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Detection of Salmonella antibodies in asymptomatic fattening pigs varies depending on the test and the matrix used

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Introduction

Human salmonellosis is a common meat-borne infection in Europe including Finland. Around 10% of the domestic cases are due to contaminated pork in Finland. *Salmonella* infection in fattening pigs is mostly asymptomatic and therefore identification of *Salmonella*-positive pigs is usually not possible at the slaughterhouse. Serological testing has been established in some European countries to identify fattening farms producing *Salmonella*-infected animals. The presence of antibodies to *Salmonella* can easily be detected by commercial ELISA kit using blood or meat juice samples collected at the slaughterhouse (Felin et al. 2015, 2019). However, reliable and comparable commercial ELISA tests are of major importance for serological monitoring (Felin et al. 2017). In this work, presence of *Salmonella* antibodies were studied in blood and meat juice samples of Finnish fattening pigs with two commercial ELISA tests.

Material and Methods

In total, 146 blood samples of fattening pigs originating from 29 farms (1-10 samples per farm) and 94 meat samples from 66 farms (1- 5 samples per farm) were selected for this study. The blood samples were collected from pigs at farm at the end of the fattening period before arrival to the slaughterhouse (Felin et al. 2019) and meat samples of diaphragm muscle were collected from fattening pigs at slaughter during *Trichinella* sampling (Felin et al. 2015). The samples were stored at -70°C until testing. Presence of *Salmonella* antibodies was studied with two commercial ELISA tests: Pigtype® Salmonella Ab (Qiagen, Leipzig, Germany) and PrioCheck® Porcine Salmonella kit (Thermo Fischer Scientific, Waltham, MA USA). Statistical analyses were performed using SPSS Statistics 24. Correlation between the ELISA tests was estimated by calculation of Spearman's rho. Additionally, Cohen's kappa value was calculated to test the level of agreement between the ELISA tests.

Results

The OD% values varied in blood samples (146) between 18 and 116 (median=29, mean=33) using Pigtype and between 1 and 70 (median=20, mean=21) using PrioCheck (Table 1). The OD% values varied in meat juice samples (N=94) between 9 and 55 (median=19, mean=21) using Pigtype and between 0 and 71 (median=9, mean=15) using PrioCheck (Table 1).

There was no correlation (P>0.05, Spearman's rho) between the tests. Using the cut-off OD% values of 20, 30 and 40, the detection rate of *Salmonella* antibodies in blood samples was clearly lower with PrioCheck compared to Pigtype (Table 2). There was a fair agreement (Cohen's kappa=0.247, P< 0.0001) between the tests when blood samples were studied using the cut-off OD% value of 30. In meat juice samples, higher detection rates were obtained with PrioCheck compared to Pigtype when the cut-off OD% values of 30 and 40 were used (Table 2).

Discussion and Conclusions

The key element of *Salmonella* control programs in Europe is the classification of fattening pig herds according to seroprevalence at slaughter measured by ELISA. ELISA test are typically reliable, accurate and cost effective. However, there is a lack of correlation between serological and microbiological results for detection of individual *Salmonella*-positive pigs and there is variability associated with the use of different ELISA kits and matrices (Mainar-Jaime et al. 2018).

In this study, we could show that blood samples had a clearly higher mean and median OD% values than meat juice samples, which also influenced the seroprevalence using different cut-off levels. This demonstrates that the matrix influences the OD% values and the seroprevalence, and therefore, the cut-off value should be adjusted depending on the matrix used. We could also show that the seroprevalence was clearly higher in blood samples with Pigtype compared to PrioCheck using cut-off OD% values of 20, 30 and 40. Blood samples were studied with a newer Pigtype test than meat juice samples. The newer Pigtype test detected antibodies to more antigens (serotypes), which may explain the higher detection rates compared to PrioCheck test, especially when the cut-off value of 40 was used. Interestingly, higher detection rates were obtained in meat juice samples with PrioCheck compared to Pigtype when cut-off OD% values of 30 and 40 were used. One reason can be the equation for the calculations of OD% values, which differs between the two tests and may influence the results. There was no clear correlation between the ELISA tests in

our study, which further complicates the comparison of serological results if they have not been studied with same methods.
This study show that the ELISA test used can strongly affect the results. This study also demonstrate that the cut-off value should be adjusted depending on the test and matrix used.

References

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Table 1: OD% values in blood and meat juice samples using Pigtype and PrioCheck commercial ELISA tests

Sample	ELISA test	Mean	Median	SD	Min	Max
Blood	Pigtype	33	29	15	18	116
(N=146)	PrioCheck	21	20	11	1	70
Meat juice	Pigtype	21	19	7	9	55
(N=94)	PrioCheck	15	9	16	0	71

Table 2: Detection rates of Salmonella antibodies in blood and meat juice of fattening pigs using different tests and OD% values

Matrix	Test	Detection rate		
		OD%≥20	OD%≥30	OD%≥40
Blood	Pigtype	95%	49%	23%
(N=146)	PrioCheck	51%	19%	3%
Meat juice	Pigtype	50%	5%	2%
(N=94)	PrioCheck	26%	14%	9%