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ScienceDirect
International Journal of Food Microbiology 111 (2006) 99–104
INTERNATIONAL JOURNAL OF Food Microbiology
www.elsevier.com/locate/ijfoodmicro

Testing of pathogenic *Yersinia enterocolitica* in pig herds based on the natural dynamic of infection

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Received 28 September 2005; received in revised form 6 April 2006; accepted 20 April 2006

Abstract

This study was performed to evaluate testing methods of pathogenic *Yersinia enterocolitica* in pigs at different ages. Relevant tools and procedures are crucial if pig herds should be declared free from pathogenic *Y. enterocolitica*.

Historical data based on serology showed that the two farms investigated in this study (herds A and B) were contaminated with *Y. enterocolitica* O:3 since at least 1995. Laboratory investigations of 60 pigs were sampled one to four times (herd A) and 20 pigs were sampled one to three times (herd B) at different ages were the basis for this report.

The following testing procedures could be used to conclude that a herd is free from pathogenic *Y. enterocolitica*:

— serological testing of pigs could be performed as a basis for categorisation for all ages from about 100 days including at slaughter when the pigs are 150–180 days old,
— bacteriological examination of faeces could be used as a basis for categorisation at all ages from 85 days until about 135 days,
— bacteriological examination of tonsils could be used as a basis for categorisation at all ages from 85 days including at slaughter when the pigs are 150–180 days old. However, due to animal welfare aspects, one should avoid sampling of tonsils. Accordingly, the serological method or bacteriological examination of faeces at relevant ages should be preferred.

One aspect related to slaughter hygiene is that in pigs slaughtered at the age of 135 days or more, the tonsils may be a more significant source of human pathogenic *Y. enterocolitica* than faeces.

Keywords: *Y. enterocolitica*; Pigs; Herd level; Serology; Bacteriology; Tonsils; Faeces; Food safety

1. Introduction

Yersinia enterocolitica is one of a few zoonotic bacteria that has a stable reservoir within the pig population in Norway (Nesbakken and Skjerve, 1996). In Belgium and Norway, case-control studies have identified raw or undercooked pork as the main source of yersiniosis in man (Ostroff et al., 1994; Tauxe et al., 1987). Healthy pigs are often carriers of strains of *Y. enterocolitica* that are pathogenic to humans, in particular strains of O:3/biovar 4 and O:9/biovar 2 (Hurvell, 1981; Nesbakken, 1992). In general, the organisms are present in tonsils of the pigs, but to a lesser degree in the intestine and faeces of pigs investigated at slaughtering (Nesbakken et al., 2003). Shiozawa et al. (1991) reported that O:3 strains were isolated from 85% of the oral swabs from 40 slaughtered pigs and presented evidence that the organisms colonize the pigs' tonsils. In Norway, a decline in human cases of yersiniosis has been recorded since the beginning of the 1990s. This decline has been attributed to implementation of

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doi:10.1016/j.ijfoodmicro.2006.04.019

improved slaughtering methods, including enclosure of the anus into a plastic bag after rectum-loosening (Nesbakken et al., 1994).

In the epidemiological study of Skjerve et al. (1998), a higher herd prevalence of antibodies against *Y. enterocolitica* O:3 was found in conventional fattening herds (86%) than in integrated (farrow-to-finish) herds (53.1%). The herd prevalence of antibodies against *Y. enterocolitica* in multiplying herds (56.1%) was similar to the level in the conventional integrated herds. This epidemiological study demonstrated that integrated herds seemed to be an important protective factor. If preventive measures could bring the prevalence further down in such herds, serological tests can be used by the abattoir as a tool for control and categorisation of *Y. enterocolitica* in the meat chain (Nesbakken, 2004). A serological investigation indicates that 14 of 15 SPF (specific pathogen free) herds examined may be free from *Y. enterocolitica* O:3 (Lium et al., 2006). This investigation also indicates that it is possible to establish a cluster of pig herds free from *Y. enterocolitica* O:3, and to keep the herds free from this pathogenic variant for many years. According to serological testing, the basic herd at the top of this SPF pyramid seem to have been free from this pathogenic variant since 1996. Before a final conclusion can be drawn, bacteriological examinations of faecal samples or tonsils from pigs in all herds have to be performed. Such bacteriological investigation is ongoing. However, basic knowledge about the dynamics of *Y. enterocolitica* O:3 infection in pigs is crucial for how and when the pigs should be tested and if a herd should be declared free from this pathogenic variant. A study of Fukushima et al. (1983) provides data about carrier rates in faeces of pigs and seroconversion during pig production. In a highly contaminated Japanese herd, the organisms were excreted in the faeces of 56 to 105 days old pigs within 7 to 21 days after entering pens that were contaminated with *Y. enterocolitica* O:3. Only eight animals were investigated, and the layout of the study does not fully reflect the European method of producing fattening pigs. The carrier rates of the tonsils at different ages were also not studied. A recent study does reflect the European pig production system based on bacteriological examination of faeces from fattening pigs during their lifetime, but serological testing of blood samples was not performed. Bacteriological examination of tonsils was only done at time of slaughtering (Gürtler et al., 2005). None of these studies have performed bacteriological examination of tonsils and faeces in addition to serological testing of the same animals at different ages. This coherence is important for the choice of relevant tests of pig herds. Accordingly, this study was performed to evaluate testing methods of pathogenic *Y. enterocolitica* in pigs at different ages. Relevant tools and procedures are crucial if herds should be declared free from pathogenic *Y. enterocolitica*.

2. Materials and methods

2.1. The herds

This investigation was performed in two multiplying herds with Norwegian Landrace (L) sows during the period from the mid-January to late April 2004. The herds were selected on the basis of data previously obtained showing a high prevalence of antibodies against *Y. enterocolitica* O:3 (Table 1).

Table 1
Historical serological data. Antibodies against *Y. enterocolitica* O:3 at farms A and B at the time of slaughtering

Year	Number of samples with antibodies against *Y. enterocolitica* O:3 / total number of samples (%)	
	Herd A	Herd B
1995	35/40 (88)	36/40 (90)
1999	6/10 (60)	6/10 (60)
2000	4/9 (44)	5/10 (50)
2001	5/10 (50)	2/10 (20)
2003	6/10 (60)	10/10 (100)
1995–2003	56/79 (71)	59/80 (74)

Herd A has 35 breeding sows and herd B 25 breeding sows which are inseminated with semen from Yorkshire (Y) boars for production of both breeding animals (LY) for sale and fatteners in the herds. In 2003, about 200 and 100 breeding gilts, respectively, were sold from herd A and B. The general health level and hygienic practice are characterized as good in both herds. The herds purchase breeding sows from two different breeding herds. The carrier rates of *Y. enterocolitica* are not known for these two breeding herds.

2.2. Collection of samples

2.2.1. Blood samples

Historical data: In the period from 1995 to 2003, blood samples were collected four times in connection with the slaughter of fattening pigs from the two farms as showed in Table 1. A total of 79 samples from fattening pigs in farm A and 80 samples from fattening pigs from farm B were collected during bleeding in the abattoir (Table 1).

Data from 2004: From herd A, 104 blood samples were collected from pigs on four different occasions. Forty animals were sampled once, three animals were sampled twice, ten animals were sampled three times, and seven animals were sampled four times. In herd B, 49 blood samples were collected from 20 animals during three visits. Three animals were sampled only once, five animals were sampled twice, and twelve pigs were sampled three times.

Blood was collected in vacutainers from *Vena jugularis externa* (Benjaminsen and Karlberg, 1979), kept at 4 °C and brought to the National Veterinary Institute of Norway, Oslo. Samples were centrifuged, the serum collected and frozen at − 70 °C until further transport to the Danish Veterinary Laboratory, Copenhagen, Denmark.

2.2.2. Tonsils

The tonsils were scraped with a long sharp spoon (Bone curette "Schroeder" 30 cm/12 " sharp/rigid tk 24448-02", Jan F. Andersen, Hønefoss, Norway). Material on the spoon was transferred to sterile swabs, previously dipped into sterile peptone water (Mölnlycke HealthCare AB, type 157300, Gothenburg, Sweden), put into sterile plastic bags intended for use in a peristaltic homogeniser ("stomacher"), placed into a box and held at 4 °C. The samples were transported to the laboratory where analysis was initiated 18 h after collection.