CORRELATION BETWEEN COLOR OF MEAT JUICE SAMPLES AND SALMONELLA ANTIBODY LEVELS IN THE DANISH MIX-ELISA

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Abstract  A mandatory salmonella control program for swine has been conducted in Denmark since 1995. Finisher herds are examined for Salmonella antibodies by use of meat juice samples taken from carcasses at the slaughter plant.

This study reports for the first time the risk of false positive meat juice samples due to dark stained meat juice samples. When the neck muscle m. sternomastoideus is used for meat juice production, a strong correlation between dark staining and seroprevalence is demonstrated. Approximately 3-5% of the samples were considered to be too dark stained. When the psoas minor muscle was used no correlation was found. The study highlights the importance of correct sampling of meat in order to avoid dark meat juice samples, and allow too dark meat juice samples to become excluded from the monitoring.

Introduction  Since 1995, a nationwide and mandatory salmonella control program for swine has been conducted in Denmark (Nielsen et al. 2001). As part of the program finisher herds are examined for salmonella antibodies by use of meat juice samples taken from carcasses at the slaughter plant.

Previous research has documented a clear correlation between antibody levels in serum and meat juice by examination in the Danish salmonella Mix-ELISA (Nielsen et al 1998). Two different muscles are normally used for meat juice production, m. sternomastoideus in the neck or m. psoas minor in the pelvis region. Serum samples are diluted 1:400 and meat juice samples 1:30 respectively, in order to obtain comparable results between serum and meat juice.

Correctly taken meat samples for meat juice shall not contain blood clotting on the surface or larger blood veins as this may affect the ELISA result, since blood contains 10 times more antibodies compared to meat juice. However, under the current practical sampling conditions 1-10% of the 580.000 annual examined samples appear by visual inspection to be more or less dark stained with blood. The prevalence of dark stained samples is highly slaughterhouse depended. These samples constitute a risk for false positive results in the surveillance. In order to examine the correlation between the degree of dark staining in meat juice due to blood contaminated samples and the ELISA result, the following investigations were carried out.

Materials and Methods  Different concentrations of meat juice were scanned in order to find the absorbance spectrum for meat juice. Using a visual wavelength at which meat juice only absorbed weakly could be used for detection of relative concentration of blood mixed with meat juice.

All routine meat juice samples received during 3 weeks in March 2005, representing all major Danish slaughter plants, were included in the study. A total of 38,532 samples were examined, 27,814 from the neck and 10,718 from the psoas. After pre-dilution 1:30 and application to ELISA wells, absorbance for meat juice samples was examined in an OD reader at 450 nm. Subsequently, the salmonella antibody results for the samples expressed as OD% were correlated with absorbance at 450 nm. Additionally, the origin of the meat sample, m. psoas minor or m. sternomastoideus was included as an explanatory factor. Statistical calculations were made using the SAS software.

Samples were divided into groups based on the absorption at 450 nm. From zero to 600 absorption units each group comprised 100 absorptions units and the last two groups were divided into 400 and 500 absorptions units due to few data in these intervals.

For each group the percent positive samples were calculated together with the percent of samples in relation to total samples. Using Excel spreadsheet the calculation and graphics were done for all samples and subsequently for the samples divided by muscle group.
The described increased seroprevalence among dark stained meat juice samples is the first report on the risk of false positive meat juice samples due to dark staining. The swine salmonella control programs in Denmark, Germany, The Netherlands, Belgium, Ireland and United Kingdom all use or may use meat juice for the monitoring of the herd seroprevalence. Additionally, meat juice may be used in some countries for monitoring of other diseases e.g. Pseudo rabies. Consequently, dark staining of meat juice may have an unwanted effect on these monitoring programs. It is not possible to predict the proportion of dark stained samples in the different programs as the Danish experience shows a significant variation between slaughterhouses and between the two investigated muscle groups.

Variation in meat juice composition is expected as meat juice may be considered as a combination of residual serum from blood vessels, intracellular liquid from blasted cells and lymph. Different muscles produce different meat juice due to the variation in muscle composition. The danger of blood polluting meat juice was recognized when the meat juice testing was developed, as blood contains 10 times more antibodies compared to meat juice. Due to this it was soon realized, that the heart and the diaphragm muscle could not be used in the ongoing monitoring, as these meat surfaces frequently are blood contaminated.

During the last year, an increased focus on the importance of correct meat sampling in the Danish salmonella control program has been established. The degree of dark stained meat juice samples was followed. Followup studies indicate that even small blood clots on the meat surface or medium size to larger blood vessels in the surface of the meat may be the cause of dark meat juice samples. Consequently, meat with this should be avoided when sampling meat for meat juice production.

The surprising difference between the neck muscle and the psoas minor muscle is yet to be explained. Apparently, even very dark samples from the psoas minor muscle have not an increased seroprevalence. The authors recognized a significant correlation as for the total samples shown in figure 1. The m. psoas minor had a significantly different correlation, Figure 2. A slightly higher seroprevalence was observed in samples with an absorbance below 250 constituting approximately 80% of the m. psoas minor samples. In contrast to the neck muscle samples, the m. psoas minor samples remained at a seroprevalence of approximately 10% independent of an increasing sample absorbance.

**Discussion**

The described increased seroprevalence among dark stained meat juice samples is the first report on the risk of false positive meat juice samples due to dark staining. The swine salmonella control programs in Denmark, Germany, The Netherlands, Belgium, Ireland and United Kingdom all use or may use meat juice for the monitoring of the herd seroprevalence. Additionally, meat juice may be used in some countries for monitoring of other diseases e.g. Pseudo rabies. Consequently, dark staining of meat juice may have an unwanted effect on these monitoring programs. It is not possible to predict the proportion of dark stained samples in the different programs as the Danish experience shows a significant variation between slaughterhouses and between the two investigated muscle groups.

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The surprising difference between the neck muscle and the psoas minor muscle is yet to be explained. Apparently, even very dark samples from the psoas minor muscle have not an increased seroprevalence. The authors recognized a significant correlation as for the total samples shown in figure 1. The m. psoas minor had a significantly different correlation, Figure 2. A slightly higher seroprevalence was observed in samples with an absorbance below 250 constituting approximately 80% of the m. psoas minor samples. In contrast to the neck muscle samples, the m. psoas minor samples remained at a seroprevalence of approximately 10% independent of an increasing sample absorbance.
nized that the study on the *psoas* minor muscle is done on a limited number of samples, and that the samples may not be sufficient representative. Additional studies need to be done before a final conclusion may be drawn.

With the current knowledge, it is possible to improve the sampling of meat for meat juice production and to exclude too dark samples from the *salmonella* monitoring.

**Conclusions** False positive results in the Danish serological *salmonella* control program may occur due to dark staining of meat juice samples. The dark staining is most likely due to blood contamination of the meat juice. Between 3% and 5% of the samples may be considered to be too dark. The study highlights the importance of correct sampling of meat in order to avoid dark meat juice samples, and allow too dark meat juice samples to be come excluded from the monitoring.

**References**
