

SUBCLINICAL SALMONELLA INFECTION IN DANISH FINISHING PIG HERDS ASSOCIATION BETWEEN SEROLOGICAL AND BACTERIOLOGICAL TESTING

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*Le programme de surveillance des salmonelles dans les troupeaux de porcs danois destinés à l'abattoir comporte un suivi sérologique des infections salmonelliques subcliniques. L'une des interventions concernant les troupeaux infectés consiste en un suivi bactériologique. L'agrément du testage bactériologique et sérologique doit par conséquent être évalué. L'objectif de la présente étude était d'évaluer l'association entre le testage sérologique et bactériologique au niveau du troupeau. 135 troupeaux ont participé à cette étude. Ils ont été étudiés à partir d'échantillons de sang, d'aliment et de loges à partir de 10 loges par troupeau. En général, les sérotypes de salmonelles isolés à partir d'échantillons d'aliment n'étaient pas des *Salmonella typhimurium* tandis que *S. typhimurium* était le sérototype dominant dans les échantillons de loges. Une relation linéaire positive entre la proportion d'échantillons de loges positifs à *S. typhimurium* et la séro-prévalence a été observée au niveau du troupeau. On n'a pas observé de relation entre les échantillons d'aliment positifs ou les échantillons de loges positifs à des « non *S. typhimurium* » et la séro-prévalence au niveau du troupeau. La probabilité de tester un troupeau positif au plan bactériologique à condition que le troupeau soit positif au plan sérologique dépendait du choix des critères pour la définition des porcs et des troupeaux positifs.*

INTRODUCTION

As part of the salmonella surveillance programme in Danish slaughter pig herds (Mousing et al. 1997), the occurrence of subclinical salmonella infection in pigs is monitored serologically at slaughter by examination of meat juice samples, using a so-called mix-ELISA (Nielsen et al. 1996). Subsequently blood samples and pen samples are used as a diagnostic follow-up in infected herds. The association between the serological and the bacteriological testing therefore needs assessment. The objective of the present study was to assess the association between serological and bacteriological testing at the herd level.

MATERIAL AND METHODS

96 herds with unknown salmonella status and 39 herds with a high sero-prevalence in the national salmonella programme were selected and visited. From each herd, 10 pens were selected and examined by a pooled pen sample (5x5 g faeces), a feed sample (50 g feed) and 5 blood samples per pen. All samples were forwarded to the Danish Veterinary Laboratory and examined by culturing or in the mix-ELISA. Hence these data included the results of bacteriological testing of pen and feed samples, stated as salmonella serotype or negative, and the result of the serological testing, stated as OD %.

When analysing the results of the bacteriological testing of feed and pen samples, salmonella serotypes were categorized as either '*S. Typhimurium*' or 'non-*S. Typhimurium*'.

When assessing the association between the serological and bacteriological tests at herd level, a herd was considered bacteriologically positive if *S. Typhimurium* was isolated from one or more pen(s). A pig was considered serologically positive when OD % > 10 (Nielsen et al. 1996) and for comparison, when OD % > 40. In order to examine the best match of tests, different herd prevalences of serologically positive pigs was used to define a serologically positive herd.

To test whether bacteriological findings would predict serological response at herd level, linear regression (SAS, ver 6.11) was performed. The significance level $\alpha = 0.1$ was used and the selection method was backwards. In order to study the agreement of tests, the positive and negative predictive values were calculated at different herd prevalence levels of seropositive pigs (range 0.02 - 0.6). The predictive values were defined as the probability of testing a herd positive or negative in one test, provided the other test was positive or negative. A herd was defined as bacteriologically positive when *S. Typhimurium* was isolated from one or more pen sample(s).

RESULTS

The results of the bacteriological testing showed no association between salmonella serotypes, isolated from pen and feed samples (Figure 1, a). Generally, the salmonella serotypes isolated from feed samples was non-*S. Typhimurium* while *S. Typhimurium* was dominating in the pen samples. No association was found between salmonella isolated from feed samples and the herd sero-prevalence (Figure 1, b).

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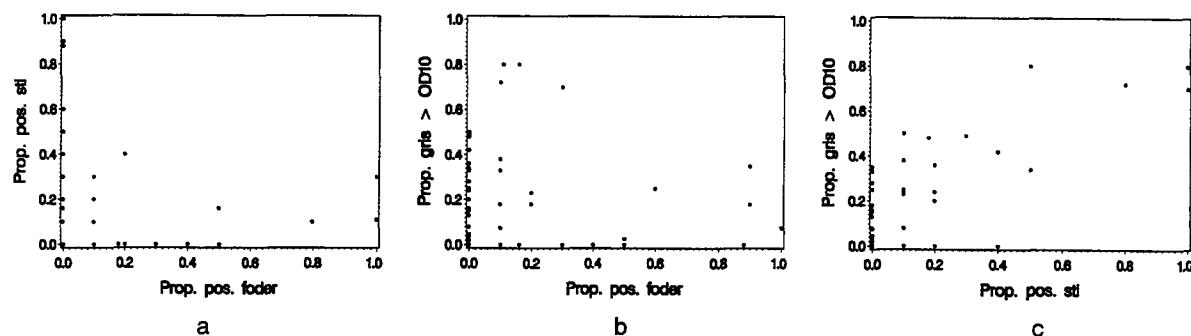
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To further test the ability of bacteriological findings to predict serological herd prevalence, a linear regression was performed. The dependent variable was $\log(\text{proportion of individual OD \%} > 10)$. The independent variables, included in the model was: Proportion S. Typhimurium positive feed samples, proportion S. Typhimurium positive pen samples, proportion non- S. Typhimurium positive feed samples and proportion non-S. Typhimurium positive feed samples. The resulting model after backwards elimination only included the proportion of S. Typhimurium positive pen samples as significant at the 0.1 level. (F statistics = 1.61) (Figure 1, c).

Figure 1

Bacteriological and serological findings in feed ($n=10$), pen ($n=10$) and serum ($n=10$) samples from 135 Danish finishing herds. a) proportion of positive feed samples and pen samples. b) proportion of positive feed samples and herd prevalence of sero-positive pigs ($OD \% > 10$). c) proportion of positive pen samples and herd prevalence of sero-positive pigs ($OD \% > 10$)



The probability of testing a herd bacteriologically positive, provided that the herd was serologically positive, ranged from approximately 0.5 at serological herd prevalence = 0.04, to 0.97 at serological herd prevalence = 0.6. Both the positive and negative predictive values of each test were calculated, and the best over-all agreement of tests results was found to be at herd sero prevalence = 0.3 (Figure 2). The same procedure was performed with pigs defined serologically positive when $OD \% > 40$. In this case the best over-all agreement of tests results was found at herd sero prevalence = 0.07 (Figure 3).

Figure 2
Agreement of serological and bacteriological determination of salmonella status. 135 Danish finishing herds were defined as bacteriologically salmonella positive if S. Typhimurium was isolated from one or more pen samples. Pigs were defined as serologically positive when $OD \% > 10$.
B- = bacteriologically negative (S. Typhimurium was not isolated from any pen(s)), B+ = bacteriologically positive, S- = serologically negative and S+ = serologically positive.

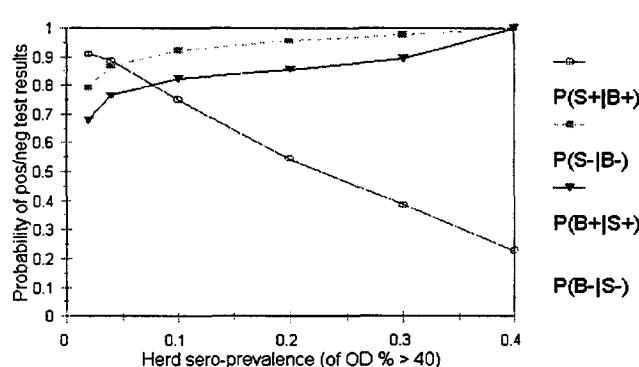
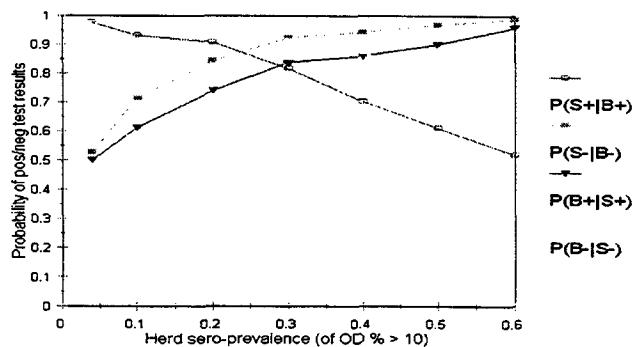


Figure 3
Agreement of serological and bacteriological determination of salmonella status. 135 Danish finishing herds were defined as bacteriologically salmonella positive if S. Typhimurium was isolated from one or more pen samples. Pigs were defined as serologically positive when $OD \% > 40$.
B- = bacteriologically negative (S. Typhimurium was not isolated from any pen(s)), B+ = bacteriologically positive, S- = serologically negative and S+ = serologically positive.

DISCUSSION

According to the results of this study, a positive linear relation between proportion of *S. Typhimurium* positive pen samples and sero-prevalence was observed at the herd level. No relation between positive feed samples or 'non-*S. Typhimurium*' positive pen samples and sero-prevalence was observed at the herd level. Since the mix-ELISA contains antigens from the most frequently isolated salmonella serotypes in Denmark (Nielsen et al. 1996) these findings indicates that *S. Typhimurium* has a different herd epidemiology than the other salmonella serotypes isolated in Denmark.

The results of calculating predictive values of serological and bacteriological tests emphasize the importance of selecting criteria for definition of seropositive pigs and herds. When defining pigs serologically positive at OD % > 10, as suggested by Nielsen et al. 1996, a herd prevalence of 0.3 indicated a probability of 0.8 for obtaining one or more *S. Typhimurium* positive pen sample(s) out of ten. When defining pigs serologically positive at OD % > 40 a herd prevalence of 0.1 indicated a probability of 0.8 for obtaining one or more *S. Typhimurium* positive pen sample(s) out of ten. These findings may be of relevance when using pen samples for bacteriologically confirmation of the serological results obtained in the salmonella surveillance programme in Danish slaughter pig herds.

REFERENCES

- Mousing, J., Thode Jensen, P., Halgaard, C., Bager, F., Feld, N., J., Nielsen, B., Nielsen, J.P., Bech-Nielsen, S., 1997. Nation-wide *Salmonella enterica* surveillance and control in Danish slaughter swine herde. *Prev. Vet. Med.*, in press.
- Nielsen, B., Ekeroth, L., Bager, F., Lind, P., 1996. Use of muscle juice as a source of antibodies for large scale serological surveillance of *Salmonella* infection in slaughter pig herds. Submitted to *Res. Vet. Sci.*
- SAS Institute Inc., SAS/STAT® User's Guide, Version 6, *Fourth Edition, Volume 1*, Cary, NC: SAS Institute Inc., 1989. 943 pp.
- SAS Institute Inc., SAS® Language: Reference, Version 6, *First Edition*, Cary, NC: SAS Institute Inc., 1990. 1042 pp.