The Prevalence Of *Yersinia enterocolitica* And Other Yersinia Species In Meat And Some Edible Offal Of Food Animals

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ABSTRACT

The objective of this study was to investigate the prevalence of *Y. enterocolitica* and other Yersinia species in meat and some edible offal samples of food animals and to determine their susceptibilities to antimicrobial agents. For this purpose, a total of 90 random samples (30 each of meat, liver and kidney) were collected from 30 animals (10 each of cattle, sheep and camel) from local butcher's shops in Sharkia province, Egypt (traditionally slaughtered animals at Zagazig abattoir). The obtained results declared that Yersinia spp. were detected in 6 out of 30 (20%) cattle samples, 7 out of 30 (23.33%) sheep samples and from 7 (23.33%) of camel samples. *Y.enterocolitica, Y.intermedia* and *Y.frederiksenii* could be isolated and identified from the examined meat, liver and kidney of the examined animals with varying percentages. Antibiotic susceptibility analysis showed that there was no difference in the susceptibilities of the three *Yersinia* species isolated from the examined samples. The results revealed that no strains were resistant to ceftriaxone, cefuroxime, ciprofloxacine, gentamicin, kanamycin, neomycin or polymyxin. In contrast, there were high levels of resistance to ampicillin, penicillin, cefalothin and amoxicillin.

INTRODUCTION

Yersiniosis is a disease that affects wild and domestic animals as well as humans. Enteric yersiniosis is caused by pathogenic *Yersinia enterocolitica.* However, human yersiniosis, which is very common in Europe, is mostly caused by *Y. enterocolitica (1).* The disease is transmitted by the faecal– oral route and typical symptoms are fever, abdominal pain and diarrhoea *(2,3).*

Y. enterocolitica is known as a psychrotrophic waterborne and foodborne enteropathogen *(4).* Outbreaks of yersiniosis are commonly associated with food vehicles such as meat (particularly pork), milk, powdered milk, cheese, tofu and raw vegetables *(5-7).* The first and definitive food-associated outbreak of yersiniosis occurred in Oneida County, New York, where over 220 individuals (primarily school-age children) were stricken with acute gastroenteritis after the consumption of contaminated milk *(8). Y. enterocolitica* has been isolated from meat, chicken, vacuum packaged meat, pork, ham, drinking water, milk and oysters *(9-12).*

Y. enterocolitica are commonly detected in meat and poultry products as well as from oysters, mussels, shrimp, blue crab, fish, salad and stewed mushrooms, and cabbage, celery and carrots. The level of this pathogen was found consistently in high numbers on vacuum-packed meats with a pH above 6 held at low temperature *(13,14).* Growth of this pathogen is enhanced in cooked meats or at low temperature whereas competitive microorganisms are inactivated.

Yersinia enterocolitica is a foodborne pathogen that can cause acute gastroenteritis and mesenteric lymphadenitis mimicking appendicitis *(2,15).* Human yersiniosis is the third most common enteric disease after campylobacteriosis and salmonellosis in many European countries *(1).* However, epidemiological data on the prevalence of pathogenic *Y. enterocolitica* in animals in EU-member states are missing as the reporting of this pathogen in animals is not mandatory in most countries.

Y. enterocolitica is commonly transmitted to humans by contaminated water and foods. Wild

and domestic animals can also act as reservoirs, and their role in the epidemiology of this infection has been studied *(16)* Previous investigations carried out in San Luis, Argentina, have shown the presence of *Yersinia* in different samples of slaughtered animals (porcine tongues the aim of this study was to investigate The prevalence of *Yersinia enterocolitica* and other Yersinia Species in meat and some edible offal of food animals and caecum contents, bovine tongues and chicken skin) *(16)* .

Seasonality seems to play a pivotal role in the incidence of *Y. enterocolitica* infection in focal geographical areas, with a higher frequency noted during cooler parts of the year. In Belgium, for instance, *(17)* noted that 'isolations peak in late autumn and early winter', which parallels the incidence noted in Italy, Czechoslovakia, and Scandinavia. Reports, however, from the United States, The Netherlands, Australia, South Africa, Canada, Japan, and Bangladesh do not show a seasonal clustering aim of work Factors.

MATERIALS AND METHODS

A total of 90 random samples (30 each of meat, liver and kidney) were collected from 30 animals (10 each of cattle, sheep and camel) from local butcher's shops in Sharkia province, Egypt (traditionally slaughtered animals at Zagazig abattoir). Each collected sample was placed in a sterile plastic bag, well identified and immediately transferred to the meat hygiene laboratory in the Food Control Dept. Faculty of Vet. Medicine Zagazig University in ice box where they were bacteriologically examined for the presence of *Y. enterocolitica* and other Yersinia species.

Y. enterocolitica and other Yersinia species were isolated using 2-stage enrichment procedures including pre-enrichment in Tripticase Soy Broth (TSB-Difco 0370-01-1) *(18-20)* and selective enrichment in Bile Oxalate Sorbose (BOS) Broth *(20,21)* Then a loopful of each sample was streaked onto Cefsulodin-Irgasan-Novobiocin (CIN) agar (Oxoid-CM 653). Five presumptive Yersinia colonies from each sample were selected for further biochemical analyses *(20,22).* Standard oxidase, catalase, Gram staining, and other biochemical tests for the isolation and identification of Yersinia spp. were performed at 25 °C as shown in Figure (1) *(14).* Then the biochemical results were assessed according to *(20,22,23).*

Pre-enrichment (cold) procedure
(25 g of examined sample + 225 ml of Tripticase Soy Broth, 4 °C for 21 d)
↓
Selective enrichment procedure
(0.1 ml Pre-enrichment sample + 10 ml of Bile Oxalate-Sorbose (BOS) Broth, 25 °C for 5 d)
↓
Inoculation onto Cefsulodin-Irgasan-Novobiocin agar
(CIN Agar suppl. SR 109, 30 °C for 24-48 h)
↓
***Yersinia* isolation procedure (on 5presumptive positive colonies)**
- Gram staining Gram negative, coccobacilli or short-bacille without spore
- Catalase positive
- Oxidase negative
- Klinger Iron Agar alkaline/acid reaction, no H_2S production
- L-Arginine decarboxylase (Moeller) negative
- Nitrate reduction positive
- Urea hydrolysis positive
- Motility positive

↓
***Y. enterocolitica* and other *Yersinia* spp. identification procedures (25 °C)**
-Voges Proskauer (VP)
-Citrate utilization
-Indole
-L-Ornithine decarboxylase (Moeller)
-Carbonhydrate fermentation
-D-Sucrose -D-Raffinose
-L-Rhamnose -D-Sorbitole
-D-Melibios -α-Methyl D-glucoside

Figure1. Isolation and Identification Procedure of *Y. enterocolitica* and other *Yersinia* spp.

Table 1. Biochemical reaction (36°C) used to differentiate the four Yersinia species

Species	Acid production form			
	Sucrose	L-Rhamnose	Raffinose	Melibiose
Y.enterocolitica	+[a]	-	-	-
Y.kristensenii	-	-	-	-
Y.frederiksenii	+	+	-	-
Y.intermedia	+	+	+	+

A symbols: +=positive within 7 days, -= negative at 7 days according to *(13, 24).*

Antibiotic Resistance

To obtain basic data for resistance monitoring programs, *Y. enterocolitica* strains isolated from the examined samples were tested for their susceptibilities to antimicrobial agents according to *(25).*

RESULTS AND DISCUSSION

Table 2. Incidence of Yersinia species isolated from the examined cattle samples (No. of samples =30).

Samples	*Y.enterocolitica*		*Y.intermedia*		*Y.frederiksenii*		Total Yersinia Spp.	
	No.	%	No.	%	No.	%	No.	%
Meat(10)	1	10	1	10	-	-	2	20
Liver(10)	2	20	-	-	1	10	3	30
Kidney(10)	1	10	-	-	-	-	1	10
Total(30)	4	13.33	1	3.33	1	3.33	6	20

Table 3. Incidence of *Yersinia* species isolated from the examined sheep samples (No. of samples = 30)

Samples	*Y.enterocolitica*		*Y.intermedia*		*Y.frederiksenii*		Total Yersinia Spp.	
	No.	%	No.	%	No.	%	No.	%
Meat(10)	2	20	-	10	1	10	3	20
Liver(10)	1	10	1	-	1	10	3	30
Kidney(10)	-	-	1	-	-	-	1	10
Total(30)	3	10	2	6.67	2	6.67	7	23.33

Table 4. Incidence of *Yersinia* species isolated from the examined camel samples (No. of samples=30)

Samples	*Y.enterocolitica*		*Y.intermedia*		*Y.frederiksenii*		Total Yersinia Spp.	
	No.	%	No.	%	No.	%	No.	%
Meat(10)	2	20	1	10	1	10	4	20
Liver(10)	1	10	-	-	1	10	2	30
Kidney(10)	1	10	-	-	-	-	1	10
Total(30)	4	13.33	1	3.33	2	6.67	7	23.33

The obtained results declared that *Y.enterocolitica, Y.intermedia and Y.frederiksenii* could be isolated and identified from the examined meat samples of cattle with percentages of 10%, 10% and 0% respectively (Table 2). Such values in sheep meat samples were 20%,0% and 10% respectively. (Table 3). Meanwhile, the rate of such isolated *Yersinia* species in the examined camel's meat samples were 20%,10% and 10% respectively.(Table 4). The highest rate of total *Yersinia* isolation in the examined meat samples of food animals was found in camel samples (40%), followed by sheep samples (30%), while the lower percentage was found in cattle samples (20%) Such finding coincide with that previously reported ***(26)***, Meanwhile, higher values were detected by ***(27,28)***.

On the other side *Y.enterocolitica, Y.intermedia and Y.frederiksenii* were isolated from the examined cattle liver with percentages of 20%, 0% and 10% respectively (Table 2). The percentages of such identified Yersinia organisms in the examined liver samples of sheep were10%, 10% and 10% respectively (Table 3). Such values for the examined camel liver samples were 20%, 0% and 10% respectively, (Table 4). The highest rate of total *Yersinia* isolation in the examined liver samples of food animals was found in cattle samples (30%), and sheep samples (30%), while the lower percentage was found in camel 'liver samples (20%).The obtained results substantiate what has been reported ***(29)***, higher ***(30)*** and lower values ***(31)*** values were mentioned.

From the results achieved in Tables 2, 3 and 4 it's evident that *Y.enterocolitica, Y.intermedia and Y.frederiksenii* were isolated from the examined cattle kidney with percentages of 10%, 0% and 0% respectively and from the examined kidney samples of sheep with percentages of 0%, 10% and 0% respectively, while they could be isolated from camel kidney with percentages of 10%, 0% and 0% respectively. Similar (29,32), lower (33,35) and while ***(36)*** mentioned higher results.

Antibiotic Resistance

Antibiotic susceptibility analysis showed that there was no difference in the susceptibilities of the three Yersinia species isolated from the examined samples. The results revealed that no strains were resistant to ceftriaxone, cefuroxime, ciprofloxacine, gentamicin, kanamycin, neomycin or polymyxin. In contrast, there were high levels of resistance to ampicillin, penicillin, cefalothin and amoxicillin.The obtained results coincide with that reported by ***(25)***.

Yersiniosis is a disease that affects wild and domestic animals as well as humans. Enteric yersiniosis is caused by pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. However, human yersiniosis, which is very common in Europe, is mostly caused by *Y. enterocolitica* ***(1)***. The disease is transmitted by the faecal –oral route and typical symptoms are fever, abdominal pain and diarrhoea ***(2,3)***.

Two major sources of bacteria causing foodborne disease in meat and meat products may be identified. The living animal carries pathogenic bacteria while the processing environment harbours them. In addition, the human being is also an important source of pathogenic bacteria, most frequently indirectly by cross contamination. Bacteria originating from the animal may during slaughter contaminate the carcass, and subsequently distributed via cut meat or meat raw material intended for further processing into meat products. Limiting the contamination and subsequent inactivation of occurring pathogenic bacteria will be decisive to the safety of meat and meat products ***(37)***.

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الملخص العربى

مدى تواجد ميكروبات اليرسينيا أنتيروكوليتكا والأنواع الأخرى فى لحوم وأعضاء حيوانات الذبح

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الهدف من هذه الدراسة معرفة مدى تواجد أنواع اليرسينيا المختلفة فى اللحوم وبعض الأعضاء لحيوانات الذبح وأيضا معرفة مدى استجابة هذه الميكروبات للمضادات الميكروبية المختلفة ، لذلك تم تجميع عدد ٩٠ عينة عشوائية من العضلات والكبد والكلى لكل من (الأبقار والأغنام والجمال) من محلات القصابيين من محافظة الشرقية بمصر .

وقد أوضحت النتائج أن أنواع اليرسينيا المختلفة تم تحديدها فى ٦ عينات بنسبة ٢٠% فى الأبقار وأيضا تم تحديدها فى ٧ عينات لكل من الأغنام والجمال بنسبة ٢٣,٣٣% وتم تقسيم العترات المعزولة إلى يرسينيا (أنتيروكوليتكا ، أنترميديا وفريد كسين) وأوضحت نتائج المضادات المكيوربية أن أنواع اليرسينيا المختلفة تأثرت بكل من سيفترياكسون ، سيفروكسيم ، سيبروفلوكساسين ، جنتاميسن ، كاناميسين ، نيومايسين والبولى ميكسين) وأن هناك مقاومة لكل من الامبيسيلين ، البنسلين ، سيفولوسين والاموكسسيللين.