

# Success-rate for eradication of *Salmonella* by cleaning and restocking pig herds and the use of antemortem-blood samples in herds after restocking.

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## Abstract

Over the period from 1995 to 1999 there has been a decrease in the number of herds assigned to level 2 or 3 in the Danish *Salmonella* Surveillance system for finishing herds. At the same time the number of herds with a zero prevalence over a 1 year period increased from 52 % in 1995 to 75 % in 1998.

This result has for the majority of herds been achieved by a reduction strategy. In contrast to this result, 349 herds have tried a depopulation - repopulation strategy to solve their problem. 52 % of these herds entered level 2 or level 3 again after a few months.

The success-rate for depopulation-repopulation was so poor, that a system for antemortem blood samples was initiated, to avoid the introduction of high-prevalence finishers to the slaughterhouse after a depopulation-repopulation. The results showed that, when blood samples were taken according to the schedule, they could be used as a predictor for meat-juice-prevalence at slaughter. The predetermined limit for entering level 3 was more than 11 % positive blood samples (individual cutoff 40 OD%). This result was found to be in accordance with the results from the ongoing surveillance system. But also in this study it was found, that a depopulation-repopulation strategy is inefficient. It should always be combined with a reduction strategy.

## Introduction

According to the Danish surveillance program, a large majority of Danish herds are monitored by serological testing of meat-juice samples and classified in relation to the risk of introducing *Salmonella* to the slaughterhouse. Following classification, medium risk (level 2) and high risk (level 3) herds are obliged to receive advisory visits by a veterinarian and a swine consultant, and to make a plan on how to reduce the salmonella-level. In addition to this, pigs from high risk herds have to be slaughtered under special hygienic precautions and tested microbiologically before shipment from the abattoir (1).

A status of the Danish *Salmonella* Surveillance Program

on May 1. 1999 showed a decline in the number of herds assigned as level 2 or 3 from a maximum of 3.4 % level 2 herds and 2.3 % level 3 herds in December 1995 to 2.5 % level 2 herds and 0.9 % level 3 herds. Also it was found, that an increasing number of herds had a zero prevalence over a 1 year period. From April 1. 1995 to April 1. 1996, 52 % of the herds had a zero prevalence. From April 1998 to April 1999, 75 % of the herds had a zero prevalence.

The majority of herds have reached this result by following a reduction strategy based on increased hygiene, change of diets and inclusion of organic acids in the diet (2, 3, 4). An alternative solution for level 2 or 3 herds was to depopulate and repopulate the herd. If the local veterinary practitioner could certify, that the herd had been depopulated totally, and the herd area had been sufficiently cleaned and disinfected, the herd status would be changed from level 2 or 3 to level 1.

After a period, the Danish Veterinary Services found, that too many of the herds entered level 2 or level 3 again after a short period. The success-rate was found to be so poor, that the system was terminated. A system of ante-mortem blood samples replaced the previous system. After depopulation, cleaning and repopulation, a minimum of 45 blood samples were collected 3-4 weeks before shipment of the first batch of finishers for slaughter. If a maximum of 5 (11 %) samples of 45 was positive, finishers from the herd was treated as level 1 pigs, until enough meat-juice-samples had been taken to classify the herd according to the surveillance system (1). If more than 5 samples were positive, the herd would be treated as a level 3 herd.

## Materials and Methods

### 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.

#### Part 1.

From the Danish Zoonosis Register, information regarding the historical and actual salmonella-status of all Danish herds delivering pigs for slaughter can be extracted. If the herd changes status due to repopulation-depopulation, the shift from level 2 or 3 to level 1 is marked, so all herds, that have changed status for that reason can be identified.

The salmonella-status of these herds for the following 6 months after depopulation was extracted from the Zoonosis Register and summarized.

#### Part 2.

At least 45 blood samples evenly distributed between finishers, that were to be slaughtered within 3-5 weeks were taken by the local vet and submitted to the Danish Veterinary Laboratory for analysis. The results of the blood samples would be sent to the Danish Veterinary Services, and the herd would be classified according to the pre-set limits.

Thirty-two herds have used the system so far. For these herds the serological results from the blood samples were extracted from the zoonosis-register. The results were compared with results from the ongoing meat-juice-surveillance from the same herds the following month. The cutoff used for the meat-juice-samples was the cutoff used in the surveillance program (salmonella-value 30 equal to OD % 40):

The association between antemortem-blood samples and meat-juice-prevalence the following month was investigated.

The statistical method was logistic regression using proc logistic (5). The general statistical model has the following form:

$$\text{Logit}(\text{positive meatjuice-samples/number of samples}) = \text{intercept} + \beta * \text{predictor}$$

The number of meat-juice-samples from the following month showed a big variation from 1 to 39. Suspected overdispersion at the herd-level was accounted for using the Williams procedure in proc logistic. This procedure is particularly well suited for data, where the number of samples per unit differs.

The results from the ante-mortem blood samples were transformed in different ways to investigate, which predictor would be the most efficient predictor. The following transformations were investigated:

- Average salmonella-value of the blood samples
- Prevalence using cutoff 0 in salmonella-value (10 OD%)
- Prevalence using cutoff 30 in salmonella-value (40 OD%)
- A class variable with 4 levels:
  - Level 1. Zero in average salmonella-value
  - Level 2. Average salmonella-value above 0, but no individual values above 30
  - Level 3. Prevalence between 0 and 11 using cutoff 30 in salmonella value
  - Level 4. Prevalence above 11 using cutoff 30 in salmonella-value

After investigating the different models, possible outliers were identified, and the veterinary or the producer was contacted in order to find out, if there was a plausible explanation for the outlier. If a plausible explanation was given, the initial models were rerun to see whether the results were improved.

## Results

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### Part 1.

In the period from February 1995 to March 1998, 349 herds of the 16500 herds taking part in the Danish Salmonella Surveillance Program for finishing pigs, applied for a change of salmonella-status from level 2 or 3 to level 1 due to the fact, that they had depopulated the herd, cleaned the herd area, and repopulated with new pigs.

183 (52 %) of the 349 herds entered level 2 or 3 again within 6 months after repopulation.

### Part 2.

The average salmonella-value of the ante-mortem blood samples varied between 0 and 57. The prevalence using cutoff 0 varied between 0 and 98 %. The prevalence using cutoff 30 varied between 0 and 71 %. The meatjuice-prevalence in pigs slaughtered in the first month after pigs were sampled for antemortem prevalence estimation varied between 0 and 100 %. 18 (56 %) of the herds had a zero prevalence in the meat-juice-samples sampled the first month.

In table 1 is shown the statistical results using different continuous predictors from the ante-mortem blood samples for the prediction of the meat-juice-prevalence the following month.

Table 1. Statistical results using different continuous predictors

Predictor	Results from all herds		Results without 1 outlier	
	OR for a 1 unit increase in the predictor (C. I)	P-value	OR for a 1 unit increase in the predictor (C. I)	P-value
Average salmonella-value (range 0-57)	1.064 (1.014-1.128)	0.02	1.076 (1.028-1.137)	0.004
Prevalence using cutoff = 0 (range 0-98 %)	1.031 (1.006-1.060)	0.02	1.036 (1.014-1.067)	0.004
Prevalence using cutoff=30 (range 0-71 %)	1.049 (1.010-1.097)	0.02	1.058 (1.021-1.105)	0.004

One severe outlier was identified. This outlier had a very low antemortem result, but a very high meat-juice prevalence. The producer admitted, that the blood samples had been taken from several age groups. The analyses were rerun omitting this outlier. Results are shown in table 1.

For all 3 predictors there was a significant association between antemortem blood samples and meat-juice prevalence at slaughter. Omitting the outlier improved the results.

In table 2 is shown the results from an analysis, where the predictor is stratified into 4 strata (see materials and methods). The results are shown for all 32 herds. High prevalence herds are defined as herds with a prevalence above 33 % in the meatjuice samples the following month. Thirty-three % is the limit for level 3 herds in the largest herd-size stratum in the Danish Salmonella Surveillance program (1).

In Table 3 are the same results shown, omitting the outlier.

Table 2. Logistic regression results for a stratified predictor. Average meat-juice-prevalences for each antemortem prevalence group and proportion of high prevalence herds. All herds.

Antemortem prevalence group	OR (C.I.)	Average meat-juice-prevalence	Number of herds	P-value	Number of high prevalence herds
Level 4	17.09 (2.29-650)	50 (30-70)	9	0.03	4 (44 %)
Level 3	0.77 (0.04-35)	4 (0.7-22)	13	0.86	0
Level 2	9.81 (1.02-400)	36 % (14-66)	5	0.09	2 (40 %)
Level 1	1	5 % (0.5-37)	5		0

Table 3. Logistic regression results for a stratified predictor. Average meat-juice-prevalences for each antemortem prevalence group and proportion of high prevalence herds. Omitting the outlier.

Antemortem prevalence group	OR (C. I.)	Meatjuice-prevalence	Number of herds	P-value	Number of high prevalence herds
Level 4	16.92 (2.54-452)	50 % (31-69)	9	0.02	5 (56 %)
Level 3	0.77 (0.04-25)	4 % (0.8-20)	13	0.85	0
Level 2	3.47 (0.21-115)	17 % (3-54)	4	0.38	1 (25 %)
Level 1	1	6 % (0.6-34)	5		0

## Discussion

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A depopulation - repopulation strategy has proven an efficient strategy for eradication of *Salmonella* Typhimurium DT 104 (6). This result is in contrast with the results found here. Depopulation - repopulation had a low chance of success compared to the cost. Less than 50 % of the herds were able to keep out of level 2 or 3 after depop.-repop.

Two factors are the probable cause of a failure. One is insufficient cleaning and disinfection, leading to reinfection of pigs after restocking. The other factor could be introduction of infected pigs after repopulation. In this study it was not possible to decide which factor that was the most important factor, but probably both situations occurred.

The difference between the eradication-protocol for DT 104 (6) and the voluntary depopulations done in this study is probably also, that a lot of resources are put into the DT 104 protocol, where the voluntary depopulations are less strictly controlled.

Contrasted to the fact, that more and more herds are reaching a level, where no positive meat-juice-samples are found over a 1 year period, and given the fact, that the majority of herds are reaching this level by introducing a reduction model including improved hygiene, changed diets and use of organic acids in feed or water (2, 3, 4), a depopulation-repopulation strategy is not an efficient tool in itself. It should always be combined with the implementation of other *Salmonella*-reducing strategies.

The discouraging results from the depop.- repop. study is to some extent also found in the second part of this paper. Approximately half the herds had a zero prevalence after repopulation.

There was a significant association between ante-mortem bloodsamples and meat-juice-prevalence.

All the continuous predictors from the ante-mortem bloodsamples were significant. Higher average OD % and higher prevalences using both cutoff 0 and 30 in *Salmonella* value were all significantly associated with higher meat-juice-prevalences.

The stratified analysis showed, that the preliminary choice of the limit of 11 % for the ante-mortem prevalence was useful. Half the herds with a meat-juice prevalence above 11 % had more than 33 % positive meat-juice-samples the first month after sampling. One out of 22 herds (4.5 %) with an ante-mortem prevalence below 11 % had a meat-juice-prevalence above 33 %.

The follow-up on the identified outlier stressed the need for following the guidelines to get a useful estimate of the ante-mortem prevalence. Testing pigs that would have more than 3-5 weeks to slaughter can produce a result, which will give a wrong prediction of the postmortem prevalence.

## References

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