Al-Ashmawy AM. *Food hygiene handbook: fluid milk dairy products, fats and oils and eggs*. Zagazig, Egypt, Zagazig University Press, 1990.
11. Seeliger HPR, Jones D. Genus *Listeria* In: Sneath PAA et al., eds. *Bergey's manual of systematic bacteriology*, Vol. 2. Baltimore, Williams and Wilkins, 1986:1235–45.
12. Stolk-Engelaar VMM, Hoogkamp-Korstanje JAA Clinical presentation and diagnosis of gastrointestinal infections by *Yersinia enterocolitica* in 261 Dutch patients. *Scandinavian journal of infectious diseases*, 1996, 28:571–5.
13. El-Gmiey SR. The prevalence of *Y. enterocolitica* in ice-cream. *Assiut veterinary medical journal*, 1995, 32(64):186–90.
14. Walker SJ, Brooks J. Survey of the incidence of *Aeromonas* and *Yersinia* species in retail foods. *Food control*, 1993. 4(1):34–40.