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INCIDENCE OF YERSINIA SPP. IN SOME RETAIL MEAT IN MANSOURA CITY EGYPT

(With 3 Tables)

By

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مدى تواجد ميكروبات البارسينيا فى بعض انواع اللحوم المباعة بمدينة المنصورة جمهورية مصر العربية

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أجرى هذا البحث على عدد ٢٠٠ عينه من اللحم البقرى المحلى والمستورد المجمد واللحم المفروم المجمد المحلى ولحم الدجاج الطازج جمعت عشوائيا من محلات الجزارة والسوبر ماركت وحوانيت ذبح الدجاج المختلفة بمدينة المنصورة - محافظة الدقهلية- ج.م.ع. لتبيان مدى تواجد ميكروبات اليارسينيا بها. وقد أظهرت نتائج الفحوص أن نسبة تواجد Yersinia spp. هى ٨,٥% من مجموع العينات المختبرة . وكانت نسبة إنتشار هذه الميكروبات متباينة بإختلاف نوع اللحم المفحوص حيث تم عزل هذه اليكروبات بنسبه ٦% و١٢% و ١٠% و٦% من عينات اللحم البقرى واللحوم المجمدة المستوردة واللحوم المحليه المفرومه المجمدة ولحوم الدجاج المذبوح غلى التوالى. وتراوحت نسب عزل جرثومة Y.enterocolitica بين ٢-٨% من العينات المختبرة هذا ولم تتمكن من عزل هذا الميكروب من اللحم البقرى. وقد أوضحت الدراسة أن نسبة عزل ميكروبات اليارسنيا الأخرى(Y. intermedia, Y. krestensenii and Y. Fredreksenii) هى ٦% من مجموع العينات المختبرة. وقد دلت الدراسة على تواجد عترتين من Y. biotype 4 enterocolitica فى عينات اللحوم المجمدة المستوردة فقط وهى من النوع الممرض . بينما كانت باقى عترات Y.enterocolitica المفحوصة من النوع الغير ممرض Biotype 1A. هذا وقد نوقشت الأهمية الصحية لهذه الميكروبات.

SUMMARY

A total of 200 samples of fresh beef meat, imported frozen meat, locally frozen minced meat and raw chicken meat (each of 50) were obtained

randomly from different butcher shops; supermarkets and chicken slaughter shops in Mansoura city El-Dakahlia province Egypt for the presence of Yersinia spp. Overall Yersinia spp. was recovered from 8.5% of the examined samples. The isolation rate was dependent on the meat type and, these bacteria were isolated from fresh beef meat, imported frozen meat; locally frozen minced meat and raw chicken meat in percentage 6%; 12%, 10% and 6% respectively. The contamination rate of Y.enterocolitica in tested meats was 2-8%, except for fresh beef meat from which no Y. enterocolitica was recovered. Other species of Yersinia (Y.intermedia; Y.Fredreksenii and Y.kristensenii) was isolated from 6% of all samples. Two of Y. enterocolitica isolates from imported frozen meat belonged to pathogenic biotype 4. Other Y.enterococlitica isolates were regarded as environmental strains which belonged to biotype IA. Also the public health inportance of these organisms were discussed.

Key Words: *Yersinia spp., Retail, Meat.*

INTRODUCTION

The genus yersinia belongs to the family Enterobacteriaceae and comprises 10 species including viz Y.pestis, Y.pseudotuberculosis; Y.enterocolitica; Y.frederiksenii; Y.kristensenii; Y.intermedia; Y.aldovae. Y.rohdei; Y.mollaretii and Y. bercoverii. Y. pestis, Y. pseudotubeclosis and few subgroups within true species Y. enterocolitica are of medical importance (Kapperud, 1991).

Y.enterocolitica is a versatile foodborne pathogen with a remarkable ability to adapt to a wide range of environmental within and outside its host. The bacteria typically access their hosts via food or water in which they will have grown to stationary phase at ambient temperature (Roy, 1997).

There has been a substantial increase in the frequency of the isolation of Yersinias from both clinical and non clinical sources. Yersinias has been isolated from humans in many countries of the world, but it seems to be found most frequently in cooler climates (WHO, 1987). In developed countries it can be isolated from 1-2% of all human cases of acute enteritis (Kapperud, 1991).

A wide range of food including raw and cooked meat, poultry, fish, shellfish, raw milk, pasteurized milk, dairy products, salads, vegetables and fruits has been shown to contain Yersinia spp. (Walker and Brooks, 1993). Several investigations have documented the growth

of Yersinias on raw and cooked meat at low temperature (Lee et al., 1981). In addition, the bacterium is also able to multiply in vacum packed meat during refrigerated storage (Hanna et al., 1976).

Gastroenteritis, the most common symptom in outbreaks of foodborne yersiniosis, with abdominal pain. fever, diarrhoea and to a variable degree, sore throat, bloody stools, rash, vomiting, and joint pain. Post infection manifestation including pseudoappendicular syndrome; arthritis and acute inflammation of the connective tissues or skin (Cover and Aber 1989).

In Dakahlia province, sporadic cases of yersiniosis were diagnosed bacteriologically from diarrhoeic children and patient suffering from appendicitis (Howaidy, 1998).

The present study was done to determine the incidence of Yersinias in fresh beef meat; imported frozen meat, locally frozen minced meat and raw chicken meat.

MATERIALS and METHODS

Sampling:

A total of 200 samples consisting of fresh beef meat; imported frozen beef meat; locally frozen minced meat and raw chicken meat (each of 50) were obtained randomly from different butcher shops; supermarkets and chickens slaughter shops in Mansoura city, El-Dakahlia province, Egypt. The samples were collected in its regular cosumers packages and other samples were collected in sterile plastic bags. All samples were dispatched directly to the laboratory, where they kept chilled and frozen samples were thawed by over- night refrigeration before being tested for occurrence of Yersinia spp.

Isolation of Yersinia spp:

A portion, 25 g. of each sample was added to 225 ml of Trypticase-soy broth and thoroughly mixed using a Moulinex - type blender equipment with metalic flask for one minute. After incubation at 25°C for 24 hours, 1 ml of this pre-enrichment culture was then added to 9ml Bile - oxalate- sorbose broth (BOS, Schiemann, 1982) and incubated at 25°C for a further 5 days. BOS enrichment culture was streaked with and without alkali (0.5% KoH solution in water) treatment (Aulisio et al., 1980) onto Yersinia Selective Agar (Oxoid CM653 Plus SR 109) plates, incubated at 25°C and examined after 24 and 48 hours. Colonies showing typical bulls eye morphology were purified on Tryptone soya agar and

tested for their Gram, catalase and oxidase reactions. Only Gram neegative rods; catalase positive and oxidase negative isolates were retained for further identification. Presumptive colonies were screened using kliglers iron agar (KIA, Oxoid); urea agar and motility test. Isolates of Yersinia spp were urease positive; motile at 25°C but not at 37°C incubation and produced an acid butt, alkaline slant with no gas or hydrogen sulphide in KIA were confirmed to species as discussed by Gilmour and Walker (1988) using the API20E (Biomerieux) incubated at 30°C for 24 hours and for their ability to produce acid from D-raffinose, and sucrose.

Isolates identified as Y. enterocolitica were grouped into biotypes on the basis of biochemical properties according to (Wauters et al., 1987).

RESULTS

Results are obtained at Tables 1 , 2 and 3.

DISCUSSION

Data in Table (1) indicate that imported frozen meats had the highest overall incidence of Yersinia spp with 12% of samples being positive. Meanwhile 6% of fresh beef meat samples harboured Yersinia spp. Higher results were reported by Kaarib and Seeger (1994) and Tassinari et al., (1994) who recorded incidence of 33.3% and 40% respectively.

On biochemical characterisation, Y.enterocolitica was the most common species of Yersinias isloated from imported frozen meat with incidence of 8% (Table 1) which is nearly agree with that reported by Saleh and Lotfy (1995). On the other hand, no Y. enterocolitica was found in all examined fresh beef meat samples and only 2% and 4% of samples were positive for Y. intermedia and Y. fredreksenii respectively (Table 1). Abroad, several surveys point to prevalence of this organism in raw beef meat samples. Inoue and Kurose (1975); Listner et al. (1975) and Karib et al.(1994) found that Y.enterocolitica was isolated from 14.6%, 10.8% and 13.3% beef meat samples respectively. Also the same investigators recorded the occurrence of Y. intermedia; Y. fredreksenii, Y. Kristansenii and other Yersinia spp. in the examined raw beef meat.

It is likly that tropical soil contains fewer cold tolerant bacteria than soil from temperate zones (Rao et al., 1998). Also breeding of pigs which are the most significant reservoirs of Y. enterocolitica (Kwaga and Iverson 1992 and Lambertz et al., 1996), in our islamic area are seldom. These reasons may explain the absence of Y. enterocolitica in examined fresh beef meat in this study.

The occurrence of Y. enterocolitica in the examined imported frozen meat may be attributed to contamination of meats in exporting countries before freezing (<-12°C) which do not allow their growth (Garcialopez et al., 1998) subsequently handling and solding at ambient temperature as in our area, appears to permit their growth.

In this survey, the incidence of Yersinia spp. intested locally frozen minced meat was 10%. Y. enterocolitica was recovered from 2 (4%) samples, while Y.intermedia and Y.Kristensenii were detected in 3 (6%) and (2%) samples respectively. In one occasion, one sample contained two Yersinia spp (Table 1).

There are relatively few surveys on the incidence of Yersinias in frozen minced beef meat have been published. Roberts et al. (1992) could isolate Y.enterocolitica and other Yersinia spp from 6% and 5% samples of minced beef meat respectively which are lower than the reported herin. Several studies also point to the occurrence of Yersinias in other meat products as hamburgers, beefburgers and sausage (Panebianco et al., 1992; Roberts et al., 1992 and Bosi et al., 1995).

In Egypt, El-Gohary et al. (1993) could detecte Y. enterocolitica from 14% and 10% of examined sausage and luncheon meat samples respectively. Moreover, Hafez (1996) found that 2.5% of examined imported frozen liver were contaminated with Y. enterocolitica.

The occurrence of Yersinia spp in raw chicken meat and skin is presented in Table (1) whereas, 6% of samples were found to contain Yersinia spp. Such incidence is lower than that reported by Quaglio et al. (1988) who reported an incidence of 10%.

In the present study, only Yersinia entercolitica and Y.intermedia were identified from examined raw chicken samples with percentages of 2% and 6% respectively. In one occasion two species of Yersinia were detected in one sample (Table 1).

The finding concerning Y. enterocolitica is in agreement to that reported by Mousa (1989), while higher result was reported by Leistner et al. (1975); Khalafalla (1990); Sharma et al. (1992); Karib et al. (1994) and Khalafalla et al. (1995) who recorded incidence of 28.9; 5.5% 6.25%; 5% and 15% respectively.

Sekhar et al. (1994) reported a case of Y. enterocolitica food poisoning probably caused by chicken in white sauce eaten in a restaurant.

Among the most important factors that explain the difference of incidence percentages between the different investigators are the differences in the geographic distribution of Yersinia spp.; variations in methods of isolation and variations in animal husbandry practices and food processing (ICMSF, 1996).

The biotyping results of Y.enterocolitica isolates in this study show that the most frequent biotype was Y. enterocolitica biotype IA which comprissed 71.4% of tested Y. enterocolitica isolates (Table 2). Also several investigators found this biotype in their examined samples (Christensen 1982; Walker & Brooks, 1993 and Bosi et al. 1995), this species is often referred to as environmental strain and generally obtained from terrestrial and fresh water ecosystems (Roy, 1997). Generally strains of Y. enterocolitica biotype IA; Y. intermedia Y.fredreksenii and Y. Krestensenii have no medical importance (Kapperud, 1991). However such environmental Yersinia strains may conceal the presence of pathogenic variants (WHO, 1987).

In this study 2 of 7 (28.6%) Y. enterocolitica isolates from imported frozen meat belonged to biotype 4 (Table 2).
Little available literatures dealing with the incidence of Y. enterocolitica biotype 4 in Egyptian meat and therefore it was hard to discuss the aforementioned results, but generally this biotype was isolated frequently from pork meat (Kwaga &Iverson 1992 and Lambertz et al. (1996). In Egypt Abd El-Monem & Saad (1998) could isolate clinical serotyps of Y.enterocolitica 0:3, 0:8 and 0:9 with low frequencey from poultry products.

It is well documented that Y. enterocolitica biotype 4 is primary pathogenic strain for human (Wauters et al., 1987). In addition this biotype has been isolated from human gastro-intestinal infection in many countries of the world (Lee et al., 1990).

The methods used for the isolation of Yersinia spp in this study had previously been reported as best methods when testing food (Gilmour and Walker 1988 and ICMSF, 1996).

It is clear from Table (3) that 11.8% of positive samples were obtained only without alkali treatment, but these were not obtained after the use of alkali treatment. While 70.6% of positive samples were recovered only after alkali treatment. It is pertinent to note that a post.

enrichment alkali treatment did increase the isolation rate of Yersinia spp and decrease the growth of competing bacteria however 3 samples only yieleded Yersinia spp before and after this treatment. Therefore samples should be plated onto selective agar both before and after alkali treatment. These results supported the findings obtained by Swaminathan et al. (1982) and Walker and Brooks (1993).

In conclusion environmental strains of Yersinia were isolated from examined samples in various percentages. In addition occurrence of Y. enterocolitica biotype 4 in imported frozen meat represented a possible public health concern. However, the failure to isolate Y. enterocolitica from fresh beef meat should not infer that this sort is not a source for Y. enterocolitica. This has emphasized the need to produce and sell meat under carefully controlled conditions.

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Table (1), Incidence of Yersinia spp in the examined samples.

Sort of meat	No. tested	Yersinia spp.		Y. enterocolitica		Y. intermedia		Y. fredreksenii		Y. Kristensenii	
		No.	%	No.	%	No.	%	No.	%	No.	%
Fresh beef meat	50	3	6	0	0	1	2	2	4	0	0
Imported frozen meat	50	6	12	4	8	2	4	0			
Locally frozen minced meat	50	5	10	2	4	3	6	0	0	1*	2
Composite of raw chicken meat and skin	50	3	6	1	2	3*	6	0	0	0	0
Total	200	17	8.5	7	3.5	9	4.5	2	1	1	0.5

* one sample contained two Y.species

Table (2) Biotyping of isolated Y. enterocolitica

Type of meat	No. tested	Biotype 1A		Biotype 4	
		No	%	No	%
Imorpted frozen meat	4	2	28.6	2	28.6
Locally frozen minced meat	2	2	28.6	0	0
Raw chicken meat	1	1	14.2	0	0
Total	7	5	71.4	2	28.6

Table (3) Efficiency of alkali treatment for the isolation of Y.ersinia spp from examined samples.

Isolation procedure	No of positive samples for			
	Y. Spp		Y. enterocolitica	
	No	%	No	%
Without alkali treatment	2	11.8	1	14.3
With alkali treatment	12	70.6	5	71.4
With or without alkali treatment	3	17.6	1	14.3