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Short communication

Assessment of factors influencing the within-batch seroprevalence of human enteropathogenic *Yersinia* spp. of pigs at slaughter age and the analogy with microbiology

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ABSTRACT

The microbiologically and serologically-based prevalence of human enteropathogenic *Yersinia* spp. at moment of slaughter varies between pig farms due to different herd-level factors. A face-to-face questionnaire concerning a broad range of farm aspects (e.g., management and housing system, biosecurity, and hygiene measurements) was performed on one hundred farms. Factors influencing the seropositivity of 7047 pigs against human pathogenic *Yersinia* spp. were determined and compared to the microbiology.

At the slaughterhouse, pieces of diafragm of on average 70 slaughter pigs per batch were sampled to determine the level of antibodies against enteropathogenic *Yersinia* spp. After univariable mixed-effect logistic regressions, variables that were related to the seropositivity ($p < 0.05$) were included in a multivariable model ($p < 0.1$). The factors remaining significantly associated in the latter model were an increasing number of piglet suppliers (zero up to eleven suppliers) (Odds Ratio = 1.4), a high density of pig farms in the area (high versus low density) (Odds Ratio = 2.3), the use of semislatted floors in the fattening pig unit (semi slatted floor versus fully slatted floor) (Odds Ratio = 3.8) and the possibility of snout contact in the fattening pig unit (snout contact or not) (Odds Ratio = 0.1).

Decreasing the risk of infection with human enteropathogenic *Yersinia* spp. at moment of slaughter or during rearing is possible by changing farm management factors.

1. Introduction

Human pathogenic *Y. enterocolitica* (98.4%) and *Y. pseudotuberculosis* (0.9%) are causing the third most important bacterial food-borne disease which leads to about 7000 confirmed human cases in the European Union each year (EFSA and ECDC, 2015). Pigs are the main reservoir of these pathogens (Thibodeau et al., 1999; Fredriksson-Ahomaa et al., 2010). The infection is mainly caused by consumption of pork and products thereof. Contamination of pork occurs by (cross-)contamination during slaughter or following steps, due to infected pigs (Laukkanen et al., 2009). A reduction of the number of infected pigs at farm-level would reduce the amount of contaminated carcasses. To obtain an indication of the number of infected pigs prior to slaughter, there are three possible samples: tonsils, faeces or blood. Microbiological isolation before slaughter requires sampling of the tonsils as pigs are intermittent shedders and most pigs no longer shed enteropathogenic *Yersinia* spp. in the faeces at slaughter age (Vilar et al., 2013), thus resulting in an underestimation of the prevalence when analyzing faecal samples. Nevertheless, sampling of tonsils in living pigs is not animal-friendly (Fukushima et al., 1983; Thibodeau et al., 1999; Nesbakken et al., 2006), so, if sampling happens before slaughter, serological analysis is the most appropriate method.

The number of studies based on the serological prevalence of *Yersinia* spp. are limited (Skjerve et al., 1998; von Altrock et al., 2011). The study of Skjerve et al. (1998) was based only on the antibodies against *Y. enterocolitica* serotype O:3 and its risk factor analysis was based on five pigs per farm from 265 slaughter pig producing farms (conventional and farrow-to-finish production) (Skjerve et al., 1998). The study of von Altrock et al. (2011) assessed the level of antibodies against both enteropathogenic *Yersinia* spp., analyzing 30 blood samples per herd in 80 herds (von Altrock et al., 2011). Snout contact, use of tetracycline, fasting pigs before slaugh-

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ter, the use of bedding material, daily observation of a cat in the stables and drinking from nipples were identified as risk factors. Common protective factors were the use of municipal water, a farrow-to-finish farm and manual feeding of slaughter pigs.

It is important to determine farm factors influencing this seroprevalence, so farmers could adapt their farm management by introducing these factors to decrease the within-batch prevalence. The aim of this study is to gain information about these farm factors to influence the within-batch seroprevalence of pigs at time of slaughter so that measurements can be taken to reduce this within-batch seroprevalence.

2. Material and methods

2.1. Study design and sample collection

Between January and December 2012, one hundred farms were ad random selected for *Yersinia* seroprevalence investigation (Vanantwerpen et al., 2014). The farms were distributed throughout Belgium, although most farms were situated in the west part of Belgium due to the high density of pig farms in that area (0.9 herds/km2). From each farm, one batch was considered at the slaughterhouse. The number of pigs sampled per batch was established very precise and accurate (number of pigs to be sampled per batch was calculated based on an expected batch prevalence of 50%, a confidence level of 95% and an accepted error of 10%). The batch size varied from 70 to 930 pigs and 41–88 pigs per batch were sampled (piece of diaphragm). A total of 7047 pig carcasses were included in this study. The amount of antibodies against *Yersinia* outer membrane proteins (YOP's) was established using an enzyme-linked immunosorbent assay (Pigtype Yopscreen, Labor Diagnostik Leipzig, Qiagen, Leipzig, Germany). The results were expressed in %OD.

2.2. Collection of questionnaire data

Two to seven days before the pigs were slaughtered and pieces of diaphragm were collected, a standardized questionnaire was completed by the same investigator. Data were obtained by interviewing the farmer and by direct observation of certain factors. The questionnaire used contained 68 questions regarding different aspects of the farm (Vanantwerpen et al., 2015). At the time of the visit, the *Yersinia* status of the farm was still unknown. In total, 59 fattening pig herds and 41 farrow-to-finish herds were included in the analysis. The number of sows in the farrow-to-finish farms varied between 80 and 750, and the size of the fattening pig farms ranged from 297 to 10500 slaughter pigs.

2.3. Statistical method

The dependent variable was defined as the infection status of the animals, specifying a positive animal having an activity value of 30OD%. Antibodies were present in 4692 (66.6%) pigs and the within-batch seroprevalence ranged from 0 to 100%. Among the farms, 44% had a high number of seropositive animal (seroprevalence > 85%) and only seven farms, presented no seropositive animal (Vanantwerpen et al., 2014). Independent variables included categorical, continuous and binomial farm-level variables. Stata/MP 12.1 (StataCorp, 2011) and Excel software were used for all analyses. An overview of the collected farm data showed that some factors were not useful for the analysis due to different reasons. If the correlation coefficient between variables was high ($r \geq |0.7|$), only one of the variables was included in the model. To decide which variable to include in the analysis depended on the biological plausibility. A factor that was only applied by five farms or less was excluded. This resulted in exclusion of 19 factors. Moreover, variables with a high number of missing values (more than 15 missing) were omitted. Some variables were pooled, e.g. the factor 'cleaning-disinfection-empty' and the 'proper use of a disinfection bath' (Vanantwerpen et al., 2015). According to the reducing measurements mentioned above, only 36 explanatory factors were retained for the analyses (Vanantwerpen et al., 2015). Independent variables were first screened in univariable analyses (mixed effect logistic regression) using a significance level of $P < 0.05$ for inclusion in the multivariable model with the farm as random effect. In this model, only factors which remained significantly associated with a positive seroresponse ($P < 0.1$) were determined as risk or protective factors. Possible interaction elements between significant independent variables were evaluated and included if they were significant ($P < 0.1$).

3. Results

The final logistic regression model yielded three risk factors, one protective factor and one significant interaction (Table 1).

The risk factors were 'the use of a semi-slatted floor in the fattening pig unit', 'presence of other pig farms in the area (closer than 500m)' and 'the number of piglet suppliers'. As protective factor there was 'snout contact possible between pens' that, in relation with the presence of pets in the stable, turned into a risk factor.

There were 41 farrow-to-finish farms included in this study, that had a mean within-batch seroprevalence of 60%. The fattening pig farms with just one piglet supplier (n = 34) had a slightly higher prevalence of 64% ($P > 0.1$). The prevalence increased for farms with two (n = 8) and three or more (up to 11 suppliers) (n = 17) piglet suppliers to 68 and 85% respectively.

The distribution and the mean within batch seroprevalence of the farms depending on the presence or absence of the risk factors is shown in Table 2.

4. Discussion

The aim of this study was to investigate the possible risks for infection with *Yersinia* spp. during the whole rearing period and compare them with the risk factors based on the microbiological prevalence at time of slaughter.

The first two risk factors, 'the use of a semi-slatted floor in the fattening pig unit' and 'presence of other pig farms in the area' could be explained easily. Since pig faeces can contain human pathogenic *Yersinia* spp., the contact between these faeces and pigs may lead to

Table 1
Final logistic regression model with a random effect for farm, of variables significantly ($P \leq 0.1$) associated with the presence of human pathogenic *Y. enterocolitica* antibodies in Belgian pig batches at slaughter ($n = 100$ farms).

Factor	Odds Ratio	P-value	95% CI[a]
Presence of semi slatted floor in fattening pig unit	3.8	0.022	[1.21; 11.82]
Presence of other pig farms in the area (closer than 500m)	2.3	0.076	[1.01; 5.31]
Number of piglet suppliers	1.4	0.003	[1.13; 1.82]
Presence of snout contact in fattening pig unit	0.1	0.001	[0.03; 0.37]
Interaction snout contact and pets	17.6	0.004	[2.56; 121.51]

[a] The 95% CI of the Odds Ratio.

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