SURVEY ON THE INCIDENCE AND TOXIGENICITY OF *YERSINIA ENTEROCOLITICA* IN SOME MEAT PRODUCTS

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ABSTRACT

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A total of 120 samples of meat products including luncheon, beef burger, sausage and basterma (30 of each) were collected from different supermarkets and shops in Assiut city to study the prevalence of *Yersinia* spp.in these products and confirmation of the isolated *Y.enterocolitica* by PCR as well as the virulence of this organism.The obtained results showed that the incidence of *Yersinaia spp*. wase 6.6, 10, 20 and 3.3% in examined luncheon, beefburger, sausage and basterma samples respectively.The recorded data revealed that the incidence of *Y.enterocolitica* in the same products was 6.7, 6.7, 13.3 and 3.3% respectively. Concerning, *Y.intermedia*, it could be isolated from 3.3% of sausage samples only while *Y.kristensenin* was isolated from beef burger and sausage in percentage 3.3% for each. The biotyping results of *Y.enterocolitica* in this study shown that the two strain of *Y. enterocolitica* isolated from luncheon and the one strain which was isolated from basterma were of biotype 3 while one strain of *Y.enterocolitica* which isolated from beefburger was Biolype 2 and the other strain was of Biotype 3. Also the four strains isolated form sausage were found to be two strains belonged to biotype 2 and the other two strains belonged to biolype3. Confirmation of *Y.enterocolitica* was done by suing PCR while the virulence was carried out by using Auto-agglutiration test. The results revealed that 4 from 9 of the isolated *Y.enterocolitica* had the ability to cause turbidity or clumbing (virulent) in percentage of 44.4%. The other 5 strains 55.5% were avirulent. The public health importance of the organism was discussed and the suggestive measures for safe healthful products were outline.

***Key words:** Meat products, yersinia entero colitica, PCR.*

دراسة مدي تواجد وسمية ميكروب اليرسينيا انتيروكوليتكا في بعض منتجات اللحوم

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أجريت هذه الدراسة على ١٢٠ عينة من منتجات اللحوم (اللانشون ، البيف بيرجر، السجق، البسطرمة) ٣٠ من كل نوع والتى جمعت من محلات البقالة والسوبر ماركت المختلفة في محافظة أسيوط لمعرفة مدى تواجد ميكروبات اليرسينيا وتصنيفها والتأكد من ميكروب اليرسينيا انتيروكولوتيكا باختبار انزيم البلمرة المتسلسل واختبار مدى سمية

هذا الميكروب المعزول: حيث تم عزل ميكروبات االيرسينيا بنسبة ٦,٧ ، ١٠، ٢٠، ٣,٣% من عينات اللانشون والبيف بيرجر والسجق والبسطرمة بالترتيب وقد أوضحت الدراسة أن نسبة عزل ميكروب االيرسينيا انتيروكوليتكا في نفس العينات كانت ٦,٧، ٦,٧، ١٣,٣، ٣,٣% بالترتيب وقد تم عزل ميكروب اليرسينيا انترميديا بنسبة ٣,٣% من عينات السجق فقط بينما أمكن عزل ميكروب اليرسينيا كريستسيناي من عينات البيف بيرجر والسجق بنسبة ٣,٣% لكل منهما وقد دلت الدراسة على أن عترتي اليرسينيا انتيروكولتيكا التي عزلتا من اللانشون والعترة التي عزلت من البسطرمة صنفوا على أنهم biotype 3 أما بالنسبة للعترات التي عزلت من البيف بيرجر فقد وجد ان عترة واحدة صنفت على أنها biotype 2 والعترة الأخرى صنفت على أنها biotype 3 بينما العترات التي عزلت من السجق (٤عترات) فقد وجد أن عترتين صنفتا على انهما biotype 2 وعترتين صنفا على أنهما biotype 3 هذا وقد تم إجراء أنزيم البلمرة المتسلسل للتأكيد من وجود ميكروب اليرسينيا انتيركولتيكا وعند اختبار مدى سمية ميكروب اليرسينيا انيتركولتيكا ا باختبار Autoaglutination test اتضح أن ٤ من ٩ من الميكروبات المعزولة حققت قدرتها على إحداث تعكير وتجلط بنسبة ٤٤,٤% بينما باقى الميكروبات المعزولة (٥ميكروبات) لم تحقق قدرتها على إحداث نتائج ايجابية بنسبة ٥٥,٥% هذا وقد تم مناقشة مدى خطورة هذا الميكروب على صحة المستهلك والطرق المقترحة للحد منه.

INTRODUCTION

Meat and meat products are subjected to contamination with several types of microorganisms from different sources during the period elapses from the time of slaughtering, preparation, processing and cooking. High incidence of bacteria food poisoning occurred in last years due to *Salmonella* and *Yersinia enterocolitica* infection (Greenwood and Hooper, 1990).

The genus Yersiniuis a member of the family *Enteriobacteriaceae*, contain 11 spp. including. *Y.pestis, Y.pseudotuberculosis* and *Y.enerocolotica* wich are pathogenic for humans and animals. *Y. frederiksenii, Y.intermedia, Y.kristensenii, Y.aldova, Y. rohdei, Y. mollareti* and *Y. bercovier* are widespread in nature but are not usually associated with diseases. *Y. ruchei* is a fish pathogen, which cause red mouth disease (Carnial and Mollaret, 1990).

Y.enterocolitica is a Gram-negative, facultatively anaerobic, rod, shaped bacterium. Optimal growth occurs at 30-37°C but the bacterium is psychrotrophic and can grow in food at refrigeration temperature (4°C) (Bercovier and Mollaret, 1984).

Yersinia species consists of pathogenic and nonpathogenic spp. The virulence of *Y.enterocolitica* is associated with biogroup, serogroup and geographic distribution. However, *Y.enterocolotica* strains have been distributed in 5 biogroup according to their biochemical properties. Strains of biogroups 2,3,4 and 5 (except 3A and 3B) are restricted to a small number of serogroups (0:1; 0:2, 0:3, 0:5, 27, 0:9) and are generally isolated from a specific host. Strains of serogroups 0:3; 0:9; and 0: 5.27 are the major causes of human infections in Europe, Japan, Southern Africa and Cardada (Carter, 1975).

Y.enterocolitica is a zoonotic microorganism capable of causing yersiniosis in humans through direct,or in direct contamination of food by faeces or urine. Meats can be contaminated by faecal materials during slaughtering and dressing (Stern, 1981; Tudor *et al.*, 2010).

The disease yersiniosis, which is caused by this orgaism is typically detected after infection by examination of a patients stool. It occurs often in children 5-15 years of age or in older adults that may have weakened immune systems. The symptoms which take about 4-7 days to manifest and can last from 1-3 weeks, are nausea, diarrhea and abdominal pain mesenteric lymphadenitis, acutentritis, arthritis and skin rash called "erythema nodosum" (Robert, 2008).

Microbac laboratories had developed an assay to quickly test for and identify *Y.enterocolitica* in client samples by using PCR technology which is based on DNA detection as a molecular techniques (Robert, 2008 and Tudor *et al.*, 2010).

Using PCR is not only the time and cost saving for detecting the pathogen but the specificity and sensitivity of the analysis which doesn't require long enrichments or confirmation step (Robert, 2008).

A simple system for determinating the

virulence of *Y.enteroclotica* based on their observation that the virulent strain of *Y.enterocolitica* agglutinated when grown on tissue culture media while avirulent strains didn't exhibit the agglutination property (Laird and Cavanaugh, 1980). Plasmids containing virulence genes may be lost during culture and conformational procedures. Temperature above 30°C, is known to cause a loss of virulence plasmids in pathogenic *Y.enterocolitica* but plasmid loss may also occur as a result of other less defined circumstances (Johnson, 1998).

Most clinical isolates of *Y.enterocolitica* produce a heat-stable enterotoxin. However, this enterotoxin probably doen't play an important role in *Y.enterocolitica* infection because most *Y.entercolotica* don't produce enterotoxin in vitro at temperature greater than 30°C and because strains that doesn't produce enterotoxin in vitro caused diarrhea in experimentally infected mice (Scheimann, 1989).

The purpose of the present investigation was designed to evaluate the prevalence of *Yersinia enterocolitica* in some meat products (sausage, beefburger, basterma and luncheon), confirmation of the isolated strains by PCR and carrying out the virulence of *Y.enterocolitica* recovered from the examined samples.

MATERIALS and METHODS

Collection and preparation of samples:
A total of 120 randam samples of meat products including30 of each luncheon, beefburger, sausage and basterma were collected from different supermarkets and shops in Assiut Governorate. Each sample was put in a sterile plastic bag. The collected samples were transferred directly to the laboratory under aseptic conditions without any delay where they were prepared for examination. Each sample was aseptically and carefully freed from its casing and mixed thoroughly in sterile mortar.

Enrichment procedure:
Twenty five grams of each sample were weighted aseptically and 225ml of trypticase soy broth were added then the mixture was blended for 2 min. The samples were incubated at 4°C for 14 days as cold enrichment as recommended by speck (1984).

Isolation procedure (Schiemann, 1979):
Loopfuls from the previously incubated enrichment broth were streaked onto Cefulodin Irgasan Novobiocin (CIN) agar and incubated at 22-23°C for 24-48 h. The colonies which showed dark red centre bulls eye" with a translucent border were picked up and subcultured on nutrient agar slants and incubated at 37°C for 24h for further confirmation and identification.

Identification of presumptive colonies:
Suspected colonies were confirmed by Gram's stain, kliger Iron Agar, Christensen's urea ager, Vogas-Proskauer,S fermentation test, Indole production test and Simmons citrate according to Speck, (1984).

Biochemical differentiation:
The following test were done:
Vogas-Proskauer, Sucrose, L.rhamnose, Dmelibiose, Indole, D.raffinose and Simmons citrate as described by Bercovier *et al.* (1980).

Biotyping of *Yersinia enteroclitica*:
Y.enterocolitica strains were biochemically differentiated into 5 categories according to Wauters, Biotype Scheme as cited in Speak (1984), which included lecithinase, Indole, xylose and trehalsoe test. It is most widely used and is helpful in tracing the source of epidemic strains.

Molecular analysis:
Total genomic DNA and PCR amplifications: (for the nine strains of confirmed isolates) were used as described by Wang *et al.* (1994) for *Yersinia enteroclitica*-specific identification, two primers pairs were used. The primer's sequence, the target, the PCR products size and the references, are listed in Table 1.

The oligonucletoide primers: were synthesized by Universal DNA Inc. (Tigard, OR, USA) and were unpurified grade.

PCR assy: PCR amplification were performed using Master Max. The reaction mixture contained a total volume of 25µL in

0.5mL tubes included: 12.5μL of Master Max., 2.0μL of primerYE-1, 2.0μL of primer YE-2,μL of free water and 6.5μL of Template DNA.

Cycling conditions:one cycle of 94°C for 15s, then35cycles of 94°C for 3s, 50°C for 10s and 74°C for 35s at the transition speed 5-9, and finaly, one cycle at 74 °C for 2min and 45°C for 2s, The PCR products (6-10μ/of each) were separated by electrophoresis in 2% agarose gels containing ethidium bromide (1μg $m/^{-1}$) (Wang *et al.*, 1994).

Detection of PCR products: The gel was stained with ethedium bromide and amplicans were visualized on UV Tran illuminator. The 1-KB was used as molecular size marker.

Table 1: Oligonucleotide sequences used for ideification of *Yersinia enterocolitica* by PCR:

Target gene	Primer sequence (5' -3')	Product size	Reference
Enterotoxin gene	YE-F,CTG TCT TCA TTT GGA GCA TTC YE-R,GCA ACA TAC ATC GCA GCA ATC	159bp	Ibrahim *et al.* (1992)

Detection of pathogenicity of *Yersinia enteroclitica* isolates recovered from the examined samples:

1- Biochemical tests:

Only 4 biochemical tests are necessary for identifying potentially pathogenic form of *Y.enterocolitica* which included Kligler Iron Agar, Urea hydrolysis, Sucrose fermentation and Salicin fermentation according to Schiemenn and Devenish, (1982).

2- Autoagglutionation test:

As recommended by A.O.A.C.,(1984) the culture of each isolate of *Y.enteroclitica,* previously grown on Trypticase Soy Agar at 22°C for 48h, was inoculated into each of two tubes containing 4 ml of UR-VR broth. One tubes was incubated at 22°C and the other tube was incubated at 35°C for 24h. Either turbidity in tubes incubated at 22°C or clumbing of bacteria along the walls of the tubes or on their bottom with clear supernatant fluid in tubes incubated at 35°C were considered positive for auloagglutination test.

RESULTS

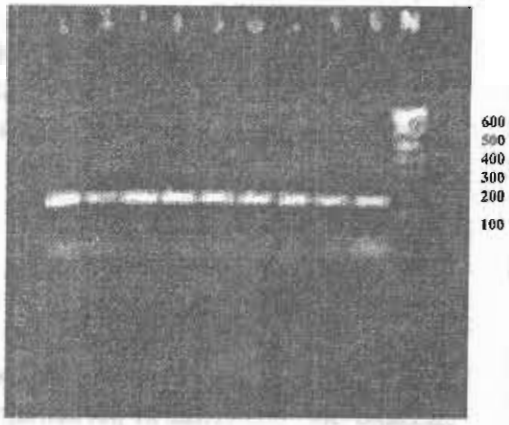
Table 2: Incidence and distribution of *Yersinia* species isolated from the esamined meat products

Types of samples	No. of exam. samples	*Yersinia* spp.		*Y.enterocolitica*		*Y.entermeida*		*Y.kristensenti*	
		+ve	%	+ve	%	+ve	%	+ve	%
Luncheon	30	2	6.7	2	6.7	0	0.0	0	0.0
Beefburger	30	3	10	2	6.7	0	0.0	1	3.3
Sausage	30	6	20	4	13.3	1	3.3	1	3.3
Basterma	30	1	3.3	1	3.3	0	0.0	0	0.0
Total	120	12	10	9	7.5	1	0.8	2	1.7

Table 3: Biotyping of the isolated *Y.erterocolitica* from the examined meat products

Types of samples	No. of +ve samples tested	Biotype 2		Biotype 3	
		No.	%	No.	%
Luncheon	2	0	0.0	2	22.2
Beef burger	2	1	11.1	1	11.1
Sausage	4	2	22.2	2	22.2
Basterma	1	0	0.0	1	11.1
Total	9	3	33.3	6	66.6

Identification by PCR:



Lane1-9: +ve for *Yersinia entrocolitica.*
M: Molecular size Marker(1-KB).

Fig. 1: Results of PCR for detection of *Y.enterocolitica* in the examined meat products.

Table 4: Pathogenicity of *Y.enteroclitica* strains isolated from the examined meat products.

Types of exam samples	No. of exam. samples	Reactions							
		+++ve		++ve		+ve		-ve	
		No.	%	No.	%	No.	%	No.	%
Luncheon	2	-	-	1	11.1	-	-	1	11.1
Beefburger	2	-	-	1	11.1	-	-	1	11.1
Sausage	4	1	11.1	-	-	1	11.1	2	22.2
Basterma	1	-	-	-	-	-	-	1	11.1
Total	9	1	11.1	2	22.2	1	11.1	5	55.5

+ + + ve = High degree of pathogenicity
+ + ve = Moderate degree of pathogenicity
+ ve = Weak degree of pathogenicity
-ve = Non pathogenic

Table 5: Incidence distribution of pathogenic and non pathogenic *Y.enteroclitica* isolated form the examined meat products

Types of samples	No. of +ve samples tested	pathogenic		Non pathogenic	
		No.	%	No.	%
Luncheon	2	1	11.1	1	11.1
Beef burger	2	1	11.1	1	11.1
Sausage	4	2	22.2	2	22.2
Basterma	1	-	-	1	11.1
Total	9	4	44.4	5	55.5

DISCUSSION

Y.enterocolitica is found world wide. The organisms has been recovered from a wide variety of animals, foods and water. The recorded data all over the world suggest that outbreaks of foodborne yersiniosis are rare (Gracey *et al.*, 1999).

1- Prevalence of *Yersinia* spp. in examined some meat products:

The finding outlined in Table 2 indicated that *Yersinia* spp. were isolated from 2, 3, 6 and 1 out of 30 examined samples from each of luncheon, beefburger, sausage and basterma with an incidence 6.7, 10, 20 and 3.3% respectively. Abdel-Malek (2001) could isolate *Yersinia* spp. from luncheon, beefburger and sausage samples in percentage 8.6, 2.9 and 14.3% respectively which disagree with the obtained results. The total incidence of *Yersinia* spp. recovered from all, the examined samples in this study was 10% which is disagree to that recorded by Dalmas and Vidon(1985) (33.3%), Tassinari *et al.* (1994) (40%)and Escudero *et al.* (1996) (75%).

The variation of incidence between the investigators may be due to the differences in the geographical distribution of *Yersinia* spp. and methods of isolation. The true incidence of yersiniosis is uncertain for various reasons: few outbreaks of foodborne illness are investigated, yersiniosis has recently known to be food or water borne and a long incubation period may be required using cold enrichment to recover certain strains from food (IC MSF, 1996).
The biochemical reaction of isolated strains of *Yersinia* spp. were differentiated into: *Y.enterocolotica*, *Y. Intermedia* and *Y.kristensenii* as shown in Table 2

I - Y.enterocolotica

The present results recorded in Table 2 revealed that *Y.entocolotica* was recovered from 2,2,4 and 1 out of the 30 examined samples from each of luncheon, beefburger, sausage and basterma in percentage 6.7, 6.7, 13.3 and 3.3% respectively with a total incidence of 7.5%. The obtained results of *Y.enterocolotica* of luncheon was nearly similar to that recorded by Abou El-Ela (1994) and Ahmed and Mohamed (1998) who recorded 6.6% and 7.5% respectively while it was higher than that recorded by Abdel-Malek (2001) (2.9%) and lower than El-Gohary *et al.* (1993) (10%).On the contrary Abd El-All (1993) Abd Eaziz *et al.* (1996) and Ahmed (1996) failed to detect *Y.enterocolotica* in luncheon.

The incidence of *Y.enterocolitica* in beefburger samples in the present study was higher than that obtained by Abd El-Monem; Saad (1988) (2%), Nortji *et al.* (1998) (3.9); Abdel-Malek (2001) (2.9%), but it was lower than Abd El-All (1993) (8%); El-Taher (1998) (20%). The lower incidence of *Y.enterocolotica* in both luncheon and beefburger may be attributed to the addition

of spices and nitrite which have a bacterostatic affect (Libby, 1975), in addition to, the curing processing of the products which play a greet effect of the survival and multiplication of the existing microorganisms (Fehlhaber 1981).

Evaluation of sausage samples for the existence of *Y.enterocolitica* in this study are nearly similar to those reported by El-Gohary *et al.* (1993) (14%); Abdel-Malek (2001) (14.3). A higher incidence was recorded by Abd El-All (1993) (20%), Khalafalla (1995) (15%); Garcia Lopez *et al.* (1998) (19.9%) whereas lower findings were detected by Nortji *et al.* (1998) (2%), Vural *et al.* (1996) (0.8%); El Taher (1998) (10%).

The presence of *Y.enterocolotica* in high incidence in the examined sausage samples may be attributed to the bad hygienic conditions during manufacturing of the product as well as contamination of raw materials, contact surfaces and casing (Abd El -Malek, 2001).

The prevalence of *Y.enterocolitica* in the examined basterma samples was nearly agreement to that recovered by Abou El-Ela (1994). However, some investigators (Abd El-Aziz *et al.*, 1996; Ahmed 1996) failed to detect *Y.enterocolitica* in the examined luncheon, beefburger, sausage and basterma collected from different shops in Cairo and Giza.

The failure to detect *Y.enterocolotica* in some food products may be due to the acidic condition of such products or presence of other microbial growth which lowered the pH because the pathogen is sensitive to acidic condition (Feeley and Schiemenn, 1984).

II- Y.intermedia:

From the data shown in Table 2, *Y.intermedia* was isolated from 1 out of 30 examined sausage samples in percentage of 3.3% with a total incidence 0.8%, wherease the pathogen could not be detected in luncheon beefburger and basterma samples. Many investigators such as Inoue and Kurose (1975), Leistner *et al.* (1975); Karib *et al.* (1994) recorded the occurrence of *Y.intermedia* in the examined beefburger, furthermore, El-Gohary *et al.* (1993) succeeded to isolated *Y.intermedia* from 4% of the examined luncheon and sausage samples.On the other hand AbdEl-Malek (2001) failed to detect *Y.intermedia* in the examined luncheon, beefburger and sausage samples.

III-Y.kristensenii:

Y.kristensenii is isolated from one sample out of 30 examined samples from both beefburger and sausage with an incidence of 3.3 and 3.3% respectively and a total incidence of 1.7%.The organism was not detected in the examined luncheon and basterma samples while AbdEl-Malek (2001) succeeded to isolate *Y.kristensenii* from luncheon in percentage 5.7% .Many researchers as Inoue and Kurose (1975), Leistner *et al.* (1975) and Karib *et al.* (1994) reported the prevalence of *Y.kristensenil* in the examined raw beef.

Morever, The variation between the present findings and those obtained by other investigators may be attributed to several factors including enrichment broth and plating media used as previously reported by Schiemann and Wauters (1992).

2- Biotyping and virulence of *Y.enterocolitica*:

A- Biotyping of *Y. enterocolitica*:

From the data outlined in Table 3, it was found that all *Y.enteroclitica* isolated from luncheon (2 strains) belonged to biotype3, while the 2 strains which were recovered from beefburger: one of them belonged to biotype2 and the other was belonged to biotype 3. Morevere the 4 strains of *Y.enterocolotica* which obtained from sausage were belonged to biotype 2 (2strains) and biotype 3 (2strains). On the other hand, the only strain of *Y.enterocolotica* which was recovered from basterma belonged to biotype 3. Iit was apparent that *Y.enterocotica* biotype 3 was the most frequent biotype which represent 66.6% of all the tested *Y.enterocolotica* isolates.

In Egypt, Fady (1993) recorded that *Y.enterocolitica* biotype 2 was isolated from fresh sausage samples. Also Abou El-Ela

(1994) identified *Y.enterocolitica* biotype 4 recovered from luncheon.

The strains which identified biochemically as *Yersinia enterocolotica* were subjected to PCR test .The result indicated that all strains were positive for *Yersinia enterocolitica.*

B- Virulence of*Y. enterocolotica* recovered from examined samples:

The results presented in Table 4 revealed that one out of two *Y.enterocoltica* strains isolated from the examined luncheon samples was regarded as of moderate degree of pathogenicity depending on their reactions on autoagglutination test and the other strain was negative for autoagglutination test (non-pathogenic). Whereas one strain of *Y.enterocototica* out of 2 two strains recovered from beefburger was pathogenic with moderate degree, while the second strain was negative for autoaglutination (non pathogenic). Concerning the virulence of *Y.enterocolitica* strains detected in sausage samples, it was found that one out of four isolated strains was of high degree of pathogenicity, one was weak degree of pathogenicity and the other two strains were non pathogenic. On the other hand the only one strain of *Y.enterocolitica* recovered from basterma samples was non pathogenic.

Regarding the total incidence of the pathogenicity of *Y.enterocolitica* strains isolated from all the examined samples, it was cleared in Table 4 that, 1 (11.1%), 2 (22.2%), 1 (11.1%) and 5 (55.5%) were tabulated as high degree (+ + +ve), moderate degree (+ +ve), weak degree (+ ve) of pathogenicity and non pathogenic (-ve) respectively.

The summarized data in Table 5 emphasized that 4(44.4%) out of 9 *Y.enterocolitica* strains recovered from the examined samples were pathogenic, whereas 5 (55.5%) were found to be non pathogenic.

In fact, it was reviewed that only *Y.enterocditica* was considered as pathogenic for humans, while the other species, such as *Y.intermedica* and *Y.kristensenii* appeared to be primarily environmental strains that do not cause human illness (Feeley and Schiemann, 1984).

The recovery of pathogenic *Y.enterocolotica* is contingent upon a number or factors including the amount and level of back ground flora coming from enrichment and plating, the level of pathogenic *Y.enteroclotica* and the numbers of non pathogenic *Y.enterocolotica* present in the sample (Johnson, 1998).

The autoagglutination test is suitable for routine use and has been described as the most useful test for identifying pathogenic strains of *Y.enterocolotica* as mentioned by Varnam and Evans (1991).

In conclusion the information given by the achieved results revealed that some meat products were contaminated by *Yersinia* spp. and this may reflect the lack of hygienic supervision. Therefore these products play a significant role in the epidemiology of yersiniosis. So strict hygienic measures during manufacturing transportation and storage, properly heated for cooked food and avoid cross contamination with animal and human feces should be recommended to avoid contamination with *Yersinia* spp.

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