MYCOPLASMA HYOPNEUMONIAE – ASPECTS OF EPIDEMIOLOGY, PROTECTION AND CONTROL

by

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Academic dissertation

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SUMMARY

Observations on the course of disease
In three newly constructed all-in/all-out finishing pig herds, one to three pigs from each pen were randomly selected at allocation (final sample size, 100). These pigs were followed throughout the entire rearing period to study the course of a natural *M. hyopneumoniae* infection (assessed by detection of antibodies and magnitude of lung lesions) among multiple source fattening pigs, and to assess the negative influence of this infectious agent on average daily gain (ADG). In two out of three herds, around 80% of pigs had indications of *M. hyopneumoniae* infection until time of slaughter. Pigs in the third herd remained infection-free. In the combined data, prevalences of pneumonia at slaughter were 38%, 73% and 95% in pigs that had developed antibodies to *M. hyopneumoniae* at 33, 61 and 92 days after arrival, respectively ($\chi^2=10.37; p<0.01$). The median magnitude (score, 0-1) of affected lungs was 0.24, 0.04 and 0.01, respectively ($p=0.14$). The ADG of pigs with a non-complicated pneumonia as compared with those which remained free from *M. hyopneumoniae* was decreased by more than 60 g per day after adjusting for herd, pen, weight and sex ($p=0.010$; total n=69).

Prevalence survey
There were 1296 pig herds with more than 3 sows in the province of Vaasa during the year 1990. Random sampling conducted in two parts was used to select 150 herds for participation in the *M. hyopneumoniae* antibody survey. 112 herds subsequently started the study. A total of 1773 colostrum samples were collected and analysed. A diagnosis was determined for 90 herds. According to a drop-out analysis, censoring 22 herds did not bias results. The prevalence of herds with antibodies was 39% (95% confidence interval (CI), 29%-49%). The seropositive herds were significantly larger than the rest of the herds (mean no. of sows, 27.9 and 18.4, respectively; $p<0.001$). On average, about 49% of the pigs delivered to specialized fattening herds were possible carriers of *M. hyopneumoniae* (95% CI, 31%-67%). Thus, in practically every fattening herd, young fatteners of different health status concerning *M. hyopneumoniae* were mixed, thereby contributing to spread of infection.

Screening of herds for *M. hyopneumoniae*
The reliability of colostrum sampling to screen herds for *M. hyopneumoniae* antibodies was studied in two cohorts: in elite breeding herds (n=183) participating in the National Pig Health
Scheme, and in conventional piglet-producing breeding herds (n=347) intended for the LSO-2000 quality chain. The total number of colostrum samples analysed was 14,919. The median prevalence of sows with antibodies to *M. hyopneumoniae* in seropositive herds was 28.2% (range, 2.7%-100%; n=42). According to clinical and pathological follow-up for one year, none of the herds with ≤10% prevalence of seropositive samples was truly infected. Thus, with a sample size of 25, detection of an endemic infection with 95% probability was possible.

The sampling strategy was shown to have an effect on test results; in herds with a high prevalence of sows with antibodies, colostrum samples collected within 2 hours after farrowing were 3 times more likely to contain antibodies than samples collected 2 to 12 hours after farrowing (odds ratio, 3.0; 95% CI, 1.4-6.6). In herds with a low pathogen load among sows, samples collected from old sows (parity no. >5) were 3.3 times more likely to contain antibodies than samples collected from young sows (odds ratio, 3.3; 95% CI, 1.5-7.5). Moreover, antibodies to *M. hyopneumoniae* were able to persist up to 3 years in some sows after a successful eradication of the infection from the herd. Thus, the parity number and the history of the sows are important when interpreting the results of an antibody assay. Furthermore, because repeated freezing or improper handling (spoilage) of the colostrum samples did not cause biologically relevant problems for the ELISA, colostral sampling is feasible for monitoring and surveillance systems and field studies.

**Studies on the transmission of protection**

The transmission of protection against *M. hyopneumoniae* from sow to offspring was studied in one satellite herd of a sow pool herd. The amount of blocking (= antibody level) in sow serum collected 19 days ante- and 3 days post-partum corresponded with 67% (p<0.001; n=25) and 53% (p<0.001; n=15) of that in colostrum samples, respectively. Seven sows (28%) were classified as having a high serum antibody level at 19 days pre-farrowing, whereas 12 sows (48%) had had a high colostral antibody level. The antibody level in the serum of piglets was related to that in the colostrum of the dam. These findings illustrated a large variation in humoral immune response of both sows and piglets to *M. hyopneumoniae* in an endemically infected herd. Three groups of 10 piglets were weaned at the age of 14 days and moved to isolation units. Piglets which had received the highest amount of antibodies to *M. hyopneumoniae* by colostrum appeared to be totally protected against *M. hyopneumoniae* to up to 14 days of age. This opens up possibilities of eradicating the disease by a shorter non-
farrowing period than generally used, provided that serological status concerning antibodies to *M. hyopneumoniae* can be controlled.

**Field trial**
A field trial aimed at eradicating *M. hyopneumoniae* from all pig herds of a region was carried out among member pig herds (153 farrowing herds + 85 farrowing-to-finishing herds + 150 specialized finishing herds) of the cooperative slaughterhouse Lihakunta. 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). 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. hyopneumoniae*. Two farrowing herds (1.3%) and 20 farrowing-to-finishing herds (23.5%) were shown to be infected with *M. hyopneumoniae*. A programme to eradicate the infection from these herds was undertaken. To follow the success of the screening and the eradication programmes, a blood sample was collected systematically from every 30th pig at the slaughterhouse (total n=509) during one month’s time. Antibodies to *M. hyopneumoniae* were not detected in 506 samples, whereas three samples were considered suspicious or positive. Accordingly, three herds were shown to be infected. One of these herds was previously falsely classified as non-infected. Two of the herds were finishing herds practising a continuous flow system (CF). Unlike finishing herds, which practice all-in/all-out management routines at herd level, CF herds do not get rid of transmissible diseases spontaneously between batches, therefore, the rest of the CF herds (total n=7) were screened. Two more infected herds were detected. Another programme to eradicate infection was instigated. In addition, a decreasing prevalence of lung lesions at slaughter (from 5.2% to 0.1%) and a lack of clinical signs indicated that all member herds were finally free from *M. hyopneumoniae* by the end of the year 2000. This result reveals that all tools needed in a regional eradication programme are available today.
LIST OF ORIGINAL PAPERS

Original papers are referred to in the text by Roman numerals I–V:


1. INTRODUCTION

The services of diagnostic veterinary laboratories have traditionally been used in connection with acute outbreaks of diseases, the most striking manifestations of infections among animals. By analysing autopsies or other samples collected from a diseased animal, a diagnostician may be able to determine the immediate cause of an outbreak. However, in preventive veterinary medicine, emphasis has also been placed on screening as well as monitoring and surveillance for diseases. Screening refers to the systematic examination of individuals or groups of individuals of a population in order to ascertain the prevalence of certain characteristics that may not be apparent in any member of the population (Toma et al. 1999). Monitoring is the practice of collecting information related to health and disease in a population in an ongoing manner. Like surveillance programmes, the purpose of monitoring may be to describe the frequency of disease occurrences or the occurrence of new diseases (Toma et al. 1999). Therefore, monitoring and surveillance programmes may be able to prevent spread of infections.

In the middle of the 1990’s, the total pork production in the former province of Vaasa (hereafter denoted as Vaasa province) corresponded to over 20% of the national production (170 million kg) in Finland. The number of pig herds was 1800 and the mean number of sows per herd was 20-25. A typical specialized fattening herd of the region comprised 200-300 pigs located in one unit and practised all-in/all-out management routines. The young fatteners (Yorkshire x Landrace) generally originated from 15 to 20 different piglet-producing herds and weighed approximately 25 kg upon arrival to the fattening herd.

In 1990, a joint project was initiated by the pig industry in Vaasa province, the aims of which were to screen the pig population for the presence of prevalent pig diseases in the region, and to increase the diagnostic ability for disease prevention purposes. According to the first survey made, swine enzootic pneumonia (primarily caused by \textit{M. hyopneumoniae}) with secondary infections was the most common cause of death of finishing pigs in Vaasa province during 1990 (Rautiainen et al. 1991). The overall mortality rate per batch of pigs, however, differed from 0 to 3.8% between herds (mean, 1.4%). Every fifth pig had lung lesions recorded at slaughter, indicating production losses during rearing. To study prevention of pneumonic outbreaks during the fattening period, half of the herd owners used in-feed medication routinely at the start of rearing.
The health status among pigs in Finland is generally considered to be high. Porcine reproductive and respiratory syndrome (PRRS), Aujeszky’s disease (AD) or swine influenza (SI), all of which are common respiratory diseases of pigs globally, have never been reported in Finland (Anon. 2000). Progressive atrophic rhinitis (PAR) has been diagnosed only sporadically (Anon. 2000). Antibodies to Actinobacillus pleuropneumoniae (serotype 2), the causative agent of fibrinous pneumonia and pleuritis in pigs, have commonly been demonstrated in Finnish pig herds, but these infections are generally subclinical (Levonen et al. 1994, 1996). Consequently, M. hyopneumoniae is considered the most important respiratory pathogen of pigs in Finland. Work against swine enzootic pneumonia (SEP) should thus be an essential part of herd health programmes which aim to decrease detrimental effects of diseases.

According to earlier studies, the importance of SEP for pig production is well known. Still, estimates of the negative effect of SEP on average daily gain (ADG) was shown to vary from negligible up to 44% in different studies (Straw et al. 1989). This raised speculations whether preventive measures against SEP could be directed towards improvements in the environment and in management instead of towards preventing infection of M. hyopneumoniae.

M. hyopneumoniae is considered ubiquitous in pig herds and latent, subclinical infections occur frequently (Ross 1999). Several health control systems aimed at eliminating M. hyopneumoniae infections have been developed (Goodwin & Whittlestone 1967; Keller 1980; Schulman 1980). Subclinical M. hyopneumoniae infections have constituted the main problem in these systems, thus indicating the need for a sensitive diagnostic test (Keller 1980). Another concern has been the high reinfection rate of herds (Goodwin 1985). In Finland, until the end of the 1990’s, transmission of infectious diseases was hampered by small herd sizes and low overall pig density. Thus, similar to in Southwestern Finland (Tuovinen et al. 1994b), a prevalence study using serology was considered necessary in sow herds of Vaasa province to stimulate discussion about different health control strategies.

Demonstration of specific antibodies in colostrum by ELISA, as a screening method for M. hyopneumoniae, was introduced by Zimmermann et al. (1986) and has also been applied in Finland by Levonen (1994). According to earlier observations, the serologic profile varied markedly between herds expressing antibodies and did not appear to be associated with the
clinical outcome of the infection. Age of the sow, time of sampling in comparison with farrowing, and improper handling of samples were speculated to have an effect on the demonstration of antibodies in colostrum and, thus, on the reliability of analysis. On the other hand, if the varying response of sows to \textit{M. hyopneumoniae} was real, it might indicate that some sows in a herd are immune while others remain in a carrier state. Consequently, a potential risk for failure exists in eradication programmes that rely on maternal protection of piglets against \textit{M. hyopneumoniae}.

Several studies were planned to find answers for the above questions. In the course of the studies, the ultimate goal was widened to include a working strategy for eradicating \textit{M. hyopneumoniae} from a particular geographical region to minimize the possibility of reinfections.
2. AIMS OF THE STUDY

An attempt was made to get new information about the prevalence of *M. hyopneumoniae* in the densely pig-populated Vaasa province as well as to document the negative influence of this infectious agent on ADG in newly constructed finishing herds. This information was used to scrutinize eventual needs for different control strategies against *M. hyopneumoniae* infections in pork production. The aims were:

1. To study the course of a natural *M. hyopneumoniae* infection (assessed by detection of antibodies and magnitude of lung lesions) among multiple source fattening pigs and to assess the negative influence of this infectious agent on ADG.

2. To estimate the prevalence of *M. hyopneumoniae* in sow herds in Vaasa province based on the demonstration of antibodies in colostrum.

3. To study the reliability of antibody analysis in colostrum in detecting the presence of *M. hyopneumoniae* in herds.

4. To study the transmission of protection against *M. hyopneumoniae* from sow to offspring, which could then be exploited in eradication programmes.

5. To determine whether the available diagnostic tools and the current eradication programmes are sufficient for systematically eradicating *M. hyopneumoniae* from an entire community.
3. LITERATURE REVIEW

3.1. *M. hyopneumoniae* in piglet-producing herds

3.1.1. Clinical outcome

Under field conditions, mycoplasmal pneumonia is characterized by a high morbidity and a low mortality. The principal clinical sign is coughing. Onset of the disease is gradual, with coughing continuing for a few weeks or even months, although some affected pigs evidence little or no coughing. Animals with secondary bacterial infections may manifest inappetence, laboured breathing, increased coughing, elevated temperatures and prostration (Ross 1999).

Goodwin (1984) reported early signs and incubation periods in 50 breakdowns of previously *M. hyopneumoniae*-free herds. The first signs were either coughing in growing pigs (52% of outbreaks), illness in adult stock (34%) or pneumonia in routinely slaughtered pigs (14%). Incubation periods of six weeks to six months or more were observed. His findings clearly expressed the difficulties in early case-finding of *M. hyopneumoniae* infections if surveillance is based on clinical signs alone.

3.1.2. Protective immunity against *M. hyopneumoniae*

All age groups have been suggested to be equally susceptible to mycoplasmal pneumonia (Piffer & Ross 1984; Kobisch et al. 1993), although the peak prevalence of the infection often takes place in growing and finishing pigs (Wallgren et al. 1993a; Yagihashi et al. 1993; Morris et al. 1995b). *M. hyopneumoniae* has been detected in lung tissue of piglets as young as 2 weeks and expressing signs of respiratory disease (Holmgren 1974). However, the age when piglets become infected by *M. hyopneumoniae* depends on the balance between protective immunoglobulins received by colostrum and the pathogen load of the herd (Wallgren et al. 1998). The amount of maternally derived serum immunoglobulins in piglets varies considerably and is associated with the immunoglobulin level in the colostrum of the dam (Kavanagh 1994; Morris et al. 1994; Wallgren et al. 1998). In some litters, these antibodies may be reduced in the serum at 2 weeks of age, in other litters they may persist up to 6.5 to 9 weeks of age (Mori et al. 1987; Morris et al. 1994; Wallgren et al. 1998).

A protective and long-lasting immunity is generally achieved among pigs that have recovered from mycoplasmosis (Goodwin et al. 1969; Kobisch et al. 1993). However, as individual pigs will be exposed to *M. hyopneumoniae* at different times during field conditions, the immune
status towards *M. hyopneumoniae* may vary within a herd. Further, general immune responses vary considerably between pigs due to genetic differences (Edfors-Lilja et al. 1991). Consequently, large variations between individuals can be seen in the course of pneumonia when pigs are exposed to *M. hyopneumoniae* (Goodwin & Whittlestone 1973). Some individuals may become immune, while others remain in a carrier state, having no clinical signs of disease. The latter category of sows has been proven capable of infecting its offspring before weaning (Goodwin 1965; Clark et al. 1991). Generally, the risk for being a carrier of *M. hyopneumoniae* is markedly reduced when an animal is over one year of age, a fact that is commonly utilized in eradication programmes (Waldmann & Radtke 1937; Zimmermann et al. 1989). Still, according to Wallgren et al. (1998), sows may become more susceptible to *M. hyopneumoniae* at the time of farrowing because protective antibodies are transferred from the blood to the udder during the last month of pregnancy (Jönsson 1973; Wallgren et al. 1998). The practical relevance of this observation in eradication programmes has, however, not been reported. In conclusion, immunity and infection status towards mycoplasmosis may vary greatly between animals within a herd.

### 3.2. *M. hyopneumoniae* in fattening herds

#### 3.2.1. Transmission of *M. hyopneumoniae* assessed by detection of antibodies

Transmission of *M. hyopneumoniae* is apparently effected mainly by direct contact with respiratory tract secretions from infected pigs. Carrier pigs are the major source of infection (Ross 1999). The use of ELISA has made it possible to follow the seroepidemiology of *M. hyopneumoniae* infection during the fattening period, the most critical time for the disease. In field conditions, detection of antibodies has been reported at 3 to 5 weeks post-exposure, with the peak occurring at 10 to 14 weeks post-infection (Sheldrake et al. 1990; Sørensen et al. 1993; Wallgren et al. 1993; Morris et al. 1995b). At the start of the fattening period, antibodies to *M. hyopneumoniae* are generally detected in less than 25% of animals and sometimes in no animals at all (Sheldrake et al. 1990; Sørensen et al. 1993; Wallgren et al. 1993; Yagihashi et al. 1993; Morris et al. 1995b). At slaughter-weight, prevalences of 75% to 100% are commonly found (Sheldrake et al. 1990; Sørensen et al. 1993; Wallgren et al. 1993; Yagihashi et al. 1993; Vraa-Andersen 1994), indicating that nearly all pigs get infected during rearing. Nevertheless, in some studies, the transmission of *M. hyopneumoniae* among fattening pigs, assessed by detection of antibodies, has been very limited (Morris et al. 1995b; Lindahl & Wallgren 1997), suggesting that management routines might effectively diminish transmission.
3.2.2. Pneumonic lesions at slaughter

Macroscopic lesions of mycoplasmal pneumonia consist of a catarrhal bronchopneumonia and are located in the ventral portions of the apical and cardiac lobes, the accessory lobe and the cranial portion of the caudal lobes of the lungs (Ross 1999). Antibodies to *M. hyopneumoniae*, assessed by a complement fixation test (CF), have been shown to be strongly associated with macroscopic pneumonic lesions of finishing pigs detected at slaughter (Van Til et al. 1991). Yet, slaughterpigs seropositive by ELISA do not always express such lesions (Wallgren et al. 1994). Furthermore, *M. hyopneumoniae* has been cultured or visualized in indirect immunofluorescent (IF)-stained lung sections from lungs that showed no macroscopic pneumonic lesions (Goodwin 1972; Armstrong et al. 1984; Clark et al. 1991). In addition, Wallgren et al. (1994) demonstrated that despite an 85% prevalence of macroscopic pneumonic lesions in detailed inspection, only a 13% prevalence was reported in routine meat inspection. These discrepancies are seemingly due to a number of variables in the environment being involved in the production of SEP (Roberts 1974; Van Til et al. 1991), and standard meat inspection being insufficient for research purposes.

When investigating the magnitude of pneumonia in affected lungs, it is necessary to use a lung scoring technique that is repeatable, appropriate for the purposes of the study and facilitates statistical analysis (Morrison et al. 1985). Several standardized techniques have been suggested. Morrison et al. (1985) proposed a technique which evaluated the percentage of each lung lobe macroscopically involved in the pneumonic process adjusted to the lobe’s relative weight in the normal lung. These percentages were then summed to equal the total percentage of lung affected by macroscopic pneumonia. Some authors have proposed a substantial agreement between rapid gross examination and detailed inspection of lungs (Hurnik et al. 1993; Davies et al. 1995), or between right lung examination and examination of the entire pluck (Mousing & Christensen 1993). Morrison et al. (1985) even proposed that assessing the prevalence of affected lungs or evaluating the maximally affected lung was equally informative as a herd-based indicator as scoring the percentage of each lung and calculating the mean. Regardless of the scoring method used, slaughter examination as such has been shown to be a poor indicator of life-time pneumonia of an individual pig, since lesions have been found to progress and regress dynamically throughout the life of a pig (Noyes et al. 1990; Kobisch et al. 1993), a result also supported by serological investigations.
Thus, slaughter examination alone is insufficient for the demonstration of the effect of *M. hyopneumoniae* infection during the fattening period.

### 3.2.3. Reduced average daily gain (ADG)

According to Pointon et al. (1985), the growth rate of pigs held in contact with *M. hyopneumoniae*-inoculated pigs was reduced by 12.7% between 50 to 85 kg bodyweight. In a field trial with multiple source finishing pigs, the negative effect of a natural *M. hyopneumoniae* infection on ADG was estimated to be 24 grams, or 2.9% (Tuovinen et al. 1994a). In numerous other studies, attempts have been made to determine the expected reduction in growth rate caused by *M. hyopneumoniae* infection that is associated with the severity of pneumonia, as determined by lesions recorded at slaughter. According to nine studies, estimates of the decrease in ADG of finishing pigs have varied broadly, ranging from 2.8% to 44.1% (median, 15.9%), as reviewed by Straw et al. (1989). However, debate has occurred as to whether ADG is more strongly correlated with life-time pneumonia assessed by radiography or clinical signs than with pneumonic lesions at slaughter (Noyes et al. 1990; Clark et al. 1993; Morris et al. 1995a; Sitjar et al. 1996). Another concern that might make estimations of decreased ADG due to *M. hyopneumoniae* infection difficult has been the effect of ambient environment on the development of pneumonia. Done (1991) concluded that the environment of each pig house comprises many components which determine whether disease occurs as well as its course, severity and outcome. Straw et al. (1990) doubted that it would be possible to separate environmental and disease effects, and they suggested that pneumonia may be a reflection of management factors in the herd. Scheidt et al. (1990) debated whether common respiratory tract diseases affect growth performance of pigs at all. Recently, clinical trials with modern *M. hyopneumoniae* vaccines have confirmed that control of the specific causative agent of SEP is often beneficial regarding ADG (Baekbo 2000).

### 3.2.4. Interaction with other respiratory pathogens

*M. hyopneumoniae* is the primary aetiological agent of SEP (Goodwin et al. 1965; Mare & Switzer 1965). In addition to complex interactions between the mycoplasma and poor management with poor environmental conditions, reduced performance associated with disease is influenced by interactions with other infections (Ross 1999). In experimental infections, *M. hyopneumoniae* has been shown to predispose to infections with *Pasteurella multocida* (Smith et al. 1973; Ciprián et al. 1988; Amass et al. 1994), and to potentiate infections with *Actinobacillus pleuropneumoniae* (Yagihashi et al. 1984), PRRS-virus
Thacker et al. 1999) and possibly pseudorabies virus (Shibata et al. 1998). These findings strongly support the suggestion that *M. hyopneumoniae* is immunosuppressive (Ross 1999), and therefore not only a threat to the lungs but also to the overall health status of pigs.

### 3.3. Aspects of diagnostic methods in screening of herds

#### 3.3.1. Basic assumptions

Surveys conducted in a variety of countries usually express the prevalence of lesions typical for SEP in slaughter-weight pigs (Ross 1999). Yet, when dealing with respiratory diseases, a “case” can either be a farm or an individual animal. In addition, the outcome of the disease can be either clinical or subclinical (Stärk 2000). Thus, selection of the unit of concern and definition of a case are basic assumptions to be made when dealing with interpretation of prevalence studies. Basically, four diagnostic approaches can be used to define a case of respiratory disease (Stärk 2000): definition by clinical signs, by lung scoring at slaughter or during post-mortem investigations, by serological analysis, or by demonstration of the agent by cultivation or by polymerase chain reaction (PCR). The usefulness of these outcomes primarily depends on the study objectives. A combination of different techniques is often required to obtain high sensitivity and specificity at diagnosing (Stärk 2000).

#### 3.3.2. Clinical and pathological investigations

According to Sørensen et al. (1993), coughing was found to be the most reliable clinical indicator of reinfection in Danish SPF production herds, but surveillance through herd inspections alone failed to detect 30% of infected herds. Goodwin (1984) reported that coughing in growing pigs was the first sign in only 52% of breakdowns in herds participating in a national health control scheme in Britain. Correspondingly, pneumonia in routinely slaughtered pigs was the first sign in no more than 14% of breakdowns (Goodwin 1984). Recently, Masserey-Wullschleger and Maurer (1998) used clinical inspection combined with pathological examination to screen health status of all breeding herds (n=122) in two pig-dense regions in Switzerland. They ended up with prevalences of 47.6% and 33.9% of endemically infected herds, respectively. These figures were used in a regional eradication programme and therefore could not be verified using other diagnostic methods.
3.3.3. Demonstration of antibodies

Recent seroepidemiological surveys for *M. hyopneumoniae* are based on enzyme-linked immunosorbent assay (ELISA), first reported by Bruggmann et al. (1977). Compared with earlier methods, such as indirect hemagglutination (IH) and complement fixation (CF) tests, the ELISA is generally more sensitive (Armstrong et al. 1983) and antibodies can be demonstrated over a longer period, *e.g.*, one year post-exposure (Armstrong et al. 1983; Bereiter et al. 1990). Several authors (Nicolet et al. 1980; Freeman et al. 1984; Mori et al. 1988) have been concerned with cross-reactions between *M. hyopneumoniae* and *M. flocculare* (Friis 1974; Strasser et al. 1992), a common non-pathogenic mycoplasma with antigenic similarities to *M. hyopneumoniae*. However, based on serodiagnostic tests, it seems likely that pigs naturally infected with *M. flocculare* will have insufficient levels of antibodies to cross-react with *M. hyopneumoniae* (Armstrong et al. 1987; Sheldrake & Romalis 1992). Moreover, by using monoclonal antibodies in a blocking ELISA (Feld et al. 1992; Le Potier et al. 1994) or a double-sandwich ELISA (Mori et al. 1987), cross-reactions between *M. hyopneumoniae* and other mycoplasmas, namely *M. flocculare* and *M. hyorhinis*, which are common inhabitants of the pig’s respiratory tract, have been markedly reduced. However, cross-reactions can not be totally excluded.

Sørensen et al. (1993) and Levonen (1994) used ELISA for screening of health-controlled herds believed to be free from *M. hyopneumoniae*. In both studies, subclinical infections were detected, which illustrates the need for serological testing in pig health control programmes aimed at documenting the absence of *M. hyopneumoniae*. Volmer et al. (1994) and Horst et al. (1997) reported on serological surveys done in conventional German pig herds based on colostral antibody assays by ELISA. The selection of herds was non-random and sample sizes per herd tended to be small (4-13 samples). Thus, the result of varying antibody prevalences (57.1%, 7.6% and 61.9%) in different populations of herds was difficult to interpret. Tuovinen et al. (1994b) used a random sample of 100 herds and a mean sample size of 22 colostrum samples per herd to study the prevalence of *M. hyopneumoniae* antibodies in farrowing herds in Southwestern Finland. Antibodies were detected in 8% of herds, which later appeared to be a fairly accurate estimate of the prevalence of herds truly infected with *M. hyopneumoniae* in that region (Tuovinen et al. 1996).
The use of sow colostrum instead of serum for antibody detection has several advantages when aiming to document the presence or absence of a disease at herd level:

1) Colostrum samples are easily collected by the herd managers and the samples can be stored frozen for later usage. In addition, colostrum sampling is considered less stressful for the animals than blood sampling (Tenhagen et al. 1995; Eichhorn & Frost 1997);

2) Antibodies to *M. hyopneumoniae*, detected by ELISA, may persist for up to one year (Armstrong et al. 1983; Bereiter et al. 1990), which makes sows a suitable target group for serological surveys;

3) Up to 90% of the immunoglobulin content of colostrum is of serum origin (Bourne & Curtis 1973);

4) At the time of farrowing, colostrum contains a higher concentration of antibodies than serum (Eberli 1987; Morris et al. 1994; Sørensen et al. 1993; Yagihashi et al. 1993). This is due to the antibody level in the serum of sows being related to the stage of pregnancy and continuously decreasing during the month prior to parturition (Jönsson 1973; Wallgren et al. 1998). Further, the IgA in colostrum (10-15% of the total colostral immunoglobulins) is for the most part produced in the mammary gland (Bourne & Curtis 1973).

However, the quality of colostrum must be taken into consideration in immunological testing. The level of IgG, the main immunoglobulin of sow colostrum, has been shown to decrease rapidly, namely by 66% and 80% during the first 12 and 24 hours after farrowing, respectively (Curtis & Bourne 1971; Klobasa & Butler 1987). Furthermore, some reports have indicated that different lobes of the mammary gland may contain different levels of immunoglobulins. However, variation of immunoglobulin levels between individual sows is obviously larger than that between different lobes (Wu et al. 1980; Klobasa & Butler 1987). Since the amount of serum proteins increases with age (Friendship et al. 1984), the age of the principals ought to be considered as well. Klobasa and Butler (1987) reported a tendency for ≥5th parity sows to have more antibodies in colostrum than younger sows. Finally, Bouwkamp et al. (1993) and Levonen et al. (1996b) have reported that spoilage of colostrum might cause false-positive reactions when analysed by blocking ELISA. In conclusion, several biological and physical properties of colostrum samples might have an effect on the reliability of test results.
3.3.4. Demonstration of *M. hyopneumoniae*

*M. hyopneumoniae* was shown to be the causative agent of SEP by Goodwin et al. (1965) and Mare and Switzer (1965). Later, recommendations to facilitate the primary isolation of the agent were given by Friis (1975). However, isolation of the organism is still considered complicated because of its fastidious nature and slow growth. Furthermore, isolation is rarely successful in samples (nasal swabs) from live animals (Mattsson et al. 1995; Sørensen et al. 1997). For these reasons, cultivation is not a sufficient method for surveillance programmes. Fluorescent antibody (FA) technique is more practical than cultivation for diagnosing acute and subacute pneumonias caused by *M. hyopneumoniae* (Meyling 1971), but is unsuitable for surveillance programmes for practical reasons.

Several DNA hybridization and polymerase chain reaction (PCR) methods for the detection of *M. hyopneumoniae* DNA have been demonstrated (Stemke 1989; Harasawa et al. 1991; Artiushin et al. 1993; Mattsson et al. 1995; Blanchard et al. 1996; Baumeister et al. 1998). PCR has proven to be a fast, sensitive and specific method when used in experimentally infected, autopsied animals at an acute stage of the disease (Mattsson et al. 1995; Sørensen et al. 1997). However, the sensitivity has been much lower when nasal swabs (Mattsson et al. 1995) or lung washings (Baumeister et al. 1998) collected from naturally infected animals have been tested. Nested-PCR has been shown to be much more sensitive than conventional PCR, and thus could be better suited for detection of *M. hyopneumoniae* from nasal swabs (Calsamiglia et al. 1999; Calsamiglia & Pijoan 2000).

3.4. Control and eradication

Done (1991) has summarized four main groups of environmental factors associated with high levels of clinical pneumonia: meteorological factors, population and social factors, management factors and airborne pollution. Both Done (1991) and Straw (1992) have listed environmental variables and management routines that can be altered to reduce the incidence of pneumonia. According to them, introduction of pigs from multiple source herds, continuous flow of pigs through facilities, and high stocking densities are particularly high-risk factors for pigs. Health-matching of purchased multiple source feeder pigs was found effective by Tuovinen et al. (1994a) in preventing *M. hyopneumoniae* infection and pneumonia.
Valnemulin (Hannan et al. 1997), tiamulin and enrofloxacin (Friis & Szancer 1994; Hannan et al. 1997) have been shown to be the most potent antimicrobials against *M. hyopneumoniae*. In addition, lincomycin, tetracycline and tylosine were able to inhibit the growth of the agent *in vitro* (Friis & Szancer 1994). Using medications, the best response in reducing pneumonia is usually experienced where treatment is introduced early either to coughing individuals (Kavanagh 1992), or to clinically ill pigs and pen-mates, parenterally and in-feed, respectively (Bousquet et al. 1998).

According to studies reviewed by Baekbo (2000), a marked and significant reduction in the prevalence and extent of pneumonic lesions and an increase in daily weight gain by 20 to 40 grams have been achieved using modern vaccines against *M. hyopneumoniae*. However, some studies have failed to demonstrate a significant positive effect of vaccination (Baekbo 2000).

Segregated early weaning (SEW) techniques aim to procure piglets free of endemic pathogens, such as *M. hyopneumoniae*, by weaning and then transfer them to new facilities at the age of 5 to 21 days, often combined with a strategic antimicrobial administration (Alexander et al. 1980; Harris 1988, 1990). Recently, it has been suggested that medication is not a prerequisite for the control of *M. hyopneumoniae* when employing SEW techniques (Clark et al. 1994; Dritz et al. 1996). In fact, the protective immunity acquired by pigs that have recovered from mycoplasmosis (= adult breeding stock) is commonly exploited in eradication programmes that are based on removal of young animals from the herd and medication of the breeding stock (Waldmann & Radtke 1937; Zimmermann et al. 1989; Wallgren et al. 1993b). Unfortunately, reinfections are likely to take place in pig-dense areas, where risk for airborne infections is high (Goodwin 1985; Stärk et al. 1992; Thomsen et al. 1992).
4. MATERIALS AND METHODS

4.1. Selection of herds and animals

The three all-in/all-out fattening herds (A, B and C) in the observational study on the course of *M. hyopneumoniae* infection (I) were selected as typical modern fattening herds of the region. Because the herds had a similar disposition, a similar initial health status was expected. All buildings were less than four years old and mechanically ventilated. Manure was handled as a liquid; thus, very little bedding was used. Pen dividers were only partly solid. The pigs were offered liquid feed 3 to 4 times per day in a trough shared by two pens, and had free access to water during the entire rearing period. Avoparcine (14 mg per kg dry matter; Avotan®, American Cyanamid, Wayne N.J., USA), but no other antimicrobial substance, was added to the feed. Anthelmintics (morantel tartrat 12.5 mg per kg body weight, Farantel®, Orion-Farmos, Turku, Finland) were administered to the feed once during the first week after arrival.

The animals (242 + 176 + 200 pigs) originated from multiple source herds and were reared from a weight of 25 to 105 kg. The pigs were segregated by body weight (but not by sex) into different pens on arrival. On day 5 after arrival to the fattening herds, 1 to 3 pigs from each pen were randomly selected to participate in the study. These pigs (n=36 per herd) were given an identity number. The total sample size of 108 was calculated according to Snedecor and Cochran (1980) to allow the detection of at least a 50 g difference in ADG between the healthy and the non-healthy pigs. A pig was defined as healthy when:

a) no macroscopic pneumonic lesions were detected at slaughter; and

b) no antibodies to *M. hyopneumoniae* were detected in any of the serum samples collected (n=5 per pig).

All other pigs were defined as non-healthy.

According to customer registers of 5 slaughterhouses, the total number of pig herds with more than 3 sows was 1296 in Vaasa province during the year 1990. Every herd was given a number. Random sampling conducted in two parts was used to select 150 herds for participation in *M. hyopneumoniae* antibody survey (II). Thirty-six herd owners from the first random sample refused to participate in the survey. Half of them had an acceptable reason (going to leave the business in near future). Therefore, a second random sample of 16 herds was taken. Information was collected for the herds about the numbers of animals, postal codes
(indicating geographical location) and reasons for a possible rejection from the survey. Finally, 112 herds participated in the study. A dropout analysis was done of those herds whose owners were unwilling to send samples during the study.

The reliability of colostrum sampling to screen herds for *M. hyopneumoniae* antibodies (III) was studied in two cohorts: in elite breeding herds (n=183) participating in the National Pig Health Scheme (Anon. 1997), and in conventional piglet-producing breeding herds (n=347) intended for the LSO-2000 quality chain (Tuovinen et al. 1996). The total number of colostrum samples collected was 14,919. Health monitoring was similar in both cohorts and was based on quarterly veterinary inspections of the breeding herds, one veterinary inspection per batch of grow-finishing pigs at phenotype testing stations or fattening herds, and inspection of lungs for lesions typical for SEP at slaughter (body weight 105 kg).

Persistence of colostral antibodies to *M. hyopneumoniae* in sows following a successful eradication programme (III) was studied in one elite breeding herd (n=35). The herd was monitored until the last sow in the programme was slaughtered, i.e. for a period of 3.5 years.

The transmission of protection against *M. hyopneumoniae* from sow to offspring (IV) was studied in a sow pool herd (Holmgren & Gerth-Löfstedt 1992) comprising 600 sows. The herd consisted of a central unit and eight farrowing units (satellite herds), and had been infected with *M. hyopneumoniae* 1.5 years before the study. By the time of the study, the breeding stock was free from clinical signs of mycoplasmosis, while clinically, pathologically and serologically verified SEP was present among fatteners originating from the pool and not being mixed with pigs from other sources. The follow-up of the study took place in one satellite herd that had received a group of sows three weeks prior to expected farrowing. Blood and colostrum samples were collected from 25 different parity sows. During the first week after farrowing, three of the smallest piglets in each litter were given an identity number. None of these piglets were cross-fostered. At a median age of 14 days (range, 11-14 days), 30 apparently healthy piglets, comprising the smallest piglets in each litter (median weight, 4.0 kg; range, 2.0-5.5. kg), were weaned. They were divided into three groups according to the level of antibodies to *M. hyopneumoniae* in the colostrum of their dams (Table 1).
Each experimental group was transported to a separate isolation unit. The pigs were slaughtered at a mean age of 136 days in a clean environment without contact with other pigs and without scalding. The littermates to the experimental pigs served as a control (n=270). They were transferred from the satellite herd to a fattening herd at an age of 12 weeks without being mixed with any other pigs.

Table 1. Immune status of the dams (assessed by antibody level in colostrum) of piglets in the experimental groups.

<table>
<thead>
<tr>
<th>Immune status for <em>M. hyopneumoniae</em> in dams</th>
<th>No. of piglets weaned from these dams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
</tr>
<tr>
<td>High responders (n=5)</td>
<td>10</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
</tr>
<tr>
<td>Low responders (n=5)</td>
<td>10</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
</tr>
<tr>
<td>A mixed response</td>
<td></td>
</tr>
<tr>
<td>High responders (n=2)</td>
<td>5</td>
</tr>
<tr>
<td>Low responders (n=2)</td>
<td>5</td>
</tr>
</tbody>
</table>

The field trial focused on eradicating *M. hyopneumoniae* from all pigs herds of a slaughterhouse company (V) was carried out among member pig herds of the cooperative slaughterhouse Lihakunta, which operates in Eastern and Northern Finland and comprises 7% of the national pork production. The numbers of farrowing and farrowing-to-finishing herds in the study were 153 and 85, respectively, and the number of specialized 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). According to a pilot study, pigs in roughly half of the finishing herds were infected with *M. hyopneumoniae*. However, before the field trial, the prevalence of piglet-producing herds, which were transmitting the infection to the finishing herds, was estimated to be as low as 4-5%.
To follow the success of the screening and the eradication programmes (V), slaughter-weight pigs were randomly sampled with the intention of detecting at least one sample with antibodies to *M. hyopneumoniae* with 99% confidence, if the prevalence of positive samples was at least 1% (Cannon and Roe 1982). Thus, a blood sample was collected systematically from every 30th pig at the slaughterhouse (total n=509) during one month’s time.

### 4.2. Weight measurements of fattening pigs

The experimental fattening pigs (I) were individually weighed on days 5, 33, 61 and 92 after arrival using standard mobile scales. The ADG for each pig and period was calculated. To estimate the final live weight of pigs slaughtered before day 92, the carcass weight was divided by 0.74 (average carcass weight was 74% of live weight).

### 4.3. Lung inspection and demonstration of *M. hyopneumoniae*

Lungs of the experimental fattening pigs (I) were individually marked at slaughter and sent to the laboratory. On the day of slaughter, the lungs were palpated and the magnitude of the condensed area was estimated visually by an examiner, who at that time was unaware of the serological status of the pigs. The total extent of pneumonic lesions (score, 0–1) was registered by a specially developed technique (I) that had been proven to have a high degree of repeatability, the limits of agreement between two consecutive inspections being -0.02 and 0.02 as calculated by Bland and Altman (1986). To confirm the diagnosis of pneumonia, all lungs with macroscopic pneumonic lesions were examined histopathologically. Tissue samples were treated using standard methods (I) and stained with hematoxylin and eosin. Samples with moderate lesions typical for mycoplasmosis (Jubb et al. 1993) were defined as being pneumonic. Pleural adhesions were recorded macroscopically and lungs were classified as having or not having pleural adhesions.

Lungs from the experimental early-weaned pigs (IV) as well as seven lungs from the control pigs were examined macroscopically for lung lesions. From each lung, tissue samples (1 cm$^3$) were collected from four standard spots (the middle of the left and right apical and cardiac lobes), as well as from areas with pathological lesions. These samples were treated and stained similarly as above, and examined histopathologically.

From each lung of the early-weaned pigs (IV), two bronchial swabs were taken from the large bronchi with sterile cotton swabs (no samples were collected from control pigs). The samples
were frozen (-18°C) in 1 ml of mycoplasma medium and later cultivated for *M. hyopneumoniae* at the National Veterinary and Food Research Institute, Department of Virology, Helsinki, using the methods described by Friis (1975) and Friis et al. (1991). The distal part of each right cardiac lobe was frozen (-18°C) and later examined by polymerase chain reaction (PCR) technique at the National Veterinary Institute, Uppsala, Sweden, using the method described by Mattsson et al. (1995) to detect *M. hyopneumoniae*.

In the field trial (V), lung lesions were registered and reported continuously for all member herds by the meat inspection team at the abattoir (Anon. 1995).

### 4.4. Collection of blood and colostrum samples

Fattening pigs (I) were bled on days 5, 33, 61 and 92 after arrival to the fattening herds. A final blood sample was collected upon exanguination at slaughter. Early-weaned piglets (IV) were bled at the ages of 22, 56 and 91 days. A final blood sample was collected upon exanguination at slaughter. Blood was collected from the sows in study IV at a median of 19 days before farrowing (n=25; range, 14-27 days), and at a median of 3 days post-partum (n=15; range, 1-7 days). These clotted blood samples were centrifuged 3500 x g for 10 minutes (Heraeus Sepatech, Megafuge 1.0, Germany), and the sera were frozen at -18°C until assayed.

Blood samples from pigs over 10 weeks of age were collected by practising veterinarians (III, V) or consulting veterinarians (V). The survey samples of slaughter-weight pigs (V) were collected at exanguination. Blood samples in studies III and V were refrigerated without separating the serum. A batch of samples was sent to the laboratory daily or every second to third day.

Colostrum samples (II, III, IV, V) without additives were collected by the herd managers into 10 ml plastic tubes on the day of farrowing. Information was collected about the identity number of the sow, the date of farrowing, the parity number, the time of the first piglet born and the time of sampling. The samples were stored in home freezers (-18°C) until being sent to the laboratory in batches of 15-30 samples. A majority of samples had been stored several months (up to 5 months) before being analysed. A sample from each sow was expected, though no more than 30 samples per herd from large herds. With this sample size, it was possible to find with 95% confidence at least one sample with antibodies in any size of herd,
if the prevalence of samples with antibodies was at least 10% (Cannon & Roe 1982). Before the analysis, all colostrum samples were centrifuged at 5500 x g (Heraeus Sepatech, Megafuge 1.0, Germany) for 15 minutes in 5 ml plastic tubes (Sarstedt®, Nümbrecht, Germany), and the fatty layer was removed by a vacuum-connected pipette.

4.5. Antibody assays

For the detection of antibodies to *M. hyopneumoniae* in serum or colostral whey, three different antibody assays were used:

1. Experiments I, III, IV, V: A monoclonal blocking ELISA (B-ELISA) described by Feld et al. (1992) was used (*Mycoplasma hyopneumoniae ELISA®, DAKO, Glostrup, Denmark*). At a cut-off value of 50% blocking, the sensitivity and specificity of the ELISA (with 95% confidence intervals) have been reported to be 100% (98-100%) and 100% (93-100%), respectively (Sørensen et al. 1997).

2. Experiment II: An indirect ELISA (I-ELISA) described by Bommeli and Nicolet (1983) was used (Chekit® Hyoptest, Dr. Bommeli AG, Liebefeld-Bern, Switzerland). While the sensitivity of the test is considered high (Zimmermann et al. 1986; Levonen 1994), the specificity is reported to be low (Levonen 1994).

3. Experiment IV: As a supplementary test to the blocking ELISA, an indirect ELISA with a tween-sonicated antigen (Tween-ELISA) was used (Bölske et al. 1990; Wallgren et al. 1992). Sera diluted to 1:100 in PBS-Tween with an optic density of ≥ 0.500 at a wavelength of 450 nm were classified as expressing antibodies. This assay was done at the National Veterinary Institute, Uppsala, Sweden. According to Wallgren et al. (1996), the Tween-ELISA was more sensitive than the B-ELISA when a group of pigs naturally infected with *M. hyopneumoniae* was blood-sampled repeatedly.

4.6. Analysis of some characteristics of colostrum samples

The influence of contaminants of colostrum on test results (III) was analysed by centrifuging samples for either 30 or 60 minutes. In addition, the effect of repeated freezing (10 times) and spoilage of colostrum samples was tested by deliberately handling samples in improper ways (III). Before the manipulations, the samples were standardized for the age of the sows from which the samples were collected, and for the infection status of the corresponding herds. The sample sizes used allowed detection of differences of at least 3-4 percentage units in the blocking values between the manipulated and the control samples (Martin et al. 1987). The influence of varying fat content, as measured by a standard line measurer (Vis, Inox, 0.05
mm), was also analysed. To prevent plate-to-plate variation, manipulated and control samples were analysed in parallel on the same microtiter plates.

4.7. Eradication programmes

The eradication programmes for individual herds (V) 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 combined with a 14 days piglet-free period (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 two batches of pigs (Goodwin 1985), when all-in/all-out management routines are practised at herd level.

4.8. Statistical methods

The 95% confidence intervals were calculated both for continuous normally distributed data (I) and for binomial data (I, III). The univariate analysis methods used included $\chi^2$-test (II, III), $\chi^2$-test for trend (I), Student’s t-test for continuous data in two groups (II), paired t-test for normally distributed data (III), Wilcoxon’s signed-rank test for non-normally distributed data (IV), two-tailed Mann-Whitney U-test for non-normally distributed data (III, IV) and Kruskal-Wallis one-way analysis of variance for non-normally distributed data (I, IV). The multivariate methods included multiple logistic regression (III) and the mixed-effects nested-design general linear model (I). Correlations were investigated by Pearson’s correlation (III, V). Calculations were mainly performed by the statistical packages Statistix 4.1 and Statistix for Windows® (Analytical Software, Tallahassee, FL). In addition, SPSS 7.5 (SPSS Inc., Chicago, IL) was used for analysis of the mixed-effects nested-design model (I), and Epi Info® 6.0 (CDC, Atlanta, GA) for the calculation of $\chi^2$-test for trend (I).
5. RESULTS

5.1. OBSERVATIONS ON THE COURSE OF DISEASE (I)

5.1.1. Transmission of *M. hyopneumoniae* infection during the rearing period

The identity of 100 out of 108 pigs was certified throughout the entire rearing period. The cumulative prevalences of pigs with serum antibodies to *M. hyopneumoniae* between arrival and slaughter in herds A and B is expressed in Figure 1.

Fig. 1. Prevalence (%) of pigs with antibodies to *M. hyopneumoniae* in herds A (♦) and B (■) during the fattening period.

Furthermore, 8 seronegative pigs in herd A expressed minor pneumonic lesions typical of SEP at slaughter. Thus, altogether 77% of pigs in herd A and 81% of pigs in herd B fulfilled the criteria for being non-healthy at time of slaughter. Hence, despite pigs’ different initial prevalences of serum antibodies to *M. hyopneumoniae* in herds A and B, a marked majority in both herds became non-healthy, which indicates the efficient transmission of *M. hyopneumoniae* among fattening pigs. In contrast, antibodies were not detected in any of the serum samples in herd C; nor were any macroscopic pneumonic lesions detected at slaughter. This finding, together with those in herds A and B, expressed the crucial role of *M. hyopneumoniae* in pneumonias.

5.1.2. Relationship between time for seroconversion and pneumonia

In the combined data (n=100), prevalences of pneumonia at slaughter were 38%, 73% and 95% in pigs that had developed antibodies to *M. hyopneumoniae* 33, 61 and 92 days after
arrival, respectively ($\chi^2=10.37; \ p<0.01$). Such a trend indicated a healing process for pneumonias acquired early in life, as suggested previously (Noyes et al. 1990; Wallgren et al. 1994). On the other hand, the median magnitude of the affected lungs for these groups were 0.24, 0.04, and 0.01, respectively ($p=0.14$).

5.1.3. Effect of a non-complicated \textit{M. hyopneumoniae} infection on ADG

In herd B, several individual pigs were treated during rearing with antimicrobials for clinical respiratory signs, and several pigs had high lung scores at slaughter. Moreover, all pigs in this herd were in-feed medicated with tylosin for 5 days (100 mg per kg dry matter, Tylan®, Elanco, Copenhagen, Denmark) due to an outbreak of a dysentery-like disease 60 days after arrival. Hence, unlike in herd A the overall health status in herd B was not considered non-complicated, and the herd was omitted from this analysis.

In this analysis, respect was paid on the defined health status of individual pigs (healthy/non-healthy), but not on the time of infection or the actual lung score. No significant interactions were shown between factors in pigs (total $n=69$) of herds A and C. Hence, variation due to all interactions – including those between random and fixed factors – were pooled with the within-cell variation (Winer 1971). Confounding was considered, and in the final model (ADG=herd + sex + health status + pen (herd) + weight on arrival + intercept), the differences between pens within herds were not statistically significant ($p=0.353$), but the difference between herds was significant ($p=0.023$). Health status had a significant effect on ADG ($p=0.010$). The ADG of non-healthy pigs decreased by 220 g per day when compared with healthy pigs (standard error, 80 g), after adjusting for herd, pen within a herd, weight on arrival and sex. Weight ($p=0.021$) and sex ($p=0.005$) also had significant effects on ADG.

5.2. PREVALENCE SURVEY (II)

5.2.1. Prevalence of antibodies to \textit{M. hyopneumoniae} in sow herds in the province of Vaasa

A total of 1773 colostrum samples were collected and analysed by I-ELISA. A diagnosis could be made for 90 herds (Table 2).
Table 2. Results of the survey for *Mycoplasma hyopneumoniae* in the province of Vaasa in 1991 based on the demonstration of antibodies in colostrum by I-ELISA.

<table>
<thead>
<tr>
<th></th>
<th>Seronegative herds</th>
<th>Seropositive herds</th>
<th>Censored from survey</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of herds</td>
<td>55</td>
<td>35</td>
<td>22</td>
<td>112</td>
</tr>
<tr>
<td>Mean no. of sows / herd</td>
<td>18</td>
<td>27</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>Mean no. of feeder pigs sold / herd in 1991</td>
<td>181</td>
<td>273</td>
<td>201</td>
<td>214</td>
</tr>
</tbody>
</table>

Twenty-two of the 112 herds which started the study were censored from the survey. Of these, 14 herds sent no samples. In eight herds, antibodies to *M. hyopneumoniae* were not detected, but in these herds samples were collected from less than 2/3 of the sows. The geographical location of the censored 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). Hence, censoring the 22 herds presumably did not bias results.

The prevalence of herds with antibodies was 39% (95% CI, 29%-49%), which was higher than expected according to a similar study in Southwestern Finland (Tuovinen et al. 1994b). Due to the high prevalence, the confidence intervals became fairly wide. The seropositive herds were significantly larger than the rest of the herds (mean no. of sows, 27.9 and 18.4, respectively; p<0.001). On average, 49% of the pigs delivered to specialized fattening herds were possible carriers of *M. hyopneumoniae* (95% CI, 31%-67%). Thus, in practically every fattening herd, young fatteners of different health status concerning *M. hyopneumoniae* were mixed, thereby contributing to spread of infection.
5.3. SCREENING OF HERDS FOR *M. HYOPNEUMONIAE* (III)

The median prevalence of sows with antibodies by B-ELISA to *M. hyopneumoniae* in seropositive herds was 28.2% (range, 2.7%-100%; n=42). This figure was clearly higher than that found in study II. According to clinical and pathological follow-up for one year, none of the herds with ≤10% prevalence of seropositive samples was truly infected.

Based on continuous health monitoring during 1996, sensitivity of the B-ELISA at herd level (mean sample size/herd, 28) was calculated to be 100% (95% CI, 87%-100%; n=34) since all herds shown to be infected with *M. hyopneumoniae* in 1995 were detected in the 1995 screening of herds by colostrum samples. Specificity at herd level was 98% (95% CI, 97%-99%), calculated as the proportion of herds not infected with *M. hyopneumoniae* (n=464) and not having antibodies in any of the colostrum samples in 1995 (n=456), i.e. (456/464) x 100%.

The sampling strategy was shown to have an effect on test results: in herds with a higher than the median prevalence of sows with antibodies, samples collected within 2 hours after farrowing were 3 times more likely to contain antibodies than samples collected at 2-12 hours (odds ratio, 3.0; 95% CI, 1.4-6.6). In herds with a lower than the median prevalence of sows with antibodies, samples collected from old sows (parity no. >5) were 3.3 times more likely to contain antibodies than samples collected from young sows (odds ratio, 3.3; 95% CI, 1.5-7.5). Moreover, antibodies to *M. hyopneumoniae* were shown to persist up to 3 years in some sows after a successful eradication of the infection from the herd.

Repeated freezing or spoilage of the colostrum samples (III) did not cause biologically relevant problems for the B-ELISA, enabling the usage of colostrum serology under farm conditions.
5.4. STUDIES ON THE TRANSMISSION OF PROTECTION (IV)

5.4.1. Antibodies to *M. hyopneumoniae* in sows and offspring
The large variation in humoral immune response (B-ELISA) of sows to *M. hyopneumoniae* in an endemically infected herd and the significant reduction in circulating antibodies prior to farrowing were well illustrated: the median blocking percentage in serum samples collected 19 days ante-partum was 25.2% (range, 7.0%-96.0%). Three days post-partum that value had decreased to 19.9% (range,1.8%-95.6%). The median blocking percentage obtained in the colostrum samples was 47.8% (range, 4.0%-97.3%). The amount of blocking of serum collected ante- and post-partum corresponded to 67% (p<0.001; n=25) and 53% (p<0.001; n=15), respectively, of that of the colostrum samples. Seven sows (28%) were classified as having a high serum antibody level (blocking percentages exceeding 50%) 19 days pre-farrowing, whereas 12 sows (48%) had a high colostral antibody level.

The median blocking percentages of serum collected from piglets of high- and low-responding sows (15 + 15 piglets in total) were 40.0% and 21.2% at 22 days of age (p<0.001), and 23.6% and 22.3% at 56 days of age (p=0.34), respectively. All piglets (n=6) that expressed a high level of serum antibodies were delivered by sows with high amounts of antibodies to *M. hyopneumoniae*. Thus, the antibody level in the serum of piglets was related to the antibody level in the colostrum of the dam. The two piglets (from the same litter) that had the highest serum antibody level at the age of 22 days had a blocking percentage just below the cut-off value when they reached 56 days (47.6% and 46.0%). At 91 days of age and at time of slaughter, the serum from all pigs expressed a low antibody level with a blocking percentage of <35%. Consequently, the passively acquired immunity of the smallest piglets in a litter depends on the humoral immune status of the dam and the age of the piglet.

5.4.2. Efficacy of passive immunity in field conditions
Three of the early weaned experimental pigs died at an age of 11.5 weeks due to oedema disease. To prevent further cases, all pigs in groups II and III were treated with intramuscular injections of enrofloxacin (5 mg per kg body weight, Baytril , Bayer AG, Leverkusen, Germany). When found dead (n=3) or at slaughter (n=27), neither macroscopic nor histopathologic pneumonic lesions were found in any of the experimental pigs. *M. hyopneumoniae* could not be demonstrated either by cultivation or by PCR. In control pigs,
mild pneumonia with infiltrating mononuclear cells around blood vessels and airways was found in one out of seven lung samples collected at slaughter.

As assessed by Tween-ELISA, absorbance values increased significantly between days 91 and 136 in pigs delivered by sows with low amounts of antibodies (B-ELISA) to *M. hyopneumoniae* (experimental group II; 0.10 unit change; *p*<0.01) and in pigs delivered by sows with low or high amounts of antibodies to *M. hyopneumoniae* that had been placed in a mixed herd at age of 14 days (experimental group III; 0.08 unit change; *p*<0.01), but not in group I, which was delivered by high-responding sows (-0.01 unit change; *p*=0.68). Consequently, at the time of slaughter, group I had the lowest median absorbance value (Tween-ELISA) of experimental groups I, II and III (*A* _{450}, 0.11, 0.21 and 0.25, respectively; *p*<0.01) with this value being lower than that of control pigs left in the satellite herd (*A* _{450}, 0.17; *p*<0.01). Among control pigs, antibodies to *M. hyopneumoniae* (*A* _{450}>0.49) were demonstrated in 3 out of 84 pigs (4%) tested at slaughter, and increased absorbance values (*A* _{450}, 0.30-0.49) were obtained in another 6 pigs (7%).

In conclusion, serological and pathological findings in control pigs indicated that the pigs that remained in the pool were truly contaminated by *M. hyopneumoniae* despite no disease outbreak occurring among these pigs. In contrast, pigs in experimental group I had seemingly been protected against *M. hyopneumoniae* infection, whereas the health status of pigs in groups II and III remained less conclusive.

5.5. FIELD TRIAL (V)

5.5.1. Effect of screening and eradication programmes on disease prevalence

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. hyopneumoniae* by B-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. hyopneumoniae*. A programme to eradicate the infection from these herds was undertaken. In addition, single positive samples were detected in 11 herds. However, based on further inspections and blood samples, no other findings indicated *M. hyopneumoniae* infection. These herds were classified as non-infected false-positive herds.
In March 2000, a total of 509 serum samples were collected from slaughter-weight pigs. Out of these, 506 samples (99.4%) were seronegative for *M. hyopneumoniae*. Two samples were considered positive and one sample suspicious. These three samples were traced back to three different herds. According to additional inspections, all of these herds were shown to be infected with *M. hyopneumoniae*. None of the herd owners had sold live animals to any other herds. Two of these herds were finishing herds practising a continuous flow system. The third one was a farrowing-to-finishing herd (no. of sows, 20) which had already had a single positive serum sample in the screening test in autumn 1999, but was then classified as a false-positive herd. On the basis of experiences received in study IV, a single rebound in making a diagnosis of a subclinical *M. hyopneumoniae* infection is not unexpected.

Because the health status of finishing herds practising a continuous flow system was unknown, all such herds were identified (n=7) and their health status was verified serologically. Antibodies to *M. hyopneumoniae* were detected in a total of four out of seven herds. The barns in these herds were emptied, cleaned and disinfected, after which all-in/all-out management routines were implemented.

The quarterly prevalence of lung lesions in all slaughtered pigs decreased from 5.2% to 0.1% during 1998-2000. The decreasing trend was statistically significant (r= -0.96; p<0.001; n=12). Antibodies to *M. hyopneumoniae* have not been detected in any of the follow-up samples taken after the completion of the eradication programmes (n=284; samples from 3 herds are still missing). Nor have any clinical or pathological findings indicated failure of the eradication programmes until completion of follow-up in February 2001 (12 months after the survey). These findings taken together with those of the survey showed that all member herds were finally free from *M. hyopneumoniae* by the end of 2000.
6. DISCUSSION

6.1. GENERAL DISCUSSION

The study was conducted because of the following observations:

a) Respiratory diseases cause marked economic and welfare problems in specialized fattening pig herds.

b) To prevent outbreaks of pneumonia during the fattening period, half of the herd owners used in-feed medication routinely at the start of the fattening period.

c) The primary infectious agent causing this respiratory disease was concluded to be \( M. \) hyopneumoniae.

Improvements of environmental and management errors are known to decrease risk for severe clinical outbreaks of pneumonia during the fattening period (Done 1991; Straw 1992). However, the influence of non-complicated mycoplasmosis on production is under debate. Furthermore, the prevalence of piglet-producing herds transmitting the infection to all-in/all-out fattening herds is unknown. Therefore, different strategies to control mycoplasmal infections (SEP) have been discussed.

The studies performed provided information on the effect of infections with \( M. \) hyopneumoniae on ADG in fattening pig herds (I) and on the prevalence of \( M. \) hyopneumoniae in different populations of pig herds (II, III, V). Further, the reliability of sow colostrum in screening of herds for \( M. \) hyopneumoniae was scrutinized (III). The transmission of protection against \( M. \) hyopneumoniae from sow to offspring was also investigated (IV), and the effect of undertaking eradication programmes in a region was documented (V). The main results and conclusions of these studies are shown in Table 3.
<table>
<thead>
<tr>
<th>NO.</th>
<th>STUDY TITLE</th>
<th>MAIN RESULTS</th>
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<tr>
<td>I</td>
<td>Varying effects of infections with <em>M. hyopneumoniae</em> on the weight gain recorded in three different multiple source fattening pig herds.</td>
<td>- <em>M. hyopneumoniae</em> was efficiently transmitted among fattening pigs&lt;br&gt;- lung lesions were more prevalent in pigs that developed antibodies late in the fattening period&lt;br&gt;- even a non-complicated <em>M. hyopneumoniae</em> infection reduced average daily gain more than 60 g</td>
<td>- to totally protect fattening pig herds from <em>M. hyopneumoniae</em> infection would be sensible&lt;br&gt;- lung inspection alone may not be an accurate tool to assess the effect of <em>M. hyopneumoniae</em></td>
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<td>II</td>
<td>The prevalence of <em>M. hyopneumoniae</em> in pig herds in Western Finland based on the demonstration of antibodies in colostrum by ELISA.</td>
<td>- more than 30% of sow herds were obviously infected with <em>M. hyopneumoniae</em>&lt;br&gt;- the seropositive herds were larger than the seronegative herds&lt;br&gt;- about 50% of feeder pigs originated from seropositive herds</td>
<td>- 2/3 of the sow herds were obviously free from <em>M. hyopneumoniae</em> infection, and should be prevented from being infected</td>
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<td>III</td>
<td>Monitoring antibodies to <em>M. hyopneumoniae</em> in sow colostrum – a tool to document freedom of infection.</td>
<td>- a high herd-level sensitivity of the ELISA was documented&lt;br&gt;- the sampling strategy and the robustness of the technique were analysed&lt;br&gt;- antibodies were shown to persist up to 3 years in some sows</td>
<td>- colostrum samples were well suited to document freedom from <em>M. hyopneumoniae</em> infection</td>
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<td>IV</td>
<td>Aspects of the transmission of protection against <em>M. hyopneumoniae</em> from sow to offspring.</td>
<td>- the concentration of antibodies in serum decreased in sows prior to farrowing&lt;br&gt;- the amount of antibodies in serum of the smallest piglets in a litter compared with the humoral immune response of the dams, and the efficacy of protection were documented</td>
<td>- a large variation was present in passive protection between litters&lt;br&gt;- in conditions similar to this study, litters of high-responding sows appeared to be totally protected from <em>M. hyopneumoniae</em> for up to 14 days of age</td>
</tr>
<tr>
<td>V</td>
<td>Regional eradication of <em>M. hyopneumoniae</em> from pig herds and documentation of freedom of the disease</td>
<td>- according to a serological survey, and a clinical and a pathological follow-up, the screening of herds and the eradication programmes were shown to be successful</td>
<td>- all the tools needed for a regional eradication programme are available today</td>
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6.2. EFFECT OF A NON-COMPLICATED *M. HYOPNEUMONIAE* INFECTION ON ADG (I)

The prevalence of pneumonic lesions at slaughter was significantly higher the later the pigs developed antibodies (B-ELISA) to *M. hyopneumoniae* (I). This indicated a healing process for pneumonias acquired early in life, as presented previously (Noyes et al. 1990; Wallgren et al. 1994). On the other hand, when early-infected pigs expressed pneumonic lesions at slaughter, these lesions tended to be larger than lesions of late-infected pigs. This was obviously a consequence of the clinically manifested secondary infections in herd B. According to Noyes et al. (1990), such early pneumonias result in high cumulative life-time pneumonia scores and a strong negative influence on the ADG of affected pigs. Similar conclusions were reported by Morris et al. (1995a), indicating that weight losses are more substantial in pigs affected early in life. Therefore, if fatteners cannot be totally protected from infections with *M. hyopneumoniae*, it appears to be beneficial to delay the onset of infection as long as possible.

The growth reduction due to *M. hyopneumoniae* infection was significant even in herd A, despite these pigs seroconverting late during the rearing period and only small percentages of their lung volumes being affected when inspected at slaughter (I). No management errors were present in the herd (n=242; 11 pigs per pen; each pig had access to an area of 0.79 m² and an air volume of 3.4 m³). No medical treatment against respiratory disease was administered and the mortality rate was minimal (0.4%). Both these factors indicate a good clinical health status. Nevertheless, the daily growth due to the infection with *M. hyopneumoniae* was estimated to be significantly reduced by more than 60 g (= the parameter estimate (220 g) minus two standard errors (80 g)), corresponding to about 6%. Consequently, even a non-complicated pneumonia acquired late in the fattening period was concluded to be able to cause a remarkable reduction in ADG. This conclusion gave a strong argument for performing health control strategies aimed at totally eliminating *M. hyopneumoniae*, such as that carried out in study V.
6.3. PREVALENCE OF *M. HYOPNEUMONIAE* ANTIBODIES IN SOW HERDS IN THE PROVINCE OF VAASA (II)

The true prevalence of herds with antibodies by I-ELISA to *M. hyopneumoniae* in Vaasa province might have been lower than the observed prevalence of 39% for two reasons: firstly, due to the seemingly low specificity of the I-ELISA used; secondly, due to a possible sampling bias from the second random sample. The median prevalence of sows with antibodies to *M. hyopneumoniae* in seropositive herds was 8.6% (II), ranging from 2.3% to 70.0%, which is in accordance with earlier reports indicating prevalences of 10% or lower (Levonen 1994; Tuovinen et al. 1994b). Martin et al. (1992) suggested that a fairly high prevalence of positive samples should be assumed in actually infected herds. This is due to the herd predictive value of a positive test result decreasing with increasing sample size if the specificity of the test is not 100%. The specificity of the I-ELISA used in this survey, when a critical number of one reactor was used to indicate the herd’s health status, seemingly was low (Levonen 1994). In study II, three of the positive herds had within-herd prevalences of positive samples lower than 5%. Thus, with no gold standard, these herds were presumed to be false-positive when interpreting the results.

A sampling bias may have taken place in the second random sample of 16 herds. Antibodies were detected in six herds, not detected in seven herds, and three herds were censored due to missing samples. In contrast to these findings, the non-participating herds (going to leave the business in near future) of the first sample were rather small herds, and therefore, less likely to be infected with *M. hyopneumoniae* according to earlier results (Tuovinen et al. 1994b). Perhaps more precise data on the non-participants should have been collected, after which a stratified random sample based on herd size and geographical location could have been taken.

After taking into account both the possible bias and the low specificity of the test, a true prevalence of slightly more than 30% remained (95% CI, 20%-40%). This prevalence is much higher than that (8%) in Southwestern Finland (Tuovinen et al. 1994b). A reason for the high prevalence might be a local tradition of liberal trading of breeding animals from herds with an unknown health status. In any case, the prevalence was much lower than that generally expected (Ross 1999). In fact, about 2/3 of the herds were obviously not infected with *M. hyopneumoniae*. This result was considered a salient addition to strategies concerning animal
health consultations. Indeed, information about infection risks and verifying health status soon became important parts of consultation in the region. However, the observation that numerous young fatteners of different health status were continuously mixed in finishing herds (I) clearly indicated the possible advantage of performing eradication strategies against *M. hyopneumoniae* in piglet-producing herds. Furthermore, the results obtained inspired one to a closer analysis of the application of colostrum serology in broad-spectrum screening of herds (III).

6.4. SCREENING OF HERDS FOR *M. HYOPNEUMONIAE* (III)

6.4.1. Some biological and physical properties of colostrum

When analysing the presence of antibodies by B-ELISA in colostrum, an early sampling (<2.0 hours after start of farrowing compared with 2.0-12.0 hours) increased the probability of detecting colostral antibodies to *M. hyopneumoniae* in infected herds (III). This is in accordance with earlier findings (Klobasa et al. 1986; Klobasa and 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. Early sampling could, therefore, be a useful tool for increasing the sensitivity of monitoring and surveillance systems, which aim to detect new infections or an increase in infectious pressure 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 among sows. Earlier reports are conflicting. Klobasa and Butler (1987) reported a tendency for ≥5th parity sows to have more colostral antibodies 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). The most obvious reason for this contradiction is that the whole-cell indirect ELISA used by Yagihashi et al. (1993) and the B-ELISA, which was used in study III and is based on monoclonal antibodies towards a 72-kDa surface antigen (Feld et al. 1992), react differently in convalescent sera as well as in colostral whey of old sows. According to Kobisch et al. (1993), booster infections with *M. hyopneumoniae* intensify the antibody production especially towards the 72-kDa antigen. Booster infections definitely take place in sows, that stay a long time in the herd.
However, a strong immunization without a booster effect had obviously occurred in the elite breeding herd, which was followed 3.5 years after a successful eradication of the infection from the herd (III): antibodies to *M. hyopneumoniae* were detected in some sows until the end of the follow-up. Hence, the serological status of sows present in herds after eradication programmes may not represent the current health status of such herds. In conclusion, the parity number of sows ought not be a criterion for testing in a survey study. However, the parity number as well as the history of the sows is important in some circumstances when interpreting the results of an antibody assay.

When colostrum samples were exposed to extreme conditions, a significant but biologically irrelevant increase in the blocking percentages was noted in repeatedly frozen samples (III). This increase did not correlate with the fat percentage of the samples. Thus, a possible misstorage of colostrum samples is not a major problem when using the B-ELISA, which makes colostrum sampling a feasible alternative in monitoring and surveillance systems and field studies. However, a good sampling practice, when not using preserving additives, assumes a clean collection followed by rapid refrigeration and freezing of the colostrum samples.

### 6.4.2. Sampling to detect disease

None of the herds with less than a 10% prevalence of positive samples was found to be truly infected with *M. hyopneumoniae* in study III. Thus, based on a minimum of 10% prevalence of positive samples in truly infected herds, a minimum of 19-25 samples would have been sufficient to declare herds with 30-100 sows free from an endemic *M. hyopneumoniae* infection with 95% confidence (Cannon & Roe 1982). The decrease in the number of samples from 30 to 25 per herd would, in addition, increase herd-level specificity by about 0.3 percentage unit (using the equations of Martin et al. 1992).

In monitoring and surveillance systems, 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; Stärk et al. 1992; Thomsen et al. 1992). Units with the highest airflow, namely, the dry sow unit in farrowing herds and the finishing unit in farrowing-to-finishing herds have an increased risk of introducing aerosols containing the infectious agent (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 1994), consistent with what was observed in the elite breeding herd in study III. Hence, if colostrum samples were analysed frequently, e.g. monthly, they would probably be sensitive samples for the detection of reinfections as well. To further increase the sensitivity in monitoring and surveillance systems, early sampling (<2 hours after the start of farrowing) is recommended (III), as suggested above.

6.5. MATERNAL PROTECTION AGAINST *M. HYOPNEUMONIAE* (IV)

As antibodies to *M. hyopneumoniae* probably play an important role in the defence against disease (Blanchard et al. 1992), the high variations in antibody activity (B-ELISA) obtained in study IV indicate that development of protective immunity differs between sows, and that some of them might stay in a carrier state for a long time after being contaminated by *M. hyopneumoniae*, as suggested earlier (Goodwin 1965; Clark et al. 1991). The decreased incidence of respiratory diseases reported among offspring from older sows (Goodwin 1965) is understandable because older sows have probably to a larger extent than younger sows been exposed to and had time to develop immunity towards microbes present in a herd. Indeed, old sows (parity no. ≥5) have been reported to have more antibodies in colostrum than younger sows (Klobasa & Butler 1987), a finding of study III as well. However, variation due to genetics does also occur (Edfors-Lilja et al. 1991).

Corresponding to earlier results (Kavanagh 1994; Morris et al. 1994; Wallgren et al. 1998), in study IV the antibody level in serum of piglets was found to be related to the antibody level in the colostrum of the dam. Thus, the passive protection of piglets against *M. hyopneumoniae* varied from litter to litter according to the immune status of the sows. The health status of piglets therefore depends on the balance between passively acquired immunity and the pathogen load of the environment. Within the investigated sow pool, this was indicated by large variations in clinical manifestations of SEP, *i.e.* cough at weaning and pneumonia detected at slaughter, recorded in different batches of fatteners despite the pathogen load for growers being minimized (age-segregated system on a building level and no mixing of groups).
In conclusion, when piglets receive a sufficient amount of antibodies to \textit{M. hyopneumoniae} by colostrum, they appear to be totally protected from \textit{M. hyopneumoniae} to up to 14 days of age in conditions similar to those of study IV (= low infectious pressure). This presents the possibility of eradicating the disease employing a shorter piglet-free period than generally used, \textit{i.e.} with lower costs, provided that piglets do not become exposed to the microbe post-weaning by older piglets. However, the humoral immune response to \textit{M. hyopneumoniae} varies between sows in endemically infected herds, and the antibody level in the serum of offspring is related to the antibody level of sow colostrum. As the smallest piglets probably receive the smallest amounts of colostrum, attention must be paid to low-responding sows and low-colostrum-consuming (runt) piglets. Due to these limitations, eradication attempts ought not be done in endemically infected herds without carefully monitoring the amounts of antibodies to \textit{M. hyopneumoniae} (either in serum or colostrum) in all those sows intended to farrow during the eradication programme. No seronegative sows should let farrow. Alternatively, attempts could be made to increase the level of antibodies in colostrum by vaccinating the sows and to do research in the efficacy of such vaccine antibodies against \textit{M. hyopneumoniae} in piglets.

6.6. SUCCESS OF THE COMMUNITY-WIDE ERADICATION PROGRAMME (V)

The number of farrowing herds shown to be infected with \textit{M. hyopneumoniae} was small, as expected according to the pilot study. This finding bolstered the conclusions drawn from studies I and II that the mixing of young feeder pigs of different health status can be disastrous for a large number of herds, even when the number of animals carrying the infectious agent is small. On the other hand, by focusing preventive measures on a relatively small number of herds (the infected farrowing herds) in study V, the health status of numerous finishing herds was improved.

One in four farrowing-to-finishing herds was shown to be infected with \textit{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 herds presented a constant risk for \textit{M. hyopneumoniae} infection for other herds through animal transport, temporary selling of feeder pigs and their close vicinity to healthy herds. Consequently, determining the health status of
such herds and eradicating \textit{M. hyopneumoniae} from those infected were found to be the most essential activities in study V.

To assess the success of the elimination programme, a survey to detect even a low prevalence of disease was carried out. The following measures were taken to increase the probability of detecting positive samples:

A ) The test used had already been shown to be very sensitive (Sørensen et al. 1997);

B ) Slaughter pigs were chosen to be the target group for sampling because of the high prevalence of antibodies in this age group, as indicated in study I and in other studies (Wallgren et al. 1993a; Yagihashi et al. 1993; Morris et al. 1995b), and because they represented the infectious status of all the source herds of the corresponding finishing herds;

C) The size of the random sample was chosen to detect at least 1% prevalence of antibodies (Cannon & Roe 1982);

D) The sampling was prolonged to several weeks to reduce clustering of the samples.

The two positive and one suspicious sample detected during the survey could all be traced back. They indeed appeared to be indications of an endemic \textit{M. hyopneumoniae} infection in three particular herds. None of these herds had been selling live animals to other herds, thus, the risk for spreading the infection was minimized. Moreover, the health status of the rest of the herds with similar production type (continuous flow system) was screened. The 506 negative samples (99.4%) indicated that the overall prevalence of \textit{M. hyopneumoniae} had been reduced to a minimum, perhaps even to zero. This trend was also expected from the significant reduction of lung lesions to around 0.1%, since high lung lesion prevalences were earlier (I) associated with \textit{M. hyopneumoniae} infection, a result supported by Tuovinen et al. (1994a). In addition, no clinical breakdowns entitling to compensations in finishing herds (V) had been reported up to the end of the follow-up in February 2001. All of these findings strongly support the success of the programme. The apparent success of the programme was further seen in daily gain, as one might expect. During 1998-2000 the average daily gain increased from 799 g to 875 g in specialized finishing herds (n=150, according to slaughterhouse records, results not shown). This increase was of the same magnitude as in study I. However, in addition to the effect of \textit{M. hyopneumoniae} freedom, the effect of freedom from sarcoptic mange was also represented in the figures for study V.
Only time will confirm the ultimate success of the programme, since some of the eradication programmes and screening tests had taken place very recently. Regarding the first aspect, earlier reports of the success of eradication programmes have claimed rates of 94% (Zimmermann et al. 1989) and 81% (Heinonen et al. 1999). As to the latter aspect, latent infections were considered to have caused several breakdowns in the regional eradication programmes in Switzerland (Masserey-Wullschleger & Maurer 1998). In a community-wide elimination and monitoring programme (V) it will, however, be possible to detect any clinical breakdown rapidly and prohibit further spread of the disease.

6.7. REMARKS ON METHODS: DEFINITION OF CASES AND NON-CASES IN FIELD STUDIES (I, III, IV)

The definition of case (or disease) was critical for the estimation of reduction of ADG due to *M. hyopneumoniae* in study I, the estimation of test sensitivity and specificity as well as sample size in study III, and the investigation of maternal protection in study IV.

**Study I**

The classification between diseased and non-diseased pigs in study I was based on a combination of diagnostic tools. To be defined as non-diseased, a pig had to be free from both macroscopic pneumonic lesions (confirmed by histology) at slaughter, and antibodies to *M. hyopneumoniae* in serum samples (n=5 per pig). Because pneumonia is known to be a dynamic process (Noyes et al. 1990; Kobisch et al. 1993), some of the lesions might have resolved before time of slaughter. Moreover, the age of pigs at slaughter differs by several weeks within herds and between herds, which further decreases the accuracy of lung inspection as a solely indicator of life-time pneumonia. Similarly, in field conditions, the time for post-exposure seroconversion varies markedly between individuals (Sørensen et al. 1993), although the majority of infected pigs are expected to seroconvert before slaughter. On the other hand, some individuals might already be convalescents before the start of fattening. In all, the chosen definition of a diseased pig was considered to cover the entire spectrum of *M. hyopneumoniae* infections during the fattening period.
Study III

Herd with no antibodies to *M. hyopneumoniae* in colostrum samples were defined as truly non-infected if they met three criteria during the following 12 months:

1. They were free from clinical signs of respiratory diseases based on quarterly veterinary inspections;
2. No signs of respiratory diseases were observed in the corresponding finishing herds (testing stations) based on veterinary inspections upon arrival of pigs and on owners’ opinions later during the rearing period;
3. No lung lesions typical of SEP were observed at slaughter by the meat inspection team.

When evaluated separately, each of the above methods has weaknesses as a surveillance technique. According to Sørensen et al. (1993), coughing was found to be the most reliable clinical indicator of infections with *M. hyopneumoniae*, but surveillance through herd inspections alone failed to detect 30% of infections. Similarly, Goodwin (1984) detected cough as the first clinical sign of disease in only 52% of infected herds. He did detect, however, clinical signs other than cough (anorexia, pyrexia, laboured breathing) in an additional 34% of herds. Lung inspection is a traditional feature in surveillance systems for *M. hyopneumoniae* (Goodwin & Whittlestone 1967; Keller 1980; Schulman 1980; Sørensen et al. 1993), although subclinical infections sometimes express no macroscopic pneumonic lesions in slaughter-weight pigs, as was found in study IV and in other studies (Goodwin 1972; Armstrong et al. 1984; Clark et al. 1991; Wallgren et al. 1994; Lindahl & Wallgren 1997). However, both specialized fattening pig production and phenotype testing in Finland typically comprise mixing of growers from multiple source herds, a management routine which predisposes to clinical outbreaks of respiratory diseases if the health statuses of the source herds differs (Zimmermann et al. 1989; Christensen et al. 1999). In addition, the herd owners’ willingness to report possible outbreaks of disease was increased by giving a guarantee of freedom from *M. hyopneumoniae* to premium-quality, higher-priced feeder pigs. In conclusion, the described disease surveillance, which was a combination of three methods and continued for one year, was regarded as reliable in detecting *M. hyopneumoniae* infection among health-classified pigs. It was thus used as a gold standard.
Study IV

No disease was recorded among the batch from which the experimental pigs originated, indicating a low pathogen load for that batch. Nevertheless, antibody detection and pathological findings of the control pigs (*i.e.* mild pneumonia with infiltrating mononuclear cells around blood vessels and airways) indicated that the pigs that had remained in the sow pool were truly contaminated with *M. hyopneumoniae*.

With respect to the experimental pigs, no signs of pneumonia were recorded at slaughter. Nor could *M. hyopneumoniae* be demonstrated in the lungs, either by cultivation or PCR. However, the question whether all these pigs really were free from *M. hyopneumoniae* remains unanswered. As for serology, while no signs of infection were recorded by the B-ELISA, a minor but significant (*p*<0.01) increase in absorbance values was noted in groups II and III, but not in group I, when measured by the Tween-ELISA. In this context, it is of interest to note that when a group of pigs naturally infected with *M. hyopneumoniae* was blood-sampled repeatedly, a significant increase in the amount of serum antibodies was demonstrated 10 to 20 days earlier with the Tween-ELISA than with the B-ELISA (Wallgren et al. 1996).

The observation that pigs in group I, which originated from dams with high amounts of antibodies to *M. hyopneumoniae*, expressed significantly lower amounts of serum antibodies to *M. hyopneumoniae* at slaughter than the other groups may indicate the efficacy of a strong passive immunity not only against disease but also against infection. According to previous observations, a maternally conferred protection against disease may persist for up to 7 weeks among offspring of high-responding sows (Wallgren et al. 1998).

The health status of the other early-weaned piglets (groups II and III) remained more speculative. These groups originated entirely (100%) or partly (50%) from low-responding sows. The efficacy of their maternal immunity may well have decreased at early weaning, as also indicated by previous results (Wallgren et al. 1998). Both these groups demonstrated elevated serum levels of antibodies to *M. hyopneumoniae* at the end of the trial. The maximum value recorded (*A*$_{450}$=0.43) was close to the cut-off value of the Tween-ELISA (*A*$_{450}$=0.50) and may indicate detection of antibodies. Bearing this in mind, one must also remember the following: 1. the low density of pigs (10 per unit) in these groups; 2. their youth at slaughter (136 days); 3. all pigs in groups II and III were treated with enrofloxacin at the
age of 11.5 weeks to prevent oedema disease. Enrofloxacin has excellent minimal inhibitory concentration (MIC) values for *M. hyopneumoniae* (Friis & Szancer 1994) and a pharmacokinetic that makes it well suited to combat this infection (Scheer 1987).

The difficulty to prove the presence of *M. hyopneumoniae* in high-health systems has been demonstrated earlier. When early signs of disease and incubation periods were evaluated in specific pathogen-free (SPF) herds attended for primary outbreaks of mycoplasmosis, a late spread of *M. hyopneumoniae* was observed by Goodwin (1984, 1985). A subclinical spread of the infection may prevent the detection of *M. hyopneumoniae* for several months following introduction into a herd (Goodwin 1984). In agreement, it has proven possible to rear piglets to market weight (107 kg body weight) without receiving any condemnations for respiratory lesions at slaughter, despite the dams harbouring *M. hyopneumoniae* (Lindahl & Wallgren 1997). Taken together, the observations made in the references above and in study IV strongly indicate that failure to demonstrate *M. hyopneumoniae* in lungs will not necessarily guarantee freedom from the infection. In this context, it is important to remember that both cultivation and PCR require a minimal amount of microbes in samples collected. Further, the lack of macroscopic manifestations of disease indicates a need for standard sampling sites rather than strategic sampling.
CONCLUDING REMARKS

1. Assessed by antibody seroprevalence and prevalence of pneumonic lesions at slaughter, *M. hyopneumoniae* was effectively transmitted among finishing pigs during the fattening period. Around 80% of slaughtered pigs had indications of infection.

2. Pneumonias acquired early in life may have healed by the time of slaughter. On the other hand, when early-infected pigs in this study expressed pneumonic lesions at slaughter, these lesions tended to be larger than lesions of late-infected pigs. Since pneumonia is a dynamic process, lung inspection alone may not be sufficiently accurate to assess the effect of *M. hyopneumoniae* infection on the health status of pigs.

3. Even a non-complicated pneumonia caused by *M. hyopneumoniae* was shown to reduce the ADG of multiple source finishing pigs by more than 60 g. This provides a strong argument for performing health control strategies aimed at totally eliminating *M. hyopneumoniae*.

4. Based on the analysis of colostral antibodies by I-ELISA, about 2/3 of sow herds were free from *M. hyopneumoniae* in the province of Vaasa in 1991. However, half of the pigs delivered to specialized finishing herds originated from seropositive herds and were possibly carriers of *M. hyopneumoniae*.

5. Twenty-five colostrum samples per herd was shown to be sufficient for the detection of an endemic infection with 95% confidence.

6. Early colostrum sampling (within 2 hours post-farrowing) could potentially be used to increase sensitivity in monitoring and surveillance systems, which aim to detect new infections as early as possible. Testing high-parity sows is another tool to increase sensitivity, but only in herds with a low pathogen load among sows.

7. Mishandling of colostrum samples was not a major problem when using the B-ELISA, making colostrum sampling an attractive prospect for monitoring and surveillance systems and field studies.
8. In an endemically infected herd, a large variation in humoral response to *M. hyopneumoniae* was illustrated in sows, indicating that the development of protective immunity differed between sows.

9. The antibody level in serum of piglets was related to the antibody level in the colostrum of dams. Thus, the passive protection of piglets against *M. hyopneumoniae* varied from litter to litter according to the immune status of the sows.

10. When piglets receive a sufficient amount of antibodies to *M. hyopneumoniae* by colostrum, they appear to be totally protected from *M. hyopneumoniae* to up to 14 days of age in conditions similar to this study.

11. All the tools needed for a successful region-wide eradication programme are available today.
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Taudinkulku
Kolmessa uudehkoksa kertatäyttöisessä lihasikalassa valittiin kasvatuskauden alussa jokaisesta karsinasta satunnaisesti 1-3 välyyksestä tullutta sikaa (otos yhteensä 100 sikaa). Tavoitteena oli seurata *Mycoplasma hyopneumoniae* -tartunnan leviämistä kasvatuskauden aikana vasta-aineiden muodostumisen ja teurastushetkellä todettavien keuhkomuutosten perusteella. Lisäksi haluttiin mitata tartunnan aiheuttamaa päiväkasvun hidastumista. Kahdella tilalla todettiin noin 80 %:n sioista saaneen tartunnan teurastukseen mennessä. Kolmannelle tilalle ei tartuntaa tullut ollenkaan. Kahden ensimmäisen tilan yhdistetyissä tuloksissa oli keuhkotulehdusten esiintyvyys 38 %, 73 % ja 95 % sellaisten sikojen joukossa, joilla todettiin *M. hyopneumoniae* vasta-aineita 33, 61 tai 92 päivää kasvatusajan alusta lukien ($\chi^2=10,37$; p<0,01). Keuhkomuutosten laajuus (asteikolla 0-1) oli näissä ryhmissä vastaavasti 0,24 , 0,04 ja 0,01. Tartunnan saaneiden sikojen päiväkasvu aleni vähintään 60 g tartunnalta säästynyt sikoihin verrattuna (p=0,010; n=69). Tilastomallissa otettiin huomioon tilan, karsinan, sikojen alkupainon ja sukupuolen vaikutus päiväkasvun.

Alueellinen kartoitus

Tilakohtainen *M. hyopneumoniae* kartoitus
Ternimaitonäytteiden luotettavuutta tilakohtaisessa *M. hyopneumoniae* -kartoituksessa selvitettiin kahdentyypissä sikoissa: säännölliseen terveysvalvontaan kuuluvissa
jalostussikaloissa (n=183) ja tavanomaisissa porsastuotantosikaloissa (n=347), jotka pyrkivät ns. LSO-2000 terveysluokkaan. Näiltä tiloilta analysoitiin kaikkiaan 14 919 ternimaitonäytettä. Seropositivisissa sikaloissa todettiin 42 ja seropositivisten emakoiden osuus (mediaani) näissä oli 28,2 % (vaihteluväli 2,7 % - 100 %). Vuoden kestäneen klinisen seurannan ja keuhkomuutosten seurannan perusteella pääteltiin, että tilat, joilla seropositivisemakoi oli ≤ 10 %, ei tosiasiassa ollut M. hyopneumoniae -tartuntaa. Johtopäätöksenä oli, että endeeminen M. hyopneumoniae -tartunta voidaan 95 % luotettavuudella todeta sikalasta 25 ternimaitonäytteen avulla.

Ternimaitojen näytteenotolla osoitautui olevan vaikutusta testitulokseen. Tiloilla, joilla vastaineita todettiin runsaasti, niitä todettiin 3 kertaa useammin niissä näytteissä, jotka oli lypsetty kahden tunnin sisällä porsimisesta kuin 2-12 tuntia porsimisesta lypsetyissä näytteissä (OR 3,0; 95 % luottamusväli 1,4 - 6,6). Sellaisilla tiloilla, joissa emakoiden tartuntapaine oli alhainen, vasta-aineita todettiin 3,3 kertaa useammin yli viisi kertaa porsineilla emakoilla kuin sitä runsommilla emakoilla (OR 3,3; 95 % luottamusväli 1,5 - 7,5). Vasta-aineet säilyivät joillakin emakoilla jopa kolme vuotta senkin jälkeen, kun tartunta oli saneerattu tilalta. Emakoiden porsimiskerralla ja lähtötilalla todettiin siten voivan olla vaikutusta ternimaitotutkimuksen tuloksiin. Ternimaitojen toistuva pakastaminen tai niiden pilaantuminen ei muuttanut merkittävästi ELISA-testin tuloksia. Loppupäätelmänä oli, että ternimaito sopii hyvin valvontaohjelmien ja kenttätutkimusten näytteeksi.

**Vastustuskyvyn siirtyminen**

Vastustuskyvyn siirtymistä emakon ja porsaiden välillä tutkittiin eräässä emakkorenkaan satelliittisikalasssa. M. hyopneumoniae -vasta-aineiden määrä oli emakoiden seerumissa 67 % ternimaidon vasta-ainemäärästä 19 päivää ennen porsimista (p<0,001; n=25), ja 53 % kolme päivää porsimisen jälkeen (p<0,001; n=15). Seitsemällä emakolla (28 %) oli korkea seerumin vasta-ainetaso 19 päivää ennen porsimista, kun taas ternimaidon vasta-ainetaso oli korkea 12 emakolla (48 %). Pikuporsaiden seerumin vasta-ainetaso oli riippuvainen ternimaidon vasta-ainetasosta. Näitä havainnot ilmensivät, kuinka niin emakoiden kuin pikuporsaiden M. hyopneumoniae -vasta-ainetaso vaihtee endeemisesti infektoituneella tilalla. Kolme 10 porsaan ryhmää vieroitettiin 14 päivän äässä ja siirrettiin muista sikaloista erillään oleviin eristysyksiköihin. Sen ryhmän porsaat, jotka olivat saaneet eniten ternimaidon M. hyopneumoniae -vasta-aineita, näyttivät olevan suojattuja tartunnalta aina 14 päivän ikään asti. Tämän havainnon perusteella M. hyopneumoniae -tartunta voisi olla mahdollista
saneerata tiloilta käyttäen lyhyempää porsitustaukoa kuin mihin on totuttu, edellytäen, että emakoiden *M. hyopneumoniae*-vasta-ainetasot tunnetaan.

**Kenttäkoe**