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YERSINIA IN SOME EGYPTIAN READY-TO-EAT MEAT PRODUCTS

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ABSTRACT

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A total of 100 samples of some Egyptian ready-to-eat meat products (70 samples of basterma, and 30 samples of luncheon) were investigated for the presence of *Yersinia*. The samples were evaluated sensorialy and their pH values and sodium chloride (NaCl) contents were estimated. The samples were randomly collected from different localities (supermarkets and mini-markets) in Assiut city, Egypt. All basterma and luncheon samples were accepted from the sensory point of view, although most of them were of medium quality. The mean pH and sodium chloride content values were 4.69 & 7.23; and 5.85 & 1.7 for basterma and luncheon samples, respectively. Of the 70 investigated basterma samples 13 (18.57%) were confirmed positive for *Yersinia*, and of the 30 luncheon samples 6 (20%) were confirmed positive. A total of 98 strains were isolated from the suspected positive samples, out of them 27 (27.55%) strains were found to belong to the genus *Yersinia*. Basterma samples contained *Y. enteroclitica, Y. frederiksenii, Y. intermedia,* and *Y. Kristensenii* at an incidence rate of 8.57, 4.29, 2.86, and 7.14%, while luncheon samples contained *Y. enteroclitica, Y. frederiksenii,* and *Y. Kristensenii* at an incidence rate of 13.33, 3.33, and 6.67%, respectively. *Yersinia enterocolitica* biovars 1, 3, and 4 were identified from basterma samples at percentages of 3, 1, and 2%, respectively. From luncheon samples biovars 1 and 4 were identified at percentages of 3 and 1%, respectively. The public health significance of the identified *Yersinia* strains was discussed.

Keywords: *Yersinia, Ready-to-eat Meat Products, Basterma, Luncheon.*

INTRODUCTION

The study of the microbial quality of ready-to-eat meat products including cured meat products is important from the public health point of view as most of these food products consumed without any need for further cooking. To ensure the safety of these foods, periodic surveillance to determine pathogenic agents is necessary (Bryan *et al.*, 1968).

The genus *Yersinia* is a typical member of the *Enterobacteriaceae* although it has a number of distinctive features. Yersinias are low temperature pathogens and are able to grow at 4°C, therefore in cold chain food products they could offer a potential food safety hazard (Varnam and Evans, 1991; Grahek-Ogden *et al.*, 2007). *Yersinia* are widely distributed throughout the environment and have been isolated from raw milk, sewage-contaminated water, soil, sea-food, humans, and many worm-blooded animals such as poultry and especially pigs (Schmidt and Rodrick, 2003).

Of the many species that comprise the genus *Yersinia*, three are important from the human pathogenicity point of view, namely *Y. pestis, Y. pseudotuberculosis, and Y. enterocolitica. Yersinia pseudotuberculosis, and Y. enterocolitica* are foodborne pathogens (Boer, 1992; Sprague and Neubauer, 2005; Tennant *et al.*, 2005). *Yersinia enterocolitica* "an important entero-pathogen" can cause acute enteritis (especially in children), enterocolitis, mesenteric lymphadenitis, and terminal ileitis (Bottone, 1997). Some other species of *Yersinia* including *Y. fredericksenii* and *Y. kristensenii* considered to be environmental organisms (nonpathogen) have not been studied extensively due to the absence of classical *Yersinia* virulence markers. They were occasionally reported to be associated with illness in human and were isolated from some patients as well as *Y. enterocolitica* considering them as opportunistic (Varnam and Evans, 1991; Sulakvelidze, 2000).

The pathogenic bacterium *Y. enterocolitica* has become increasingly important as a food contaminant. Of special significance in food hygiene is the ability of *Y. enterocolitica* to grow in refrigerated foods at temperatures close to 0°C

(Kapperud, 1991). Worldwide surveillance data show an extensive increase in the number of non-outbreak-related isolates and cases of yersiniosis reported. This notice has led to the referral of *Y. enterocolitica* as a potential emerging enteric human pathogen worldwide (Ostroff, 1995; Tauxe, 1997).

In most developing countries, little is known about the incidence of foodborne diseases or pathogens and associated foods. Therefore, the determination of prevalence of different foodborne pathogens in foods will indicate the potential health risks to consumers (CDC, 1983).

In Egypt, basterma and luncheon, ready-to-eat meat products, are kept in the refrigerators during their storage in the markets especially after the packages is being opened. As *yersinia*e are widely distributed in the environment, they can contaminate such meat products especially with poor food handling procedures, and they are able to proliferate at 4°C, which may constitute a public health hazard. Therefore the present study was conducted to investigate *Yersinia* in those meat products.

MATERIALS and METHODS

1. Sample collection:
A total of 70 basterma and 30 luncheon samples were randomly collected from different localities (mini-markets, supermarkets) in Assiut city, Assiut, Egypt. The samples were dispatched directly to the laboratory where they were investigated with a minimum of delay.

2. Sample preparation:
At laboratory each sample was divided into two parts. One part used for the sensory and the chemical assessments and the other part used for the bacteriological (*Yersinia*) investigation.

3. Sensory and chemical assessments:
Organoleptic assessment of the samples was done using 9-point hedonic scale according to Meilgaard *et al.* (2007). The samples pH were detected according to Lyhs *et al.* (1998) using EC-pH Scan 2 (EUTECH INSTRUMENTS 59000-20) at room temperature. Sodium chloride contents of the samples were determined according the method described by AOAC (1995) using one gram of the thoroughly mixed sample.

4. *Yersinia* isolation:
4.1. Cold and selective enrichment:
The sample was thoroughly mixed in a sterile mortar. Ten grams of the sample were weighed under sterile conditions and inoculated into 90ml of sterile trypticase soya broth (TSB) supplemented with yersinia selective supplement (Oxoid, SR0106E); mixed well and incubated at 4°C for up to 14 days for cold and selective enrichment of *Yersinia* (Greenwood and Hooper, 1989).

4.2. Selective plating:
Isolation of *Yersinia* was done by streaking a loopful from the previous enriched broth onto the surface of selective agar media (Fredriksson-Ahomaa and Korkeala, 2003). Yersinia isolation agar (HIMEDIA, M564) was used for plating and the inoculated plates were incubated at 30°C for 24 – 48 hours. Plates showing colonies resembling *Yersinia* (well established colonial growth and/or red colonies surrounded by a transparent border "bull eye like") were considered suspected positive for *Yersinia*. The negative plates show no or weak bacterial growth (non-characteristic).

4.3. Strains purification:
Colonies resembling *Yersinia* on the selective agar plates (at least 2 colonies from each plate) were sub-cultured onto nutrient agar plates, incubated at 28°C for 24 hours for purification. Separate colonies were picked up from the previous plates and sub-cultured onto nutrient agar slants, incubated at 28°C for 24 hours, then kept in the refrigerator for further identification.

5. Confirmation of colonies from selective agar plates:
To confirm the obtained isolates as *Yersinia*, all the pure isolates on the agar slants were sub-cultured for refreshment and examined for gram staining, urease activity, Kligler's Iron agar reaction, and utilization of glucose (gas production) (Krieg and Holt, 1984).

6. Biochemical characterization of *Yersinia* strains:
All the isolates which were confirmed as the genus *Yersinia* were submitted to further biochemical testing to identify its species. Activities of lysine decarboxylase (Møller), ornithine decarboxylase (Møller), urease, and utilization of rhamnose, sucrose, cellobiose, sorbitol, and raffinose were evaluated. Further analyses were also conducted applying esculin hydrolysis, indole and Vogus-Proskauer tests (25°C) (Krieg and Holt, 1984).

7. Biotyping of *Yersinia enterocolitica*:
Yersinia enterocolitica isolates were biotyped according to lipase activity (hydrolysis of Tween 80), Nitrate reduction, indole production, and utilization of D-xylose and D-trehalose (acid production) (Varnam and Evans, 1991).

RESULTS

Sensory evaluation of the examined basterma and luncheon samples revealed that all the examined samples were organoleptically accepted (data not showen).

The results recorded in Table 1 revealed that, the pH values of the examined basterma samples ranged from 3.3 to 6.1 with a mean value of 4.69±0.12. Luncheon samples had pH values ranged from 4.5 to 6.9 with a mean value of 5.85±0.1. Concerning the sodium chloride contents of the samples, they ranged from 4.07 to 12.34 and from 0.65 to 2.93 with a mean value of 7.23±0.21 and 1.7±0.09 for basterma and luncheon samples, respectively (Table 1).

The data presented in Table 2 declared that, out of the 100 examined samples, 39 were suspected positive for *Yersinia* (out of them only 19 were confirmed positive), and 61 were negative. Out of the 70 examined basterma samples 28 (40%) were suspected positive and 42 (60%) were negative. However, only 13 (18.57%) out of the 70 basterma samples were confirmed positive for *Yersinia*. On the other hand, 11 (36.67%) out of the 30 examined luncheon samples were suspected positive and 19 (63.33%) were negative. Only 6 (20%) of the examined luncheon samples were confirmed positive for *Yersinia*.

A total of 98 suspected *Yersinia* isolates were obtained from the suspected samples (74 isolates from basterma samples and 24 from luncheon samples). Out of them, only 27 (27.55%) isolates were confirmed morphologically and biochemically as *Yersinia* and the other 71 (72.45%) isolates were identified biochemically as non-*Yersinia* strains. Of the 27 isolates confirmed as *Yersinia*, 19 were obtained from basterma samples and 8 from luncheon samples (Table 2).

The 27 isolates of *Yersinia* were further characterized into *Y. enterocolitica* (10 strains), *Y. frederiksenii* (5 strains), *Y. intermedia* (2 strains), and *Y. kristensenii* (10 strains). Of the 19 strains from basterma 6, 4, 2, and 7 strains were identified as *Y. enterocolitica*, *Y. frederiksenii*, *Y. intermedia*, and *Y. kristensenii*, respectively. The 8 luncheon isolates were identified as *Y. enterocolitica* (4 strains), Y. *frederiksenii* (1 strain), and *Y. kristensenii* (3 strains), as shown in Table 3.

Yersinia enterocolitica showed the highest incidence from the investigated samples. It was found in 6 (8.57%) and 4 (13.33%) of the examined basterma and luncheon samples, respectively. However, *Y. frederiksenii* was detected in 3 (4.29%) basterma and 1 (3.33%) luncheon samples. *Yersinia intermedia* was detected in 2 (2.86%) of the basterma samples but not in any of the luncheon samples. On the other hand, *Y. kristensenii* was isolated from 5 (7.14%) basterma and 2 (6.67%) luncheon samples. In a total, *Y. enterocolitica* was detected in 10% of the all examined samples, while *Y. frederiksenii*, *Y. intermedia*, and *Y. kristensenii* were detected in 4, 2, and 7% of the examined samples, respectively (Table 4).

The 10 identified *Yersinia enterocolitica* strains from the examined samples were further biotyped into 6, 1, and 3 strains of biovars 1, 3 and 4, respectively. The 6 strains from basterma were biotyped into biovarl (3 strains, 50%), biovar 3 (1 strain, 16.67%) and biovar 4 (2 strains, 33.33%). Likewise, the 4 *Y. enterocolitica* strains from luncheon samples were biotyped into 3 (75%) strains of biovar 1and 1 (25%) strain of biovar 4, as declared in Table 5.

As shown in Table 6, the incidence rates of *Y. enterocolitica* biovars from the examined samples were 6, 1, and 3% for the biovars 1, 3, and 4, respectively. From basterma samples the rates were 4.29% for the biovar 1, 1.43% for the biovar 3, and 2.86% for the biovars 4. While, form luncheon samples, the rates were 10 and 3.33% for the biovars 1 and 4, respectively. The biovar 3 could not be identified from any of the examined luncheon samples.

Table1: Statistical traits for the pH values and the sodium chloride contents of the examined basterma and luncheon samples

Character	Basterma (70)*		Luncheon (30)*	
	pH	NaCl	pH	NaCl
Minimum	3.3	4.07	4.5	0.65
Maximum	6.1	12.34	6.9	2.93
Mean	4.69	7.23	5.85	1.7
SE	0.12	0.21	0.1	0.09

* Number of samples
SE= standard error

Table 2: Occurrence of *Yersinia* in the examined samples

Meat products	Number of samples	Growth on Yersinia isolation agar						Bacterial isolates					
		Suspected positive		Negative		Confirmed positive		Suspected *Yersinia* isolates		Confirmed as *Yersinia*		Non *Yersinia*	
		No	%	No	%	No	%	No	%	No	%	No	%
Basterma	70	28	40	42	60	13	18.57	74	75.51	19	25.68	55	74.32
Luncheon	30	11	36.67	19	63.33	6	20	24	24.49	8	33.33	16	66.67
Total	100	39	39	61	61	19	19	98	100	27	27.55	71	72.45

Table 3: Biochemically characterized *Yersinia* isolates from the examined samples

Meat product	Number of isolates	*Y. enterocolitica*		*Y. frederiksenii*		*Y. intermedia*		*Y. kristensenii*	
		No	%	No	%	No	%	No	%
Basterma	19	6	31.58	4	21.05	2	10.53	7	36.84
Luncheon	8	4	50.00	1	12.5	-	-	3	37.5
Total	27	10	37.04	5	18.51	2	7.41	10	37.04

Table 4: Incidence of the identified *Yersinia* strains from the examined samples

Meat product	Number of samples	*Y. enterocolitica*		*Y. frederiksenii*		*Y. intermedia*		*Y. kristensenii*	
		Positive samples	%	Positive samples	%	Positive samples	%	Positive samples	%
Basterma	70	6	8.57	3	4.29	2	2.86	5	7.14
Luncheon	30	4	13.33	1	3.33	-	-	2	6.67
Total	100	10	10	4	4	2	2	7	7

Table 5: *Yersinia enterocolitica* biovars

Meat product	Number of isolates	Biovar					
		1		3		4	
		No	%	No	%	No	%
Basterma	6	3	50.00	1	16.67	2	33.33
Luncheon	4	3	75.00	-	-	1	25.00
Total	10	6	60.00	1	10.00	3	30.00

Table 6: Incidence of the *Yersinia enterocolitica* biovars from the examined samples

Meat product	Number of samples	*Yersinia enterocolitica* biovar					
		1		3		4	
		Positive samples	%	Positive samples	%	Positive samples	%
Basterma	70	3	4.29	1	1.43	2	2.86
Luncheon	30	3	10	-	-	1	3.33
Total	100	6	6	1	1	3	3

DISCUSSION

Ready to eat meat products consumed without any further cooking may constitute a potential health hazards transmitting many pathogens to humans.

The pH of the food and its content of sodium chloride are among the factors that limit the bacterial growth and determine which species that can grow in it. *Yersinia* can grow at pH range from 4–9 and at sodium chloride concentrations up to 7% (Varnam and Evans, 1991).

The pH mean value for basterma samples "4.69", in the current study, was lower than those detected by Aiedia (1995) "5.40", El Khateib (1997) "5.70", and Abd El-Gafar (1999) "5.32". Concerning their sodium chloride content mean value "7.23%", nearly similar results were detected by Mousa *et al.* (1993), Aiedia (1995) and Abd El-Gafar (1999). However, higher mean values "8.3% and 8.1%" were reported by El Khateib (1997) and Hassan (1997), respectively. As for luncheon samples, higher pH mean value (6.2) and lower sodium chloride content mean value (1.2) were detected by Abd El-Aziz (2004) in 50 samples of luncheon collected at Assiut city, Egypt.

The pH values (3.3 – 6.1) and sodium chloride contents (4.07 – 12.34) of basterma samples seemed to be less suitable for the growth and multiplication of *Yersinia.* However, the pH "4.5 - 6.9" and sodium chloride contents "0.65 – 2.93" of luncheon samples seemed to be more suitable. This may explain the lower incidence of *Yersinia* from basterma (18.57%) than from luncheon (20%) samples. Likewise, it may explain the low percentage of *Yersinia* isolates from basterma (25.68%) than from luncheon (33.33%) samples. The presence of garlic in the coat layer of basterma may also play a role.

Food-borne pathogenic *Yersinia* is facultative anaerobic, gram-negative *Enterobacteriaceae* and is isolated frequently from soil, water, animals, and foods (Ackers *et al.*, 2000; Sharma *et al.*, 2003). The consumption of food contaminated with *Yersinia* can lead to gastrointestinal infections. Cold chain food products could offer a potential food safety hazard as *Yersinia* is able to grow at 4°C "psychrophilic organism" (Aulisio *et al.*, 1983; Grahek-Ogden *et al.*, 2007).

The total incidence of *Yersinia* from the examined samples in the current study was 19%. This is at

variance with findings recorded by Tassinari *et al.* (1994) "40%", Abd El-Malek (2001) "11.4%", and Mohamed *et al.* (2012) "10%" in a variety of meat and meat products samples. Lower incidence of *Yersinia* was recorded by Mohamed *et al.* (2012) in basterma (3.3%), and by the same authors and by Abd El-Malek (2001) (6.7% and 8.6%, respectively) in luncheon samples collected at Assiut city.

The variation of incidences between the investigators may be due to the differences in the geographical distribution of *Yersinia* spp., methods of isolation and diversity of kind of samples. The true incidence of yersiniosis is uncertain as few outbreaks of food-borne illness are investigated and a long incubation period may be required using cold enrichment to recover certain strains from food (ICMSF, 1996).

Yersinia enterocolitica is thought to be a significant food-borne pathogen although the incidence of the pathogenic isolates in food is low (De Boer, 1992; Fredriksson-Ahomaa *et al.,* 2001). Its ability to multiply at refrigeration temperatures makes it of significant importance in food hygiene (Lee *et al.,* 1981). In the current study, *Y. enterocolitica* was detected at an incidence rate of 10% in the examined samples, which disagree with Hudson *et al.* (1992), who found 3.4% of 203 samples of ready-to-eat fleshfoods purchased from retail outlets at Hamilton, New Zealand, positive for *Y. enterocolitica.* The authors assumed that in the case of cooked fleshfoods, most contamination occurs post-cooking and contamination rates are increased by poor food handling procedures.

In basterma samples the incidence rate of *Y. enterocolitica* was 8.57%. Lower incidences were recorded by Abou El-Ela (1994) and Mohamed *et al.* (2012). For luncheon samples, the obtained rate was 13.33%, which is higher than those reported by El-Gohary *et al.* (1993), Abou El-Ela (1994), Ahmed and Mohamed (1998), Abd El-Malek (2001), and Mohamed *et al.* (2012) who reported rates of 10%, 6.6%, 7.5%, 2.9%, and 6.7%, respectively.

The variation between the present findings and those obtained by other investigators may be attributed to several factors including differences in isolation and identification methods and diversity of hygienic condition and competing microflora of samples (Güven *et al.*, 2010).

Yersinia enterocolitica is a common food-borne enteric pathogen found in water, dairy products, and meats (Tauxe *et al.,* 1987; Ackers *et al.,* 2000). It causes human infections whose symptoms include diarrhea, terminal ileitis, mesenteric lymphadenitis, arthritis, and septicemia (Krieg and Holt, 1984).

Biotyping of *Y. enterocolitica* is of considerable importance as a means of discriminating within the species (Varnam and Evans, 1991). *Yersinia enterocolitica,* is highly heterogeneous species and can be divided into several bioserotypes, only a few of which are known to associate with human disease (Bottone, 1997; Robins-Browne, 1997). Most *Y. enterocolitica* strains associated with human yersiniosis belong to bioserotypes 1B/O:8, 2/O:5,27, 2/O:9, 3/O:3, 2/O:5,27 and 4/O:3 with bioserotype 4/O3 dominated globally (Brubaker, 1991; Bottone, 1999). Environmental biovar 1 of *Yersinia enterocolitica* were isolated from water, soil, food, faeces, humans and animals, and they are nonpathogenic (Varnam and Evans, 1991).

In the current study, biovars 1, 3, 4 were detected from basterma samples, while biovars 1, 4 were obtained from luncheon samples at varying rates. Biovar 1 was the highest in number (6 strains) and incidence rate (6%), while biovar 3 was the lowest (1 strain; 1%). Mohamed *et al.* (2012) detected biovar 3 in 11.1 and 2.9% of examined basterma and luncheon samples, respectively, which is higher than the obtained results. Abd El-Malek (2001) isolated biotype 3 at an incidence rate of 22.2% from examined luncheon samples. In the present investigation biovar 3 could not be detected in any of the examined luncheon samples. Abou El-Ela (1994) identified *Y. enterocolitica* biovar 4 recovered from luncheon samples at percentage of 6.6% that seemed higher than the present findings (3.33%).

Yersinia frederiksenii, Y. intermedia and *Y. kristensenii* are considered to be environmental organisms. However, they described as opportunistic occasionally associated with illness in humans. *Yersinia frederiksenii* was isolated from some patients as well as *Y. enterocolitica* (Varnam and Evans, 1991).

Yersinia intermedia and *Y. kristensenii* failed to be detected in any of examined basterma and luncheon samples collected at Assiut city by Mohammed *et al.* (2012), which is partially at variance with the current findings. Abd El-Malek (2001) could not detect *Y. intermedia* in any of the examined luncheon samples collected at Assiut city, which is in agreement with the obtained results; however he could detect *Yersinia kristensenii* in luncheon samples at an incidence rate of 5.7% that is seemed slightly lower than the present findings (6.67%). El-Gohary *et al.* (1993) detected *Y. intermedia* at a rate of 4% in 50 samples of luncheon collected from Alexandria Governorate.

Yersinia frederiksenii, and *Y. intermedia* has been isolated mainly from fresh water sources, fish, foods,

and occasionally from sick and healthy humans. Strains of *Y. kristensenii* have been isolated mainly from soil, from various environmental sources (fresh water, foods) and rarely from healthy or sick man and animals (Krieg and Holt, 1983).

On conclusion, the results of this study emphasized that basterma and luncheon available in the markets were contaminated with *Yersinia*, which may reflect the lack of hygienic supervision. For that, these products may constitute a potential health hazards to human causing sporadic non-outbreak illness of yersiniosis. Good sanitary precautions, hygiene and proper food handling may be necessary to prevent contamination of such products with *Yersinia*, especially pathogenic ones, and to control the disease. As well, proper heat treatment before consumption "if possible" may be helpful to control the disease.

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اليرسينيا في بعض منتجات اللحوم المصرية سابقة التجهيز

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اجريت هذه الدراسة لتقييم تواجد ميكروب اليرسينيا في بعض منتجات اللحوم المصرية سابقة التجهيز. تم جمع عدد ١٠٠ عينة من المنتجات (٧٠ عينة من البسطرمة ، و ٣٠ عينة من اللانشون) بطريقة عشوائية من الاماكن المختلفة (محلات البقالة ، والسوبر ماركت) بمدينة اسيوط. تم فحص العينات حسيا ، كما تم قياس تركيز ايون الهيدروجين وتحديد نسبة ملح الطعام في العينات. اظهر الفحص الحسي ان جميع العينات مقبولة من الناحية الظاهرية. كانت متوسطات تركيز ايون الهيدروجين ومحتوى ملح الطعام هي ٤,٦٩ ، ٧,٢٣ ، و ٥,٨٥ ، ١,٧ في عينات البسطرمة واللانشون على التوالي. تواجد ميكروب اليرسينيا في ١٣(١٨,٥٧%) عينة من البسطرمة ، و ٦(٢٠%) عينات من اللانشون. تم عزل اجمالى عدد ٩٨ عترة من عزلات البكتريا وجد منها عدد ٢٧(٢٧,٥٥%) عترة تنتمي لميكروب اليرسينيا. تواجدت ميكروبات *Yersinia enterocolitica,* في عينات البسطرمة بمعدل ٨,٥٧ ، ٤,٢٩ ، ٢,٨٦ ، و ٧,١٤ % ، بينما *Y. frederiksenii, Y. intermedia,* and *Y. kristensenii* تواجدت ميكروبات *Yersinia enterocolitica, Y. frederiksenii,* and *Y. kristensenii* في عينات اللانشون بمعدلات ١٣,٣٣ ، ٣,٣٣ ، و ٦,٦٧ % ، على التوالي. تم تصنيف عترات *Yersinia enterocolitica* والتي عزلت من عينات البسطرمة إلى biovars ١، ٣، و ٤ بمعدلات ٤,٢٩ ، ١,٤٣ ، و ٢,٨٦% ، على التوالي. كما تم تصنيف العترات التي عزلت من عينات اللانشون إلى biovars ١ و ٤ بمعدلات ١٠ و ٣,٣٣%، على التوالي. تم مناقشة الأهمية الصحية لميكروبات اليرسينيا التي تم التعرف عليها.

الكلمات الدالة: اليرسينيا ، منتجات اللحوم سابقة التجهيز ، البسطرمة ، اللانشون