Yersinia enterocolitica Prevalence in the French Pig And Pork Industry: First results of the French Task Group

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Introduction

Yersinia enterocolitica is the third cause of gastro-intestinal diseases transmitted by contaminated food-stuffs consumption in Europe (Efsa, 2009). Pig is considered to be the main animal reservoir of human pathogenic Yersinia enterocolitica strains (Ostroff et al., 1995). The bacterium can be isolated from its tongue, tonsils or feces. In France, while the main pathogenic biotypes are known for humans (4/O:3, 2/O:9 and 3/O:5,27), few data are available regarding their prevalence in the pork chain production.
In 2008, a French task group which aimed at developing common detection and identification procedures for Y. enterocolitica was set up by Ifip, Anses and Aérial. In 2009, a prevalence study was initiated in three slaughter-houses localized in two different French areas (Brittany and Alsace), in order to get preliminary Y. enterocolitica prevalence data on tonsils.

Materials and methods

Sampling was performed from January to Mai 2009 on tonsils before carcass refrigeration. A total of 140 (7 batches of 20 pigs), 139 and 132 (6 batches of 20 pigs and 1 batch of 12 pigs) swabs were analysed in the brittany slaughter-house n°1, the brittany slaugher-house n°2 and the alsatian slaughter-house respectively. The same microbiological method using an enrichment in ITC broth (Irgasan, Ticarcillin, Potassium chlorate) (48h, 25°C) and streaking on CIN (Cefsulodin, Irgasan, Novobiocin) agar plates (24h, 30°C) was used by all three laboratories involved. Typical colonies of *Yersinia enterocolitica* were confirmed by using Api 20E or 32E strips (Biomérieux) or by using the following biochemical tests: glucose degradation, lactose, urea and deamination of tryptophan.

Pathogenic and non pathogenic strains biotypes were either determined by multiplex PCR or biochemical testing. By PCR multiplex, we combined the method of Thisted-Lambertz and Danielsson-Tham (2005) targeting the three virulence genes ail, virF and rfbC, with the method of Arnold et al., (2004), which targets the *Yersinia enterocolitica* species specific 16s rRNA gene. By biochemical testing, esculin, indole, xylose and trehalose tests were used as described in the ISO 10273 standard, as well as the tween esterase test.

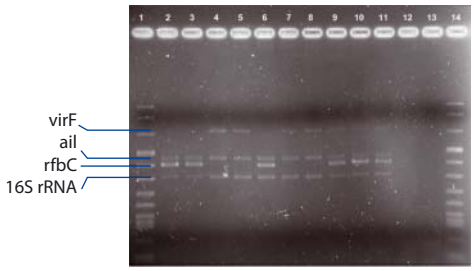
Results

1/Prevalence estimate

	Samples		Prevalence (%)	Inter batches prevalence (%)
	analysed	positive		
Brittany slaughter-house n°1	140	33	26,6 [17,3-31,3]	100
Brittany slaughter-house n°2	139	11	8 [4,5-13,6]	/
Alsatian slaughter-house	132	42	31,8 [24,5-42,2%]	85,7 [47,3-96,8]

The compilation of the results shows that the overall prevalence perpig can be estimated at the slaughter house before cooling to20.9% [17.3 to 25.1%].

2/Biotypes identification



By PCR (figure 1), we showed that 96,3% of strains isolated from the alsatian slaughter-house belonged to the pathogenic biotype 4/O:3 while 3,7% belonged to the non-pathogenic biotype 1A (amplification of the 16S rRNA coding gene only).

In the brittany slaughter-house n°2, 18,2%, 63,6% and 18,2% of isolated strains belonged to biotypes 4/O:3, 2/O:9 or 3/O:5,27 and 1A respectively.

Using biochemical testing, we showed that, in the brittany slaughter-house n°1, 67% of isolated strains belonged to biotype 4 while 33,3% belonged to biotype 3.

Figure 1: Example of identification of biotypes of strains of *Y. enterocolitica* by multiplex PCR

Lanes 1, 14: Ladder VIII (Roche); 2, 3, 6, 9, 10, 11: Y. enterocolitica biotype 4/O:3; 4, 5, 7,8 : Y. enterocolitica biotype 2/O:9 or 3/O:5,27; 12 : Citrobacter freundii, 13: water negative control.

Conclusion

This study has provided a first glimpse of the level of prevalence of *Y. enterocolitica* in pig tonsils in France. A large variability of results between slaughter-houses has been observed.

The overall prevalence estimate is lower than that determined by other European countries (32% in Switzerland, 56% in Finland). 96.3%, 100% or 81.8% of strains isolated from the brittany slaughter-house n°1, the brittany slaugher-house n°2 and the alsatian slaughter-house were pathogenic respectively.

Considering the preliminary results of prevalence obtained, this study demonstrates the value of establishing the epidemiological situation of *Y. enterocolitica* in pigs in France.

Citations: Arnold et al., 2004. J. Vet. Med., B51, 23-27 - Efsa, 2009. The EFSA journal, 223 pages - Ostroff S.M., 1995. Contri. Immunol., 13, 5-10 - Thisted-Lambertz S.T., Danielsson-Tham L.M., 2005. Appl. Environ. Microbiol., 71, 3674-81.