Acta vet. scand. 2000, *41,* 213-225.

Monitoring Antibodies to *Mycoplasma hyopneumoniae* in Sow Colostrum – a Tool to Document Freedom of Infection

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Rautiainen E, Tuovinen V, Levonen K: Monitoring antibodies to Mycoplasma hyopneumoniae in sow colostrum – a tool to document freedom of infection. Acta vet. scand. 2000, 41, 213-225 – In a survey in Finland in 1995, 14919 colostral whey samples from 530 farrowing herds were analysed by a monoclonal blocking-ELISA to detect antibodies to *Mycoplasma hyopneumoniae (M. hyopneumoniae).* Antibodies were detected in 274 (1.8%) samples and in 42 herds (7.9%). The median prevalence of sows with antibodies in seropositive herds was 28.2% (range, 2.7-100%). According to clinical and pathological follow-up in finishing herds in 1996, all of the farrowing herds which were seronegative in 1995, were truly non-infected with *M. hyopneumoniae.* In acutely infected herds, samples collected earlier than 2 h after farrowing were 3 times more likely to contain antibodies than samples collected 2-12 h after farrowing (odds ratio, 3.0; 95% CI, 1.4-6.6). Repeated freezing or spoilage of the colostrum samples did not cause biologically relevant problems for the ELISA. Antibodies to *M. hyopneumoniae* were shown to persist up to 3 years in some sows. As a conclusion, colostrum samples were very sensitive samples for the screening of herds for *M. hyopneumoniae* infection and possibly also for a regular surveillance.

monoclonal; blocking-ELISA; survey; sensitivity; specificity; sample size; sampling scheme; persistence; antibodies; health; control; programme.

Introduction

Mycoplasma hyopneumoniae (M. hyopneumoniae) is the causative agent of swine enzootic pneumonia, one of the most common and economically important diseases occurring in swine (*Ross* 1999). Demonstration of specific antibodies in colostrum, as a control method for *M. hyopneumoniae,* was introduced by *Zimmermann et al.* (1986) and applied by *Levonen* (1994a). The use of sow colostrum instead of serum for antibody detection has several advantages: 1. Antibodies to *M. hyopneumoniae,* detected by the enzyme-linked immunosorbent assay (ELISA), may persist at least one year (*Armstrong et al.* 1983, *Bereiter et al.* 1990), which makes sows a suitable target group for serological surveys; 2. Of the immunoglobulin content of colostrum, 90% is of serum origin (*Bourne & Curtis* 1973); 3. At the time of farrowing, colostrum contains a higher concentration of antibodies than serum (*Eberli* 1987, *Yagihashi et al.* 1993, *Sørensen et al.* 1993, *Morris et al.* 1994). This is due to the fact that the antibody level in serum of sows is related to the stage of pregnancy and continuously decreases during the month prior to parturition (*Wallgren et al.* 1998). Further, the IgA

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in colostrum (10%-15% of the total colostral immunoglobulins) is for the most part produced in the mammary gland (*Bourne & Curtis* 1973); 4. Finally, colostrum samples are easily collected by the herd managers and the samples can be stored frozen in the herds for later usage. In addition, colostrum sampling is less stressful for the animals than blood sampling.

In this paper, we report the large scale usage of colostrum samples in Finland as a part of 2 health control programmes which aimed to eliminate *M. hyopneumoniae* from elite breeding herds and conventional sow herds, respectively. During 1995, there were 161 000 sows in Finland (*Anon.* 1997a) and the production was concentrated to the western and the southwestern parts of the country. The mean number of sows per herd was 35. Two thirds of the fattening pigs were raised in specialized fattening herds. Such herds typically had 200-300 pigs located in one unit and practised all-in/all-out management systems. The growers (Yorkshire x Landrace) generally arrived at the weight of approximately 25 kg and originated from 15-20 different farrowing herds.

Swine enzootic pneumonia with secondary infections was the most common cause of death in the fattening herds during 1990 and in-feed medications were commonly used to prevent acute outbreaks of disease during the fattening period (*Rautiainen et al.* 1991). The prevalence of *M. hyopneumoniae* in sow herds was 30% in the Western Finland (*Rautiainen* 1998) and 8% in the Southwestern Finland (*Tuovinen et al.* 1994). Antibodies to *Actinobacillus pleuropneumoniae* (serotype 2) were common even in the elite breeding herds, but the infections were almost entirely subclinical (*Levonen et al.* 1994b). Porcine reproductive and respiratory syndrome (PRRS) has never been reported in Finland (*Anon.* 1998).

The objective of this study was to determine, whether farrowing herds can be documented free of *M. hyopneumoniae* infection by monitoring antibodies in sow colostrum. Respect was paid to the size of the herds when the sample sizes required were defined. In addition, the significance of the age of the sampled sows, as well as the time of sampling in comparison to farrowing were validated. Further, the robustness of the sampling strategy was tested by deliberately handling samples in improper ways. Finally, the persistence of antibodies to *M. hyopneumoniae* following eradication was studied in one herd.

Materials and methods

The health control programme for the elite breeding herds

According to the national health control programme (Anon. 1997b), the elite breeding herds in Finland are declared free of *M. hyopneumoniae* and of the following other pathogens: Toxigenic *Pasteurella multocida, Serpulina hyodysenteriae, Clostridium perfringens* type C, *Sarcoptes scabiei* var. *suis* and all serotypes of salmonella. In addition, a clinical freedom of *Actinobacillus pleuropneumoniae* (serotype 2) is claimed. The herd monitoring of *M. hyopneumoniae* is based on clinical veterinary inspections every third month and on colostrum serology every sixth month. Further, clinical inspections and individual slaughter inspections of pigs delivered to the fenotype testing stations are performed. Annually, 1%-2% of the breeding herds get infected with *M. hyopneumoniae* (*Rautiainen et al.* 1996) and are excluded from the programme. The number of the herds participating in the national health control programme in 1995 was 183 and most of the herds were established breeding herds.

The LSO-2000 quality chain

The LSO-2000 quality chain, started in 1994 by HK Foods Ltd., Turku, includes a health control programme for conventional sow herds in the

Southwestern Finland (*Tuovinen & Heinonen* 1997). The health monitoring is similar to that of the breeding herds and same diseases are monitored excluding the clostridial diarrhea of piglets. The sow herds are classified into a health class or a standard class. The growers of the health class herds are transported to separate all-in/all-out finishing herds without mixing them with standard growers. Their health status is followed clinically and by lung examination at slaughter. The health class herds (n = 347 during 1995) are paid more for their growers than the standard class herds.

Colostrum samples

Colostrum samples without additives were collected by the herd managers into 10 ml plastic tubes. The samples were collected during farrowing or as soon as possible after it. The managers were asked to include the following information of the sows with each sample: the identity number of the sow, the date of farrowing, the parity number, the time of the first piglet born and the time of the sampling. The samples were stored in home freezers (-18 °C). Batches of 15 to 30 samples, wrapped in paper and packed in card board boxes, were sent to the laboratory. The majority of the samples arrived at the laboratory within 24 h after dispatch. In 1995, the total number of samples was 14 919 originating from 530 herds. In small herds, the number of samples per herd corresponded to the number of sows. From large herds, not more than 30 samples were expected. With this sample size it was possible to find at least one sample with antibodies with 95% confidence in any size of herds, if the prevalence of samples with antibodies was at least 10% (*Cannon & Roe* 1982).

Detection of antibodies to M. hyopneumoniae

Before the analysis, all samples were centrifuged at 5500 x g (Heraeus Sepatech, Megafuge 1.0, Germany) for 15 min in 5 ml plastic tubes (Sarstedt®, Nümbrecht, Germany), and the fatty layer was removed by a vacuum connected pipette. The colostral whey was analysed by a monoclonal blocking-ELISA (*Mycoplasma hyopneumoniae* ELISA®, DAKO, Glostrup, Denmark) in single wells to detect antibodies to *M. hyopneumoniae.* ELISA results were expressed as percentages of blocking of the monoclonal antibody used in the assay. A sample with a blocking-percentage over 50% at 492 nm wave length was classified as having antibodies (positive). All other samples were classified as negative. At the cut-off value of 50%, the sensitivity and the specificity of the ELISA (with 95% confidence intervals) have been reported to be 100% (98% to 100%) and 100% (93% to 100%), respectively (*Sørensen et al.* 1997).

Classification of herds

A herd was classified as non-infected during 1995 if it was serologically negative, free from clinical respiratory symptoms according to regular veterinary inspections and feed-back from the corresponding finishing herds (testing stations) and slaughterhouse did not indicate *M. hyopneumoniae* infection during 1996. In addition, more colostrum samples were analysed during 1996 to ascertain the seronegativity of the herds.

A herd was classified as truly infected with *M. hyopneumoniae,* if antibodies were detected in 3 or more samples. Since the true specificity of the test used in this study was 99% in individual serum samples (*Sørensen et al.* 1992, *Sørensen et al.* 1993, *Sørensen et al.* 1997) and the number of samples tested per herd was at most 30, the probability of getting 3 or more positive samples in a non-infected herd was only 0.3% as calculated according to the upper-tail binomial probability distribution (*Casella & Berger* 1990).

If antibodies were detected in less than 3 colostrum samples in a herd, additional clinical inspections were made by the local veterinarian. If respiratory symptoms indicating *M. hyopneumoniae* infection were detected, the herd was classified as possibly infected and could not join the health control programme.

If no respiratory symptoms were detected, blood samples were collected to detect serum antibodies to *M. hyopneumoniae* in growing pigs. Animals under 10 weeks of age were not sampled, because antibodies rarely are found in that age group of pigs (*Wallgren et al.* 1998). If no antibodies were detected, herds were classified as non-infected provided that no signs of *M. hyopneumoniae* were reported during the finishing period or at slaughter during the subsequent year. In addition, more colostrum samples were analysed.

Influence of parity number and time of sampling

The influences of the parity number of a sow and of the time of sampling (in hours) on the test results were analysed with the samples of the truly infected herds.

The effect of fat and contaminants

In order to analyse any major influence of fat and contaminants of colostrum samples on the test results, 22 colostrum samples were chosen. To prevent bias caused by herds or by the age of the sows, every sample was from a different herd and the samples were evenly chosen from first, second, third and fourth parity sows.

Each sample was divided into subsamples 1A and 1B. The samples 1A were centrifuged 5500 x g for 30 min at room temperature. The samples 1B were centrifuged 4000 x g for 60 min at constant +20 °C temperature (Hettich, Rotanta/RPC, Tuttlingen, Germany). The fat content (%) was estimated using a standard line measurer (Vis, Inox, 0.05 mm) and the fatty layer was removed. Both samples were analysed for antibodies to *M. hyopneumoniae* in dublicate wells on the same microtiter plate.

The effect of repeated freezing and spoilage

The influence of repeated freezing of colostrum samples on the test results was analysed in 44 samples. Twenty-two of the samples originated from 3 herds with no antibodies to *M. hyopneumoniae.* The other 22 samples originated from 6 herds with antibodies to *M. hyopneumoniae* in 3 or more samples. All samples were divided into subsamples 2A and 2B, and then frozen at -18 °C. The samples 2B were thawed in room temperature (for 1.5 h) and frozen again 10 times. Then, both 2A and 2B were thawed and centrifuged 5500 x g for 30 min. The fat-% was measured, the fat was removed and the samples were analysed for antibodies to *M. hyopneumoniae* in dublicate wells on the same microtiter plates.

In order to analyse the influence of spoilage of colostrum samples on the test results, the samples 2B were finally left in room temperature for 65 h (2.5 days). Thereafter, the samples, now named 2C, and the samples 2A were centrifuged 4000 x g for 60 min at constant +20 °C temperature. Both samples were analysed parallel as described above.

Persistence of antibodies following an eradication programme

An acute *M. hyopneumoniae* infection was diagnosed clinically, pathologically, and serologically in one elite breeding herd (herd X) with 35 sows during October 1994. An eradication programme was introduced in June 1995 using a method of partial depopulation combined with medication (*Zimmermann et al.* 1989). In order to follow the serological status of the sows after the eradication programme, a total of 211 colostrum samples were collected at regular intervals between May 1996 and October

1998 for detection of antibodies to *M. hyopneumoniae.* Thirty-nine of these samples originated from 18 breeding sows born before the eradication programme. From 7 of these sows there were samples from 2 successive farrowings. The remaining 172 samples originated from 105 replacement gilts and sows born after the eradication programme.

The statistical analyses

The sensitivity of the ELISA on herd level was defined as the proportion of herds infected with *M. hyopneumoniae* in 1995 that had one or more colostrum samples with antibodies. The specificity was defined as the proportion of herds not infected with *M. hyopneumoniae* in 1995 that did not have antibodies in any of the colostrum samples. The 95% confidence intervals (CI) for binomial data were calculated according to *Casella & Berger* (1990). The blocking-values of different groups of sows were compared using the 2-tailed Mann-Whitney U-test.

The associations between seropositivity and parity number of the sow, seropositivity and the time of sampling in relation to farrowing as well as seropositivity and complete vs. incomplete information of parity number and time of sampling were analysed using chi-square tests. For further analyses, the parity number of the sow and the time of sampling were used as dichotomous variables. The herds were divided into 2 groups based on the median prevalence of sows with antibodies. Multiple logistic regression was used to assess the strength of association between the seropositivity and the parity number of the sow and also the time of sampling in the full data and in the data stratified by the 2 groups of herds. Interactions were evaluated with previously selected variables forced in the model.

The difference between the blocking-values of the subsamples 1A and 1B was tested with a paired t-test to analyze whether the mean difference of the pairs was different from zero. The correlation between the difference and the fat-% was tested with Pearson correlation. Also differences between the subsamples 2A, when compared with subsamples 2B and 2C, respectively, as well as the correlation of the differences obtained with the fat-% were calculated using these analysing methods. Based on 95% confidence level (Z_{α}=1.96), 80% power (Z_{β}= -0.84) and 5%-units expected standard deviation (s) in any of the differences (X_1-X_2), a 4%-units difference would be detected with the sample size of 22 (n), and a 3%-units difference with a sample size of 44 based on the following equation: $n = 2[(Z_{\alpha}-Z_{\beta})s / (X_1-X_2)]^2$ (*Martin et al.* 1987).

The difference between the blocking-percentages of the samples of the 2 successive farrowings in the elite breeding herd X was tested with a paired t-test.

The statistical package used in the analyses was Statistix® 4.1 (Analytical Software, Tallahassee).

Results

Detection of antibodies to M. hyopneumoniae

Antibodies to *M. hyopneumoniae* were detected in 274 (1.8%) samples and in 42 herds (7.9%). The median prevalence of sows with antibodies in seropositive herds was 28.2% (range, 2.7%-100%). In the remaining herds (488 herds), no antibodies were detected.

By the definitions, 31 herds were truly infected with *M. hyopneumoniae* (4 elite breeding herds, 27 LSO-2000 herds). In 11 herds with a maximum of 2 positive sows additional inspections were made. Respiratory symptoms were recorded in 3 of the herds, and these herds were excluded from the study (Table 1). The remaining eight herds were concluded non-infected by the additional tests (Table 1). In these herds, the mean blocking-percentages (B) were higher

Table 1. The herds with one or 2 colostrum samples with antibodies to *Mycoplasma hyopneumoniae* detected in blocking-ELISA in a survey of 530 herds in Finland in 1995. The herds (n = 31) with 3 or more samples with antibodies were classified as truly infected (not shown in the table).

Herd number	No. of colostrum samples	No. of samples with antibodies (%)	Mean blocking-% of the samples with antibodies[a]	Respiratory symptoms detected	No. of additional blood samples (no antibodies)	Follow-up in 1996: Feed-back from the finishing herds indicating SEP[b]	Follow-up in 1996: No. of colostrum samples (no antibodies)	Classification of the herds
1	37	1 (2.7)	66.3[c]	no	14	no	42	
2	30	1 (3.3)	51.9	no	10	no	30	
3	30	1 (3.3)	53.4	no	10	no	30	non-
4	30	1 (3.3)	59.6[c]	no	4	no	30	infected
5	15	1 (6.7)	50.1	no	10	no	30	herds
6	15	1 (6.7)	58.3[c]	no	30	no	31	
7	10	1 (10.0)	50.7	no	7	no	17	
8	20	2 (10.0)	60.5[c]	no		no	23	
9	15	2 (13.3)	53.3	yes				possibly
10	15	2 (13.3)	64.4	yes				infected
11	6	2 (33.3)	74.9	yes				herds

[a] The cut-off value for a positive result was 50%.
[b] SEP = swine enzootic pneumonia.
[c] The sows with antibodies not born in the herd.

(p=0.02) among sows purchased (B_{492}=61.0%, standard deviation (SD) 3.1%) than among sows originating from the herds (B_{492}=51.5%, SD 1.5%).

In total, 496 (488+8) herds were tested free from *M. hyopneumoniae* during 1995 and a majority (N = 464 herds, including the 8 herds with false positive reactions) of the herds still joined a health control programme the following year, which enabled a long-term monitoring of these herds. Most herds (462 herds) remained free from *M. hyopneumoniae,* while a *M. hyopneumoniae* infection was diagnosed in 2 of the 464 herds during 1996. On the basis of the clinical and the serological findings of a regular monitoring, it was concluded that these 2 herds experienced a recent infection and not a chronic, earlier non-detected *M. hyopneumoniae* infection.

From 32 out of 496 herds, neither regular nor clinical serological samples were received in 1996. In 1997, twelve of these herds delivered colostrum samples again and no antibodies were detected.

Sensitivity and specificity

From the results given above, the sensitivity of the ELISA on herd level was calculated to 100% (95% CI, 87%-100%; n = 34) since all herds shown to be infected with *M. hyopneumoniae* during 1995 were detected in the survey performed that year. The specificity on herd level was 98% (95% CI, 97%-99%) calculated as the proportion of the herds not infected with *M. hyopneumoniae* (n = 464) that did not have antibodies in any of the colostrum samples in 1995 (n = 456), i.e. (456/464)*100%.

The effect of the parity number and the time of sampling

One of the herds (herd no. 13) truly infected with *M. hyopneumoniae* was censored from this analysis. That herd was a large, newly established multi-site farrowing herd and was, thus,

Table 2a. The crude associations of seropositivity to *Mycoplasma hyopneumoniae* in colostrum with the age of the sow and with the time of sampling in comparison to farrowing in samples of 30 truly-infected herds according to chi-square tests. The parity number and the time of sampling were known of 610 and 403 samples, respectively.

Parity number	No. of samples	No. of positive samples (%)	Time of sampling (hours)	No. of samples	No. of positive samples (%)
1	178	60 (34)	<1	116	52 (45)
2	115	31 (27)	1	104	38 (37)
3	99	42 (42)	2	69	34 (49)
4	64	22 (34)	3	29	4 (14)
5	44	12 (27)	4	32	8 (25)
6	33	20 (61)	5	11	4 (36)
7	31	14 (45)	6	18	7 (39)
>7	46	21 (46)	7	10	7 (70)
			>7	14	2 (14)
	Overall chi-square	19.3		Overall chi-square	23.1
	P-value	0.007		P-value	0.003

not considered to be a conventional farrowing herd.

From 648 samples collected from 30 infected herds, 230 (36%) contained antibodies to *M. hyopneumoniae.* The parity number was recorded from 610 sows (Table 2a). The mean time from farrowing to sampling was 1.9 h (median, 1.0 h; range, 0-12.0 h; n = 403) and 99% of the samples were collected within 8 h from farrowing. The longest recorded time from farrowing to sampling of a colostrum sample with antibodies was 8.5 h. Both the parity number of the sow and the time of sampling were known for 388 samples, which originated from 28

Table 2b. The association of seropositivity to *Mycoplasma hyopneumoniae* in colostrum with the age of the sow and the time of sampling in comparison to farrowing, according to multiple logistic regression analyses in the full data and in the data stratified by the median prevalence (36%) of sows with antibodies in 28 truly-infected herds.

		Coefficient	P-value	Odds ratio (95% limits)
Full data	CONSTANT	-1.037	<0.001	
(no. of samples	OLD[a]	0.794	0.005	2.2 (1.3-3.9)
388)	SOON[b]	0.619	0.012	1.9 (1.2-3.0)
High prevalence	CONSTANT	-0.631	0.087	
herds (no. of	OLD	0.118	0.769	1.1 (0.5-2.5)
samples 161)	SOON	1.104	0.006	3.0 (1.4-6.6)
Low prevalence	CONSTANT	-1.240	<0.001	
herds (no. of	OLD	1.206	0.004	3.3 (1.5-7.5)
samples 227)	SOON	0.056	0.864	1.1 (0.6-2.0)

[a] OLD = 1: parity number higher than 5 (n = 62); OLD = 0: parity number lower than or equal to 5 (n = 326)
[b] SOON = 1: time of sampling shorter than 2.0 h (n = 278); SOON = 0: time of sampling 2.0-12.0 h (n = 110)

Table 3. The influence of extended centrifugation, repeated freezing and spoiling on the blocking-values of colostrum samples in a monoclonal blocking-ELISA for the detection of antibodies to *Mycoplasma hyopneumoniae*.

	No. of samples	Blocking-%		Difference of the manipulated and the control samples			
		median	range	mean	SD	range	p-value[d]
Control: samples 1A	22	23.1	9.6-67.1				
Extended centrifugation[a]: samples 1B	22	23.8	9.5-70.1	-2.0	5.2	-10.1-7.0	0.079
Control: samples 2A	44	38.4	0-95.5				
Repeated freezing[b]: samples 2B	44	44.5	0-95.3	2.2	4.7	-6.9-13.7	0.004
Control: samples 2A	42	37.8	0-95.1				
Spoiling[c]: samples 2C	42	35.8	0-95.1	-1.3	6.3	-17.9-10.9	0.193

[a] Samples 1B centrifuged 4000 x g for 60 min, samples 1A centrifuged 5500 x g for 30 min.
[b] Samples 2B frozen 10 times repeatedly in successive work days.
[c] Samples 2C same as samples 2B, but left in room temperature for 65 h. Two samples were inadequate for analysis.
[d] Paired t-test.

herds. The samples with incomplete information were significantly less often seropositive than the samples with complete information ($p = 0.03$).

The crude associations between seropositivity and the parity number of the sows, as well as the time of sampling in comparison to farrowing are shown in Table 2a. Seropositivity was associated with the parity number of the sows ($p = 0.007$), as well as with time of sampling in comparison to farrowing ($p = 0.003$). However, there was no linear dose-response relationship between either of the variables and the seropositivity. Consequently, the time of sampling was converted into a dichotomous variable SOON (1: time <2.0 h; 0: time ≥2.0 h) and the parity number was converted into a dichotomous variable OLD (1: parity number >5; 0: parity number ≥5). According to the median prevalence of sows with antibodies (36%), the herds were divided into 2 groups. The associations between the variables were analysed in 3 models (Table 2b) (the non-significant interaction terms were not included). Seropositivity as the dependent variable, the odds ratio was 3.0 (95% CI, 1.4-6.6) for samples collected soon after the farrowing (SOON = 1) as compared to samples collected later in herds with a high prevalence of sows with antibodies. The odds ratio was 3.3 (95% CI, 1.5-7.5) for samples of old sows (OLD = 1) as compared to samples of young sows in herds with a low prevalence of antibodies.

Effect of the extended centrifugation

The mean fat-% of the samples was 9.9% (SD, 4.2%; range, 4.0-23.0%). The blocking-percentages between the subsamples 1A and 1B did not differ significantly (Table 3).

Effect of the repeated freezing and the spoilage

The mean fat-% of the samples was 10.8% (SD, 4.2%; range, 2.2-21.2%). The blocking-percentages of the subsamples 2B were significantly higher than those of the subsamples 2A (Table 3). The fat-% of the samples did not cor-

Table 4. The detection of colostral antibodies to *Mycoplasma hyopneumoniae* in blocking-ELISA in 18 sows from an infected herd born before completion of an eradication programme during summer 1995. Antibodies were not detected in 105 replacement gilts and sows born after completion of the eradication programme.

Sow number	Before the eradication programme	Time after effectuating the eradication programme (months)					
		7-12	13-18	19-24	25-30	31-36	37-42
1	-	-	-		-		
2	-					+	
3	+		+				
4	+		+				
5	-	+	+			+	+
6	-	-					
7	-	-		-	-		-
8	+			+	+	+	
9	+	+	+				
10	-	+	+	+	+		
11	-		+	+	+		
12	-	+	+	+			
13	+	+	+		+		
14		+					
15	+	+					
16	+	+			-		
17	-				-		
18	-						-

+antibodies detected
- antibodies not detected

relate with the difference of the blocking-values ($p = 0.95$).

The blocking-percentages of the subsamples 2A and 2C did not differ significantly (Table 3). Two samples were excluded from the final analysis because of too little colostral whey left.

Persistence of antibodies following an eradication programme

Antibodies to *M. hyopneumoniae* were not detected in any of the 172 samples collected from the 105 replacement gilts and sows born after the eradication programme. According to the health control programme, herd X was declared free from *M. hyopneumoniae* in the beginning of 1996.

Antibodies to *M. hyopneumoniae* were detected in 13 of the 18 breeding sows born before the eradication programme. From 7 of the seropositive sows there were samples from 2 successive farrowings. The mean blocking-values of the former and the latter samples were 78.3% and 78.2%, respectively ($p = 0.98$).

Antibodies have been detected in colostrum more than 3 years following the eradication programme (Table 4).

Discussion

In this study, herds with no antibodies to *M. hyopneumoniae* in colostrum samples were defined as non-infected with *M. hyopneumoniae,* if they were free from clinical signs of disease during the following 12 months and no signs of infection were observed in the corresponding finishing herds (testing stations) or in the slaughterhouses. This monitoring was regarded

reliable in detecting an infection if present, because it was continued for a year and carried out in 3 different sites. In addition, mixing of growers from multiple sources is a known risk factor for respiratory diseases if the health status of the source herds differs from each other (*Zimmermann et al.* 1989, *Christensen et al.* 1999). Therefore, this monitoring was used as gold standard for evaluation of the sensitivity of the test at the herd level. It turned out that the ELISA detected all infected herds in 1995, since all of the serologically negative herds were defined as truly non-infected. This corresponded to a herd level sensitivity of 100% (CI, 87%-100%), which made the test very suitable for use as a screening test.

Antibodies to *M. hyopneumoniae* were detected in 42 herds. Herds, that were classified as truly or possibly infected herds were excluded from the health control programmes. The numbers of the excluded herds in Southwestern Finland (27/347) and breeding herds (4/183) corresponded well to the numbers observed earlier (*Tuovinen et al.* 1994, *Rautiainen et al.* 1996). None of the herds with less than 10% prevalence of positive samples was found truly infected with *M. hyopneumoniae* (Table 1). Thus, based on a minimum of 10% prevalence of positive samples in truly infected herds, a minimum of 19-25 samples would have been enough to declare herds with 30-100 sows free from *M. hyopneumoniae* infection with 95% confidence (*Cannon & Roe* 1982). The decreasing of the number of samples analysed per large herds (with 30-100 sows) would, in addition, increase the herd-level specificity by about 0.3 %-units (using the equations of *Martin et al.* 1992).

Early sampling (<2.0 h after the start of farrowing compared to 2.0 to 12.0 h) increased the probability of detecting colostral antibodies to *M. hyopneumoniae* in infected herds (Table 2b). This was in accordance with earlier findings (*Klobasa et al.* 1986, *Klobasa & Butler* 1987). However, when the data was stratified by the status of infection of the herds, early sampling increased the number of positive samples only in herds with a high prevalence of positive samples, i.e. in recently infected herds. Early sampling could, therefore, be a useful tool for increasing the sensitivity in surveillance programmes, which aim to detect an infection as early as possible. Testing high parity sows (parity number >5) was also a tool to increase the number of positive samples, but only in herds with a low prevalence of positive samples, i.e. in herds with a low pathogen load with respect to sows. Earlier reports are conflicting. *Klobasa & Butler* (1987) reported a tendency for ≥5th parity sows to have more antibodies in colostrum than younger sows, while a gradual decrease in the prevalence of positive samples was observed with increasing parity number in another study (*Yagihashi et al.* 1993). Obviously, farm to farm variation exists and the parity number of sows ought not be criterion for testing in a survey study.

The parity number, still, as well as the history of the sows is important in some circumstances. In our study, antibodies to *M. hyopneumoniae* were found up to 3 years after the infection was eradicated. Hence, the serological status of sows present in herds after eradication programmes may not represent the current health status of such herds. A similar situation was faced in the survey of the study. In 4 out of the 8 false positive herds, the seropositive sows were purchased animals. The mean blocking-percentage of the purchased animals was much higher than that of the seropositive sows in the other 4 herds (61% and 52%, respectively; $p = 0.02$). Therefore, it is possible that the ELISA did correctly identify true seropositive purchased sows which, however, had not transmitted the infection into the new herds. If that was the case, the herds were in fact not antibody

"false-positive" herds.

When colostrum samples were exposed to extreme conditions a significant but biologically irrelevant increase in the blocking-percentages was noted in the repeatedly frozen samples. That increase did not correlate with the fat-% of the samples. It was probably due to changes of the colostrum induced by the treatment, which caused a blocking effect on the wells in the same way as the antibodies. Similar findings have been reported earlier (*Bouwkamp et al.* 1993, *Levonen et al.* 1996). The full spoilage of the samples in our study did, however, not alter the test results, possibly due to the centrifugation of the samples executed before the final analysis. The centrifugation probably eliminated any newly-formed substances (coagulations, bacterial mass, etc.) as also indicated by the centrifugation tests performed (Table 3). As a conclusion, a possible misstorage of colostrum samples is not a major problem when using the ELISA. However, a good sampling practice, when not using preserving additives, assumes a clean collection followed by a rapid refrigeration and freezing of the colostrum samples.

In a regular surveillance, a test should detect a recent infection as early as possible. Several authors have described risk factors for the reinfection of a herd indicating the importance of airborne transmission of *M. hyopneumoniae* between herds (*Goodwin* 1985, *Thomsen et al.* 1992, *Stärk et al.* 1992). Units with the highest airflow have increased risk of introducing aerosols containing the infectious agent, that is, the dry sow unit in farrowing herds and the finishing unit in farrowing-to-finishing herds (*Laube et al.* 1996). Furthermore, antibodies to *M. hyopneumoniae* have been detected in colostrum samples even weeks before the clinical outbreak of an acute disease (*Sørensen et al.* 1993, *Levonen* 1994a). That happened also in the elite breeding herd X in our study (data not shown). Hence, if colostrum samples were analysed frequently, e.g. monthly, they would probably be sensitive samples to detect also a recent infection.

Acknowledgements

This study was supported by the Finnish Veterinary Foundation. The authors thank the breeding herds that cooperated in this study, as well as the laboratory stuff. The authors also thank Mari Heinonen for providing information of the LSO-2000 quality chain, as well as Per Wallgren and Anna-Maija Virtala for critical reading of this paper and for excellent comments.

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Sammanfattning

Analys av antikroppar gentemot Mycoplasma hyopneumoniae - er metod att dokumentera infektionsfrihet

I en kartläggning i Finland år 1995 analyserades 14 919 råmjölksprov från 530 besättningar för antikroppar mot *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*). En monoklonal blocking-ELISA användes. I småbesättningarna analyserades prov från varje sugga. I de stora besättningarna analyserades 30 prov. Alla besättningarna var med i något hälsoövervakningsprogram. Antikroppar mot *M. hyopneumoniae* konstaterades i 274 prov (1.8%) och i 42 besättningar (7.9%). Medianprevalensen av suggor med antikroppar i seropositiva besättningar var 28.2% (variation, 2.7%-100%). Enligt uppföljning i år 1996 var alla besättningarna, som var seronegativa i 1995, faktiskt infektionsfria avseende *M. hyopneumoniae.* I akut infekterade besättningarna var prov som hade mjölkats tidigare än 2 timmar efter grisningen, 3 gånger oftare seropositiva än prov som hade mjölkats 2-12 timmar efter grisningen (odds ratio, 3.0; 95% konfidens intervall 1.4-6.6). Upprepad frysning eller förskämning av råmjölksprov påverkade inte betydligt resultaten av ELISA testen. Efter en lyckad sanering påvisades antikroppar mot *M. hyopneumoniae* upp till 3 år efter saneringen bland suggor som var födda före saneringen. Sammanfattningsvis; råmjölksprov kan effektivt användas för kartläggning av suggbesättningar avseende *M. hyopneumoniae* infektion. Råmjölksprovtagning förefaller användbar också för regelbunden kontroll för nyinfektioner.

(Received February 1, 1999; accepted January 4, 2000).

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