Monitoring changes in the association between salmonella-serology and microbiology over time

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Abstract: We found a strong correlation between seroprevalence in finishing herds and the number of positive pen samples in pig herds in level 2 or 3 in the Danish Salmonella Surveillance program. Several times during the period we observed, that the association, although always present, changed from one month to the following month. We found indications of a seasonality in the association, indicating, that late winter we would isolate more Salmonella for a given seroprevalence compared to late summer. Herds using home-mixed meal feed had less positive pen faecal samples for a given seroprevalence than herds using pelleted, purchased feed.

Keywords: Seroprevalence, meal, pellets.

Introduction: In most ongoing surveillance systems there is a need for verifying, that the association between the surveillance results and the true prevalence is constant over time. There are ways of doing this, but they are expensive, and will have to be performed several times each year to be useful for validating the system. Here we suggest using material sampled for other purposes to validate changes in the association between microbiology and serology over time.

Materials and Methods: From August 1996 to April 2001, we compared serological results from the ongoing surveillance systems with microbiological results from pen faecal samples obtained from herds assigned to level 2 or 3 according to the Danish surveillance scheme. Serological results were obtained by using the Danish Salmonella-Mix-Elisa on meat-juice-samples. A positive sample was defined as a sample with an OD% above 20. Pen faecal samples consisted on pooled samples of 5 droppings sampled from 5 different areas within one pen. Between 1 and 100 samples were obtained pr herd, with an average of 12 samples pr herd. In total we used microbiological and serological results from 6317 sampling rounds. Some herds were sampled several times in the period.
We used a logistic regression model to model the association between serology and microbiology, using seroprevalence in the same month, the previous month and sampling month as predictors for the microbiological results. Williams method was used to adjust for overdispersion. To investigate whether there were changes in association within a short time, we compared results month by month for the entire period.

From 735 of the herds we obtained results on which type of feed, that had been used in the herd. Some of the herds were sampled several times, in total 1371 sampling rounds were included. For the statistical analysis we used a logistic regression model, including a repeated statement to account for the fact, that some herds were sampled several times.

**Results:** There was a strong association between serological results and microbiological results. A 1 percent rise in seroprevalence in the same month results in an increase in microbiological prevalence of 1.4 percent (p<0.0001). A 1 percent rise in seroprevalence in the previous month results in an increase in microbiological prevalence of 1.01 percent (p>0.0001). There was a strong effect of sampling month (p>0.0001) indicating that the association between serology and microbiology, although strong, and always positive, cannot be assumed to be constant over time. In figure 1 we show the OR for a positive pen faecal sample, given constant seroprevalence, compared to results from April 2001 and the seroprevalence month by month in the period from August 1996 to April 2001.

It is seen, that the OR of isolating *Salmonella* from a pen faecal samples, given constant seroprevalence, varies considerably over time. The highest risk of isolating *Salmonella* for a given seroprevalence is in March, and the lowest risk is in July. The OR in March is 1.7 compared to July. 7 times during the period, we recorded a change in association between one month and the following month.

OR for isolating *Salmonella* in pen faecal samples from herds using pelleted feed was 1.25 compared to herds using home-mixed meal, given they had the same seroprevalence, indicating that for a given seroprevalence, we found app. 25 % more positive pen faecal samples, if the herd used pelleted feed compared to home-mixed meal.

**Discussion and conclusion**
The highly significant association between serology and microbiology shown here is in concordance with several other studies. In all surveillance systems there is a need for evaluating the association between the results from the diagnostic system.
and the true level in the population. Even the most thorough quality control systems cannot assure that this association is stable over time.

![Graph showing OR for isolation of Salmonella in a sample, adjusted for effect of seroprevalence.](image)

Figure 1. OR for isolation of Salmonella in a sample, adjusted for effect of seroprevalence

The observed changes in association over time can either be attributed to changes in sensitivity or specificity of the serological test or the microbiological test. We cannot be sure, which is correct. And it is important to realise, that this is not necessarily due to laboratory failure, but could also be attributed to unknown or unobserved changes in the biology of the production system under observation.

It has been suggested, that the differences observed in several studies in Denmark between serological results from pigs or herds given different types of feed were due to differences in the serological results, and not reflected in microbiological differences.

The results of this study suggest, that this difference in serological results would be even more substantial, if we used microbiology as the outcome for studies into the effect of different feed type.

Based on other studies and these results, we can conclude, that not only do herds using home-mixed meal have a lower risk of being classified as a high seroprevalence herd, but even when they are classified as a high seroprevalence herd, they will have less microbiologically positive pen faecal samples than similarly classified herds, that uses pelleted feed.