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Occurrence of *Yersinia enterocolitica* and *Campylobacter* spp. in slaughter pigs and consequences for meat inspection, slaughtering, and dressing procedures

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Abstract

The purpose of the present investigation was to assess the occurrence of *Yersinia enterocolitica* and *Campylobacter* spp. in the lymphoid tissues and intestinal tract in pigs and the risk for contamination during the compulsory meat inspection procedures and the procedures during slaughtering and dressing. Another objective of the investigation was to compare traditional isolation methods, the use of a polymerase chain reaction (PCR) method (BUGS'n BEADS™ bacterial DNA isolation kit) and an ELISA method (VIDAS CAM) as tools in risk management in the slaughterhouse. The results indicate that the compulsory procedure for the incision of the submaxillary lymph nodes represents a cross-contamination risk for virulent *Yersinia*. In the screening of 97 animals in 1999, 5.2% of the samples were positive, and by the sampling of 24 samples in 2000–2001, 12.5% of the samples were positive. In the last case, *Y. enterocolitica* O:3 was found in the kidney region in one of the subsequent carcasses that was only touched by the meat inspection personnel before sampling. In addition, incision of the mesenteric lymph nodes might represent a cross-contamination risk since 8.3% of the samples were positive. The association between antibody titres and the occurrence of virulent yersiniae in the tonsils (21–18) was striking, with virulent yersiniae found in the tonsils in most pigs with high titres. The contents of the stomach, ileum, caecum, and colon also represent contamination risks for *Y. enterocolitica* O:3 if the slaughterhouse personnel cuts into the viscera with their knives by accident; the frequency of virulent *Yersinia* varied from 4.2% to 16.7% within these sections. *Campylobacter* was detected in the gastrointestinal tract of all pigs, and the high contamination of tonsils (66.7%) and intestinal tract (100%) might represent an occupational health hazard. There was no statistical difference between the traditional method for isolation of *Y. enterocolitica* [International Organization for Standardization, 1994. Microbiology—General Guidance for the Detection of Presumptive Pathogenic *Yersinia enterocolitica* (ISO 10273). International Organization for Standardization, Genève, Switzerland (16 pp.)] and the BUGS'n BEADS™ detection method for virulent *Y. enterocolitica*. Likewise, there was no statistical difference between the traditional method for isolation of *Campylobacter* spp. [Nordic Committee on Food Analysis, 1990. *Campylobacter jejuni/coli*. Detection in Food. Method No. 119, 2nd ed. Nordic Committee on Food Analysis,

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Esbo (7 pp.)] and the BUGS'n BEADS™ detection method or the VIDAS CAM method for detection of *Campylobacter* spp.
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1. Introduction

Healthy pigs are often carriers of strains of *Yersinia enterocolitica* that are pathogenic to humans, in particular strains of O:3/biovar 4 and O:9/biovar 2 (Hurvell, 1981; Kapperud, 1991; Schiemann, 1989). The organisms are present in the oral cavity, particularly on the tongue and in the tonsils, and in the intestine and faeces. Shiozawa et al. (1991) reported that O:3 strains were isolated from 85% of oral swabs from 40 freshly slaughtered, healthy pigs, and presented evidence that the organism colonised the pigs' tonsils. Strains of O:3 have been detected frequently on the surface of freshly slaughtered pig carcasses (in frequencies up to 63.3%) (Andersen, 1988; Nesbakken, 1988). This is probably the result of spread of the organism via faeces, intestinal contents, or contamination from the oral cavity during slaughter and dressing operations. The association between yersiniosis in man and the consumption of pork in Belgium (Tauxe et al., 1987) and Norway (Ostroff et al., 1994) identified raw or undercooked pork as the main source of infection. The apparently low incidence of the infection in Moslem countries (Samadi et al., 1982), where consumption of pork is restricted, also points to pork as a source of infection with *Y. enterocolitica*.

It is impossible to reject pigs contaminated with *Y. enterocolitica* at traditional postmortem meat inspection. Pig slaughter is an open process with many opportunities for the contamination of the pork carcass with *Y. enterocolitica*, and it does not contain any point where hazards are completely eliminated (Borch et al., 1996). Meat inspection procedures concerning the carcass head also seem to represent a cross-contamination risk: Incision of the submaxillary lymph nodes is a compulsory procedure according to the EU regulations (European Commission, 1995). This may, however, result in the bacterium being transported from the tonsillar region to other parts of the carcass by the knives and hands of the meat inspection personnel (Nesbakken, 1988). In view of the fact that the incidence of tuberculosis in pigs and humans has been reduced to a very low level in many parts of the world, it may be possible to reconsider regulations that require incision of the submaxillary lymph to detect tubercular nodes by meat inspectors.

Campylobacter spp. is a frequent cause of enteritis with *Campylobacter jejuni* more commonly implicated than *C. coli* (Stern and Kazmi, 1989). In 1999, 2027 culture-confirmed cases of *Campylobacter* infection were recorded in Norway (National Institute of Public Health, 2000), a 19% increase from 1998. Thus, *Campylobacter* strengthened its position as the most frequently reported cause of acute bacterial gastroenteritis in Norway as in many other rich and industrialised countries in the world. Campylobacters are often found in the intestinal tract of pigs, and here *C. coli* is the more common (Oosterom et al., 1985). Pig carcasses are more frequently contaminated with campylobacters than cattle or sheep carcasses (Franco, 1988). However, the reported rate of contamination from pig carcasses varies widely from 2.9% in Poland (Kwiatek et al., 1990) to 95% in Sweden (Svedhem and Kaijser, 1981).

There were two objectives to the present investigation. The first was to assess the occurrence of *Y. enterocolitica* and *Campylobacter* spp. in lymphoid tissues and the intestinal tract in pigs, together with the risk for contamination of carcasses, during the compulsory meat inspection procedures and the procedures during slaughtering and dressing. The second purpose of the study was to compare traditional isolation methods, the use of a polymerase chain reaction (PCR) method (BUGS'n BEADS™ bacterial DNA isolation kit) and an ELISA method (VIDAS CAM) as tools for risk management in the slaughterhouse.

2. Materials and methods

2.1. Collection of samples

All samples were collected from one federally inspected slaughterhouse in southeastern Norway, at

which about 90 pigs were slaughtered per hour. The slaughtering process in this abattoir is based on a traditional mechanised slaughter line with vat scalding (62 °C), dehairing, singeing/flaming, and polishing. Enclosure of the rectum with a plastic bag is a common practice during evisceration. When unclean working operations have been performed, the knife is rinsed and decontaminated in hot water (82 °C) before the next operation. A manually operated electric saw was used to split the carcass. The pigs weighed from 78.4 to 90 kg (mean 84 kg) after slaughtering and dressing.

2.1.1. Screening: July 1999

Submaxillary lymph nodes were collected from 97 randomly selected, freshly eviscerated, healthy slaughtered pigs. The samples originated from 12 different conventional slaughter pig herds, each represented by 2–15 (mean 8.1) individuals. Seven herds totalling 63 pigs were categorised as herds with a specialised slaughter pig production, while five herds with 34 pigs were farrow-to-finish herds.

2.1.2. Investigation of pigs from three selected specialised slaughter pig herds

During November 2000 and January and February 2001, blood samples, tonsils, submaxillary lymph nodes, mesenteric lymph nodes, contents of stomach, ileum, caecum, colon faeces, and carcass samples from a total of 24 freshly eviscerated, healthy slaughtered pigs were collected. Eight individuals represented each herd. The samples originated from three different conventional slaughter pig herds, which were selected after isolation of *Y. enterocolitica* O:3 from tonsils of slaughter pigs slaughtered from these herds a few weeks earlier. Blood samples were taken during bleeding of the animals and delivered to the National Veterinary Institute of Norway, Oslo. Samples were centrifuged and the serum was collected and frozen at − 70 °C until further transport to the Danish Veterinary Laboratory, Copenhagen, Denmark. When the pigs had been processed to the point just subsequent to evisceration, the stomach, the small intestine, and the large intestine were removed and placed into plastic bags. The materials were transported at ambient temperature to the National Veterinary Institute of Norway. Samples from mesenteric lymph nodes, contents of stomach, last section of ileum, caecum, colon, and faeces were aseptically collected and put into sterile plastic bags within 3 h after collection. The tonsils together with the tongue were separated from the gullet and put into sterile plastic bags. Submaxillary lymph nodes (not incised) were removed from one of the carcass halves and put into sterile plastic bags. Carcass surface samples were collected just subsequent to meat inspection before removal of the head and the final dressing of the carcass. Each of four sample sites, a total of 200 cm^2 per carcass (or 100 cm^2 from each half carcass), from the ham, pelvic duct, kidney region, and medial neck (Fig. 1) of the carcasses was rubbed thoroughly with sterile swabs dipped into sterile peptone water (Mölnlycke Health-Care, type 157300, Gothenburg, Sweden) and put into small, sterile plastic bags. The samples were transported at ambient temperature to the laboratory where analysis was initiated within 2 h after collection.

2.2. Serological methods

Sera were analysed for antibodies against *Y. enterocolitica* O:3 by an indirect pig immunoglobulin (Ig) lipopolysaccharide enzyme-linked immunosorbent assay (LPS-ELISA) (Nielsen et al., 1996). A basic

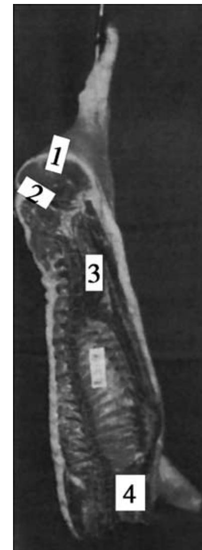

Fig. 1. Pig carcass sampling sites: a total of 200 cm^2 per carcass (or 100 cm^2 from each half carcass) from: (1) ham, (2) pelvic duct, (3) kidney region, and (4) medial neck.

cut-off of optical density (OD%) of 10 was used to maximise the specificity of the ELISA.

2.3. Isolation and detection

Swabs for *Campylobacter* and *Y. enterocolitica* were diluted in 10 ml peptone water (Oxoid L34, Oxoid, Basingstoke, Hampshire, England), homogenised in a peristaltic blender for 30 s, and decimal dilutions were prepared for plating or enrichment.

2.3.1. Y. enterocolitica

Samples were analysed by an International Organization for Standardization (1994) method (ISO 10273). Samples of tonsils (10 g), lymph nodes (surface sterilized by flame) (5 g), and gastrointestinal tract contents/faeces (10 g) were diluted 1:1 with peptone water (Oxoid L34), homogenised in the peristaltic blender for 30 s, and 1 ml was transferred to 99 ml Irgasan–Ticarcillin–potassium chlorate (ITC) enrichment broth (Irgasan DP 300, Ciba-Geigy, Basle, Switzerland; Ticarcillin, Beecham Research Laboratories, Brentford, England; Wauters et al., 1988). The ITC enrichment broth was incubated for 2 days at 25 °C when a 10-µl volume was streaked for selective isolation on *Salmonella–Shigella*–desoxycholate–calcium chloride (SSDC, Yersiniaagar; E. Merck AG, Darmstadt, Germany, 1.11443) and Cefsulodin–Irgasan–Novobiocin (CIN) agar (Oxoid CM653, SR109) plates. SSDC were incubated for 24 hours and CIN plates for 18–22 h at 30 °C. Colonies characteristic for *Yersinia* were biochemically confirmed, first selecting only lactose-negative, urease-positive colonies, and later with Vitek (Bio-Merieux, Marcy l'Etoile, France) using the revised bio-grouping scheme for *Y. enterocolitica* (Wauters et al., 1987) as a key and serologically for O:3 and O:9 reactivity (Sanofi Diagnostics-Pasteur, 63501, 63502, Marnes la Coquette, France).

2.3.2. Campylobacter

Samples of tonsils (10 g), lymph nodes (surface sterilized by flame) (5 g), and gastrointestinal tract contents/faeces (10 g) were analysed using the Nordic Committee on Food Analysis No. 119 (NCFA, 1990) and VIDAS CAM (BioMerieux) in parallel. The initial sample enrichment in Preston broth (Oxoid CM67, SR48, SR117, SR84) was used for both methods. After 24 h of incubation, a 10-µl volume of the enrichment was streaked for selective isolation on Preston agar (Oxoid CM689, SR48, SR204E) and *Campylobacter* blood-free selective (CBFS) medium (Oxoid CM739, SR155E) according to NCFA. The enrichment broths were reincubated for an additional 24 h and tested as prescribed by the VIDAS CAM method. Typical colonies were confirmed as specified by NCFA.

2.3.3. Detection of pathogenic Yersinia and Campylobacter spp. using BUGS'n BEADS™ bacterial DNA isolation kit (Genpoint, Oslo, Norway)

According to the instruction manual, 100 µl of enriched sample was added to a 1.5-ml Eppendorf tube containing 800 µl of binding and washing buffer (BW) and 200 µg magnetic Bacteria Binding Beads (Chemagen Biopolymer-Technologie, Baesweiler, Germany). Contents were mixed by pipetting and left at room temperature for 5 min. The tube was subsequently placed in a magnetic separator (ABgene®, Epsom, UK) to facilitate the separation of beads (with bound bacteria) from the medium. After careful removal of the supernatant by pipetting, 50 µl of lysis buffer was added to the bacteria–bead complex followed by incubation at 80 °C for 5 min. Released DNA was then precipitated onto the beads by addition of 150 µl refrigerated 96% ethanol and incubation another 5 min at room temperature. To wash the DNA–bead complex, the supernatant was removed using the magnet, followed by two additions of 1 ml 70% ethanol (using the magnet in between). The DNA–bead complex was finally resuspended in 40 µl sdH_2O and incubated at 80 °C for 10 min to evaporate remaining ethanol.

Table 1
Occurrence of *Y. enterocolitica* in the submaxillary lymph nodes in 97 pigs from 12 randomly selected herds (screening: July 1999)

Herd type	Number of animals/ number of herds	Number of positive animals (%)	Number of positive herds (%)
Specialised slaughter production	63/7	5 (7.9)	3 (42.9)
Farrow-to-finish herds	34/5	0	0
All herds	97/12	5 (5.2)	3 (25.0)

Table 2
Occurrence of antibodies to *Y. enterocolitica* O:3 in blood, *Y. enterocolitica* O:3 isolated by ISO 10273 (marked as "O:3"), and virulent yersiniae detected by BUGS'n BEADS™ (marked as "+") from lymphoid tissues, intestinal tract, and carcass surfaces from three selected specialised slaughter pig herds

Pig number	Herd number	Serology titres[a]	Lymphoid tissues			Intestinal tract					Carcass surface sites			
			Tonsils	Submaxillary lymph nodes	Mesenteric lymph nodes	Stomach	Ileum	Caecum	Colon	Faeces	Ham	Pelvic duct	Kidney region	Medial neck
1	1	65	+											
2	1	0	+											
3	1	0												
4	1	108	O:3, +											
5	1	4												
6	1	150	O:3, +	+				O:3, +	O:3, +	O:3, +				
7	1	94	O:3, +											
8	1	40		+										
9	2	98	O:3, +											
10	2	68	O:3, +		+		O:3, +	O:3, +		O:3, +				
11	2	89	O:3, +				O:3, +							
12	2	96	O:3, +				O:3, +	O:3, +	O:3, +					
13	2	105												
14	2	106	O:3, +				O:3, +	+						
15	2	92	O:3, +											
16	2	100	O:3, +											
17	3	39	+											
18	3	88	O:3, +			O:3								
19	3	88								O:3, +				
20	3	105	O:3, +											
21	3	118	O:3, +	O:3, +	O:3, +									O:3
22	3	114	O:3, +									O:3		
23	3	83											O:3	
24	3	91	O:3, +											

[a] Antibodies to *Y. enterocolitica* O:3. Positive titres >10 OD%.

Table 3
Comparison of the International Organization for Standardization (ISO 10273, 1994) isolation procedure and the modified BUGS'n BEADS™ method for detection of virulent yersiniae from various sources of slaughter pigs

Procedure	Lymphoid tissues ($n=72$)	Intestinal tract contents ($n=120$)	Carcass surface sites ($n=96$)	Total ($n=288$)
ISO 10273	17 (23.6)	13 (10.8)	3 (3.1)	33 (11.5)
BUGS'n BEADS™	23 (31.9)	13 (10.8)	0	36 (12.5)
Summary of both methods	23 (31.9)	14 (11.7)	3 (3.1)	40 (13.9)

Number of positive samples (%).

PCR assay of genus *Campylobacter* was based on a ribosomal gene (23S rDNA), whereas that of *Yersinia* targeted the virulence plasmid of *Yersinia* species (Cornelis et al., 1998; Roggenkamp et al., 1995). *C. jejuni* and *C. coli* were specifically identified using primers that are a property of Genpoint.

The PCR reactions (50 μl) contained 3 μl of DNA–bead complex, 1× buffer (1.5 mM $MgCl_2$), 200 μM each dNTP, 10 pmol each primer, and 1 U of DyNAzyme™ Thermostable DNA polymerase (Finnzymes Oy, Espoo, Finland). The amplification profile was as follows: 37 cycles of 94 °C for 15 s, 58 °C for 30 s, 72 °C for 1 min, with a prior denaturation at 94 °C for 4 min, and a final extension at 72 °C for 7 min. PCR products were visualised by 2% agarose gel electrophoresis, stained by ethidium bromide, and analysed using a GeneGeniusgel documentation system (Syngene, Cambridge, UK).

2.4. Statistical analyses

Methods were compared using simple tabular analysis and simple and multiple logistic regression using the statistical package Intercooled Stata for Windows 7.0.

3. Results

3.1. *Y. enterocolitica*

Specific antibody titres against *Y. enterocolitica* O:3 were found in 22 (91.7%) of the 24 samples investigated, with 21 animals showing an OD% >10 (Table 2). Virulent yersiniae were detected in the tonsils of one animal with negative titres, but not in any other tissue sample from negative animals. *Y. enterocolitica* O:3 was not isolated from the tonsils,

Table 4
A summary of occurrence of different species of campylobacters in 24 pigs isolated by the Nordic Committee on Food Analysis No. 119 (NCFA, 1990), detected by BUGS'n BEADS™ and VIDAS CAM

Methods	Lymphoid tissues			Intestinal tract					Carcass surface sites			
	Tonsils	Submaxillary lymph nodes	Mesenteric lymph nodes	Stomach	Ileum	Caecum	Colon	Faeces	Ham	Pelvic duct	Kidney region	Medial neck
VIDAS CAM	10	0	5	15	23	22	24	24	7	13	4	4
NCFA	11	0	7	15	23	23	23	20	9	16	5	4
C. coli	9	–	7	13	23	22	19	18[a]	9	13	5	4
C. jejuni	1	–	–	2	–	–	–	–	–	–	–	–
C. lari	1	–	–	–	–	1	4	3[a]	–	3	–	–
BUGS'n BEADS™	16	0	5	19	22	24	24	24	6	13	5	4
C. coli	10	–	5	14[b]	20[c]	19[c]	13	19	6	13	4	4
C. jejuni	1	–	–	2[b]	2[c]	2[c]	–	–	–	–	–	–
Campylobacter spp.	5	–	–	4	2	5	11	5	–	–	1	–

[a] One pig had both *C. coli* and *C. lari*.
[b] One pig had both *C. coli* and *C. jejuni*.
[c] Two pigs had both *C. coli* and *C. jejuni*.

Table 5
Comparison of the Nordic Committee on Food Analysis No. 119 (NCFA, 1990) isolation procedure, the BUGS'n BEADS™ method, and the VIDAS CAM method for detection of *Campylobacter* spp. from various sources of slaughter pigs

Procedure	Lymphoid tissues ($n=72$)	Intestinal tract contents ($n=120$)	Carcass surface sites ($n=96$)	Total ($n=288$)
VIDAS CAM	15 (20.8)	108 (90.0)	28 (29.2)	151 (52.4)
NCFA	18 (25.0)	104 (86.7)	34 (35.4)	156 (54.2)
BUGS'n BEADS™	21 (29.2)	113 (94.2)	28 (29.2)	162 (56.3)
Summary of all methods	23 (31.9)	115 (95.8)	35 (36.5)	173 (60.1)

Number of positive samples (%).

but from the carcass of one animal and from faeces of another one with positive titres.

Y. enterocolitica O:3 was isolated from submaxillary lymph nodes in animals from three out of seven specialised slaughter herds, but not from any animal from five farrow-to-finish herds. Details of results are shown in Table 1.

In the more detailed follow up of three selected specialised slaughter pig herds in 2000–2001, virulent yersiniae were detected in tonsils from 18 (75%) of the 24 animals. As shown in Table 2, virulent yersiniae were also detected on various other sites of many animals. Cross-contamination was illustrated by the fact that *Y. enterocolitica* O:3 was also isolated from one carcass where O:3 was not detected in tonsils, lymph nodes, or gastrointestinal tract.

The ISO method and BUGS'n BEADS™ were compared lumping results from lymphoid tissues, intestinal tract, and carcass together as shown in Table 3. Adjusting for sampling sites, there was no statistical difference between the methods in multiple logistic regression. The odds ratio for BUGS'n BEADS™ was 1.10 (95% CI = 0.67–1.91).

3.2. Campylobacter

Table 4 shows details of results for analysing different tissues in the 24 pigs from the three *Yersinia*-positive herds for *Campylobacter* spp. The clear majority of campylobacters detected belonged to *C. coli* ($n=155$), but some *C. jejuni* ($n=6$) and *C. lari* ($n=12$) were also found. Campylobacters were found most frequently in the lower intestinal tract. However, campylobacters were also frequently found in the stomach, tonsils, and carcass surface, while no animals were positive in submaxillary lymph nodes.

Table 5 shows the same results, but with results lumped together for lymphoid tissues, intestinal tract contents, and carcass surface. Adjusting for sampling tissue, the BUGS'n BEAD™ found slightly more campylobacters than the others, but the difference was not statistically valid, with an odds ratio of 1.26 (0.86–1.86) for BUGS'n BEADS™ compared to VIDAS CAM and 1.10 (0.75–1.62) for NCFA compared to VIDAS CAM.

4. Discussion

The results indicate that the compulsory procedure of incisions of the submaxillary lymph nodes (European Commission, 1995) represents a cross-contamination risk for virulent *Yersinia*. In the screening of 97 animals in 1999, 5.2% of the samples were positive and by the sampling of 24 pigs in 2000–2001, 12.5% of the samples were positive. In the last case, *Y. enterocolitica* O:3 was found in the kidney region in one of the subsequent carcasses, which was only touched by the meat inspection personnel before sampling. In addition, incision of the mesenteric lymph nodes might represent a cross-contamination risk since 8.3% of the samples were positive. These lymph nodes should also be investigated when the meat inspection personnel has specific reasons to do so (European Commission, 1995).

The association between positive titres and the occurrence of virulent yersiniae in the tonsils (21 to 18) was striking. Virulent yersiniae were found in the tonsils in pigs with positive tires except for four cases. However, in one of the four pigs, virulent *Yesiniae* was found in the submaxillary lymph node. Only three pigs did not have positive titres. In one of these pigs, virulent yersiniae were detected in the tonsils.

Pedersen (1979) has also showed no absolute correlation between a titre against *Y. enterocolitica* O:3 and occurrence in the tonsils. This can be most probably attributed to contamination of the oral cavity a short time before sampling. Fukushima et al. (1990) has described *Yersiniae enterocolitica* infection of pigs in the lairage. Danish investigations indicate that the tonsils and tongue appear to be important sources of contamination for the liver, diaphragm, and head (Andersen, 1988). Observation and videotaping of the slaughtering and dressing procedures also revealed the carcass saw might be contaminated during splitting of the head and might cross-contaminate other regions of the carcass. Accordingly, it is important that the operator, who is responsible for the red offal evisceration, removes the tonsils together with the tongue and the gullet. In this way, no tonsillar material will follow the head, and direct contamination of the saw from the tonsils will be avoided and additional procedures for the removal of remaining tonsillar material from the head will not be necessary. The dressing procedures after meat inspection are important in connection with cross-contamination of the carcass. At this stage in the process, the personnel do not have to decontaminate their knives and equipment between each carcass (European Commission, 1995), and these procedures might cause contamination of other parts of the carcass.

In our study, the relative difference of finding virulent yersiniae in the tonsils and the faeces was 18 to 3 (or 6 to 1). This is in accordance with Pedersen (1979), Schiemann (1980), and Wauters (1979), who reported that the frequency of virulent yersiniae in tonsils was approximately 10 times greater from the tongues or tonsils than obtained from faeces. The relatively low level of *Y. enterocolitica* O:3 in the faeces in our study (12.5%) also agrees with Nielsen et al. (1996). They showed that the number of *Y. enterocolitica* O:3 in faeces declined dramatically 30–70 days after feeding piglets with the bacterium. These results should be taken into account in the discussion of slaughtering younger boars with lower weight to avoid the problems with boar taint (Hennessy et al., 1995). Actually, such animals might represent a greater risk for contamination of carcasses with *Y. enterocolitica* than older animals.

Stomach, ileum, caecum, and colon contents also represent contamination risks for *Y. enterocolitica* O:3 if the slaughterhouse personnel cut into the viscera with their knives by accident; the frequency of virulent *Yersinia* varied from 4.2% to 16.7% within these sections. From the caecum contents, 16.7% of the samples were positive for virulent yersiniae. Shiozawa et al. (1991) showed that 24.3% of 140 pigs were carriers of the organism in the caecum, with counts ranging from fewer than 300–110,000 *Y. enterocolitica* per gram of caecal contents. In this context, withholding feed from the pigs the evening before slaughter is relevant. An empty intestinal tract facilitates the job for the slaughterhouse personnel.

Campylobacter spp. was detected in the gastrointestinal tract of all pigs. The high contamination rate of tonsils (66.7%) and intestinal tract (100%) might represent an occupational health hazard because the infectious dose of humans may be as few as 500 cells (Black et al., 1988). The expressed reduction in the occurrence of campylobacters seen after blast chilling with temperatures about -25 °C is probably due to the sensitivity of the bacterium to freezing and drying. This has been shown by Oosterom et al. (1985) who isolated *Campylobacter* spp. from 9% and 0% of the carcasses before and after chilling, respectively. This is also in accordance with data in a study performed in August 2000 (Nesbakken et al., in preparation). Thirty-four (56.7%) of 60 carcass samples, which were collected just subsequent to meat inspection after removal of the head and the final dressing of the carcass, contained *C. coli*. Only 1 (1.7%) of the 60 samples from the same carcasses after blast-freezing contained campylobacters. After deboning and grounding of trimmings from the same carcasses, none of these samples from ground pork contained *Campylobacter* after cooling with CO_2. In a study in the mid-eighties, *Campylobacter* was not detected in 152 samples of retail pork products (Nesbakken et al., 1985).

In conclusion, pork at the retail level does not seem to represent a risk for campylobacteriosis among consumers and cannot explain the rise in the number of human cases the last years (National Institute of Public Health, 2000). However, the isolation of *Y. enterocolitica* O:3 from ground pork from the same carcasses in the study above (Nesbakken et al., in preparation) shows that preventive measures in the slaughterhouse are important for the protection of the consumers against yersiniosis linked to pork. The

results of Nesbakken et al. (1994) showed that 10% of the pig carcasses were contaminated with *Y. enterocolitica* O:3. When the rectum was sealed off with a plastic bag immediately after it had been freed, the bacterium was recovered from only 0.8% of the carcasses when the plastic bag technique was employed. The sealing off the rectum with a plastic bag immediately after it has been freed can significantly reduce the spread of *Y. enterocolitica* to pig carcasses (Andersen, 1988; Nesbakken et al., 1994). According to data from the National Institute of Public Health (2000), the occurrence of human yersiniosis has dropped by about 30–40% after the introduction of the plastic bag technique in about 90% of the pig slaughterhouses in Norway. A similar drop in yersiniosis (about 30%) has also been seen in Sweden after the introduction of this technique (Swedish Institute for Infectious Disease Control, 1995).

The slaughterhouse may use serological tests as a tool for the control of *Y. enterocolitica*. Only *Y. enterocolitica*-free herds should be in contact during transport and in the lairage and slaughtered at the same time. Carrier herds of *Y. enterocolitica* should be separated from the *Y. enterocolitica*-free ones and slaughtered at a separate time. Adjustment of the price paid to the farmer (incentive bonus to carrier-free herds) might be used to encourage farmers to join a herd sanitation programme (Skjerve et al., 1998). Meat originating from *Y. enterocolitica*-negative herds may be used as raw material for fresh meat, while meat from positive herds should be used for products to be heat-treated.

The PCR procedure, in connection with BUGS'n BEADS™, cannot differentiate among virulent *Y. enterocolitica*, *Y. pseudotuberculosis*, or *Y. pestis*, as it is based on a DNA sequence that is conserved in all these species (Kapperud et al., 1990; Skurnik and Wolf-Watz, 1989). This scarcely represents a problem in the context of the present investigation, as we have never been able to demonstrate virulent *Y. pseudotuberculosis* (or *Y. pestis*) in tonsils or on pig carcasses in Norway (Nesbakken, 1985, 1988; Nesbakken and Kapperud, 1985; Nesbakken et al., 1994). There was no statistical difference between the traditional method for isolation of *Y. enterocolitica* (International Organization for Standardization, 1994) and the BUGS'n BEADS™ detection method for virulent *Y. enterocolitica*. Likewise, there was no statistical difference between the traditional method for isolation of *Campylobacter* spp. (Nordic Committee on Food Analysis, 1990) and the BUGS'n BEADS™ detection method or the VIDAS CAM method for the detection of *Campylobacter* spp. In conclusion, the BUGS'n BEADS™ detection method for *Campylobacter* spp. and *Y. enterocolitica* and the VIDAS CAM method for the detection of *Campylobacter* spp. are time-saving methods in comparison with the traditional isolation methods and might be useful in connection with evaluation and verification of HACCP systems in the slaughterhouse. The limitation of the VIDAS CAM method is that it cannot distinguish between the different *Campylobacter* species.

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