Regional Eradication of *Mycoplasma hyopneumoniae* **From Pig Herds and Documentation of Freedom of the Disease**

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> Rautiainen E, Oravainen J, Virolainen JV, Tuovinen V: Regional eradication of Mycoplasma hyopneumoniae from pig herds and documentation of freedom of the disease. Acta vet. scand. 2001, 42, 355-364. - The objectives of this study were to 1) screen all sow herds in a region for M. hyopneumoniae, 2) to effectuate an eradication programme in all those herds which were shown to be infected with *M. hyopneumoniae*, and 3) to follow the success of the screening and the eradication programmes. The ultimate goal was to eradicate *M. hyopneumoniae* from all member herds of a cooperative slaughterhouse (153 farrowing herds + 85 farrowing-to-finishing herds + 150 specialised finishing herds) before year 2000. During 1998 and 1999, a total of 5067 colostral whey and 755 serum samples (mean, 25 samples / herd) were collected from sow herds and analysed for antibodies to M. hyopneumoniae by ELISA. Antibodies were detected in 208 (3.6%) samples. Two farrowing herds (1.3%) and 20 farrowing-tofinishing herds (23.5%) were shown to be infected with M. hyopneumoniae. A programme to eradicate the infection from these herds was undertaken. During March 2000, a survey was made to prove the success of the screening and the eradication programmes. In total, 509 serum samples were collected randomly from slaughtered finishing pigs. Antibodies to M. hyopneumoniae were not detected in 506 of the samples, whereas 3 samples were considered suspicious or positive. Accordingly, 3 herds were shown to be infected. One of the herds was previously falsely classified as non-infected. Two of the herds were finishing herds practising continuous flow system (CF). Unlike finishing herds which practice all-in/all-out management routines on herd level, CF herds do not get rid of transmissible diseases spontaneously between batches, for which reason a screening was made in the rest of the CF herds (total n = 7). Consequently, 2 more infected herds were detected. In addition to the results of the survey, a decreasing prevalence of lung lesions at slaughter (from 5.2% to 0.1%) and lack of clinical breakdowns indicated that all member herds were finally free from M. hyopneumoniae in the end of year 2000.

> ELISA; colostrum; antibodies; all-in/all-out; lung lesions; screening; sampling; survey.

Introduction

Mycoplasmal pneumonia of swine (swine enzootic pneumonia; SEP) caused by *Mycoplasma hyopneumoniae (M. hyopneumoniae)* is one of the most common and economically important diseases among pigs. Economic losses due to SEP are often associated with secondary infections, poor management and poor environmental conditions (*Ross* 1999). In Finland, the negative effect of *M. hyopneumoniae* infection on mean daily gain (MDG) of finishing pigs has been estimated to be 24 g (*Tuovinen et al.* 1994a) and 60 g (*Rautiainen et al.* 2000b). In contrast to most other countries, *M. hyopneumoniae* is not ubiquitous in Finnish sow herds; the prevalence varies between 8% (*Tuovinen et al.* 1994b) and 30% (*Rautiainen* 1998) in different parts of the country. However, until recently a majority of finishing herds have been filled with feeder pigs some of which have been carrying the infection (*Tuovinen et al.* 1994b), *Rautiainen* 1998).

Finland is free from all major epidemic pig diseases, so called list A diseases of OIE (Office International des Epizooties). In addition, porcine reproductive and respiratory syndrome, Aujeszky's disease or swine influenza have never been reported in Finland (Anon. 2000). Furthermore, elite breeding herds are declared free from M. hyopneumoniae and from the following infectious agents: Serpulina hyodysenteriae, toxigenic Pasteurella multocida, Clostridium perfringens type C, Sarcoptes scabiei var suis, and all serotypes of salmonella (Anon. 1997). In order to prevent these infections in production herds as well, health classification (HC) of farrowing herds followed by health matching of multisource feeder pigs was introduced in Finland in 1994 (Tuovinen et al. 1996). The producers of the health class feeder pigs received a premium price. Feeder pigs from herds with different health status were transported separately. The health class pigs were given a guarantee for freedom from M. hyopneumoniae.

Eradication of *M. hyopneumoniae* from infected herds without total depopulation, *i.e.* with reasonable costs, has been reported repeatedly (*Waldmann & Radtke* 1937, *Zimmermann et al.* 1989, *Wallgren et al.* 1993b). Since the start of HC, freedom of *M. hyopneumoniae* has become an economically tempting goal for many herds still infected with this particular infectious agent, and dozens of eradication programmes have been effectuated (*Tuovinen & Heinonen* 1997, *Heinonen et al.* 1999).

In Britain, reinfections with M. hyopneumoniae were shown to occur in enzootic-pneumoniafree pig herds without simple explanations and in spite of zootechnical precautions of high standard (Goodwin 1985). Consequently, Goodwin (1985) suspected that airborne transmission of this infectious agent was possible between neighbouring herds. This view was later shared by others (Stärk et al. 1992, Thomsen et al. 1992). From this point of view, only regional freedom from disease would effectively prevent the majority of reinfections. Attempts to create regions free from M. hvopneumoniae have recently been made in 2 pig dense areas in Switzerland (Masserev-Wullschleger & Maurer 1998).

The cooperative slaughterhouse Lihakunta operates in Eastern and Northern Finland to supply meat for the meat packing company Atria Ltd. In 1999, a total of 156 000 pigs were slaughtered which corresponded to about 7% of the national production. A typical specialised finishing herd of the region comprises 200-300 pigs located in one unit and practises all-in/all-out management routines. The finishers (York-shire x Landrace) generally originate from 15 to 20 different piglet producing herds and arrive at the weight of approximately 25 kg. The overall pig density is considered low.

In 1995, a preliminary survey was made to get an overview of the prevalence of *M. hyopneumoniae* in the farrowing herds of Lihakunta. During one week, 41 blood samples were collected from slaughter pigs of 7 randomly chosen finishing herds. These samples were analysed for antibodies to *M. hyopneumoniae* with an ELISA described in the chapter Materials and methods. Antibodies were not detected in 4 of the herds, whereas in the remaining 3 herds antibodies were detected in a total of 10 samples indicating *M. hyopneumoniae* infection in those finishing herds. Thus, according to the survey, pigs in roughly one half of the finishing herds were infected with *M. hyopneumoniae*. Still, the prevalence of piglet producing farrowing herds which were transmitting the infection to the finishing herds could have been as low as 4-5 percentages. The following formula was used in the calculations (*Snedecor & Cochran* 1980):

$P=1-(1-p)^{\rm N}$

where P(0.50) is the probability of a finishing herd to get infected pigs from the source herds; N(15) is the average no. of the source herds per batch of finishers; p is the prevalence of the source herds infected with M. hvopneumoniae. The objectives of this study were to screen all farrowing and farrowing-to-finishing herds of Lihakunta for M. hyopneumoniae; to effectuate an eradication programme in all those herds which were shown to be infected with M. hyopneumoniae: to follow the success of the screening and the eradication programmes. The ultimate goal was to eradicate M. hyopneumoniae from all member herds of Lihakunta before year 2000 (including specialised finishing herds).

Materials and methods

The member pig herds of Lihakunta

The number of farrowing and farrowing-to-finishing herds in this study was 153 and 85, respectively, and the number of specialised finishing herds was 150. The median number of sows per herd was 38 (range, 1-120), and the median number of finishing pigs per herd was 300 (range, 50-1800).

Screening for the health status

During 1998 and 1999, all sow herds were assumed to document their health status concerning *M. hyopneumoniae*. The health status was verified serologically using either sow colostrum samples (*Rautiainen et al.* 2000a) or blood samples mainly from finishing pigs collected at slaughter. The expected number of samples was 30 (or the corresponding no. of sows in herds with less than 30 sows). With this sample size, it was possible to detect at least one sample with antibodies with 99% confidence, if the prevalence of positive samples was at least 10% (*Cannon & Roe* 1982). The costs for the analyses were paid by the slaughterhouse company.

If antibodies to M. hvopneumoniae were not detected in the collected samples, a herd was classified as non-infected. If, on the other hand, antibodies were detected, additional clinical inspections were made by the local veterinarian. If respiratory signs (cough in weaners and young finishers) and/or substantial number of lung lesions at slaughter were detected, the herd was classified as infected. Such herds were provided an eradication plan by the slaughterhouse company for free. If, on the other hand, no confirmatory signs indicating M. hyopneumoniae infection were detected, additional blood samples were collected from pigs aged 10 weeks or more (Wallgren et al. 1998) to detect serum antibodies to *M. hyopneumoniae* in growing pigs. If antibodies were not detected in those samples, herds were classified as non-infected (false-positive herds). In specialised finishing herds, no screening was performed because the health status of such herds was dependant on that of the piglet producing 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 that. 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 from the sending.

Blood samples

The blood samples from pigs over 10 weeks of age were collected from *vena cava cranialis* (pigs up to 35 kg of weight) or *vena jugularis dexter* (pigs >35 kg of weight) using evacuated glass tubes without additive. The samples were collected by the local practitioners or by the second or third author. The samples were sent to the laboratory on the same day as collected without separation of serum. At the slaughterhouse, blood samples were collected from finishing pigs at exsanguination. All samples were refrigerated without separating the serum. A batch of samples was sent to the laboratory daily or every second or third day.

Detection of antibodies to M. hyopneumoniae

Before the analysis, all colostrum samples were treated as described earlier (Rautiainen et al. 2000b). The colostral whey (diluted 1:10 in sample diluent) 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 blockingvalue over 50% at 492 nm wave length was retested in duplicate wells and then classified as having antibodies (positive), if the blockingvalue still exceeded 50%. 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).

The blood samples were centrifuged $3500 \times g$ for 10 min and the sera were analysed similarly as the colostral whey samples with one excep-

tion: Samples with a blocking-value between 35% and 50% were classified as suspected, and the corresponding herds were treated similarly as the herds giving positive results.

Eradication programmes and the follow-up

The eradication programmes for individual herds were planned by a consulting veterinarian of the slaughterhouse Lihakunta in collaboration with the local practitioners and the herd owners. The programmes were based on removal of the young animals from the herds and medication of the breeding stock (Waldmann & Radtke 1937, Zimmermann et al. 1989, Wallgren et al. 1993b). In addition, medication for the eradication of Sarcoptes scabiei var suis, the causative agent of sarcoptic mange, was given to the breeding stock in several herds according to Hogg (1989). The eradication of M. hyopneumoniae from specialised finishing herds was based on the assumption that the infectious agent will not survive in the environment between 2 batches of pigs (Goodwin 1985), when all-in/all-out management routines are practised at herd level.

To follow the success of the programme in an individual herd, 15-20 blood samples were collected for the detection of antibodies to M. hyopneumoniae. The target group for sampling were pigs born after the effectuation of the programme and aged from 10 weeks to 6 months. The sample size was convenient, since it often happened that the number of pigs of the right age group was very limited at the time when the first pigs borne after the eradication programme were ready to be sold to specialised finishing herds. In addition to serology, the absence of both cough during rearing and lung lesions indicating SEP detected at slaughter were used as indicators of freedom from M. hyopneumoniae in finishing herds. Lung lesions were reported continuously for all member herds by the meat inspection team.

Herd	No. of sows	Production type	No. of colostral whey or serum samples		eradication	The follow-up serum samples after eradication		
			positive ^b	total	(month/year)	positive ^b	total	Notes
Kar	24	В	5	18	-	-	-	finished all production turned to finishing herd resigned membership
Lin	15	А	18	28 ^a	-	-	-	
Mäk	14	A	3	15 ^a	-	-	-	
Tuo	28	В	11	24	9 / 98	0	20	
Pie	48	А	4	30	12 / 98	0	20	
Hei	50	А	9	28 ^a	4 / 99	0	25	
Rah	46	А	25	30	4 / 99	0	30	
Mie	30	А	5	15	5 / 99	0	15	
Mar	25	А	8	10	5 / 99	0	20	
Kär	19	А	4	15	5 / 99	0	20	
OjH	30	А	11	20	6 / 99	0	10	
OjR	30	А	8	16	6 / 99	n.a.	n.a.	n.a. = not analysed
Eur	170	А	7	12 ^a	7 / 99	0	20	
Ant	35	А	13	18	8 / 99	0	11	
Kej	20	А	18	20	9 / 99	0	20	
Par	25	А	9	11 ^a	9 / 99	0	11	
Vou	38	А	8	21	9 / 99	0	15	
Meh	26	A	13	19	12/99	0	10	
Oll	10	A	6	15	12/99	n.a.	n.a.	
Pak	12	A	6	10 ^a	12/99	0	16	
Ras	60	A	5	23 ^a	12/99	0 0	11	
Ten	25	A	14	29 ^a	12/99	0	10	
Tas	20	Ac	8	15 ^a	9 / 00	n.a.	n.a.	
Aht	- 20	Cc	8	10 ^a	6 / 00		11.d.	
Tur	-	C° C°	7	7a	7 / 00	-	-	
Vit		C°	1	7 ^a	7 / 00	-	-	
Kol	-	C ^c	9	15 ^a	11/00	-	-	

Table 1. Pig herds of cooperative slaughterhouse Lihakunta with antibodies to *Mycoplasma hyopneumoniae* and which were later shown to be infected with the agent. The time of eradication of the agent is shown, too.

All sow herds were screened for *M. hyopneumoniae* antibodies during 1998 and 1999. In addition, a survey of all pig herds was done in March 2000 based on randomly collected blood samples (n=509) from slaughtered finishing pigs. Simultaneously, all type C herds were screened.

 \hat{A} = farrowing-to-finishing herd; B = farrowing herd; C = finishing herd practising continuous flow system

^a Serum samples ^b Corresponding to antibodies to M. hyopneumoniae by ELISA. ^c Indication of infection was found only during the survey or the screening of type C herds.

Survey to detect antibodies in finishing pigs at slaughter

In order to follow the success of the screening and eradication programmes, a survey was carried out during March 2000 to detect antibodies to *M. hyopneumoniae* in finishing pigs of Lihakunta (including pigs from both farrowingto-finishing herds and all kinds of specialised finishing herds). The monthly number of slaughtered finishing pigs was about 15000. With a sample size of 459 (*Cannon and Roe* 1982), it was possible to detect at least one positive sample with 99% confidence, if the prevalence of positive samples was at least 1%. Thus, to get a total of 500 samples a blood sample was collected systematically from every 30th pig.

Statistical analysis

The prevalence of lung lesions detected at slaughter was recorded quarterly during 1998-2000. The analysis of trend was made using Pearson correlation for continuous data. The programme used in the analyses was Statistix for Windows[®] (Analytical Software, Tallahassee, FL).

Results

Screening of herds

During 1998 and 1999, a total of 5067 colostral whey samples and 755 serum samples (mean, 25 samples / herd) were analysed for antibodies to M. hvopneumoniae by ELISA. Antibodies were detected in 208 samples (3.6%). Two farrowing herds (1.3%) and 20 farrowing-to-finishing herds (23.5%) were shown to be infected with M. hvopneumoniae (Table 1, Fig. 1). In addition, single positive samples were detected in 11 herds, however, without any other findings indicating M. hyopneumoniae infection according to the additional inspections and blood samples. These herds were classified as non-infected false-positive herds. In all, only few herds were unwilling to cooperate with the screening. Samples from such herds were entirely collected at the slaughterhouse.

Eradication programmes

One owner of a herd infected with *M. hyopneumoniae* refused to eradicate the infection and resigned the membership. One herd finished pig production and another one changed the production type from integrated to fattening (Table 1). In the remaining herds, an eradication programme was effectuated according to the timetable shown in Table 1. Antibodies to *M. hyopneumoniae* have not been detected in any of the follow-up samples (n=284) taken in the herds after the completion of the eradication programmes (Table 1). Nor have any clinical or pathological findings indicated failures of the



Figure 1. A map of Finland showing the regional distribution of member pig herds of cooperative slaughterhouse Lihakunta which were shown to be infected with *Mycoplasma hyopneumoniae* (marked with stars).

eradication programmes so far (February 2001).

Survey to detect antibodies in finishing pigs at slaughter

A total of 509 serum samples were collected. Antibodies to *M. hyopneumoniae* were not detected in 506 samples (99.4%). Two samples were considered positive and one sample suspicious. These 3 samples were traced back to 3 different herds and, according to additional inspections, all herds were shown to be infected with *M. hyopneumoniae*. Two of these herds

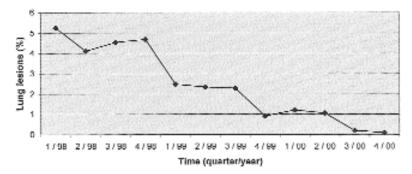


Figure 2. The quarterly prevalence of lung lesions of all slaughtered pigs during 1998-2000.

were finishing herds practising a continuous flow system. The third one was a farrowing-tofinishing herd (no. of sows, 20) which had had a single positive serum sample already in the screening test in autumn 1999, but was during that time classified as a false-positive herd. None of these 3 herds had sold live animals to any other herds.

It appeared during the survey that the health status of finishing herds practising a continuous flow system was unknown. Therefore, all such finishing herds were identified (n=7) and their health status was verified serologically. Consequently, antibodies to *M. hyopneumoniae* were detected in 4 out of 7 herds. These infected herds were emptied, cleaned and disinfected after which all-in/all-out management routines were implemented.

Lung lesions at slaughter

The quarterly prevalence of lung lesions of all slaughtered pigs decreased from 5.2 % in 1998 to 0.1% in 2000 (Fig. 2). The decreasing trend was statistically significant (r = -0.96; p < 0.001; n=12).

Discussion

The number of farrowing herds shown to be infected with *M. hyopneumoniae* was small as expected according to the pilot study. Thus, this finding together with that of the pilot study clearly visualised the experience that mixing of young feeder pigs of different health status can be disastrous for a large number of herds even if the number of animals carrying the infectious agent is small. On the other hand, by focusing the preventive measures towards just a relatively small number of herds (the infected farrowing herds) it was possible to improve the health status of numerous finishing herds.

On the contrary, altogether every fourth of farrowing-to-finishing herds was shown to be infected with M. hyopneumoniae. This, together with the finding that more than one half of finishing herds practising continuous flow system were infected, expressed the vulnerability of continuous flow systems in relation to transmissible infectious diseases. These types of herds made a constant risk for M. hyopneumoniae infection to other herds through animal transport, temporary selling of feeder pigs, and close neighbourhood to some herds. Consequently, finding out health status of such herds and eradication of M. hyopneumoniae from infected ones turned out to be the most essential activity in this study.

To follow the success of the screening and the eradication programmes, a survey was performed aiming at finding even a low prevalence of disease. Following measures were taken to

increase the probability of detecting positive samples: The test used was shown to be very sensitive (Sørensen et al. 1997); Slaughter pigs were chosen to be target group for sampling because of high prevalence of antibodies in this age group (Wallgren et al. 1993a; Yagihashi et al. 1993, Morris et al. 1995, Rautiainen et al. 2000b) and the fact that they represented the infectious status of all the source herds of the finishing herds; The size of the random sample was aimed to detect at least 1% prevalence (Cannon & Roe 1982); To reduce clustering of the samples, the sampling was prolonged to several weeks. The 2 positive samples and the one suspicious sample detected during the survey could all be traced back. It appeared that they were, indeed, indications of an endemic M. hyopneumoniae infection in 3 particular herds which, however, had not been selling live animals to other herds. In addition to these findings, one more screening was made in the rest of the specialised finishing herds, which practised continuous flow system, in order to find all potential residual infections of M. hyopneumoniae (2 more infected herds were found). On the other hand, the 506 negative samples (99.4%) indicated that the overall prevalence of M. hyopneumoniae had been reduced to a minimum, perhaps even to zero. That trend was also expected from the significant reduction of lung lesions to around 0.1%, since high lung lesion prevalences have been shown to be highly dependant on M. hyopneumoniae infection in Finland (Tuovinen et al. 1994a, Rautiainen et al. 2000b). In addition, it is important to notice that clinical breakdowns entitling to compensations in finishing herds have not been reported. Consequently, all these findings support the idea of success of the programme. However, only the near future will confirm the ultimate success, since some of the eradication programmes and the screening tests had taken place only very recently. Latent infections were considered hav-

shi et1998-2000 the mean daily gain increased fromet al.799 g to 875 g in specialised finishing herds

(n=150, according to slaughterhouse records, not shown in the results). This increase is in the same magnitude as in an earlier study (*Rautiainen et al.* 2000b). However, in addition to the effect of *M. hyopneumoniae* freedom, also the effect of freedom from sarcoptic mange was within the figures of the present study.

ing caused several breakdowns in the regional

eradication programmes in Switzerland (Mas-

The apparent success of the programme was seen in daily gain, too, as expected. During

serev-Wullschleger & Maurer 1998).

During the survey, it appeared that one farrowing-to-finishing herd, which had had a single positive serum sample of a finishing pig in the screening had been falsely classified as non-infected. It is known from earlier studies that problems in making a definitive diagnosis may arise with sub-populations of animals in individual herds with low pathogen load (Clark et al. 1991, Lindahl & Wallgren 1997, Rautiainen & Wallgren 2000). However, in an earlier survey, which covered 530 herds and was based on colostrum samples, no such problems existed (Rautiainen et al. 2000a), which expressed the superiority of colostrum samples for the screening of herds. The reason for the high sensitivity of colostrum samples is that when the pathogen load in a herd is low, sows have a longer period than finishing pigs to develop humoral immune response to M. hyopneumoniae which, in addition, is known to persist even for years (Rautiainen et al. 2000a).

Only a few member herds raised opposition to the screening of health status. The good motivation of the others can be seen as a result of the following modes of action: Veterinary consultation to the herd owners concerning eradication programmes was free of charge, as was the analysis of the samples; The field trial had welldefined epidemiological and economical targets, which were openly declared; Respect was paid to the good collaboration between the local practising veterinarians and the slaughterhouse company. Many of these principles were stressed already by *Waldmann & Radtke* (1937), and later by *Masserey-Wullschleger & Maurer* (1998).

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Sammanfattning

Regional sanering av Mycoplasma hyopneumoniae från svinbesättningar och dokumentation av sjukdomsfriheten.

Syftet för denna undersökning var 1) att analysera (fastställa) infektionsstatus gällande M. hyopneumoniae i alla suggbesättningar i en region, 2) att sanera varje besättning som bedöms vara infekterad med M. hvopneumoniae, 3) att följa analysernas och saneringarnas framgång. Det slutgiltiga målet var att utrota M. hyopneumoniae från alla medlemsbesättningar av ett andelsslakteri (153 smågrisproducerande besättningar + 85 integrerade besättningar + 150 specialiserade slaktsvinsbesättningar) före år 2000. Under år 1998 och 1999 analyserades totalt 5067 råmjölksprov samt 755 serumprov (i medeltal 25 prov/besättning) för antikroppar mot M. hvopneumoniae med ELISA. Antikroppar konstaterades i 208 prov (3.6%). Två smågrisproducerande besättningar (1.3%) och 20 integrerade besättningar (23.5%) bedömdes vara infekterade med M. hyopneumoniae. För dessa besättningar planerades och genomfördes ett saneringsprogram. I mars år 2000 kartlades analysernas och saneringarnas framgång. Totalt undersöktes (analyserades) 509 slumpmässigt tagna serumprov från slaktsvin. Antikroppar mot M. hyopneumoniae konstaterades inte i 506 prov, men tre prov bedömdes vara positiva eller svagt positiva. Det visade sig att 3 besättningar faktiskt var infekterade med M. hvopneumoniae. En av besättningarna visade sig vara en integrerad besättning, som tidigare felaktigt bedömts vara icke-infekterad. Två av besättningarna var slaktsvinsbesättningar med kontinuerlig produktion (KP). I motsats till slaktsvinsbesättningarna med all-in/all-out produktion på besättningsnivån, blir KP besättningarna inte spontant fria från smittsamma sjukdomarna mellan uppfödningspartier. Därför analyserades infektionsstatus också i resten av KP besättningarna (total n = 7). Sålunda konstaterades ännu 2 infekterade besättningar. Resultatet av kartläggningen tillsammans med en förminskning av prevalensen av lungskador vid slakt (från 5.2% till 0.1%) samt brist på kliniska sjukdomsutbrott tyder på, att alla besättningar var slutligt fria från M. hyopneumoniae i slutet av år 2000.

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