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International Association for Food Protection
2000

Journal of food protection. 2000. 63: 31-35

http://hdl.handle.net/1975/763

Journal of Food Protection, Vol. 63, No. 1, 2000, Pages 31–35

Contamination of Carcasses, Offals, and the Environment with *yadA*-Positive *Yersinia enterocolitica* in a Pig Slaughterhouse

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MS 99-114: Received 27 April 1999/Accepted 24 June 1999

ABSTRACT

This study was carried out in order to evaluate the contamination of the pig-slaughtering line with pathogenic *Yersinia enterocolitica* carrying the *yadA* gene. A total of 292 samples were collected from the slaughterhouse; 131 swab samples from pig carcasses, ears, livers, kidneys, and hearts; 89 swab samples from the environment; and 72 sedimentation samples from the air. All surface samples were studied with both the polymerase chain reaction (PCR) and culture methods. The contamination rate of edible pig offals was high with both methods. Using PCR, the detection rates of *yadA*-positive *Y. enterocolitica* for livers, kidneys, and hearts were 38, 86, and 63%, respectively, and using the culture method, the detection rates were 31, 69, and 50%, respectively. Pathogenic *Y. enterocolitica* was also detected from different environmental sites in the slaughterhouse. Using PCR, 13% of the surface samples from the environment were contaminated with *yadA*-positive *Y. enterocolitica.* PCR-positive samples were found on the brisket saw, the hook from which the pluck set (heart, lungs, esophagus, trachea, diaphragm, liver, kidneys, and tongue with tonsils) hang, the knife used for evisceration, the floors in the eviscerating area and the weighing area, the meat-cutting table, the aprons used by trimming workers, the computer used in the meat-inspection area, and the coffeemaker used by slaughterhouse workers. The respective detection rate (6%) was considerably lower when we used the culture method. Pathogenic *Y. enterocolitica* was isolated from the air in the bleeding area. Bioserotype 4/O:3 was the only pathogenic bioserotype isolated in this study. A total of 113 isolates of type 4/O:3 were characterized with pulsed-field gel electrophoresis using *Not*I and *Xba*I digests. By combining these profiles, nine different pulsotypes were obtained, the most common of which (1a) was found in 19 (61%) of 31 samples from different sites. This is the same type that has dominated in pig tonsils, which suggests that tonsils may be the source of *Y. enterocolitica* contamination in the slaughterhouse. The four pulsotypes (1a, 4g, 6g, and 19q) found on edible offals were the same as those found in tonsils, which supports our hypothesis that tonsils are the contamination source for the liver, heart, and kidneys.

In 1997, 704 yersinie gastroenteritis cases, 87% of which were caused by *Yersinia enterocolitica,* were reported in Finland *(19).* The epidemiology of this bacterium is still quite obscure. It is believed that *Y. enterocolitica* is a foodborne pathogen, even though pathogenic isolates are seldom found in foods *(2, 8, 15).* Pathogenic isolates have been recovered frequently only from pig tongues *(9, 12, 27, 28).* The main sources of the infections are thought to be undercooked pork and untreated water *(25, 31).*

We know that pigs harbor pathogenic *Y. enterocolitica* in the tonsils *(7, 29)* and that they excrete pathogenic isolates in their feces *(13, 23).* The main contamination source in the slaughterhouse is believed to be the feces *(1, 5, 22).* Pathogenic yersinia will spread from the intestines to the carcass, mainly during the loosening of the rectum *(1, 22).* Another potential source of contamination is the tonsils. When pig tonsils are removed along with the pluck set (tongue, esophagus, trachea, lungs, heart, diaphragm, kidneys, and liver), pathogenic yersinia may spread from the tonsils to the carcass and the pluck set.

When the pluck set and intestines are removed, tools and equipment may become contaminated with pathogenic yersinia. Only a few studies on yersinia contamination in pig slaughterhouses have been reported *(17, 20, 26).* From the environment, pathogenic *Y. enterocolitica* has only been recovered occasionally *(20).* The prevalence of pathogenic yersinia in the environment may be greater, because the traditional isolation methods used in the previously cited studies tend to underestimate the prevalence of this bacterium, compared to DNA-based detection methods *(12, 21).*

DNA-based typing methods are widely used in epidemiologic and contamination studies *(10).* There are several molecular methods for typing *Y. enterocolitica,* and pulsed-field gel electrophoresis (PFGE) is one of the most sensitive methods available *(14).* Using PFGE with *Not*I and *Xba*I, *Y. enterocolitica* bioserotype 4/O:3, originating from 61 pig tonsils, has been subdivided into 26 different pulsotypes *(11).*

The aim of this study was to examine the contamination of the pig-slaughtering line with *yadA*-positive *Y. enterocolitica* using both the polymerase chain reaction (PCR) and culture methods. In particular, we studied offals as a source of *Y. enterocolitica.* The pathogenic isolates were characterized with PFGE in order to obtain information on the distribution of different fingerprints in the slaughterhouse.

MATERIALS AND METHODS

Sampling. A total of 292 samples were collected from a pig slaughterhouse, which was visited five times during the period

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TABLE 1. *Pathogenic* Yersinia enterocolitica *detected on pig carcasses and offals with polymerase chain reaction (PCR) and culture method*

Samples	Number of samples	PCR-positive samples	Culture-positive samples
Carcass	80	17	5
Ear	17	4	2
Liver	13	5	4
Kidney	13	11	9
Heart	8	5	4
Total	131	42	24

TABLE 2. *Pathogenic* Yersinia enterocolitica *detected in the pig slaughterhouse environment*

Sampling sites	Number of samples	PCR-positive samples	Culture-positive samples
Brisket saw	5	1	1
Splitting saw	3	0	0
Hooks	8	1	1
Knives	8	1	1[a]
Knife sheaths	6	0	0
Refrigerator	8	0	0
Floors	7	2	0
Meat containers	2	0	0
Handrails	8	0	0
Meat cutting tables	7	1	0
Aprons	12	2	0
Hands	3	0	0
Computer	6	2	2
Coffeemaker	6	2	0
Total	89	12	5

[a] *yadA*-negative *Y. enterocolitica.*

extending from December 1997 to January 1998. Of these 292 samples, 131 were surface samples from pig carcasses and offals, including ears, livers, kidneys, and hearts; 89 were environmental surface samples from different sites, including a brisket saw, a splitting saw, hooks, knives, knife sheaths, refrigerator, floors, meat containers, handrails, meat-cutting tables, aprons, hands, a computer, and a coffeemaker (used by the slaughterhouse workers); and 72 were air samples from bleeding, eviscerating, meat-inspection, offal-harvesting, weighing, trimming, head-cutting, meat-cutting, and chilling areas.

We sampled the surfaces by swabbing them with a 7.5 by 7.5-cm, gauze-covered cotton-wool pad moistened with 10 ml of Trypticase soy broth (TSB; Difco Laboratories, Detroit, Mich.). The surface samples from the carcasses and offals were collected from the chilling rooms. The swabbed area consisted of the split surface and the shoulder from half of the carcass. The entire surface of the offals was swabbed, so that one sample contained five swabbed surfaces. The environmental surface samples were obtained by swabbing an area of approximately 15 by 15 cm, including equipment, structures, workers, and the coffeemaker, with a moistened 7.5 by 7.5-cm, gauze-covered cotton-wool pad. The swabs were then placed in sterile plastic bags and transported to the laboratory in chilled insulated boxes; samples were studied promptly upon arrival. The cotton-wool pad, moistened with 10 ml of TSB, was transferred to 90 ml of TSB, shaken vigorously, and then studied using both the PCR and culture methods.

The sedimentation method was used to find *Y. enterocolitica* in the air. Open cefsulodin-irgasan-novobiocin (CIN) agar plates (Yersinia selective agar base, Oxoid, Basingstoke, UK) and MacConkey agar plates (Difco) were exposed for 4 h at nine different sites: bleeding, eviscerating, meat-inspection, offal-harvesting, weighing, trimming, head-cutting, meat-cutting, and chilling areas.

Detection of *yadA*-positive *Y. enterocolitica* from surface samples using PCR. The surface samples were enriched overnight in TSB at room temperature before they were examined with PCR. The nested-PCR targeting the *yadA* gene on the virulence plasmid *(16)* was used, with minor modifications, as described by Fredriksson-Ahomaa et al. *(12).*

Isolation and identification of pathogenic *Y. enterocolitica.* The surface samples were examined for *Y. enterocolitica* using the culture method according to the Nordic Committee on Food Analysis *(24),* as modified by Fredriksson-Ahomaa et al. *(12).* The air samples on CIN and MacConkey agar plates were incubated at 30°C for 18 to 20 h. The lactose-negative colonies on MacConkey agar plates were streaked onto CIN agar plates, and all colonies of typical "bull's eye" appearance on the CIN agar plates were identified as described by Fredriksson-Ahomaa et al. *(12).* *Y. enterocolitica* isolates were biotyped according to the revised scheme by Wauters et al. *(32)* and were serotyped by agglutination using O:3, O:5, and O:9 antisera (Denka Seiken, Tokyo, Japan). The pathogenicity of the isolates was confirmed with the PCR targeting the *yadA* gene on the virulence plasmid, as described by Fredriksson-Ahomaa et al. *(12).*

DNA isolation, restriction enzyme digestions, and PFGE. A single colony grown on blood agar was inoculated into 5 ml TSB. Cells were harvested from 2 ml of TSB following overnight enrichment at room temperature. DNA isolation was performed according to Maslow et al. *(18),* with the modifications described by Björkroth et al. *(4),* omitting mutanolycin from the lysis solution. The DNA was digested with two rare-cutting enzymes, *Not*I and *Xba*I (New England Biolabs, Beverly, Mass.). The samples were electrophoresed at 12°C through a 1% agarose gel in 0.5× TBE (Amresco, Solon, Ohio) at 200 V in a Gene Navigator system (Pharmacia, Uppsala, Sweden) with a hexagonal electrode. The pulse times were ramped from 1 to 18 s for 20 h and from 1 to 15 s for 18 h for *Not*I and *Xba*I, respectively. Low-range PFG marker was used as a fragment size marker (New England Biolabs). The gels were stained with ethidium bromide and photographed under UV translumination.

RESULTS

A total of 21% of pig carcasses, 24% of ears, 38% of livers, 86% of kidneys, and 63% of hearts were contaminated with *yadA*-positive *Y. enterocolitica* (Table 1). Pathogenic *Y. enterocolitica* was also detected in the slaughterhouse environment (Table 2). Using PCR, 13% of surface samples were *yadA*-positive, those being the brisket saw, the hook from which the pluck set (heart, lungs, esophagus, trachea, diaphragm, liver, kidneys, and tongue with tonsils) hung, the knife used for evisceration, the floors in the eviscerating area and the weighing area, the meat-cutting table, the aprons used by trimming workers, the computer used in the meat-inspection area, and the coffeemaker used by slaughterhouse workers. The detection rate was higher with

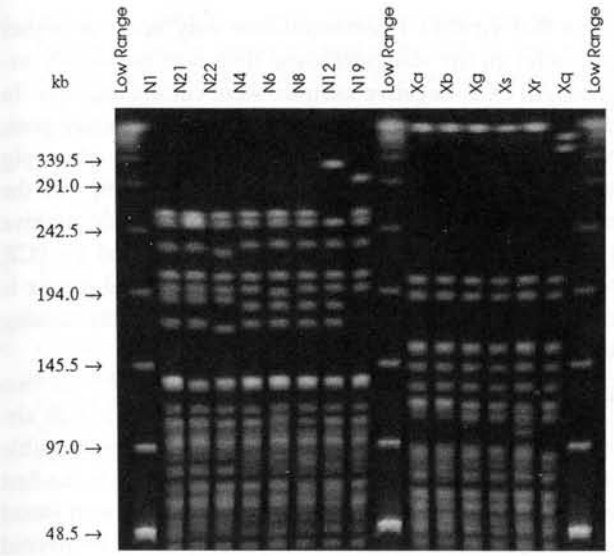

FIGURE 1. *Different NotI and* Xba*I profiles of* Yersinia enterocolitica *isolates found in the slaughterhouse.* Not*I profiles: N1, N21, N22, N4, N6, N8, N12, and N19.* Xba*I profiles: Xa, Xb, Xg, Xs, Xr, and Xq.*

the PCR compared with the culture method. All PCR-negative samples were also culture negative.

With the traditional culture method, pathogenic *Y. enterocolitica* carrying the *yadA* gene could be isolated once on the brisket saw and the hook from which the pluck set hangs and twice on the computer used in the meat-inspection area. When CIN agar plates were used, pathogenic isolates could also be recovered from two of four air samples in the bleeding area.

A total of 113 *Y. enterocolitica* isolates from 31 samples were bioserotyped and serotyped; the result was that all belonged to bioserotype 4/O:3. The *yadA* gene was detected with PCR. In 24 samples, all isolates studied were carrying the *yadA* gene; in six samples, both *yadA*-positive and negative isolates were observed; and in only one sample (the knife used in evisceration), all four isolates were *yadA* negative.

Y. enterocolitica isolates belonging to bioserotype 4/O:3 were characterized using PFGE. The 113 isolates yielded eight PFGE profiles (N1, N4, N6, N8, N12, N19, N21, and N22) with *Not*I and six (Xa, Xb, Xg, Xq, Xr, and Xs) with *Xba*I enzymes (Fig. 1), and by combining the various *Not*I and *Xba*I digestion profiles, nine different pulsotypes (1a, 21a, 22b, 4g, 6g, 8s, 12g, 12r, and 19q) were obtained (Table 3). The most common pulsotype, 1a, was found on 19 (61%) of 31 samples, including carcasses, ears, livers, kidneys, hearts, brisket saw, hook, knife, and air (Table 4). More than two different pulsotypes (1a, 21a, 22b, 4g, 6g, 8s, 12g, or 19g) were observed on samples of livers, kidneys, hearts, and the computer.

TABLE 3. *The pulsotypes obtained by combination of different PFGE patterns using Not*I *and Xba*I *digests of 113* Yersinia enterocolitica *bioserotype 4/O:3 isolates*

Pulsotypes	Number of isolates	PFGE patterns	
		*Not*I	*Xba*I
1a	59 (19)[a]	N1	Xa
21a	10 (4)	N21	Xa
22b	4 (1)	N22	Xb
4g	10 (4)	N4	Xg
6g	2 (1)	N6	Xg
8s	12 (4)	N8	Xs
12g	4 (1)	N12	Xg
12r	8 (2)	N12	Xr
19q	4 (1)	N19	Xq
Total	113 (31)		

[a] Number of samples is in parentheses.

DISCUSSION

Pathogenic *Y. enterocolitica* carrying the *yadA* gene was detected on pig carcasses and offals and in the pig-slaughterhouse environment. The contamination rate of pig kidneys, hearts, and livers with pathogenic *Y. enterocolitica* was high using both the PCR and culture methods (Table 1). A major contamination source of the edible offals may be the tonsils, which are frequently known to be yersinia positive. In Finland, the prevalence of *yadA*-positive *Y. en-*

TABLE 4. *Distribution of the different pulsotypes in the slaughterhouse*

Samples	Number of isolates	Pulsotypes								
		1a	21a	22b	4g	6g	8s	12g	12r	19q
Carcasses	18 (5)[a]	10 (3)							8 (2)	
Ears	6 (2)	6 (2)								
Livers	16 (4)	8 (2)	6 (2)				2 (1)			
Kidneys	36 (9)	16 (5)	2 (1)		4 (1)		10 (3)			4 (1)
Hearts	11 (4)	5 (2)				2 (1)		4 (1)		
Brisket saw	4 (1)	4 (1)								
Knives	4 (1)	4 (1)								
Hooks	2 (1)	2 (1)								
Computer	8 (2)		2 (1)	4 (1)	2 (1)					
Air	8 (2)	4 (2)			4 (2)					
Total	113 (31)	59 (19)	10 (4)	4 (1)	10 (4)	2 (1)	12 (4)	4 (1)	8 (2)	4 (1)

[a] Number of samples is in parentheses.

terocolitica has been 37% in pig tonsils *(11)*. The dominating pulsotype, 1a, was found on the carcasses and offals; this is the same pulsotype common in pig tonsils *(11)*, which suggests that tonsils are an important contamination source for carcasses and offals. Three other pulsotypes (4g, 6g, and 19q) found on edible offals were the same types found in tonsils, which lends further support to our hypothesis that tonsils contaminate the liver, heart, and kidneys. Tonsils are removed along with the pluck set, including tongue, esophagus, trachea, lungs, heart, diaphragm, kidneys, and liver, and are then hung on a hook. In place of hooks, conveyer belts are also used, but in either case, the spread of pathogenic yersinia from the tonsils to the pluck set is unavoidable.

The yersinia contamination from the oral cavity could largely be avoided if the head, including the tonsils and tongue, was removed before evisceration and was handled separately. According to legislation *(3)*, this cannot be done, because the head, including the tonsils and tongue, needs to be inspected in conjunction with the carcass before the carcass can be deemed fit for human consumption. Christensen and Luthje *(6)* investigated an evisceration technique in which the tongue and tonsils were left in the head and in which the head was not split during the slaughter process. This modified pluck removal led to a reduction in the spread of *Y. enterocolitica* onto the carcass, liver, and diaphragm. In addition, Andersen *(1)* and Nesbakken et al. *(22)* have demonstrated in their studies that the spread of *Y. enterocolitica* bioserotype 4/O:3 could be considerably reduced by sealing off the rectum with a plastic bag immediately after it has been freed. This method is commonly used in Denmark, Norway, and Sweden *(5)*. In order to decrease the contamination of carcasses and offals with yersinia, both the removal of the head and the sealing off of the rectum should be used.

Pathogenic *Y. enterocolitica* carrying the *yadA* gene was for the first time detected to the reported extent within the environment. *Y. enterocolitica* bioserotype 4/O:3 could be isolated on brisket saw, hooks, and knives. The pulsotype (1a) was the same as that was found on the carcasses and offals, which indicates that tools and machines may spread yersinia to other parts of the carcass and to the pluck set. It also suggests the importance of disinfecting equipment after every carcass in order to avoid cross-contamination. Pathogenic *Y. enterocolitica* was further detected on the aprons used by trimming workers, on the computer used by meat inspectors, and on the coffeemaker used by slaughterhouse workers. This indicates that workers can transfer yersinia via cloths, hands, and utensils. Some of the pulsotypes (21a and 4g) found on the computer were also observed on livers and kidneys, which reinforces the assumption that meat inspectors spread pathogenic *Y. enterocolitica* from offals with their hands. PCR-positive samples were found on the floor in the eviscerating and weighing areas and on the table in the meat-cutting area, which shows that the pig-slaughterhouse environment is contaminated with pathogenic *Y. enterocolitica*.

The PCR method yielded significantly more positive samples than did the traditional culture method, which indicates that virulent *Y. enterocolitica* may occur at higher frequencies in the slaughterhouse than was previously assumed. All PCR-negative samples were culture negative. In the study by Fredriksson-Ahomaa et al. *(12)*, culture-positive but PCR-negative samples were obtained when pig tongues were studied using destructive sampling. In the present study, samples were examined using nondestructive swabbing, which can be a better sampling method for PCR than destructive sampling, with which more food matrix is present in the enrichment medium, thereby possibly causing false-negative results via the PCR method.

yadA-Positive *Y. enterocolitica* was isolated from two air samples in the bleeding area, which suggests that airborne contamination with this pathogen may be possible during slaughter. It is interesting to note that this is the first time—to our knowledge—that any yersinia has been found in air samples. Pathogenic *Y. enterocolitica* was recovered on CIN agar when MacConkey agar was not selective enough, in spite of the findings of Sierra et al. *(30)*, which indicated that this agar should be sufficient for isolation of yersinia. Two different pulsotypes (1a and 4g) were isolated from the air, and the same types were also identified from other sites in the slaughterhouse, which indicates that airborne contamination may occur, but more information is needed about the role of pathogenic *Y. enterocolitica* as it relates to this form of contamination.

The only pathogenic bioserotype of *Y. enterocolitica* recovered in this study was type 4/O:3. This bioserotype has been the only pathogenic type isolated from tongues and minced meat at the retail level *(12)*, and it is also the main bioserotype isolated from humans in Finland *(19)*. Using the PCR method, the *yadA* gene was confirmed in one to four isolates of type 4/O:3 recovered from each positive sample. In a single sample, the knife used in evisceration, all four isolates were *yadA* negative, which indicates that most of the samples were contaminated with virulent isolates.

The edible pig offals were highly contaminated with *yadA*-positive *Y. enterocolitica*. A major contamination source may be the infected pig tonsils, since the spread of this bacterium is unavoidable during the removal of the tonsils. The only way to prevent this contamination route is to modify legislation to allow the head, with the tonsils and tongue, to be removed prior to evisceration and to inspect it in a separate room. Moreover, in order to decrease the spread of pathogenic yersinia via feces, the plastic bag technique *(22)*, in which the rectum is sealed off with a plastic bag, is recommended.

ACKNOWLEDGMENTS

We thank Sirkku Ekström for technical assistance. This work was supported by the Technology Development Centre (TEKES), Finland.

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