

Meat Juice serology underestimates prevalence of Salmonella in pig herds

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Abstract

Salmonella serology is used for classifying pig herds in risk categories in several national quality programs. Meat juice is used as test matrix in most of these programs. Two studies were done to compare the salmonella ELISA test results from meat juice with blood serum as a reference.

Pig blood and meat samples for these studies were collected in one slaughterhouse. ELISA tests were done with a commonly applied commercial test. In the first study paired blood serum and meat juice samples from 182 pigs were collected and tested in two different laboratories. In the second study meat and blood samples were collected from 470 herds, over 20.000 samples for each matrix.

The first study showed a linear relation between all matrices, but the OD values in meat juice were significantly lower than in blood serum. To obtain comparable outcomes in serum and meat juice, the blood serum OD%-values had to be reduced with 20 to 40%, depending on the lab that applied the test. This underestimation was confirmed in the second study. When the diagnostic cut off, OD10%, was applied on the blood samples, over 57% of the tested pigs showed antibodies and none of the slaughtered herds had fully negative serology, whereas with meat juice and a cut off at OD40% only 7,5% pigs were positive.

It is concluded that meat juice testing for Salmonella antibodies can heavily underestimate the proportion of pigs that have encountered a salmonella infection. Consequently, pigs from herds that are categorised as low risk may be infected with salmonella. These pigs may therefore contaminate the lairage and the slaughter line. Monitoring results based on blood serology can not be compared with results based on meat juice, without taking care of the observed differences.

Introduction

Serology is used to determine the Salmonella status of pig herds as part of control strategies in national control plans in some northern European countries. In the Danish, Dutch and German programmes, pig herds are grouped in three to four distinct risk groups.

To detect the serological response following infection several Salmonella ELISA's are on the market. These are mixed LPS ELISA's that detect Salmonella seroconversion due to infections with O-serogroups B (for example *S. Typhimurium*, *S. Derby*, and *S. Brandenburg*) and C1 (*S. Choleraesuis* and *S. Infantis*). The experimental cut off of these tests is at OD 10%. In the national monitoring programmes the cut-off is mostly increased towards 40% to obtain a manageable, i.e. not to high number, of herds that need to take corrective intervention actions (Mousing et al 1997).

In a recent ring trial where meat juice was tested meat juice results were highly variable (Berk, 2008). Berk (2008) concluded that serology on meat juice is therefore not a suited method for target setting in an EU-wide control strategy. More variability may occur in meat juice due to difference in drip loss, which differs depending on the muscle used, the pH of the meat which is partly depending on stress before slaughter, sampling treatment, etc.

The number of scientific publication comparing the performance of commercially available test on blood and meat juice are limited and the number of samples tested are limited. Aim of the present study was to compare results from Salmonella serology in blood serum and meat juice under routine circumstances and see whether outcomes are equivalent. This is a preliminary presentation of the results.

Materials and Methods

In Study I a paired blood and meat sample were collected from the same animal. Pigs were randomly selected from 25 herds at one slaughter day in a German slaughterhouse. In total 182 paired blood and meat juice samples were available.

Meat for meat juice was collected from the neck and from the diaphragm.

The ELISA tests were performed in two commercial labs.

In study II, routine blood samples and neck meat samples were collected. Serum and meat juice were prepared and tested on Salmonella in one lab. The samples were collected with comparable numbers from the same herds, paired on supply level, not on pig level.

Blood was collected before entering the scalding tank. Blood was collected in treated test tubes (12 ml) for serum collection (KABE-Labortechnik), stored and transported at 4 °C and was send to the lab three times a week.

At the lab serum was prepared. In study I the serum was split in two. One sample was send to the other lab, at 4°C.

Meat was collected at the meat inspection platform. The pigs arrive at the sampling place 40 minutes after sticking. Meat (size about 1.5x1.5x1.5 cm) of neck (Study 1 and 2) or diaphragm (only in Study 1) muscle was collected in the SALMOSTORE meat juice container (Labor Diagnostik GmbH Leipzig). The samples were immediately deep-frozen at the slaughterhouse and stored at -20°C up to one week. Serum was harvested in the SALMOSTORE meat juice container after 1-7 days by thawing. After collection of the meat juice the tubes were sent to the laboratory with the blood stored at 4°C. In study 1 the samples were split here and sent to the research lab like the serum samples.

Both labs did the SALMOTYPE® Pig Screen Elisa. In these tests blood serum is diluted 1:100 and meat juice is diluted 1:10 according to the SALMOTYPE® Pig Screen Manual.

Results

In table 1 the number of positive tests at cut-offs OD% 10, 20 and 40 are shown. The tests on meat juice led to lower number of positive samples compared to blood serum at all three cut-offs in both laboratories. The proportion of positives was higher in meat juice from diaphragm than from neck muscle.

The differences between labs was much bigger for meat juice than for blood.

Table 1: Proportion Salmonella positive serological tests at different cut-offs in different matrices in two laboratories

OD%	lab 1			lab 2		
	Blood	Neck meat juice	Diafragm meat juice	Blood	Neck meat juice	Diafragm meat juice
>10	52,7%	51,1%	58,8%	47,8%	24,2%	37,4%
>20	34,6%	23,6%	23,6%	29,7%	15,4%	19,8%
>40	13,7%	8,2%	9,9%	13,7%	7,7%	9,3%

In Study II samples were collected during the routine monitoring at the slaughterhouse where pigs are sampled at random, the blood and the neck-meat-for-juice sampling were running in parallel. The number of tested samples of neck meat juice was higher than of blood serum (respectively 28.182 and 23.021 samples).

The number of ELISA positives in neck meat juice was lower than in blood, at different cut off levels (table 2). The OD value of all blood analyses result was corrected by multiplying the OD with 0,6. In that case meat juice and blood became comparable.

Table 2: proportion positive samples in Salmonella ELISA at three cut-off values tested on meat juice and blood serum (corrected and not corrected) – Study II

OD%	Meat juice	Blood, standard	Blood, corrected*
>10	37,0%	57,7%	44,2%
>20	19,9%	37,9%	21,9%
>40	7,5%	17,0%	7,3%

* OD values were corrected by multiplying with 0.6.

Categorisation of the herds based on the tests with meat juice and blood, and blood after correction of the outcome was done according to the German QS regulations. The results are shown in table 3.

Categorisation of herds based on meat juice leads to an underestimation of category 2 and 3 farms when blood is the reference. Reducing the OD values of blood by correcting the blood OD with a factor 0.6 makes blood and meat juice comparable. A cut-off of OD65% for blood made the results also comparable, although there were still little more herds in category 2.

Table 3: Categorisation of pig herds bases on corrected and non-corrected test results

Cut Off	Meat juice	Blood			
	OD%40	OD% 40	OD% 40	OD%65	OD%10
Correction:	-	-	OD% x 0.6	-	-
cat 1	90,4%	67,9%	90,2%	89,1%	0,4%
cat 2	8,3%	24,3%	8,5%	9,6%	8,7%
cat 3	1,3%	7,9%	1,3%	1,3%	90,9%

Discussion

Serum of blood is in general the standard matrix for serological arrays. However, salmonella serology applying meat juice is standard in many European countries. The present study was done in a slaughterhouse that changed from meat juice to blood serum for the routine monitoring. This raised the question whether farmers supplying pigs to this slaughter house could expect to get other Salmonella testing results and whether more supplier of the slaughterhouse would be classified in a higher risk category. The data definitely show that changing from meat juice to blood will results in more positive tests. The proportion of ELISA positives based on blood serum was significantly higher than the proportion based on meat juice. This would lead to higher proportions of pig herds being classified in high risk categories. The outcomes of serum tests and meat juice test could be made comparable by applying a correction factor of 0.6 for the blood ODs. This indicates that ELISA on meat juice underestimates the antibody concentration with 40%.

The results of diaphragm meat juice were closer to the blood serum value than those of neck meat juice.

The outcomes also confirm earlier studies (e.g. Berk, 2008), which showed that between lab variability is much bigger for meat juice than for blood.

The acquired data show that many of the pig herds had encountered Samonella, about 60% of the samples were positive at a cut off of 10%, which is the diagnostic cut off of commercial tests.

Other studies, with smaller numbers and under experimental conditions, showed much better correlation between meat juice and serum .Therefore no correction was considered in any other study, except Wilhelm (2007). The present results were equal to the normal daily practise in this slaughter house. This underlines that underestimation in meat juice may be sampling (i.e. slaughterhouse) and lab dependent.

Variability, discrepancy and not full correspondence between meat juice and blood serum have been reported before. But a comprehensive understanding of the causes hereof are not given (Wilhelm 2007, Nielsen 1998) Meat juice is intrinsically less robust and vulnerable to sampling variability, physiological changes in the meat of the sampled animal, etc.. Categorisation of pig herds is used to control Salmonella by implementing hygienic measures at primary production level and to steer logistic slaughtering. The present study shows that herd categorisation based on serology has serious limitations that need to be taken into account before taking conclusion from a monitoring programme that applies serology with particular cut offs. For meat juice this even more true than for blood. Serology can not prevent that finally pigs from so called low category herds excrete Salmonella and contaminate lairage and slaughter line (Van der Wolf, 2001). With the uncertainties of test outcomes, and additionally the high levels of infection in slaughter pigs, as well as the evidence that contamination of carcasses is mostly depending on hygienic slaughterhouse (Swanenburg, 2001; Van der Gaag, 2004) logistic slaughtering based on categorisation by means of serology has to be done with care.

Conclusion

Routine testing of meat juice can seriously underestimate the real Salmonella serological prevalence. The study confirms that variability between labs is much higher for meat juice than for blood. Serology on blood is more robust than serology on meat juice as sampling can be better standardized and physiological changes in meat influences the composition of meat juice stronger.

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