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ISOLATION OF YERSINIA ENTEROCOLITICA FROM PORK MEAT

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Summary

Yersinia was isolated from 89 (38.7%) of 230 samples (pork head muscle: m. Digastric). Thirty eight samples were contaminated with Yersinia enterocolitica which are considered as human pathogenic.

A 100% correlation was obtained between the pyrazinamidase test and the different bioserogroups.

Most of the pathogenic bioserogroups were confirmed to be virulent by the autoagglutination test and the calcium dependency test. With the latex particle agglutination strains of biotype 1, know to be nonpathogenic, were found to be virulent (false-positive results).

Because of the presence of human pathogenic Yersinia enterocolitica in pork head meat, it is recommended to remove pork heads from the carcass as quickly as possible. Moreover this meat should be processed in plants processing heat treated meat products.

From the 3 in vitro virulence tests, autoagglutination and calcium dependency seem to be valuable methods to detect virulence in Yersinia enterocolitica.

INTRODUCTION

The number of human Yersinia enterocolitica infections is still increasing; in 1986 about 1500 cases were reported. An epidemiological study investigating the eating habits of young children could link yersiniosis to the consumption of raw pork (6). There is bacteriological evidence that this meat is contaminated with Yersinia enterocolitica. Preliminary results of a current study by Wauters (7) reveal that 24% of retail minced meat containing pork, is contaminated with pathogenic strains of Yersinia enterocolitica. The origin of this contamination is still uncertain. Until now, pigs and more especially the oral cavity of these animals have been identified as a natural reservoir of pathogenic Yersinia enterocolitica. It can be presumed that during slaughter and further processing pork becomes cross-contaminated.

Head meat from pork is frequently used to prepare mixed minced meat. The purpose of this study was to investigate to what extent this meat acts as a source of Yersinia enterocolitica in raw minced meat.

MATERIALS AND METHODS

Sampling

Samples were collected in 2 slaughterhouses and 2 local meat processing plants. At the slaughterhouse pork heads were sampled a few hours after slaughtering and at the meat processing plants following 3 days of cold storage. From each pork head one of the 2 Digastric muscles were removed aseptically, transferred in a Stomacher bag and stored in a cooling box for transportation.

Methods

At the laboratory 10g of the muscle tissue was added to 20 ml of 0.1% peptone water. After homogenizing in a Stomacher (Colworth) for 1 min, 10ml of the homogenate was transferred to 90ml of the enrichment broth Irgasan-Ticarcillin-Chlorate (ITC)(8). After incubation at 23-25°C for 3 days, all enrichment broths were streaked onto the following selective agar plates: Cefsulodin-Irgasan-Novobiocin Agar (CIN), Desoxycholate-Citrate-Mannitol Agar according to Saari and Jansen (YM) and Salmonella-Shigella-Desoxycholate Agar (SSD). The plates were incubated at 37°C during 24 h. Suspicious colonies were biochemically characterized.
All Yersinia strains were tested for the presence of pyrazinamidase activity (3). Isolates belonging to Wauters' biotype 2 and 4 were serotyped by slide agglutination. Virulence testing of the isolates was carried out by 3 in vitro virulence tests: autoagglutination at 37°C in Tryptic Soy Broth (1,5), calcium dependency at 37°C on Magnesium Oxalate Agar (MOX)(2) and latex particle agglutination test (4).

RESULTS AND DISCUSSION

The contamination of the pork head muscle m. Digastric with Yersinia spp. is considerable: 89 (38.7%) of 230 samples were found positive. Nine samples harboured 2 biochemically distinct strains of Yersinia (Table 1). Yersinia enterocolitica biotype 1 was the most common species (54 samples or 60.7%), isolated from the muscle, followed by Yersinia enterocolitica biotype 4 with 37 (41.6%) positive samples. One sample was contaminated with strains belonging to Yersinia enterocolitica biotype 2 (1.1%). Yersinia frederiksenii and Yersinia intermedia were isolated from 5 (5.6%) and 1 (1.1%) samples respectively.

The investigation yielded 292 strains of Yersinia spp. Serotyping of all strains classified as Yersinia enterocolitica biotype 2 and 4, showed that they belonged to serotype O_9 and O_3 respectively.

The pyrazinamidase test allowed to distinguish correctly all strains into the 2 different groups of Yersinia: 1/ the bioserogroups of Yersinia enterocolitica which usually harbour the virulence plasmid (Pyr^-) and 2/ the bioserogroups, without the naturally occurring plasmid, and related species (Pyr^+)(Table 2).

In order to predict the potential virulence of the Yersinia isolates the autoagglutination at 37°C, calcium dependency at 37°C and latex particle agglutination were used. With the exception for latex particle agglutination, all 292 strains were tested. Only Yersinia enterocolitica strains belonging to biotype 2, serotype O_9 and biotype 4, serotype O_3 were found to be virulent with the autoagglutination test and/or the calcium dependency test (Table 2). Hundred twenty isolates were positive , whereas 17 were negative for both tests. A few strains were positive for one of

the two tests: 3 strains autoagglutination positive and 2 strains calcium dependency positive (Table 3). Latex particle agglutination was performed with 45 strains of biotype 1 and with 57 strains of biotype 4. For several strains the results were in discordance with the other virulence tests. Fourteen strains belonging to the nonpathogenic group of biotype 1 were positive (Table 4). On the other hand, 9 strains of biotype 4 showed to be potentially virulent, were negative.

CONCLUSIONS

Pork head meat is found to contain frequently Yersinia. An important number of the strains are human pathogenic Yersinia enterocolitica. The processing of this meat in raw minced meat leads unavoidably to the contamination of the final product. Also, a transmission of Yersinia enterocolitica to other products by cross-contamination seems to be obvious. Therefore, it is recommended to separate pork heads from the carcasses as quickly as possible and to allow further treatment exclusively in meat processing plants, preparing heat treated meat pruducts.

Autoagglutination at 37°C and calcium dependency at 37°C were only positive for these biotypes/serotypes of Yersinia enterocolitica known to harbour pathogenic strains. Both tests seem to be valuable methods to detect potential virulence in Yersinia enterocolitica strains. With latex particle agglutination a number of false-positive as well as false-negative reactions can be expected.

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Table 1: Presence of Yersinia enterocolitica and related species on 230 pork head meat samples.

Number of samples	with Yersinia	Total number of samples	with Yersinia
48	enterocolitica biotype 1	54 (60.7%)	enterocolitica biotype 1
6	enterocolitica biotype 1 + 4		
28	enterocolitica biotype 4	37 (41.6%)	enterocolitica biotype 4
1	enterocolitica biotype 4 + intermedia (1.1%)*		
2	enterocolitica biotype 4 + frederiksenii		
3	frederiksenii	5 (5,6%)	frederiksenii
1	enterocolitica biotype 2 (1.1%)		

* Percentage of total number of positive samples

Table 2: Pyrazinamidase activity and virulence properties of 292 strains Yersinia spp. isolated from pork head meat.

Yersinia species	Biotype	Number of strains tested	Number of positive reactions by following tests*: CD	AAG	PYZ
enterocolitica	1	143	0	0	143
	2	3	3	1	0
	4	139	119	122	0
intermedia		2	0	0	2
frederiksenii		5	0	0	5

* CD: calcium dependence; AAG: autoagglutination; PYZ: pyrazinamidase test

Table 3: Comparison of the autoagglination and the calcium dependency test.

Autoagglutination test Numbers of strains	Calcium dependency test Numbers of strains +	Calcium dependency test Numbers of strains -
+	120	3
-	2	17

Table 4: Evaluation of the latex particle

Yersinia enterocolitica biotype	Numbers of strains tested	Calcium dependency and autoagglutination test	Latex particle agglutination Numbers of strains +	Latex particle agglutination Numbers of strains -
1	45	-	14	31
4	57	+	48	9