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CROSS-SECTIONAL EPIDEMIOLOGICAL ANALYSIS OF THE RELATIONS BETWEEN DIFFERENT HERD FACTORS AND SALMONELLA-SEROPOSITIVITY

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Une analyse épidémiologique des associations entre différents facteurs d'élevage et la séropositivité salmonellique a été effectuée à partir des informations provenant du Registre danois des zoonoses et des producteurs de coopératives de deux abattoirs danois importants. Les données concernant les fermes ont été collectées pendant la période octobre-décembre 1995, à l'aide d'un questionnaire envoyé par courrier et renseigné par les fermiers. Les résultats sérologiques ont été obtenus du Registre danois des zoonoses pendant la même période. Nous avons pu associer les données provenant de ces deux sources pour 3.785 troupeaux. Des réponses correctes à toutes les questions étaient disponibles pour 1.850 troupeaux.
Une régression logistique multivariée, avec correction pour surdispersion, a été réalisée pour 29.121 échantillons provenant des 1.850 troupeaux, et pour les facteurs d'élevage suivants, une association significative entre le facteur et la séropositivité salmonellique a été trouvée : alimentation avec aliment sec versus aliment humide fermenté pour les porcs à l'engrais augmente le risque : OR : 2,4 ; aliment acheté versus aliment préparé sur place : OR : 2,96 ; un doublement de la taille du troupeau (porcs à l'engrais) augmente le risque : OR : 1,31 ; la lumière du jour dans l'unité de finition réduit le risque : OR : 0,59 ; un statut sanitaire conventionnel augmente le risque par rapport à un statut SPF : OR : 2,08. Un statut sanitaire MS (troupeaux SPF réinfectés avec Mycoplasma hyopneumonia) augmente le risque par rapport à un statut SPF : OR : 2,20.
Aucune association significative entre la séropositivité et le facteur n'a été trouvée pour les facteurs suivants : type de sol, utilisation de paille, accès à une aire extérieure, engraisseur ou naisseur-engraisseur, enclos par porc avant la mise-bas.

The fact that human salmonellosis had been verified in an increasing number for the last decade urged the Danish Ministry for Agriculture and Fisheries to launch a program for control and reduction of salmonella in pork products. An integrated and important part of this program is a serological surveillance program for all Danish pig herds with a deliverance over 100 finishers a year (Mousing and others 1997). Approximately 15000 herds are tested each month. The test is performed on meat-juice obtained by freezing and thawing a small sample of pork. All serological results are registered in a central data-base (The Danish Zoonosis Register), identified by the Central Herd Register-code and the date of sampling.

Based on these results, herds are classified in 3 groups based on 3 months sampling: Level 1 herds with a zero or low level, level 2 herds with an intermediate level, these are obliged to try to lower their level, and level 3 herds with a high level, these herds are obliged to lower their level and the pigs have to be slaughtered under special hygienic precautions. Level 3 herds are financially penalized, if unable to lower their level after a period.

A good knowledge of risk-factors for high levels of salmonella is essential to be able to reduce the level in herds with salmonella-problems, and maybe more important, to be able to avoid herds without problems to become problem-herds.

Field-reports indicated, that herds, that used purchased feed instead of home mixed feed were more often diagnosed as having salmonella-problems. Also it was reported, that the use of fermented wet-feed seemed to be protective. These casuistic reports needed to be substantiated by epidemiological and experimental data.

MATERIALS

In the period from October first to December 31. 1995, 2 major Danish slaughterhouse-cooperatives sent out questionnaires to all producers. The purpose of the questionaire was to assemble producer-databases for the slaughterhouse cooperatives.

Data validation was not performed, except for an extensive post-hoc control performed by the author. Approximately 20 herds known by the author were checked, all results from these herds were correct. After the analyses had been performed, 4 herds with large deviations from the expected value obtained from the model were contacted. For these herds the results were valid.

A subset of 1850 herds, which had given consistent answers to all questions, was created.

Serogical results from the Danish Zoonosis Register were collected from the same period as the questionnaires were collected (October 1. 1995 to December 31. 1995).

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 (Mousing and others, 1997). The test measures an optical density in per cent of a known positive control (OD-%). Samples with

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a OD% over 40 were considered positive in this study. Between 3 and 105 samples were obtained pr herd from 3785 herds.

STATISTICAL METHODS

A series of cross-tabulations were made for each variable. Unit of observation was defined as the individual sample (pig).

A logistic regression analysis was performed using the logistic procedure of the Statistical Analysis System (1996). The Williams method was used to account for overdispersion in the data.

The analysis was performed in 2 steps:

Step 1. A model with all 10 factors was build . A backward elimination process was used based on the likelihood-ratio chi-square statistics. For herd size a transformation to natural logarithm was used in the analysis. This created a model with a better fit than using herd size. A side-effect is, that the parameter-estimate can be transformed and interpreted in OR for doubling herd size (2). All factors with a P-value under 0.05 were used for further analysis.

Step 2. 2 factor-interactions were created between all remaining factors. A backward elimination process was performed for 2 factor-interactions as described for step 1. 2-factor interactions were eliminated, if the P-value exceeded 0.10.

2 alternative methods of handling overdispersion were performed.

Method 1. Herds were divided into 2 groups (McDermott and others, 1993), herds with a seroprevalence over 0.33 and herds with a seroprevalence under 0.33. This reduces the number of observations to one per herd. The value 0.33 was chosen, because this is the limit between level 2 and level 3 herds with a yearly production above 5000 finishers. Logistic regression was performed using the logistic procedure of the Statistical Analysis System (1996).

Method 2. Beaudeau and others (1996) described a method of handling overdispersion by performing 10 repeated logistic regressions, allowing only 1 observation from each herd to be used. The first 10 samples from each herd were numbered. Logistic regressions were performed on all number 1 samples, number 2 samples and so on. Herds with less than 10 observations were only included in the analysis, until they had no more observations. If the variable was found significant at 10 % level in 4 out of 10 models, it would be significantly associated to salmonella-prevalence at the 5 % level (Beaudeau and others, 1996).

RESULTS

Table I shows the resulting relative risks of the cross-tabulations and the OR from the logistic regressions performed with williams method for handling overdispersed data and the 2 alternative methods. Only results from significant factors are shown. The overall prevalence was 4.3 percent seropositive. No interactions reached the 10 percent level.

No significant associations between seropositivity and the factor were found for the following factors: Type of flooring, use of straw, access to outdoor-area, finisher or farrow-to-finisher operation, pen-area pr pig before delivery.

Table I
Crude RR from cross-tabulation and OR with confidence interval from 3 methods

	Variable	Number of herds	No of samples	Crude RR	Williams method OR (C. I.)	Method 1 OR (C.I.)	Method 2 Interval of OR
Feeding system	Dry feed	1545	22318	2.38	2.40[3](1.62-3.68)	6.13[1](1.82-38.3)	2.1-4.2[1]
	Wet feed	305	6803	1	1	1	1
Source of feed	Purchased feed	679	10562	3.39	2.96[3](2.34-3.76)	4.93[3](2.78-9.24)	3.0-3.9[1]
	Home mixed feed	1171	18559	1	1	1	
Health status	Conventional	1262	18650	2.05	2.08[2](1.26-3.70)	3.13[4](0.93-19.5)	0.9-5.4[4]
	MS	443	7939	2.38	2.20[2](1.30-3.99)	3.14[4](0.87-20.1)	1.4-7.2[4]
	SPF	145	2532	1	1	1	1
Daylight	Yes	1672	25895	0.60	0.56[2](0.43-0.81)	0.54[4](0.28-1.18)	0.5-0.8[4]
	No	178	3226	1	1	1	
Herd size (no of finishers	> 2000	33	1780	0.87	1.31[3](1.17-1.46) for doubling herd size	1.33[1](1.03-1.73) for doubling herd size	1.1-1.90[1] for doubling herd size
	1001-2000	124	3871	1.48			
	501-1000	445	8493	1.97			
	101-500	1084	14006	1.13			
	<100	164	971	1			
Total		1850	29121				

[1]$p \leq 0.05$ [2]$p \leq 0.01$ [3]$p \leq 0.001$ [4]n. s.

DISCUSSION AND CONCLUSION

The use of questionnaire-based epidemiological analysis are subjected to various problems. These problems become even more prominent, when using a data-base collected for other purposes. In this case, a number of questions were formulated, so the producer should answer yes, if the factor was present in the herd. If the factor was absent, the producer just went on to the next question. This means, that if for some reason, a producer did not want to answer a question, then the missing answer will be registered as a no. An example of this problem is, that the producers were asked, if they had wet feed or dry feed-systems. They could answer yes to both questions, yes to one, or not answer any of the questions. In this case, we can state, that no answer to both questions can be registered as a missing value. But if the producer is asked if his pigs has access to outdoor area, then a missing yes will be registered as a no.

This will inevitably lead to misclassification-bias in this survey. Kleinbaum and others (1982) state, that if the misclassification is non-differential and independent, then the bias will be towards the null. In this study, the 2 databases were collected individually and paired afterwards. The producers did not know, that farm-data would be used for this analysis. So the farmers knowledge of own salmonella-status should not influence his answers to the questionnaire. Therefore it seems reasonable to assume, that the misclassification is non-differential and independent. Based on these assumptions, it can be postulated, that effects found significant in this analysis are valid, whereas effects found non-significant could be interesting anyway.

Clustering or overdispersion is probable in this survey, since it can be anticipated, that individual results are correlated on the herd level. If clustering is present on the herd level, then parameter variance estimates can be too small, so that the null hypothesis is too easily rejected. For handling overdispersion, Williams procedure was applied. Williams procedure is to be preferred, when the number of samples pr observation/herd are not approximately constant (SAS/STAT, 1996).

2 other methods were applied (se methods). These methods resulted in a substantial loss of information. Use of dry feed versus wet feed, purchased versus home mixed feed and increasing herd size were the only significant risk factors in all 3 methods. Conventional health status and MS health status were risk factors when compared to SPF-status in the method using Williams procedure, while daylight in the finishing unit was a protective factor.

Prohaszka (1990) demonstrated, that high levels of undissociated volatile fatty acids inhibited the growth of salmonella in sterilised caecal content. This could explain the protective effect of wet feed. In most wet feeding systems, a natural fermentation process results in growth of lactic-acid producing bacteria and yeasts.

The protective effect of home-mixed feed is surprising. Purchased feed in Denmark is salmonella-controlled, and the number of salmonella in feed is low. The most prevalent serotype in Danish pig herds and in Danish pork, *Salmonella* Typhimurium is never isolated in feedstuff in Denmark (Mousing and others, 1996).

It seems appropriate to assume, that the salmonella-level in Danish herds is not as much related to salmonella in feed, as to other characteristics of the feed. Purchased feed and home-mixed feed differs in general on some physical characteristics. Home-mixed feed is normally served as meal, and at least the cereal part is not heat treated. In general it will be less finely grinded than purchased feed. Purchased feed is in most cases sold in pellets, and all ingredients has been heat treated. Whether these differences can explain the biological effect is not known at present. Probably the introduction of exotic (feed-borne) serotypes is less important, than the effect feed can have on the spread of non-feed-borne types (as *S.* Typhimurium).

The risk of seropositivity increases with increasing herd-size. Increasing herd-size is a well known risk factor for many diseases. Table 1 demonstrates that the crude RR risk for the largest herds are lower than for middle-sized herd. This can be explained by the more frequent use of wet feeding systems and home-mixed feed in larger herds.

Conventional and MS-herds have a higher risk than SPF-herds. This could be explained by common risk-factors for introduction of salmonella and other diseases. MS-herds are in most cases former SPF-herds, which have become infected with Mycoplasma hyopneumonia. Probably some of the risk-factors for reinfection with Mycoplasma hyopneumonia to some extent are the same as for introduction of *Salmonella* Typhimurium.

The effect of daylight is difficult to explain. No daylight in the finishing unit could be a proxy for other risk factors.

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REFERENCES

Beaudeau, F., Fourichon, K., Frankena, K. (1996) Regression analysis with nested effects in epidemiological studies: Assesment of a method eliminating one level of clustering. Preventive Veterinary Medicine. 25, 315-325.

Kleinbaum, D. G., Kupper, L. L., Morgenstern, H, (1982) Epidem. Research, Principles and Quantitative Methods.

McDermott, J. J., Schukken, Y. H., Shoukri, M. M. (1993) Study design and analytical methods for data collected from clusters of animals. Preventive Veterinary Medicine. 18, 175-191.

Mousing, J., Jensen, P. T., Halgaard, C., Bager, F., Feld, N., Nielsen, B., Nielsen, J. P., Bech-Nielsen, S. (In press). Nation-wide Salmonella enterica surveillance and control in Danish slaughter swine herds. Prev. Vet. Medicine.

Prohaska, B. M. , Jayarao, B. M., Fabian, A., Kovacs, S. (1990) Th Role of Intestinal Volatile Fatty Acids in the Salmonella Shedding of Pigs. Journal of Veterinary Medicine. B 37, 570-574.

Statistical Analysis System Inc. (1996) SAS/STAT Changes and Enhancements. p. 383.