

# The relation between Salmonella-shedding and the Danish Salmonella-Mix-ELISA on the pig-level.

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Dahl, J.

DANISH BACON AND MEAT COUNCIL, Axelstorv 3, Copenhagen, Denmark

## Abstract

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In this study we found a strong association between serological results and shedding of Salmonella or Salmonella-positive cecal samples on the individual level.

The results indicated, that there was a positive correlation between both serological reaction and shedding or positive cecal sample. When using seropositivity as a predictor; OR for shedding in data set 1 was 2.8. For data set 2 OR for positive cecal sample was 3.0. Both were highly significant. Using OD% or square root of OD% were also significant predictors, but were not superior to using seropositivity.

The results clearly demonstrates a strong correlation between serology and microbiology on the individual level, but it also demonstrates, that it cannot be used for picking out individual pigs in an infected herd. The serological test is to be seen as a predictor of risk, not as a statement of absolute microbiological negativity or positivity.

As such it can be used as a tool in clinical trials, replacing microbiology as the dependant variable, because microbiology is expensive and impractical for large scale trials.

## Introduction

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The Danish Salmonella Mix-ELISA was developed as a tool for the Danish Salmonella surveillance program. Christensen (1) showed, that Salmonella were more often isolated from high seroprevalence herds than from moderate seroprevalence-herds. Nielsen (2) found, that the MIX-ELISA was a powerful diagnostic test, when used on the group level. Based on laboratory trials with Salmonella inoculated pigs and negative controls, the cut-off of the serological method was estimated to 10 OD%. Based on experiences from the initial period of the salmonella-program, the cut-off for the herd surveillance was arbitrarily set at 40 OD% (3).

No data has so far been published, whether the test could be useful on the pig level, in groups of naturally infected pigs. This could be of value in clinical trials, replacing microbiology, which is an expensive method with a low sensitivity. The objective of this study was to evaluate, whether the test could be used for this purpose.

Two questions needed to be answered, before accepting the serological test as an outcome in clinical trials:

1. Was high OD%-values indicative of higher risk of shedding or salmonella-positive cecal sample even within infected groups?

2. If the OD% was transformed into seropositive - seronegative, would the cutoff used in the surveillance system (40 OD%) be a reasonable choice ?

## Material and methods

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### Part 1.

From 144 naturally infected pigs, serum and fecal samples were obtained. Each pig was sampled 3 times, day 0, day 14 and at slaughter. Fecal samples were taken directly from the rectum and examined microbiologically using standard microbiological methods. Blood samples were analyzed using the Danish MIX-ELISA.

### Part 2.

From 25 high prevalence herds (level 3 in the Danish Salmonella Surveillance system), paired cecal samples and meat-juice-samples were sampled at the slaughterhouse. From each herd, approximately 40 pigs were sampled, divided into 4 batches, and slaughtered on 4 separate days with at 14 day interval between each batch. The total number of examined pigs was 959 pigs.

Cecal samples were analyzed using standard microbiological methods. Meat-juice samples were analyzed using the Danish Salmonella MIX-ELISA.

### Serology.

Serological examination for specific antibodies to *Salmonella* was performed by means of an indirect enzyme linked immuno sorbent assay, designated MIX-ELISA. The tests includes the *Salmonella* LPS-antigens 1,4,5,6,7 and 12, representing approximately 90% of the *Salmonella*-serotypes isolated in Danish pigs (1). The test measures an optical density (OD %) in per cent of a known positive control. In the Danish Salmonella Surveillance program a cutoff of 40 OD % is used for the surveillance of the

finishing herds.

For practical purposes this OD % is transformed into a salmonella-value, which is the OD % - 10.

### Statistical methods

Logistic regression models with random-effects (glimmix macro, SAS) were used for the analyses.

Seropositivity (OD%>40), OD % and squareroot of OD% was used as predictors for shedding or for positive cecal sample.

For part 1, pig, pen and unit were entered as random effects and sample round as a fixed effect. For part 2, date of slaughter and herd was entered into the model as random effects. Interactions between random effects and predictors were investigated in random coefficients models.

## Results

### Part I.

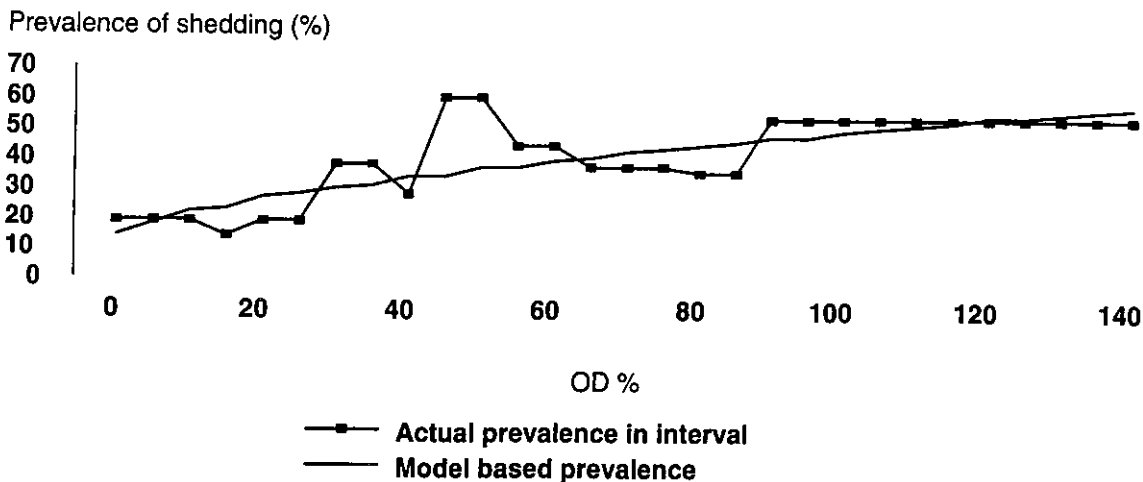
In table 1 is shown the association between serologically positive and microbiologically positive pigs.

Table 1. Serologically and microbiologically positive pigs using cutoff 40 OD % for serology for part 1.

	Microbiologically positive	Microbiologically negative	Sum	Risk for Microbiological positivity	Relative risk
Seropositive	99	117	216	0.46	2.00
Seronegative	48	159	207	0.23	
Sum	147	276	423		

In figure 1 is shown the percent pigs shedding Salmonella as a function of OD. The model based predicted line is for the model using the square-root transformation.

Figure 1. Percent pigs shedding Salmonella as a function of OD %.



Results from logistic regressions using seropositivity (cutoff OD % = 40), OD % or squareroot of OD% as predictors showed, that all were significant predictors (table 3). But the effect of using seropositivity was the predictor with the highest p-value. The random coefficient models had to be simplified by removing non-significant random effects, to achieve convergence. This is not surprising, since the final random effects model had 6 levels. The models showed, that there was a statistically significant influence by the random parameters, but there were no statistically significant interactions between the random effects and the predictors. The reduced models showed, that the pig-level was significant (0.77790, p-value 0.02), but the interaction between pig and OD % was negligible. The effect of sampling round was

significant, but did not show a significant interaction with the predictors. Looking at the different OR's here should be done with care. The magnitude of the OR does not reveal anything about the strength of the individual predictor, since the OR is expressed as the OR for the increase of 1 unit in the predictor, and since the range of the predictors are quite different (ie. from 0 to 140 for OD %, but only from 0 to 1 for seropositivity).

**Part 2.**

In table 2 is shown the association between serologically positive and microbiologically positive pigs.

Table 2. Serologically and microbiologically positive pigs using cutoff 40 OD% for serology for part 2.

	Microbiologically positive	Microbiologically negative	Sum	Risk for microbiological positivity	Relative risk
Seropositive	223	281	504	0.44	2.32
Seronegative	88	367	455	0.19	
Sum	311	648	959		

Results from logistic regressions using seropositivity (cutoff OD % = 40), OD % or squareroot of OD% as predictors showed, that all were significant predictors, but there was no real difference between the ability of the 3 predictors to predict the microbiological status of the cecal samples (table 3). Random coefficient models showed, that there was a statistically significant influence by the random parameters (herd and weekly deliverance from herd), but there were no

statistically significant interactions between the random effects (herds and weekly deliverance from herd) and the predictor (table 4). Random effect parameters are only shown for the model, where OD % was used as the predictor, since there were no differences between the random effect estimates using other predictors and the OD %. Like mentioned above, the magnitude of the OR is not a measure of the strength of the predictor.

Figure 2. Percent positive cecal samples as function of

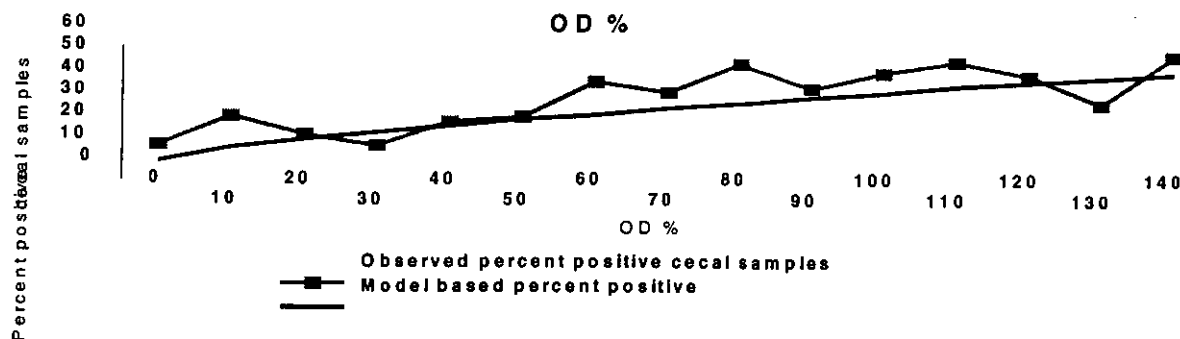


Table 3. Results from logistic regressions.

Predictor	Part 1		Part 2	
	OR for a 1 unit increase	P-value	OR for a 1 unit increase	P-value
OD %	1.011	0.04	1.014	0.0001
Squareroot of OD%	1.167	0.01	1.210	0.0001
Seropositivity (40 OD %)	3.014	0.0001	2.869	0.0001

Table 4. Random effect parameters for a model using OD % as a predictor for part 2..

Random parameter	OD%*weekly deliverance	OD%*herd	Weekly deliverance	Herd
Estimate	0.00001566	0.00000000	0.91237340	0.67065085
P-value	0.70	1	0.07	0.003

## Discussion

Random effects models are useful for exploring effects, that can work on several levels of the material. Here they were used to explore the usability of serology to predict microbiological status measured as microbiologically positive fecal or cecal samples. The interpretation of the significant random effects and the non-significant interactions between predictors and random effects, is, that the estimated OR's are valid at all levels. But the prediction of actual risk is to some extent depending on which herd, unit or pen we are investigating. Care should therefore be taken in comparing serological results between pigs in different units and herds. The reason for this is, that serology to some extent is a historical diagnostic. And for some pigs, they will reach the maximum OD % after being microbiologically negative.

The effect of pig and round found in part 1 indicates, that also time is important. Comparing results between samples taken at different times can lead to wrong results.

The practical implication of this result is, that when using serological results in clinical trials, pen, unit, herd and sampling period should always be entered into the analysis as either random or fixed effects.

Comparing across different units, herds or periods will give considerably less strength to the analysis without this approach, and might even lead to wrong conclusions in some situations.

The results of the different predictors from the 2 studies are remarkably equal, giving more credibility to the results.

There was not much gained by using OD % or squared OD % as predictors compared to using seropositivity based on cutoff 40 OD %. This could merit the more simple interpretation achieved by using a positive/negative outcome in clinical trials. On the other hand, using a continuous outcome makes model validation easier. And especially when using random effects-models, using a continuous outcome can be beneficial, since the theory behind random effects-models is better developed for normally distributed data.

The conclusion is, that serology can be used as an outcome in clinical trials, since it is associated with microbiological status. This makes large scale trials much easier, since sampling and analysis is less difficult and less expensive.

## References

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