Can on-farm serology predict the risk of *Salmonella* shedding at slaughter in fattening pigs?

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Abstract

The objective of this study was to assess whether on-farm serology and other farm characteristics could be used as predictors of *Salmonella* shedding at the abattoir. For that, 1,500 pigs from 30 pig fattening units were selected. One month before slaughter, 50 pigs from each pig unit were tagged and bled for the detection of antibodies against *Salmonella*. Pooled floor fecal (PFF) samples from 10 pens were also collected for *Salmonella* detection in each pig unit. At the slaughter, colon content of each pig was collected for *Salmonella* shedding detection. *Salmonella* was present in 13 (43.3%) of the farms. Mean herd seroprevalence (OD%≥40) was 31.7% (95%CI= 21.8-41.0). A total of 316 pigs (23.6%) shed *Salmonella* at slaughter. A random-effects logistic regression analysis showed that serology, the percentage of *Salmonella*-positive pens on the farm, and the internal biosecurity score predicted reasonably well (Area under the curve -AUC-= 0.76; $P<0.05$) whether a pig would shed *Salmonella* at slaughter or not, with estimates of sensitivity (Se) and specificity (Sp) of 71.6% and 73.6%, respectively.

Introduction

Salmonellosis remains one of the most frequent foodborne zoonoses in the EU, and contaminated pork is a significant source of human infection. In the last years, amongst the most reported serovars were *S*. Enteritidis, *S*. Typhimurium, and the monophasic variant of *S*. Typhimurium (mST), the latter two mainly associated with pig and pork sources (EFSA, 2022). Asymptomatic *Salmonella*-infected pigs are commonly arriving at the abattoir for slaughter (EFSA, 2011) and become a major source of abattoir environmental *Salmonella* contamination, which is likely the main source of carcass contamination (Argüello *et al*., 2013). Few EU countries have established National Control Programs (NCPs) against pig salmonellosis, most of them based on the serological monitoring of a relatively small number of pigs per batch slaughtered. In general, they failed to attain a significant reduction in *Salmonella* infection or the number of human cases related to pork consumption, with overall *Salmonella* seroprevalence remaining stable (Correia-Gomes *et al*., 2021). Had the major objective of an NCP to reduce the incidence of human salmonellosis, finding alternative ways to minimize *Salmonella* abattoir contamination may be more effective than trying to halt the infection within the pig farms. Being able to predict the likelihood that a pig will shed *Salmonella* upon its arrival at the abattoir may be the first step to reaching this objective. In this study, it was assessed whether serology and other farm characteristics (*Salmonella* pen contamination, farm biosecurity, etc.) could be used as predictors of *Salmonella* shedding at the abattoir.

Material and Methods

A total of 1,500 pigs from 30 representative pig fattening units were selected. A questionnaire on biosecurity (https://biocheckgent.com/en) was administered to each farm. Approximately one month before slaughter, 50 pigs/farm were selected from different pens within a pig unit, ear-tagged, and bled for detection of antibodies against *Salmonella* (Herdcheck Swine

Salmonella test, IDEXX Laboratories, Westbrook, ME, USA). Pooled floor fecal (PFF) samples from 10 pens were also collected for *Salmonella* detection at each farm unit. At slaughter, colon content (CC) was collected from these pigs for *Salmonella* shedding assessment. For *Salmonella* identification the standard ISO 6579-1:2017 method was followed. A colony from each *Salmonella*-positive culture was further selected for PCR identification of the two major serotypes of concern, *S*. Typhimurium and mST (Tennant *et al*., 2010).

The predictive model for *Salmonella* shedding at slaughter was built with 2/3 of the pigs (selected randomly) for which farm and abattoir information was collected. A random-effects logistic regression analysis was built in which *Salmonella* shedding was the dependent variable, and serology, farm biosecurity (overall, internal and external), *Salmonella*-positive pens, season, and distance and transport time to slaughter the independent ones. The farm was included the as a random (grouping) variable. Data from the remaining 1/3 of the pigs were used for model validation. The Receiver Operating Characteristic (ROC) curve and its area under the curve (AUC), as well as estimates of sensitivity and specificity, were estimated for both models and compared.

Results

The mean overall biosecurity score for these farms was 73.31% (ranging from 51% to 84%), and the mean internal and external biosecurity scores were 67.80% (40% to 82%) and 78.98% (62% to 89%), respectively. *Salmonella* was detected in 13 (43.3%; 95%CI= 27.4-60.8) of the sampled farms, with a mean pen prevalence among the *Salmonella*-positive herds of 36.9% (95%CI= 22.6-51.2). The mean seroprevalence (based on an ELISA cut-off value $OD_{650nm} \geq 40\%$) among the 30 farms was 31.7% (95%CI= 21.8-41.0). Only 6 of these farms showed seroprevalences below 10%. Out of the 1,500 pigs selected at the farm, 1,341 (89.4%) were identified at slaughter, and CC samples were collected (44.7 pigs/farm). A total of 316 pigs (23.6%; 95%CI= 21.4-25.9) were shedding *Salmonella* at slaughter (range 0% to 79.4%). mST was isolated in 151 samples (47.8%), *S*. Typhimurium in 71 (22.5%), and other serotypes in 94 (29.7%). A total of 885 animals were used for building the prediction model. Three variables resulted significantly associated with *Salmonella* shedding at the abattoir. The model predicted whether a pig would shed *Salmonella* at slaughter or not reasonably well (AUC= 0.76; $P<0.05$) (Table 1). The best cut-off value for maximizing Se and Sp was 25.9%, and the associated diagnostic Se and Sp for that cut-off value were 71.6% and 73.6%, respectively.

Table 1. Results of the random-effects logistic regression analysis to predict *Salmonella* shedding at the slaughterhouse*.

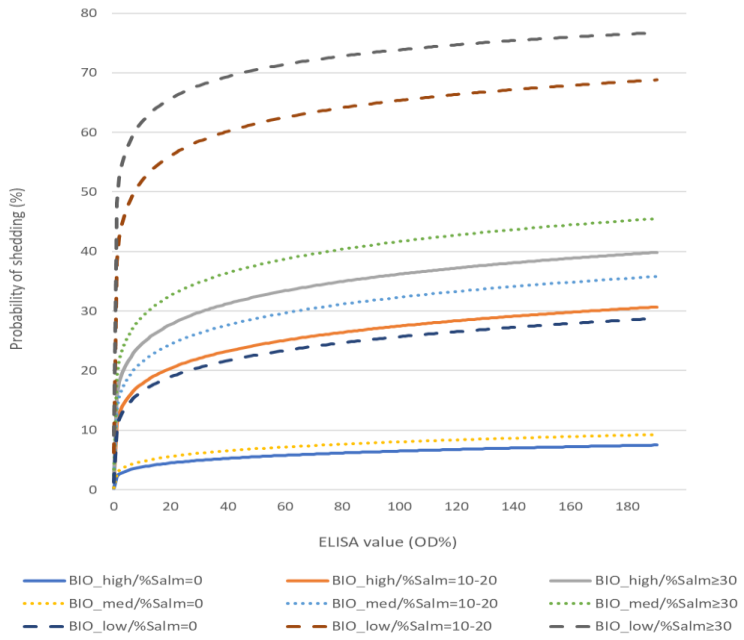
		Odds Ratio (OR)	*P*-value	95% CI
Serology (as LogOD%)		1.74	0.028	1.06-2.87
% *Salmonella*-positive pens	0%[1]	1	-	-
	10-20%	5.46	0.029	1.19-24.95
	≥30%	8.18	0.003	2.07-32.33
Internal biosecurity (score)	<64%[1]	1	-	-
	64-77%	0.25	0.043	0.07-0.96
	>77%	0.20	0.050	0.04-0.99
Constant		0.11	0.005	0.03-0.52

* Area under the curve -AUC-=0.76.

[1]Reference category. Intraclass correlation coefficient (ICC): 0.33 (95%CI= 0.19–0.50).

Figure 1 shows the predicted probability of shedding *Salmonella* at abattoir according to serological values after considering the proportion of *Salmonella* positive pens in the farms (3 categories) and their internal biosecurity scores (3 categories). In two scenarios serology would not add significant information for considering whether a pig would shed *Salmonella* at the abattoir: in case of farms with high or medium internal biosecurity with no *Salmonella*-positive pens or in case of farms with low internal biosecurity with at least one *Salmonella*-positive pen. The model was rerun using data from the one-third left of the pig population (N= 456) for validation purposes. No significant differences were found between both models.

Figure 1. Probability of *Salmonella* shedding at the abattoir as a function of serology (ELISA OD% values), farm internal biosecurity -BIO- (high, medium and low), and the percentage of *Salmonella*-positive pens in the farm -%Salm- (0%, 10-20% and ≥30%).



Discussion

Despite the good level of biosecurity of the farms, prevalence, and seroprevalence results suggested that *Salmonella* was widely spread within most farms. Herd prevalence remained high and similar to that 15 years ago (García-Feliz *et al*., 2007), despite pig salmonellosis being considered a public health concern and contaminated pork and products thereof an important source of human salmonellosis (EFSA, 2022). Although the biosecurity level of the farms is considered beneficial in reducing bacterial transmission, it appears that it cannot reduce *Salmonella* prevalence by itself (Youssef *et al*., 2021). The implementation of efficient on-farm *Salmonella* control measures will strongly depend on farmers' perception of the disease and their motivation to maintain them on an ongoing basis (Marier *et al*., 2016), and pig salmonellosis is usually asymptomatic, that is, of low concern for farmers and veterinarians. In this study, neither the overall nor the external biosecurity scores related to a reduction of *Salmonella* shedding at the abattoir, but internal biosecurity did (Table 1). More worrisome was the high proportion of pigs shedding *Salmonella* at the abattoir (23.6%), with some pig batches reaching up to 80%, and with zoonotic *S*. Typhimurium and mST being the predominant serotypes. Finding ways of preventing this shedding should be considered of utmost importance. Interestingly, as indicated by the random-effects logistic model, the proportion of pig shedding *Salmonella* at the abattoir was strongly related to the *Salmonella*

pen prevalence on the farm. The prediction model that was built with serology, farm internal biosecurity, and *Salmonella* pen prevalence factors, showed an acceptable ability to predict whether an animal will shed *Salmonella* at the abattoir. Thus, a pig with a predicted probability of shedding *Salmonella* greater than 26% would have a probability of being a true shedder of 71.6%. If the predicted probability was ≤26%, the animal would have a 73.6% probability of being a true non-shedder. Therefore, estimating the proportion of animals with a model probability higher than 26% in a given batch of pigs intended for slaughter would allow for assessing the overall risk of shedding for that batch. Once the potential risk of *Salmonella* shedding has been assessed, stakeholders could act in accordance to the results obtained. Nevertheless, it appears that the value of serology for predicting shedding depends upon the context in which it is used, and factors such as internal biosecurity and the presence of *Salmonella* in the farm may play an important role in its proper interpretation.

Conclusion

This study highlights the importance of the context where serology is used for pig salmonellosis control. Using it in the interface farm-abattoir may be helpful to identify batches of pigs at risk of shedding *Salmonella* upon their arrival in the abattoir. In some cases, being aware of the farm's internal biosecurity level and performing a bacteriological sampling of a representative number of pens would suffice to estimate the level of risk of *Salmonella* shedding for a given batch of pigs ready for slaughter. In others, serology would be required for a more accurate interpretation of the results, but in both situations, an acceptable level of knowledge on the risk of *Salmonella* shedding for a given batch of slaughtered pigs could be achieved. Reducing the likelihood of *Salmonella* shedding at this stage would be a significant step for reducing *Salmonella* carcass contamination.

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