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The Prevalence of *Mycoplasma hyopneumoniae* in Pig Herds in Western Finland Based on the Demonstration of Antibodies in Colostrum by ELISA

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Rautiainen E: The prevalence of *Mycoplasma hyopneumoniae* in pig herds in Western Finland based on the demonstration of antibodies in colostrum by ELISA. Acta vet. scand. 1998, 39, 325-330. – Swine enzootic pneumonia causes heavy economical losses in fattening herds. The aim of this study was to get an estimate of the prevalence of *Mycoplasma hyopneumoniae* in the province of Vaasa in Western Finland. There were 112 farrowing herds randomly chosen for a survey. Farmers were asked to milk colostrum samples from every sow. Owners of 22 herds did not send enough samples for a herd diagnosis. Altogether 1773 samples (mean 18.1 samples/herd) were analysed in an indirect ELISA for antibodies to *Mycoplasma hyopneumoniae.* Thirty-five herds were concluded to be infected with *Mycoplasma hyopneumoniae.* In these herds the mean prevalence of samples with antibodies was 16.3% (range 2.3%–70.0%, median 8.6%). The infected herds were significantly larger than the rest of the herds (no. of sows 27.0 vs. 18.4 respectively). The true prevalence of *Mycoplasma hyopneumoniae* infection in the province of Vaasa was estimated to be somewhat lower than the apparent prevalence of 39%. The application of colostrum serology is discussed.

swine enzootic pneumonia; survey; carrier; bias; sensitivity; specificity; positive predictive value.

Introduction

According to *Ross* (1992) swine enzootic pneumonia caused by *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) is one of the most common and economically important diseases occurring in swine. In a survey concerning 33 all-in/all-out fattening herds in Western Finland (*Rautiainen et al.* 1991) enzootic pneumonia with secondary infections was the most prevalent cause of death (mean total mortality rate 1.4% per batch, range between herds 0%-3.8%). Additionally, 50% of all dead pigs had pneumonia as a primary or a secondary finding. Several of these farms needed mass medications to control respiratory infections. At slaughter, about 20% of pigs were registered as having pneumonia, which also indicated economical losses due to growth retardation. Serum antibodies to *M. hyopneumoniae* were common in pigs that had died of pneumonia. All these observations indicated the importance of controlling *M. hyopneumoniae.*

In Western Finland, a typical specialized fattening herd consists of 200-300 pigs located in one unit. All-in/all-out management is practised. Pigs are generally received from 15-20 different farrowing herds at the weight of approximately 25 kg. Under such circumstances carrier swine certainly are the main source of infection

(*Ross* 1992). Therefore, the prevalence of the infection in farrowing herds is of importance for the spread of *M. hyopneumoniae* to fattening herds. The aim of this study was to estimate the prevalence of *M. hyopneumoniae* antibodies in sows.

Materials and methods

Selection of herds

The study was performed in pig herds with more than 3 sows in the province of Vaasa in Finland. The number of herds was 1296 according to customer registers of 5 slaughterhouses operating in the province. No respect was paid to whether the herds sold the piglets as feeder pigs or raised all or part of them to market weight in their own herd. The annual number of sold piglets was known for every herd. For the random selection procedure every herd was given a number. Then 134 herds were selected to participate in the study using random numbers between 1 and 1296. Thirty-six herds refused to participate, half of which had an acceptable reason (going to finish piglet production). Therefore, a second random sample of 16 herds was taken. Finally, 112 herds started the study. The mean number of sows per herd was 22 (range 3-66, standard deviation 11, median 20). The herds were divided into 5 geografical areas based on postal code numbers.

Colostrum samples

Farmers were asked to collect a sample from every sow, though not from more than 30 sows in large herds. Colostrum samples were collected in 10 ml plastic test tubes with a screw stopper and without additives. Farmers were asked to milk the sample before farrowing or as soon as possible after the start of farrowing. The samples were stored in home freezers (–18 °C) before being sent to laboratory in batches of about 10-30 samples. On receipt the samples were frozen again (–18 °C) until analysed. A majority of samples had been stored several months (up to 5 months) before being analysed.

Detection of antibodies to M. hyopneumoniae in colostral whey

Before analysis the samples were centrifuged at room temperature 6000 g for 15 min, after which the fatty layer was removed by a vacuum-connected pipette (50-1000 μl capacity, Plastibrand®, Wertheim/Main, Germany). Samples were analysed in duplicate wells for the presence of antibodies to *M. hyopneumoniae* by an indirect ELISA previously desribed by *Bommeli & Nicolet* (1983). The ELISA kit (Chekit® Hyoptest, Dr. Bommeli AG, Liebefeld-Bern, Switzerland) was used according to the instructions of the manufacturer. All colostrum samples in which antibodies were found were analysed a second time for confirmation to reduce the number of possible false positive samples. A herd was considered as infected, if antibodies were found in one or more samples also in the second assay.

Statistics

Student's *t*-test was used to compare the mean number of sows and chi-square -test to compare the geografical location between different groups of herds.

Results

Colostrum samples of 1773 sows were collected and analysed. In the first assay antibodies were found in 131 samples. In the confirmative assay 98 samples remained positive, 23 samples turned to suspicious and 18 samples turned to negative. Consequently, 10 herds with a single positive sample and 1 herd with 2 positive samples in the first assay changed their definition from infected to non-infected herd. The final results are shown in Table 1. According to tube

Table 1. *Mycoplasma hyopneomonia* antibodies detected in pig herds by colostrum-ELISA in a survey in 1991 in Western Finland.

	Non-infected herds	Infected herds	Censored from the survey	All herds
No. of herds	55	35	22	112
No. of sows	1 011	944	518	2473
Mean no. of sows/herd	18.4	27.0	23.5	22.1
Total no. of samples	934	781	58	1773
Mean no. of samples/herd	17.0	22.3	7.3[a]	18.1[b]
Total no. of positive samples	0	98	0[a]	98
Mean no. of positive samples/herd	0	2.8	0[a]	1.0[b]
Total no. of feeder pigs sold in 1991	9978	9545	4426	23949
Mean no. of feeder pigs sold/herd in 1991	181.4	272.7	201.2	213.8

[a] no. of herds 8.
[b] no. of herds 98.

markings, the mean colostrum milking time was 1.5 h since start of farrowing (range 0-5.5 h, median 1.0 h).

Twenty-two herds were censored from the final analysis. Of them 14 herds did not send any samples at all. In 8 herds samples were collected from less than ⅔ of the sows and no antibodies were found. The geographical location of these herds did not differ significantly from the rest of the herds ($p>0.2$ in 3 separate comparisons). Neither was the mean number of sows statistically different (23.5 and 21.7 respectively; $p = 0.5$).

The apparent prevalence of infected herds was concluded to be 39% (95% confidence interval 29%-49%). In these herds, the mean prevalence of sows with antibodies was 16.3% (range 2.3%-70.0%, median 8.6%). The infected herds were significantly larger than the rest of the herds (mean number of sows 27.0 and 18.4 respectively; $p = 0.0004$).

According to the apparent prevalence, during the year 1991 about 49% (95% confidence interval 31%-67%) of pigs delivered to specialized fattening herds originated from infected herds and were thus possible carriers of *M. hyopneumoniae.*

Discussion

Colostrum serology was chosen as a diagnostic tool to monitor the prevalence of herds infected with *M. hyopneumoniae* because samples were easy to collect and store by herd owners. When effectuating the study, promising results of the method chosen had been reported from Switzerland (*Zimmermann et al.* 1986) and Finland (*Levonen* 1990). However, today there are somewhat contradictory reports about specificity of the ELISA kit that was used in this study. *Zimmermann et al.* (1986) analysed 525 colostrum samples from 8 high health herds and 11 obviously *M. hyopneumoniae* infected herds. In their material both herd-level sensitivity and specificity were 100%. However, *Levonen* (1994a) analysed 6256 colostrum samples and found positive samples (mostly single reactors) from obviously not infected high health breeding herds (15/177). According to this, the herd-level specificity would only be 91.5%.

The herd-level sensitivity was high in both studies. However, the prevalence of samples with antibodies in infected herds varied between 5%-100% (*Zimmermann* 1986) and 4%-55% (*Levonen* 1994a), which indicates that in some infected herds nearly all sows should be tested to detect antibodies. Therefore, censoring of 8 herds with less than ⅔ of sows tested was motivated in this study.

According to *Martin et al.* (1992) the herd predictive value of a positive test result decreases with increasing sample size, if the specificity of the test is not 100%. They suggested that a fairly high prevalence of positive samples should be assumed, if a herd actually is infected. In this study the in-herd prevalence ranged between 2.3%-70.0%. Applied to this ELISA-test and this data, probably a minimum prevalence of 3.9% samples with antibodies should be assumed. According to this assumption, 3 herds (not shown in results) shall be concluded to be false positive herds. The herd-level specificity will thus be equal to 94.8% (55/58×100%). In spite of this, infected herds were still significantly larger than non-infected herds (no. of sows 25 vs. 20, respectively; $p = 0.048$).

The true prevalence of infected herds compared to the apparent prevalence may also be lower due to a possible sampling bias from the second random sample of 16 herds. Of these herds 6 were considered to be infected, 7 not infected, 1 did not get a result, 2 did not participate. In contrast, the non-participating herds of the first sample were obviously quite small herds and thus less likely to be infected. To have avoided that possible bias, more precise data of the non-participants should have been collected, after which a stratified random sample based on herd size and geographical location should have been made. However, in consideration of both the possible bias and the low specificity of the test, a true prevalence of slightly more than 30% still remains (95% confidence interval ± 10%). In other words, the most important result is that about ⅔ of the herds are not infected by *M. hyopneumoniae.* Information about infection risks might prevent further spread among farrowing herds.

In fattening herds, on the contrary, mixing of pigs from infected and non-infected herds (about 50% and 50% on the average) has obviously been the cause of high mortality due to pneumonias as presented earlier (*Rautiainen et al.* 1991). To prevent *M. hyopneumoniae* infections in fattening herds slaughterhouse activities are needed (see *Tuovinen et al.* 1994 and 1996).

Colostrum serology seems to be a useful method for herd screenings. Today, there are several reports about the use of colostrum serology for herd health surveillances with good results (*Zimmermann et al.* 1986, *Bouwkamp et al.* 1993, *Levonen* 1994a, *Levonen et al.* 1994b, *Volmer et al.* 1994, *Tuovinen et al.* 1994, *Rautiainen et al.* 1996). One reason is surely the practical way of receiving samples, although *Barfod* (1990) has expressed some doubts about the reliability of samples taken by herd owners. That could, of course, become a problem in health schemes, if colostrum serology was not combined with other surveillance methods.

Another advantage in colostrum serology is the seemingly high sensitivity. Several authors have reported that colostrum, defined mostly as milk collected within 24 h after farrowing, contains a higher concentration of antibodies than serum (*Eberli* 1987, *Yagihashi et al.* 1993, *Sørensen et al.* 1993, *Morris et al.* 1994), although the level of antibodies in serum is dependent on the stage of pregnancy (*Wallgren et al.* 1997). Taking that into consideration, colostrum serology would probably benefit from tests with a high specificity and with a somewhat lower sensitivity. For example, by using monoclonal antibodies

in a blocking ELISA (*Feld et al.* 1992, *Le Potier et al.* 1994) or in a double-sandwich ELISA (*Mori et al.* 1987) cross-reactions between *M. hyopneumoniae* and other mycoplasmas, especially *M. flocculare* and *M. hyorhinis,* which are common inhabitants of pig's respiratory tract, have been markedly reduced.

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Sammanfattning

Prevalensen av Mycoplasma hyopneumoniae i suggbesättningar i västra Finland baserad på demonstrationen av antikroppar i råmjölk med ELISA.

Tillsammans 112 suggbesättningar valdes slumpmässigt till kartläggning för prevalensen av Mycoplasma hyopneumoniae i Vasa län i västra Finland. Undersökningen utfördes med råmjölksprov. Tjugotvå besättningar sände inte tillräckligt många prov för ett pålitligt resultat. Tillsammans 1773 prov analyserades (som medeltal 18,1 prov per besättning). Trettiofem besättningar bedömdes vara infekterade. I dessa besättningar var medelprevalens av prov med antikroppar 16,3% (variationen 2,3%-70,0%, median 8,6%). Dessa besättningar var signifikant större än resten av besättningarna (suggantalet 27,0 vs. 18,4 respektivt). Den faktiska prevalensen evaluerades att vara något lägre än den synbara prevalensen 39%. Användning av råmjölkserologin diskuterades.

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