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Eradication of Aujeszky´s Disease (Pseudorabies) Virus from Pig Herds: Alternatives to Depopulation

Marie Engel

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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In the unvaccinated herds it was demonstrated that it is difficult to avoid contact between infected and uninfected animals under normal conditions, and that contact was frequent in these herds. Even so, transmission from latently infected animals to susceptibles appeared to be limited, as all the unvaccinated study herds experienced long periods without seroconversion and 3 herds became free from ADV during the study. However, once transmission takes place among unvaccinated animals, there is always a risk that this may lead to an outbreak. Three of the largest unvaccinated herds did experience severe outbreaks and vaccination was subsequently applied in these herds.

All 7 herds which applied vaccination eradicated ADV. In these herds, no outbreaks occurred and the incidence of seroconversion was very low, except for one herd where gilts were placed in direct contact with unvaccinated fattening pigs.

Today, vaccination against AD using marker vaccines is the main method of control. However, it appears that vaccination may promote risk behaviour in herd managers and it is important that basic principles of biosecurity are not neglected during a vaccination programme.

Key words: Aujeszky's disease, pseudorabies, serological study, transmission, disease control, eradication, vaccination programme, risk behaviour.

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To my parents

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Contents:

Abbreviations, 10

Background to the thesis, 11

Introduction, 12
History, 12
Aujeszky's disease virus, 13
Latent infection, 14
Transmission, 14
Infective dose
Virus excretion
Transmission of virus within a herd, 16
Descriptive studies
Analytical studies
Intervention studies
Reproductive rate
Pathogenesis and clinical signs, 19
Diagnosis, 20
Serological tests
Glycoprotein E ELISA
Vaccines, 20
Clean-up of pig herds, 21
Clean-up without use of vaccine
Clean-up with temporary use of vaccine
Pig production and history of Aujeszky's disease in Sweden, 23
Pig production in Sweden
Aujeszky's disease in Sweden

Aims of the thesis, 26

Materials and methods, 27
Animals and herds, 27
Diagnostic methods, 28
Serological tests
Virus isolation
Vaccines, 28
Field studies: 1. Eradication of ADV by replacement with uninfected animals, 29
Serological study in nine weaner pig-producing herds (I)

Quantification of opportunity of contact between infected and uninfected animals (II)
Serological study in a farrow-to-finish herd (V)

Field studies: 2. Eradication of ADV by vaccination and replacement with uninfected animals, 30
Eradication programme with killed vaccine, initially based on an attempt to distinguish between infected and uninfected animals by the difference in duration of antibody titers (III)
Eradication programme with killed vaccine based on distinguishing between infected and uninfected animals by the use of gE ELISA (IV)
Eradication programme with live gE-/TK- vaccine and gE ELISA (V)

Results, 32
Eradication of ADV by replacement with uninfected animals, 32
Serological study in nine weaner pig-producing herds (I)
Quantification of opportunity of contact between infected and uninfected animals (II)
Serological study in a farrow-to-finish herd (V)

Eradication of ADV by vaccination and replacement with uninfected animals, 33
Eradication programme with killed vaccine, initially based on an attempt to distinguish between infected and uninfected animals by the difference in duration of antibody titers (III)
Eradication programme with killed vaccine based on distinguishing between infected and uninfected animals by the use of gE ELISA (IV)
Eradication programme with live gE-/TK- vaccine and gE ELISA (V)

Discussion, 35
Unvaccinated herds, 36
Patterns of seroconversion
Contact pattern
Transmission of reactivated virus
Vaccinated herds, 38
Vaccination and eradication
Methods for differentiation
Effectiveness of vaccination programmes
Implications and speculations

References, 42

Acknowledgements, 48

Appendix

Papers I-V

The present thesis is based on the following papers, which will be referred to by Roman numerals.

I Engel, M., Wierup, M. and Novén, B., 1999.

Patterns of seroconversion to Aujeszky's disease virus in nonvaccinated chronically infected Swedish weaner pig-producing herds. J. Vet. Med. B. In press.

II Engel, M., Hird, D.W., Carpenter, T.E., and Wierup, M., 1995.

Long-term lack of transmission of Aujeszky's disease virus (ADV) in a chronically infected Swedish weaner pig-producing herd. Prev. Vet. Med. 24, 245-252.

III Engel, M. and Wierup, M., 1989.

Vaccination and eradication programme against Aujeszky's disease in Sweden, based on a gI ELISA test. Vet. Rec. 125, 236-237.

IV Engel, M. and Wierup, M., 1999.

Vaccination and eradication programme against Aujeszky's disease in five Swedish pig herds with special reference to herd owner attitudes. Acta Vet. Scand. 40, 213-219.

V Engel, M. and Wierup, M., 1997.

Eradication of Aujeszky's disease virus from a Swedish pig herd using gI-/TK- vaccine. Vet. Rec. 140, 493-495.

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Abbreviations

AD	Aujeszky's disease
ADV	Aujeszky's disease virus
CDI	Central Veterinary Institute in The Netherlands
CNS	Central Nervous System
DIC	Days-In-Contact
DPC	Daily-Pig-Contacts
ELISA	Enzyme-Linked Immunosorbent Assay
gI	glycoprotein I (today named gE)
gII	glycoprotein II (today named gB)
gE	glycoprotein E (formerly named gI)
gB	glycoprotein B (formerly named gII)
HSV	Herpes Simplex Virus
PI	Post Infection
R	Reproduction rate
SN	Serum Neutralisation test
SPF	Specific Pathogen Free
SVA	National Veterinary Institute in Sweden
TK	Thymidine Kinase

Background to the thesis

At the inception of the studies presented in the thesis, in 1985, Aujeszky's disease (AD) had become one of the economically most important diseases in swine in countries with industrialised pig production. During the 1970's there had been a marked increase in incidence and severity of the disease in the Western world.

In Europe, many countries with a dense pig population were vaccinating in endemically infected regions to control clinical disease. However, vaccination was associated with some major disadvantages. It was not possible to distinguish vaccinated animals from infected with the available serological methods. To vaccinate indefinitely was very costly. Experience from the European continent showed that AD-virus (ADV) continued to spread in spite of vaccination and that the fattening herds suffered from more severe outbreaks when only breeding herds were vaccinated.

Some countries, where AD was not yet widespread, were in the process of eradicating the disease. East Germany had achieved eradication and eradication programs were running in Great Britain and Denmark. The latter two countries were geographically isolated and vaccination was prohibited. The situation in Sweden was similar to that in Great Britain and Denmark. However, there were no public funds available to start an eradication programme.

In Sweden AD was first diagnosed in 1965 and for the next 15 years outbreaks were predominantly mild and occurred mainly in weaner pig-producing herds. However, in the beginning of the 1980's the incidence started to increase and peaked in 1985 (Figure 1). In some cases, fattening herds were also involved.

As a first step towards a national control programme, a compulsory control programme for the nucleus herds was instituted in 1973. In 1987, this was followed by a voluntary control programme for the multiplier herds. The next step needed to be taken would be to extend the voluntary programme to the weaner pig-producing herds. However, as there was no financial support, the owner of an infected herd would have to meet all the costs involved in the cleaning-up of his herd. The available methods for herd clean-up at that time, were either total depopulation or immediate test and slaughter. The first method would involve heavy expenses for the herd owner and the second method could be costly for herds with a high seroprevalence of ADV.

Thus, in order for a non-state-funded control programme to be successfully carried out in weaner pig-producing herds, it was necessary to find less expensive alternatives to these methods. The work presented in this thesis, was initiated to study alternative methods that were possible at that time and during the study

new methods were adopted, including the application of marker vaccines and marker tests.

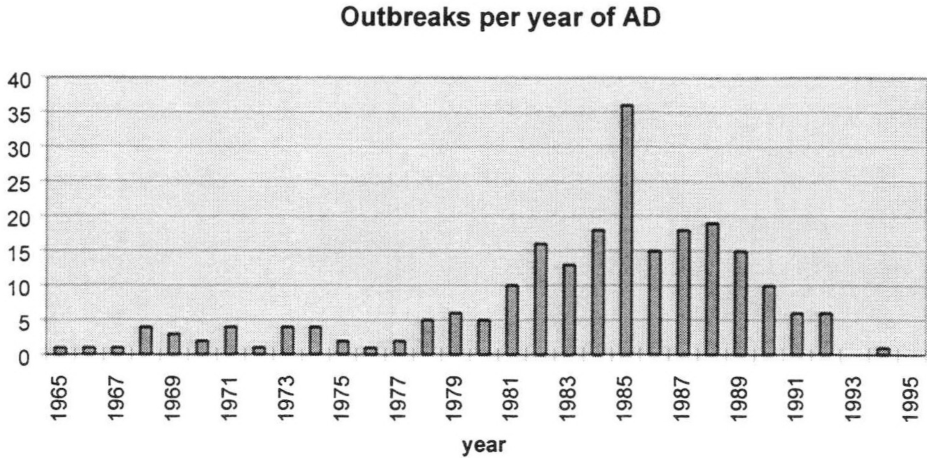

Figure 1. Number of clinical outbreaks of AD in pig herds in Sweden 1965-1995.

Introduction

History

There exists a description of a contagious condition in Southern Europe in the early Middle Ages, suggesting clinical signs of Aujeszky's disease (Skoda 1976). During the 1800's, cows with severe itching were observed in USA and Switzerland and the disease was mistaken for rabies (Kluge et al. 1999).

The Hungarian veterinarian Aladar Aujeszky described the illness, which he called pseudorabies, in 1902 and distinguished it from rabies (Aujeszky 1902). Dr. Aujeszky discovered that the disease is not caused by bacteria and proposed its viral origin, which was confirmed by a co-worker, Dr. Schmiedhoffer. The latter carried out the first isolation of the virus in 1910 (Bartha 1994).

AD in pigs was first described in 1920 (Wittman & Rziha 1989). Before the 1960's the disease in pigs was important in Eastern Europe only, but subsequently the number of outbreaks increased in Western Europe and the USA (Basinger 1979). The increase in incidence and severity of symptoms has been attributed to an increase in virulence in some strains of ADV as well as the intensification of the swine industry in the countries involved (Kluge et al. 1999).

AD has a world-wide distribution (Animal Health Yearbook 1994). Within the European Union, Finland has never been reported to be infected, some member states have eradicated the disease and the remaining states are in different stages of eradicating AD (Moynagh 1997, Westergaard 1999). Today, all the Scandinavian countries have AD-free status.

Aujeszky's disease virus

The formal taxonomic name given to Aujeszky's disease virus (or pseudorabies virus) is *Suid Herpesvirus 1 (SHV-1)*. It belongs to the subfamily *Alphaherpesvirinae* within the family of the *Herpesviridae* (Roizman et al. 1992). Other members of the subfamily *Alphaherpesvirinae* are e.g. the human herpes simplex virus (HSV) and varicella-zoster virus (VZV), bovine herpesvirus 1 (BHV-1) and equine herpesvirus 1 (EHV-1).

The structure of the herpesvirus particle is complex. The central core contains DNA wrapped around protein. The core is surrounded by the capsid, which is a protein shell composed of 162 capsomeres, arranged in icosapentahedron symmetry. Outside the capsid is the tegument, a region filled with amorphous protein. On the outside of the particle is the envelope, consisting of lipoproteins and glycoproteins that project from the surface. The diameter of the enveloped particle is 150 - 180 nm (Wittman & Rziha 1989, Roizmann et al. 1992).

The genome of ADV consists of a linear double-stranded DNA of approximately 150.000 base pairs, sufficient to encode at least 70 proteins. It is divided into a unique long (U_L) and a unique short (U_S) part. The U_S region is bracketed by inverted repeat sequences (Mettenleiter 1991).

The glycoproteins of the envelope are essential for virus adsorption to the host cell and the immunogenicity of the virus. To date, 11 glycoproteins have been found in ADV, which share similar properties with proteins found in other herpesviruses. Since 1993 the nomenclature of the glycoproteins of the alphaherpes viruses follow those of herpes simplex virus-1. The functions of the identified glycoproteins have been described by Nauwynck (1997, 1999) and Mulder and others (1997).

There is only one serotype of ADV, but strains vary considerably in virulence (Pol et al. 1989, Mc Cullough 1989). Virulence is determined multigenetically and the proteins of ADV that have been proposed to determine the virulence of the virus can be divided into three groups (Mulder et al. 1997): a) envelope glycoproteins that mediate virus entry and virus spread in the host, b) virus encoded enzymes involved in DNA metabolism or phosphorylation, and c) capsid proteins involved in virion assembly.

Belonging to the first group is glycoprotein E (gE, named gI previous to 1993) which was one of the first ADV glycoproteins described (Hampl 1984). The

absence of gE results in a reduction of virulence (Kimman et al. 1992, Jacobs et al. 1993). Vaccines with a deletion for gE were the first so called marker vaccines for which an accompanying diagnostic test was available (Van Oirschot et al. 1986). Today, only gE deleted ADV vaccines are allowed to be applied in pigs within the European Union (Moynagh 1997).

Belonging to the second group is the enzyme thymidine kinase (TK) which was among the first virulence functions recognised in ADV (Kit et al. 1985). Lack of the TK gene drastically reduces virulence, and thus most genetically engineered marker vaccines have that gene deleted (Mettenleiter 1994).

Latent infection

Following acute infection, ADV can persist in a latent state in different tissues, especially in the CNS (Sabó & Rajcani 1976, Beran et al. 1980, Rziha et al. 1984). It has been proposed that most, or all, swine that survive infection with virulent ADV are carriers and have the potential to shed virus (Mengeling 1992).

Immunosuppression or natural stress can cause reactivation of the latent virus and infectious virus particles are shed in nasal secretions. One hypothesis is that the incidence of reactivation may be directly correlated to the concentration of latent ADV in tissues (Maes 1997) which, in turn, depends on viral strain, dose and route of infection (Vilnis 1998). It has also been suggested that virulent virus may be more readily reactivated than strains of mild or low virulence (Van Oirschot 1994).

The incidence of natural reactivation is not known. The frequency of reactivation reported from experimental studies using corticosteriods ranges from 20 to 100% in various studies (Wittman et al. 1983, Van Oirschot & Gielkens 1984, Cowen et al. 1990, Schoenbaum et al. 1990, Mengeling et al. 1992, Brockmeier et al. 1993).

In the field, the frequency of reactivation can be estimated indirectly from the incidence of seroconversion in susceptible contact animals in herds where reintroduction of virus or continuous virus circulation is not taking place. There are very few such longitudinal studies, especially in unvaccinated herds.

Transmission

The pig is the only natural host for ADV. Many other animals are susceptible (including e.g. rats, mice, cats, dogs, sheep, goats and cattle) but are usually dead-end hosts. Humans are generally considered to be not susceptible.

Survival of ADV in the environment is dependent on the combined effects of pH, temperature and humidity (Davies & Beran 1981, Schoenbaum 1990*b*). Under optimal conditions the virus may survive up to 120 days (Davies & Beran 1981).

However, the survival of virus in infectious concentrations outside the host is thought to be very limited (Thawley & Torrison 1990, Kluge et al. 1999).

There are several possible routes of infection such as by fomites, transplacental transfer, breeding, artificial insemination or ingestion of infected tissue or milk (Beran 1993). Virus can also be transmitted by aerosols within a herd (Donaldson et al. 1983, Gillespie et al. 1996) or between herds (Gloster et al. 1984, Scheidt et al. 1991, Christensen et al. 1993). Introduction of ADV into a herd most commonly results from movement of infected pigs (Beran 1993) and the most common manner of transmission is by direct nose-to-nose contact between pigs (Gustafson 1986). Thus, as for most infectious diseases, the opportunity of direct contact between infected and susceptible pigs is important for the transmission of the virus. Whether or not transmission takes place during an encounter depends on the dose and strain of the virus, the age and immune status of the pigs and previous exposure to infection and/or vaccine.

Infective dose

It has been shown that piglets need less virus than adult pigs to become infected intranasally (Wittman 1991). For vaccinated animals the infective dose required is 100- to 1000-fold higher than for unvaccinated (Wittman et al. 1982). Also, the infectivity varies between different viral strains; it seems that the higher the virulence of the virus, the lower is the necessary dose to infect a susceptible animal (Van Oirschot 1988, Mc Cullough 1989).

Virus excretion

Shedding starts before or coincides with the onset of clinical symptoms (Kluge et al. 1999). Viral shedding via oropharyngeal secretions usually begins within 24 hours of infection and rises to a peak concentration in 3 to 6 days post-infection (Van Oirschot 1994). Virus can be isolated from nasal secretions for 8 to 17 days and from oropharyngeal secretions for 18 to 25 days (Wittman 1991). Continuous excretion has been reported in at least one case up to 6 months after recovery (Thawley et al. 1980). The duration of virus shedding is influenced by the strain (Maes et al. 1983).

After vaccination, a 100- to 1000-fold reduction in excreted virus titers has been observed (Pensaert et al. 1990), as well as a reduction in the duration of the shedding (Donaldson et al. 1984). Even so, the total amount of virus shed is claimed to be high enough to infect unvaccinated and vaccinated animals by contact (Wittman & Rhiza 1989).

After reactivation of latent virus, it appears that shedding is of shorter duration and lower concentration compared to during the acute phase of the disease (Wittman 1991). However, experimental studies have shown that this amount can be sufficient to infect other swine in contact (Van Oirschot & Gielkens 1984).

Transmission of virus within a herd

Once a herd has become infected, the two major ways for virus to persist in the herd is either by reactivation and shedding of latent virus or by continuous virus circulation (if there is a sufficient number of susceptible animals).

It has been stated that eradication may occur spontaneously in smaller pig herds when no control measures are applied (Beran 1986, Kluge et al. 1999). However, it is not clear to what extent these self-cleaned herds have been vaccinated. This distinction is important to make as it may not be appropriate to extrapolate findings on transmission in vaccinated herds to unvaccinated ones. For example, the finding that a large proportion of predominantly vaccinated breeding herds under quarantine in Minnesota had a low seroprevalence of ADV (Morrison et al. 1991), may not be representative for unvaccinated herds. Smith and Grenfell (1990) simulated the spread of ADV in unvaccinated breeding herds and found that ADV would not persist in herds with less than 66 sows. However, this model was not validated on field data.

Various approaches have been used to study transmission of ADV in herds, these include descriptive, analytical and intervention studies.

Descriptive studies

There are few studies of unvaccinated herds where individual animals have been followed serologically. Howarth (1969) sampled pigs introduced into a fattening herd with continuous production and found that seroconversion, which occurred in 50% of the retested animals, had a predominantly seasonal pattern. Maes and Pensaert (1984) monitored 15% of incoming piglets in 1-5 units in five fattening herds with all-in-all-out production. The pigs were followed during the fattening period and seroconversion occurred in pigs of two of the herds, in association with outbreaks in these herds. Medveczky and others (1990) tested 80 gilts in different stages of the breeding cycle. Seroconversion was only recorded after mating, in four gilts. Duffy and others (1991*a*) followed 20 gilts introduced into a breeding herd of 150 sows and observed seroconversion in three. The reported incidence rate was 19.6 per 100 gilt-years at risk.

Since the introduction of marker vaccines and marker tests, it has become possible to measure the incidence of seroconversion also in vaccinated animals. In vaccinated herds individual breeding animals have been followed serologically, either during a vaccination/eradication programme, or with the objective to document transmission in a vaccinated herd (Van Oirschot et al. 1990*b*, Duffy et al. 1991*a*, Stegeman et al. 1994, Van Nes et al. 1996 and the studies presented in this thesis). In most of the herds seroconversion in susceptible animals was very limited.

Descriptive studies like these produce incidence data and generate hypotheses, but have limited usefulness when making inferences from results with regard to risk factors.

Analytical studies

Analytical studies to identify factors influencing transmission of ADV within breeding herds have been performed e.g. in USA (Duffy et al. 1991*b*, Morrison et al. 1991, Weigel et al. 1992), Germany (Leontides et al. 1994, Leontides et al. 1995) and The Netherlands (Stegeman et al. 1995*a*). However, most of these studies are cross-sectional, based on point prevalence data of the herds and no conclusions can be drawn about causality, except for those factors that do not change over time (Frankena & Thrusfield 1997). Only one study is based on incidence data (Duffy et al. 1991*b*) and in one study the design is case-control, comparing infected with non infected herds (Leontides et al. 1994). It must also be noted that all or the majority of the herds in each study were vaccinated.

There is no general agreement between the studies regarding herd size as a risk factor. In three studies (Duffy et al. 1991*b*, Morrison et al. 1991, Leontides et al. 1994), large herd size was associated with either increased incidence, or high seroprevalence or seropositivity, whereas in two studies (Leontides et al. 1995, Stegeman et al. 1995*a*) small herd size was associated with high seroprevalence, and in one study no effect of herd size was shown (Weigel et al. 1992).

Factors positively associated with high seroprevalence or incidence were the presence (Stegeman et al. 1995*a*, Leontides et al. 1995) and serological status (Duffy et al. 1991*b*, Morrison et al. 1991) of fatteners in a herd and keeping pigs in indoor confinement (Morrison et al. 1991, Weigel et al. 1992). The fact that local pig density was identified as a risk factor for high seroprevalence (Weigel et al. 1992, Leontides et al. 1995, Stegeman et al. 1995*a*), points out that in some areas, factors influencing transmission between herds may be just as important as factors influencing transmission within a herd.

Stegeman and others (1995*a*) also examined the influence of vaccination schedule on the seroprevalence and found that vaccination of sows during nursing was associated with a higher seroprevalence than vaccination during late gestation or simultaneous vaccination of all sows every 5 months. There was also a difference in seroprevalence depending on which strain was used in the vaccine.

Intervention studies

The influence of different vaccination regimes on the spread of ADV among fattening pigs has been evaluated in field-based intervention studies. In a randomised controlled field trial, Stegeman and others (1995*b*) found that vaccination twice of fatteners reduced incidence significantly in comparison to

vaccination once. The reproductive rate (see below) was estimated to be 3.4 and 1.5, respectively, for once- and twice-vaccinated groups.

De Smet and others (1992) compared seroprevalence among fatteners in farrow-to-finish herds where sows were either vaccinated simultaneously every 4 months with live vaccine or during each lactation with subunit vaccine. Fatteners were vaccinated with live vaccine twice, once or not at all. Best results were obtained in herds where both the sows and the fatteners were intensively vaccinated with live vaccine.

The influence of different vaccination regimes on virus excretion has been studied in breeding sows which were vaccinated in the field but challenged under controlled conditions (Nauwynck et al. 1997). Two killed vaccines were compared to a live strain, suspended either in saline or oil-in-water. Sows that had been revaccinated 8-10 times were compared to sows revaccinated 1-3 times. A booster effect was recorded only for the live vaccine suspended in oil-in-water. Virus excretion was significantly lower in sows vaccinated with live vaccine compared to sows vaccinated with killed vaccine.

Reproductive rate

One important parameter for infectious diseases is the reproductive rate (R) which is the potential for a contagious disease to spread from individual to individual in a population. R is defined as the *average* number of individuals directly infected by an infectious case during its entire infectious period. The expression *basic* reproductive rate (R_o) is used when an infectious case enters a totally susceptible population. In general, for an epidemic to occur in a susceptible population, R must exceed 1. When R equals 1, the infection is endemic and when transmission is reduced to below 1, the infection dies out. The principal determinants of the reproductive rate, according to Giesecke (1994), are: a) the probability of transmission in a contact between an infected individual and a susceptible one, b) the frequency of contacts in the population, c) how long an infected individual is infectious, and d) the proportion of already immune individuals in the population.

An experimental method to quantify R for ADV in pigs has been demonstrated by De Jong and Kimman (1994), who compared transmission of a mildly virulent strain of ADV virus within vaccinated and unvaccinated groups of SPF pigs. R was estimated to 10 for the unvaccinated groups and 0.5 for the vaccinated groups.

R was estimated to be 0.7 based on field data from 98 breeding herds, in which the sows were vaccinated three times a year with live vaccine (Van Nes et al. 1996). Thus, with this vaccination regime, no major outbreak should be expected.

Pathogenesis and clinical signs

Many studies have been performed over a long period of time regarding the pathogenesis and clinical signs of ADV. For extensive reviews see Pensaert and Kluge (1989) and Pensaert and others (1991). The following summary is based on these reviews.

The pathogenesis and clinical signs of AD in pigs may differ depending upon the age of the pig, the dose and strain of virus and the route of infection. Most commonly, pigs are infected by direct nose contact with infected animals or by inhaling air containing the virus. Oral infection can also occur, but then larger quantities of virus are needed.

The nasal epithelium is especially susceptible to infection and productive for virus replication. Other sites of primary replication are the pharyngeal and ethmoidal mucosae, tonsillar tissues and lungs. From the primary sites of infection, the virus frequently invades the central nervous system (CNS), via neural pathways. Virus can also be transported with the lymph to the regional lymph nodes, where the virus replicates. Infected mononuclear cells may enter the blood-stream and thus disseminate the virus to other tissues and organs including the uterus. In pregnant animals this may lead to abortion.

The generalised infection is characteristic for more virulent strains, whereas low virulence strains are strictly neurotropic and do not cause lesions outside the CNS. More virulent strains may also have a more pronounced affinity for the respiratory tract.

The age of the pig strongly influences the type and severity of the clinical signs. In piglets, ADV infection causes extensive and progressive lesions in the CNS, resulting in neurological signs and death. Mortality is close to 100% in piglets less than 2 weeks of age, and decreases to 50-70% in 3 to 4 week old piglets. In older pigs mortality is usually below 5% and the disease is characterised by fever, loss of appetite, respiratory signs and only occasionally neurological signs.

In fattening swine, infection with ADV is often complicated by other concurrent respiratory infections. This has been suggested to be promoted by the decreased capacity of alveolar macrophages, which results from ADV infection (Iglesias 1989). Reduced weight gain is an economically important sequel to ADV infection in fatteners.

In breeding animals, where symptoms are often subclinical, the most important consequences of ADV infection are interruption of pregnancy in sows and infertility in boars.

Diagnosis

Serological tests

Several serological tests have been developed to detect antibodies to ADV. The most widely used are the serum neutralisation (SN) test and the enzyme-linked immunosorbent assay (ELISA).

The neutralising antibodies, which are responsible for humoral immunity, can be detected by the SN test from 9 or 10 days post-infection (PI) and peak between 14 and 21 days PI (Wittman & Rziha 1989).

Immunoglobulins M (IgM) and G (IgG) can be detected with the ELISA test from 5 to 6 days PI. IgM peaks 9 to 10 days PI and then decreases, whereas IgG peaks between 10 and 15 days and then may last for life (Wittman & Rziha 1989).

Glycoprotein E ELISA

The first serological test to differentiate antibodies to vaccine virus from antibodies to wild-type virus was introduced by Van Oirschot and others (1986). They developed a competitive enzyme immunoassay for detecting antibodies to glycoprotein E (then named gI), using a monoclonal antibody directed against gE. This glycoprotein is found within the envelope of field strains of the virus, but is lacking in some vaccine strains.

Later, a blocking ELISA, which was more practical for screening large numbers of samples, was constructed. This ELISA used two monoclonal antibodies directed against two separate epitopes on gE (Van Oirschot et al. 1988). With this test, antibody titres to gE can be detected 2 weeks PI, peak at 4 to 5 weeks PI and remain stable for at least 32 weeks (Van Oirschot et al. 1990*a*). Field observations have shown that antibodies to gE can persist for at least 2 years (Van Oirschot et al. 1990*b*).

Vaccines

Whereas vaccination against ADV has for many years been in use continuously to protect especially piglets from clinical disease and to prevent growth retardation in fatteners, it has, since the introduction of ADV marker vaccines (such as the gE deleted vaccines), also become a tool to achieve eradication. Even though ADV can infect vaccinated animals it has been shown that vaccination reduces both the susceptibility and the infectivity in vaccinated animals. Thus, today, the main objective for the use of ADV vaccines is to reduce transmission of virus in a population.

The influence of vaccination on the establishment of latency, reactivation and shedding after reactivation is not fully understood. It has been shown that vaccination usually does not prevent establishment of latency by field virus

(Mock et al. 1981). However, the level of colonisation attained by different live vaccines varies with strain, dose and route of administration and there are reports that certain live vaccines reduce the field virus latency load in trigeminal ganglia (Vilnis et al. 1998). Results are inconsistent regarding to what degree vaccination prevents shedding of reactivated virus (Schoenbaum et al. 1990, Mengeling et al. 1992).

When discussing vaccination in general, there are numerous factors to consider. The efficacy of vaccination can vary considerably depending on, for example, if the vaccine is live or killed, which adjuvant has been used, how the vaccine has been handled, vaccination interval, route of vaccination, the strain and dose of vaccine virus, status of the vaccinated pig (age, breed and immunological status) and type of challenge (dose and strain of virus, route of infection). The various aspects of vaccine efficacy have been discussed by van Oirschot (1992) and Stegeman (1995).

There are two broad categories of vaccines, live/attenuated vaccines and killed/inactivated vaccines. The virus in live vaccine is able to replicate in the pig. Since no replication takes place after vaccination with killed vaccine, a booster dose is necessary and also an adjuvant is added to improve immunogenicity.

Live ADV marker vaccines, especially when suspended in oil-in-water emulsion, are usually better at reducing challenge virus excretion than killed vaccine (Pensaert et al. 1990, Nauwynck et al. 1997). There are also some reports where ADV vaccination completely prevented a challenge infection (Van Oirschot 1988, Van Oirschot 1991).

Even though certain live vaccines are superior at reducing excretion of challenge virus, there remains a hesitation to use live vaccines because of the possible risks involved. A live vaccine may become contaminated with alien viruses during the manufacturing process, e.g. pestivirus (Van Oirschot 1994). There is also a risk that the vaccine virus will revert to greater virulence during replication in the host animal. Furthermore, there is concern that the vaccine virus will recombine either with a virulent field virus strain or with another vaccine strain (Henderson et al. 1991) thus resulting in the creation of virulent strains with the same negative immunologic markers as vaccine strains. It has also been suggested that gE-negative vaccine virus may persist and spread within pig populations (Christensen et al. 1992).

Clean-up of pig herds

The choice of which method to use for the clean-up of a herd from ADV, is based on several factors, the main ones being the seroprevalence in the herd, the financial need to clean-up quickly, the costs involved and the prevalence in the area (Morrison 1994).

Clean-up without use of vaccine

Up till the mid-80's the three basic strategies available for the clean-up of herds from ADV were depopulation-repopulation, offspring segregation and test and removal (Thawley et al. 1982). Vaccine was, in principle, only used to protect herds from clinical symptoms and not for the clean-up of herds from ADV, as vaccinated animals could not be distinguished from infected.

If a herd was heavily infected, it could either be cleaned-up by slaughter of the complete herd (total depopulation) or, if genetic qualities had to be salvaged, by rearing the weaned piglets intended as replacements in a separate facility (offspring segregation). Total depopulation was the most costly alternative as the farm was left unproductive for a period until repopulation could take place and the new stock was in production (Zimmerman et al. 1989).

Partial depopulation was carried out only when the seroprevalence was low in a herd, preferably below 25% (Thawley & Morrison 1988). Either the infected sows were culled directly after the test (immediate test and removal) or after weaning (phased test and removal).

The eradication programmes in Great Britain and Denmark were carried out without any use of vaccine. In Denmark, and to some degree in Great Britain, partial depopulation was implemented in low prevalence herds (Wyllie 1991, Andersen et al. 1989).

Clean-up with temporary use of vaccine

Before the era of marker vaccines, there were some reports that eradication had been achieved in herds by suppressing virus circulation within these herds with a short-term vaccination programme. Following this, the vaccinated breeding animals were replaced with unvaccinated, uninfected animals (Zuffa 1975, Mc Cracken et al. 1984, Vannier et al. 1984).

In 1984, Hogg reported on the clean-up of two vaccinated herds, based on the principle that antibody titers are short-lived (<6 months) in animals that have been vaccinated with a killed vaccine, in contrast to the long duration of titers resulting from infection with field virus or vaccination with live vaccine (Hogg et al. 1984, Hogg 1986). In these herds, the original breeding animals were rotated out by culling, and the entire herds were tested with a delay of 6 months after the last vaccination. Any animals with serum antibody titers were considered to be infected and were culled.

Completely new perspectives for the eradication of ADV opened up with the development of a complementary vaccine and test system. Van Oirschot and co-workers were the first to develop an ELISA to detect antibodies to gE, a glycoprotein which is absent in several vaccine strains. (Van Oirschot et al.

1988). Thus, pigs vaccinated with gE-deleted vaccine were negative in the gE ELISA whereas pigs infected with field virus were positive. As a direct consequence of this, herds with a large proportion of infected animals could be cleaned-up under cover of vaccine, either by test and removal or even by simply waiting until all positive animals had been rotated out of the herd without any premature culling.

Today, vaccination of the breeding herd with marker vaccines is the main method used for control of ADV (Morrison 1994). The first reported herd clean-ups based on marker vaccines (Van Oirschot & De Waal 1987, Van Oirschot et al. 1990 and paper III in this thesis) have been followed by several other reports.

Whichever clean-up program is chosen, it is necessary to know the status of neighbour herds. If they are infected it is not advisable to carry out a costly programme, due to the risk of reinfection. In endemically infected, swine-dense areas, the currently most practical and economical option is to vaccinate all herds simultaneously (Stegeman 1995, McInerney & Kooij 1997).

Pig production and history of Aujeszky's disease in Sweden

Pig production in Sweden

In Sweden, pig production is concentrated in the southern and south-western parts of the country. The two southernmost counties Skåne and Halland harbour 46% of the pig population in 4% of the total area of Sweden. Pig density in Halland is 261/100 hectares agricultural land and in Skåne 164/100 hectares agricultural land (Statistical Reports 1997). Yet, density in this area is low in comparison to e.g. the south of the Netherlands and the Flanders, with 1990 and 1020 pigs respectively per 100 hectares agricultural land (Nagel 1996). Between 3 and 4 million pigs are reared for slaughter annually in Sweden.

The number of pig herds in Sweden is continuously decreasing, while the size of the units are growing. In 1985 (at the inception of the work presented in this thesis) there were 15000 weaner pig-producing herds and 286400 breeding sows. In 1992 (at the conclusion of the studies) there were 9900 weaner pig-producing herds and 253800 breeding sows. Today (1998) there are 5200 herds and 260100 breeding sows. In 1985, 92% of the herds had less than 50 sows and 71% less than 20 sows. In 1992, the corresponding proportions were 87% and 64% respectively and for 1998, 75% and 57% respectively (*personal communication* Swedish Meats 1999).

The breeding structure for Swedish pig production can be described as a pyramid, where the top level comprises the nucleus herds which produce elite breeding boars (pure-bred Landrace, Yorkshire, Hampshire or Duroc). The second level consists of multiplier herds which produce hybrid gilts (crossbred Landrace and

Yorkshire). The next lower level of the pyramid contains the weaner pig-producing herds, where hybrid gilts are mated with pure-bred boars. Approximately one-third of these herds are farrow-to-finish herds. The bottom level of the pyramid consists of specialised fattening pig-producing herds. Animal movements in the pyramid occur only in a downward direction.

Since the 1950's a pig health control has been in function for the pig industry. The Swedish Animal Health Service routinely visits multiplier, weaner pig-producing and fattening herds to inspect and give advice on the clinical health status of the herds.

Aujeszky's disease in Sweden

The first recorded outbreak of AD in Sweden occurred in 1965 (Estola et al. 1965). The disease has since been notifiable, based on isolation of the virus. For 15 years (1965 to 1980), the incidence was limited to 1 to 6 outbreaks per year. However, during the succeeding decade (between 1981 and 1990) the incidence increased to between 10 and 36 outbreaks per year. Of 216 outbreaks reported until the end of 1990, approximately 80% had occurred in the last decade (see Figure 1). The majority of the outbreaks involved weaner pig-producing herds. In a survey conducted in 1988/89 to estimate the prevalence of AD in weaner pig-producing herds in Sweden, test positives were found only in the south of Sweden; in Skåne 19% and in Halland 12% of the herds were infected (Wahlström et al. 1990).

Twenty-seven field isolates of ADV collected between 1966 and 1989 from clinical outbreaks in geographically diverse locations in Sweden, have been examined by restriction enzyme cleavage, using Bam HI and Kpn I (F. Kovacs, unpublished data). The strains were found to be highly homologous and could be clearly distinguished from isolates from Denmark and Hungary with which they were compared.

In 1991 a national eradication programme was introduced. This was, in fact, a continuation of a compulsory control programme that had been in place in the nucleus herds since 1973, and a voluntary control programme for the multiplier herds since 1987. The eradication programme was supported by a grant from the government and was operated by the Swedish Animal Health Service (Robertsson & Wierup 1994). The programme was open to all pig-producing herds and participation in the programme was optional. However, there was a strong incentive to join it. Towards the end of the programme the industry refused to handle or slaughter pigs coming from non-participating herds and insurance companies did not pay compensation to herds outside the programme.

By September 1995 all herds had been tested at least twice and declared officially AD-free. In summary, of 8800 herds (with 240000 sows) tested, 362 (4.1%) were

found to be infected and 3097 infected pigs (1.3%) were sent to slaughter (Robertsson & Wierup 1999).

Sweden was officially declared free of AD in 1996 (Commission Decision 96/725/EC).

Aims of the thesis

The overall aim of the present thesis was to obtain experience with and knowledge about Aujeszky's disease in weaner pig-producing herds as a basis for the future National eradication programme in Sweden.

In order to establish alternative methods to depopulation, the following aims were set:

- to investigate whether unvaccinated herds could become free from ADV by using only seronegative animals for replacement (I);

- to quantify the seroconversion rate to ADV in seronegative breeding and replacement animals in unvaccinated herds (I);

- to quantify exposure of ADV-seronegative to seropositive animals and to relate this to seroconversion to ADV in an unvaccinated herd (II);

- to investigate whether herds could become free from ADV by using only seronegative animals for replacement during a period when the herds were vaccinated with either killed (III, IV) or live (V) gE-negative vaccine used in combination with a gE ELISA test.

Materials and methods

Animals and herds

The present work was carried out in 14 pig herds in the south of Sweden; 9 of these are described in paper I, 4 in paper IV and 1 in paper V. Two herds appear in more than one paper; herd A of paper I is also described in paper II and, as herd III, in paper IV and herd B of paper I is also described in paper III (for an overview see Figure 2). All herds had experienced one or more outbreaks of Aujeszky's disease during the 1980's.

Eleven herds produced weaner pigs to be sold at approximately 25 kg live weight to specialised fattening herds, and 3 herds were farrow-to-finish. The herd size ranged from 20 to 400 breeding animals. The majority of the sows and gilts were hybrids (crossbred Yorkshire-Swedish Landrace) and most boars were of the Hampshire breed. All herds but two bought all their replacement gilts from external sources.

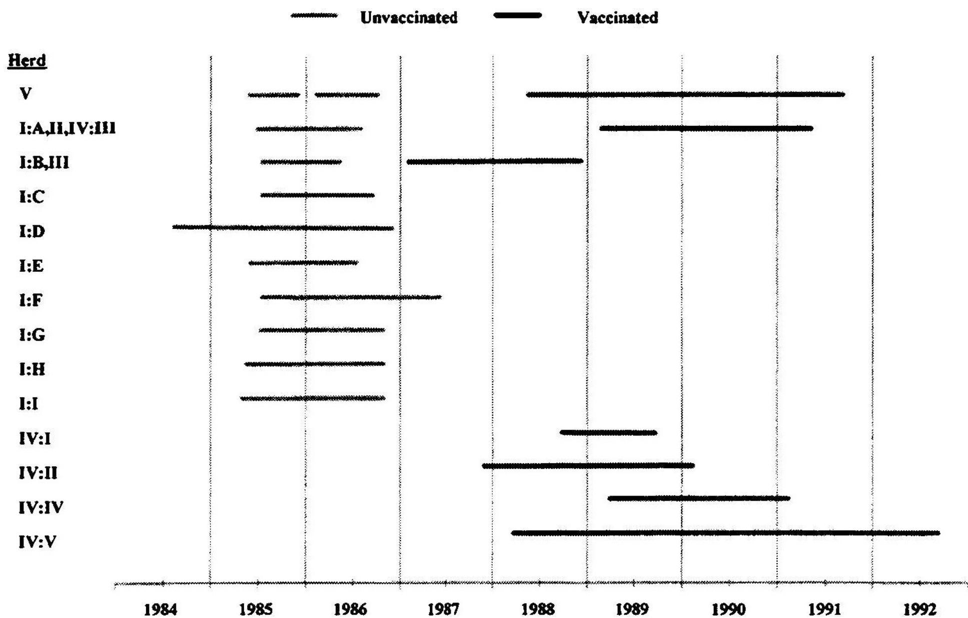

Figure 2. Time frame of the studies. Each herd is indicated by the Roman number of the paper in which it appears, and (for paper I and IV) by the herd designation given in the actual paper.

Diagnostic methods

Serological tests

As described in papers I-V, the following serological tests were applied in the studies. Unless otherwise specified, tests were performed at the National Veterinary Institute (SVA) in Uppsala, Sweden.

Blood samples taken from unvaccinated pigs (including the pre-vaccination tests) were analysed by the ELISA methods, used for routine diagnosis at SVA. Until June 1986 an indirect ELISA, developed at SVA, was used. From November 1986 a blocking ELISA (Sorensen & Lei 1986), was applied in routine diagnosis. Doubtful results were confirmed by the serum neutralisation test, performed according to standard procedures. From June until November 1986, all samples were analysed by serum neutralisation only.

Blood samples from the final test of the herd in paper V, after vaccinated animals had been rotated out, was analysed with the official test used in the National eradication programme in Sweden, which detected antibodies to glycoprotein B (formerly named glycoprotein II) (SVANOVIR PRV-gII-Ab; SVANOVA Biotech).

Blood samples taken from vaccinated pigs (papers III, IV, V) were analysed for antibodies to gE (formerly named glycoprotein I). This was either done at the Central Veterinary Institute (CDI) in Lelystad, The Netherlands, as described by Van Oirschot and others (1988), or with commercially available gE ELISA test kits. Either of two test kits were used (Suvaxyn gI test; Duphar and HerdChek Anti-ADV gI; IDEXX) in accordance with the manufacturers recommendations. Any doubtful samples from the test kit analysis were reanalysed at CDI.

Virus isolation

Tissue specimens of brain, tonsils, lung, liver and spleen were collected at necropsy from dead piglets and frozen until analysed. Virus was isolated at SVA according to standard virological methods and the isolates were identified by virus neutralisation using a known ADV antiserum.

Vaccines

Vaccines were applied intramuscularly by either the author, the herd veterinarian or the herd manager under supervision of the herd veterinarian.

A killed vaccine, PR Vac Killed (Norden), was used in the herds of papers III and IV. However, due to problems with delivery of this vaccine, two of the herds of paper IV also used Auskimmune K (SmithKline). Both vaccines contained the same gE- negative strain of virus derived from the BUK strain.

The live vaccine used in the herd in paper V was Nobi Porvac Aujeszky Live (Intervet) dissolved in Diluvac. This vaccine contained the live virus strain Begonia, in which the genes encoding for gE and thymidine kinase (TK) had been deleted.

Field studies: 1. Eradication of ADV by replacement with uninfected animals

Serological study in nine weaner pig-producing herds (I)

In order to quantify the seroconversion rate to ADV and to investigate whether it would be possible for unvaccinated ADV-infected herds to eradicate the virus by replacing animals seropositive to ADV (on ELISA) with seronegative, nine weaner pig-producing herds in the south of Sweden were studied. Breeding animals testing negative at the initial test, along with replacement animals, were followed serologically every second or third month. The herd owners were advised to keep seropositive and seronegative animals separate whenever possible and seropositive animals were marked by red eartags. Herd owners were also advised to use sanitary measures aimed at protecting the herd against reintroduction of virus.

For each seronegative pig, the *period at risk for seroconversion* was determined (expressed as "pig-days at risk"). The starting point was either the date of the initial herd test or the date when a pig was first tested after it had been introduced into the herd. The period at risk ended on the test date when a pig seroconverted or, for seronegative pigs, the test date after it had been culled, or the date of the final herd test.

The *incidence rate per 100 pig-years at risk* for each herd was calculated as:

$$\frac{\text{Total number of pigs seroconverting during the study} \times 100}{(\text{Total number of pig-days at risk} \ / \ 365)}$$

The *proportion of susceptible pigs seroconverting* was calculated as:

$$\frac{\text{The total number of pigs seroconverting during the study}}{\text{The total number of pigs at risk of seroconverting}}$$

Quantification of opportunity of contact between infected and uninfected animals (II)

In order to quantify exposure of seronegative animals to seropositive and relate this to seroconversion, one of the herds of paper I was subjected to a more detailed study, in which the location of animals was recorded on stable maps by the herd owner every one or two weeks, during a period of 319 days. The opportunity of nose contact between the two categories of pigs, as reconstructed from the stablemaps, were expressed as Days-In-Contact (DIC) and accumulated Daily-Pig-Contacts (DPC). One DIC was counted for each day a seronegative pig

had the opportunity of nose contact with at least one seropositive pig in the same or adjoining pen.

For each seronegative pig, the number of seropositive pigs that were within contact distance per day, was also determined. One DPC was counted for each seropositive pig per day with opportunity for nose contact with seronegative pigs. The accumulated DPCs were categorised according to whether opportunity of contact involved pigs within the same pen or pigs in neighbouring pens, i.e. pens which shared a common wall which allowed nose contact through iron bars.

Serological study in a farrow-to-finish herd (V)

Replacement gilts in a farrow-to-finish herd were followed for two periods with serological testing every second to third month. During the first observation period, three groups of gilts (in total 73 gilts) which had arrived during 3 consecutive months, were followed for 6 months. No interventions were carried out regarding management or hygiene, apart from the immediate culling of two gilts and a teaser boar which tested positive at the first test.

The following year, the study was repeated and four groups of gilts (in total 70 gilts) which had arrived during 4 consecutive months, were followed for 6 to 8 months. One gilt which tested positive at the first test was immediately culled. On this occasion, strict measures were taken to try to prevent contact transmission of virus to the replacement animals. Only seronegative boars were used for mating gilts. Measures were also taken to prevent nose contact between replacement animals and the other animals in the herd. The gilts were moved via the outdoor yard between the different units, instead of (as previously) through units containing infected animals. The dry sow unit was divided by a temporary wall to provide the gilts with a separate unit. In the farrowing units, the monitored gilts were occupying either a whole unit of 10 pens, or at least one side (5 pens) of a unit.

Field studies: 2. Eradication of ADV by vaccination and replacement with uninfected animals

The purpose of these studies was to find out if it would improve prospects for eradication of ADV from infected herds, if virus circulation in the herd was reduced by vaccination, during the period when animals seropositive to ADV in ELISA were replaced with seronegative.

Eradication programme with killed vaccine, initially based on an attempt to distinguish between infected and uninfected animals by the difference in duration of antibody titers (III)

One weaner pig-producing herd which had failed to be cleaned-up (paper I) was subject to a vaccination/eradication programme in which differentiation between infected and vaccinated uninfected animals was intended to be based on the

difference in duration of antibody titers between the two. To render this possible, the maximum number of vaccinations allowed for each animal was 3. In order to achieve this, the entire herd was scheduled to be replaced within 18 months. Replacement animals were vaccinated once with a killed vaccine and boostered in connection with the revaccination of the entire herd every 6 months. In order to follow seroconversion in seronegative animals, blood samples were collected in conjunction with the two herd revaccinations. Blood samples were analysed for ADV antibodies with the ELISA used in routine diagnostics at SVA. When it was discovered during the study that this ELISA could not be used to differentiate between vaccinated and infected animals, the blood samples were thereafter analysed with a gE ELISA.

Eradication programme with killed vaccine based on distinguishing between infected and uninfected animals by the use of gE ELISA (IV)

Three weaner pig-producing and two farrow-to-finish herds participated in a vaccination/eradication programme, based on the use of gE-negative killed vaccine and the gE ELISA. To begin, the entire breeding herds were tested for antibodies to ADV using the routine ELISA, and vaccinated. Animals that tested negative in the first test were given a booster dose after approximately 4 weeks and were thereafter revaccinated together with the entire breeding herd. All breeding animals of the herds were revaccinated simultaneously every 4 months. Replacement animals were vaccinated at arrival and boostered after 3 to 4 weeks. In one of the two farrow-to-finish herds, a limited number of fatteners, born to seronegative sows, were vaccinated during a period when there still remained progeny of seropositive sows in the fattening units. During the vaccination programme, sanitary measures were taken to prevent reintroduction of virus. The herd owners were also advised to keep seropositive and seronegative animals separate whenever possible and to clean and disinfect pens before they were used by seronegative animals. It was also recommended that owners selectively cull seropositive animals, when economically feasible, and keep the population density lower than normal. When all seropositive breeding animals had been rotated out of the herd, the programme ended after two negative tests of the breeding animals.

Eradication programme with live gE-/TK- vaccine and gE ELISA (V)

In a large farrow-to-finish herd with a long history of ADV infection, a live gE-negative/TK-negative vaccine was used for the breeding animals only. The vaccination schedule was the same as in paper IV. No fatteners were vaccinated. Blood samples were collected from previously seronegative animals on five herd visits. When all seropositive breeding animals had been rotated out of the herd, the programme ended after two negative tests of all the breeding animals and finding no positives in a sample of the fatteners.

Results

Eradication of ADV by replacement with uninfected animals

Serological study in nine weaner pig-producing herds (I)

The serological examinations carried out in nine weaner pig-producing herds in which only uninfected animals were used for replacement, revealed that all the herds experienced long consecutive periods (≥4 months, median 9 months) without seroconversion in monitored animals. ADV was eradicated from 3 of the herds during the study. In 2 of these herds no seroconversion was observed. The third herd, which experienced recurrent seroconversions, became free after increased culling of seropositive sows. There was no accelerated culling of seropositive sows in any other herd during the study. However, the culling of seropositive boars was increased in most of the herds. In the 7 herds where seroconversion occurred, between 9 and 86 percent of the susceptible pigs became infected and the herd-specific incidence rates varied between 14 and 156 newly infected pigs/100 pig-years at risk. A predominantly sporadic pattern of seroconversion was observed in 3 herds. In 4 herds, epidemic seroconversion occurred; in the 2 largest herds this was associated with a high piglet mortality and depression in adult animals. In the 2 smallest herds, reintroduction of virus was suspected.

Quantification of opportunity of contact between infected and uninfected animals (II)

In a weaner pig-producing herd where exposure of seronegative animals to seropositive was quantified, the accumulated Daily-Pig-Contacts over 319 days between 53 uninfected and 43 ADV-infected swine was 35660 and the total Days-In-Contact was 10809. Seventy-five percent of the monitored seronegative pigs were within nose contact distance to one or more seropositive pigs for at least 110 days and 50 percent for at least 222 days.

In spite of frequent contact, none of the animals seroconverted during the first 8 months of observation. At the test carried out 10 months after the start of the study, 1 animal was classified as "suspect" (in the subsequent test it was positive). A clinical outbreak occurred in the herd during the 12th month of observation. At the final test, carried out 13 months after the start, 45 of 52 animals had seroconverted.

Serological study in a farrow-to-finish herd (V)

Seroconversion occurred in both cohorts of replacement gilts which were followed up in a farrow-to-finish herd. In the first cohort of 73 gilts, which were not subject to any changes in management, 1 animal had seroconverted at a test carried out 4 months after the start. During the following month, a clinical

outbreak of AD occurred in the breeding herd. At the test performed 6 months after the start of the study all the remaining 57 animals had seroconverted. The second cohort of 70 gilts, where biosecurity was implemented, also experienced seroconversion although no clinical AD was observed during the study. At the test carried out 5 months after the start, 40 animals had seroconverted. At the next test, 3 months later, another 14 animals out of 26 remaining seronegative animals had seroconverted. Incidence rates of seroconversion in the first and second cohorts were 170 and 173 respectively per 100 pig-years at risk.

Eradication of ADV by vaccination and replacement with uninfected animals

Eradication programme with killed vaccine, initially based on an attempt to distinguish between infected and uninfected animals by the difference in duration of antibody titers (III)

In the vaccination/eradication programme, initially intended to be based on the difference in duration of antibody titers on ELISA between infected and uninfected animals which had been vaccinated, it was discovered that it was not possible to distinguish between the two categories of animals by this method. At the first test carried out on replacement animals which had been vaccinated only once, it was found that 18 of 22 animals and 20 of 26 animals vaccinated 6 and 3 months respectively before the test were positive in the routine ELISA. Thus it was not possible to tell whether the positive results were due to vaccination or to field infection.

However, since a gE-negative vaccine had been used, it was feasible to reanalyse the samples with a gE ELISA. All samples were negative to gE. The vaccination programme then continued, based on the gE ELISA, and was completed 22 months after the start of the programme. No seroconversions to gE took place during this period.

Eradication programme with killed vaccine based on distinguishing between infected and uninfected animals by the use of gE ELISA (IV)

Of 5 herds participating in a vaccination/eradication programme, no seroconversion to gE was observed in 2. In one herd (herd IV) the vaccination programme started during a clinical outbreak and 15 animals which seroconverted after the first test were probably infected from the start but without detectable antibodies. In another herd (herd II) a replacement gilt seroconverted. Thus, there was no or very limited spread in 4 of the herds and the programme lasted 12 to 26 months in these herds. More extensive seroconversion occurred in a farrow-to-finish herd (herd V) where no isolation unit for the replacement animals was available until 2 years into the programme. When all known infected animals had been culled, 3 ½ years after the start of the programme, the entire breeding herd was tested. Of previously seronegative animals which still

remained in the herd, 23 of 72 which had not been isolated at arrival, had seroconverted. This herd was declared gE-negative 53 months after the start of the vaccination programme.

Although the vaccination schemes were followed in all 5 herds, the ambition of the herd owners to achieve eradication of ADV varied. In the herd with the most motivated owner (herd I), strict separation was maintained between old and new pigs and the turnover of animals was accelerated so that the programme could finish within 12 months. In contrast was herd V where economic problems took priority over the ambitions to clean-up the herd. For 2 years there was no isolation unit available and it sometimes happened that replacement animals were placed at arrival in the same section as the unvaccinated fattening pigs. The turnover of breeding animals was slow and the initial time plan for the programme was not followed.

Eradication programme with live gE-/TK- vaccine and gE ELISA (V)

At the start of the vaccination/eradication programme in a large farrow-to-finish herd, virus was circulating in both the breeding and the fattening sections. Of the 11 breeding animals that seroconverted to gE during the programme, 7 were present in the herd from the beginning and were possibly infected at the first test but without detectable levels of antibodies. Four sows bought during the programme seroconverted. During the second year of the programme, culling of seropositive sows was accelerated and 22 months into the programme only 8 seropositive sows remained in the herd. Although only breeding animals had been vaccinated, both the breeding and the fattening herds became gE-negative during the programme which lasted for 39 months.

Discussion

The work in this thesis has shown that ADV can be eradicated from pig herds by gradual replacement of seropositive sows with uninfected gilts, with or without a concomitant vaccination programme. In the unvaccinated herds it was demonstrated that it is difficult to avoid contact between infected and uninfected animals under normal conditions, and that contact was frequent in these herds. Even so, transmission from latently infected animals to susceptibles appeared to be limited, as all the unvaccinated study herds experienced long periods without seroconversion. However, once transmission takes place among unvaccinated animals, there is always a risk that this may lead to an outbreak and three of the largest unvaccinated herds of the study did experience severe outbreaks. When vaccination was subsequently applied in these three herds ADV was eradicated.

In the vaccinated herds, no outbreaks occurred and the incidence of seroconversion was very low, except for one herd where gilts were placed in direct contact with unvaccinated fattening pigs. It appears that vaccination may promote risk behaviour in herd managers and it is important that basic principles of biosecurity are not neglected during a vaccination programme.

The work in this thesis provides information which would have been difficult to obtain in countries with high pig density and continuous vaccination. Here, it was feasible to compare herds before and after vaccination. It was also possible to study herds in which reintroduction of virus was not likely and seroconversion was indicative of the reactivation of latent virus. Although the work of the present thesis was descriptive and limited to 14 herds, it demonstrates what may happen in other similar herds and situations.

The results from any study should be interpreted with due regard to the country and the herds in which it is performed, and, in particular, to factors such as management, herd size and virulence of the virus that may influence the results. The present study was not designed to evaluate the influence of management or to find risk factors for the spread of ADV - there were too few herds to do this and furthermore the use of control herds would have been necessary. As regards virulence, all herds of the study had experienced clinical outbreaks of AD with CNS symptoms and increased mortality in piglets and abortions in sows (see table I in study I and table 1 in study IV) and in some herds depression was also observed in adult animals.

Unvaccinated herds

Patterns of seroconversion

The present results show that smaller herds (<50 breeding sows) may achieve freedom from ADV without the use of any radical control methods, by gradual replacement of infected animals with uninfected, when there is no continuous virus circulation in the herd or reintroduction of virus from outside (I).

A major concern in unvaccinated herds is that the risk of an outbreak increases when the proportion of susceptible animals increases and herd immunity decreases. However, even though seroconversion occurred in most of the herds which were subject to serological follow up, this did not necessarily escalate to outbreaks. With the exception of two herds, in which reintroduction was strongly suspected, major outbreaks occurred only in the three largest herds (I, V).

It is likely that herd size influences the risk of an outbreak in an unvaccinated population, since the opportunity for transmission to take place increases with the number of infectious and susceptible animals in a population (Anderson & May 1991). In large unvaccinated herds, where a high number of susceptible animals may become involved, the infection may easily escalate to an outbreak. In addition to transmission by direct contact, high concentrations of virus may build up in the air and spread to animals in other parts of the herd. Furthermore, with an increasing dose of virus the clinical signs may also be aggravated (Gustafson 1986).

An important finding of our study was that all herds experienced long consecutive periods without seroconversion, which implied that no transmission was taking place during these periods. In order to investigate the transmission of ADV it is important to quantify contact and incidence of reactivation of latent virus.

Contact pattern

The frequency of direct nose-to-nose contact between pigs is of interest, as such contact is considered to be the most common manner for transmission of ADV (Gustafson 1986). Pigs have a very well developed sense of smell and the contact nose-to-nose is one of the most important behaviours of social interaction between pigs (Jensen 1997). In a study of social interaction patterns in dry sows, nose contacts for each sow within the pen with other pigs in the pen was 5 per hour (Jensen 1984). From this it can be inferred that several contacts will occur within a day, wherever there is an opportunity for contact.

The opportunity of contact was estimated in a more detailed study (II) of one of the nine herds participating in study I. All animal movements were recorded during 319 days and the accumulated daily opportunity of contact between 53

uninfected and 43 infected swine was 35660 days. In spite of this, no transmission took place in this herd for at least 8 months. There was no indication that the opportunity of contact in this herd was more frequent than in any of the other herds of study I. Thus, it is remarkable that long periods passed in all these herds without transmission of ADV.

Transmission of reactivated virus

There could be two explanations why no transmission occurred over long periods, in spite of frequent contact. Either the reactivation of latent virus is infrequent and/or the excreted dose of reactivated virus is rarely sufficient to infect animals in contact.

The rate of spontaneous reactivation of ADV in the field is not known. If the recurrence rate of ADV in pigs is comparable to that of the closely related herpes simplex virus in humans, it would be quite frequent. In studies of humans with clinical recurrence of HSV, a mean recurrence rate of 4.8 episodes per person per year has been reported for herpes labialis (HSV-1) (Laerum et al. 1991) and for herpes genitalis (HSV-2) a median recurrence rate of 4 episodes per person and year (Benedetti et al. 1994).

An indirect measure of the frequency of reactivation could be the incidence of episodes of seroconversion (involving one or more animals) between periods without seroconversion. In the five herds observed for 10-23 months (study I) in which no reintroduction or continuous circulation of virus was suspected, there were 9 episodes of sporadic or epidemic seroconversion, in between silent periods, that were presumably set off by reactivation in one animal (see Figure 1 in paper I). However, as each reactivation event does not always result in transmission, this must be regarded as a conservative estimate of the rate of reactivation.

The lack of transmission over long periods, in spite of frequent contact and possibly frequent reactivation, suggests that the infectious dose may be insufficient. The amount of virus shed after reactivation is low and shortlasting (Wittman 1991) and adult pigs in contact require a considerable amount of virus to become infected. It has been reported that contact pigs may become infected by an animal shedding after experimental reactivation, (Van Oirschot & Gielkens 1984). However, this may not be representative of field conditions. The latency load and the amount and duration of excreted virus may be higher in the experimental situation, as experimental pigs are often inoculated with high doses of virulent strains of virus (Mengeling et al. 1992). In the first documented case of spontaneous reactivation and shedding of ADV, in a sow shortly after farrowing, the two sentinel animals in contact with the sow did not become infected (Davies & Beran 1980). Nor did any infection occur in 12 unvaccinated sentinel piglets, in several breeding herds, which spent 2-8 weeks within the same

box as infected, vaccinated sows that had recently farrowed (Maes & Pensaert 1984).

When it is taken into account that for many years a large number of gilts and weaner pigs in Sweden where sold from infected herds, it is remarkable that only 4.1% of the herds were actually found to be infected during the National eradication programme. This also implies that transmission from latently infected animals is limited.

Vaccinated herds

Vaccination and eradication

The present studies show that ADV can be eradicated from pig herds by intensive vaccination and gradual replacement with uninfected gilts. All seven herds taking part in the vaccination/eradication programme achieved gE-negative status and were reconfirmed free of ADV when retested within the National eradication programme after all vaccinated animals had been rotated out of the herd. This has also been found when these methods have been applied on an area-wide basis, especially in the Netherlands (Stegeman 1997, Bol et al. 1998), Germany (Bätza 1999) and France (Vannier et al. 1999).

It has been proposed that individual herds that have been cleaned-up under cover of vaccine, might have achieved the same results by spontaneous elimination (Stegeman 1995). However concerning at least three of the herds of the present study, spontaneous elimination would not have been a likely event. These three relatively large herds (100-400 breeding animals) with a long history of recurring problems with AD, were followed up serologically previous to being vaccinated and failed to become free by gradual replacement with ADV-free replacement animals (I, V).

Methods for differentiation

The first method that attempted to distinguish between infected and vaccinated pigs was based on the duration of antibody titers as described by Hogg (1986). However, even though the pigs in the herd of study III had not been vaccinated more than once with killed vaccine at the time of testing, differentiation between sera from the two categories was not possible with the Swedish routine ELISA. This may have been due to a difference in sensitivity between the tests used in Sweden and USA. Also, it has been shown that the pig breed may have an influence on antibody titers after vaccination (Rotschild et al. 1984).

All the vaccination/eradication programmes of the present thesis (papers III, IV and V) were subsequently carried out based on the use of marker vaccines and marker tests for the differentiation between infected and vaccinated pigs.

Effectiveness of vaccination programmes

Vaccine or vaccination programmes that are proven to be efficacious under experimental or highly controlled conditions may or may not be effective in the field. It has been experimentally demonstrated that vaccination reduces transmission by increasing the dose of virus needed to infect an animal (Wittman et al. 1982) and by reducing the excretion of virus in an already infected animal (Pensaert et al. 1990). The results of the present field studies (III, IV, V) strongly implies that vaccination also reduces transmission in the field. There was no or very limited seroconversion in 6 of the 7 herds during the programme. In the seventh herd, a farrow-to-finish herd, extensive seroconversion occurred among gilts which were placed within the same room as unvaccinated fatteners (IV). Limited seroconversion has also been recorded in other longitudinal studies of vaccinated herds where individual breeding animals have been followed (Van Oirschot et al. 1990*b*, Duffy et al. 1991*a*, Stegeman et al. 1994, Van Nes et al. 1996). However, from those studies it is not possible to quantify the reduction in seroconversion following vaccination.

Unique for the present study, is that three of the herds (two herds of study I: A & B, and the herd in study V) had been followed serologically before they were vaccinated and thus provide an estimate of the reduction in the rate of seroconversion after vaccination. During the serological study in herd A and B (I), 77% and 86% respectively of the susceptible pigs seroconverted during the serological study whereas none of the pigs seroconverted during the vaccination/ eradication programme. In the farrow-to-finish herd (V) 77% and 100% respectively, of gilts in two cohorts seroconverted during the serological study, whereas after vaccination of the breeding herd less than 3% of the susceptible animals seroconverted.

This drastic reduction in incidence of seroconversion implies an effectiveness of close to 100% for the vaccination programme in the three herds, providing that conditions in the herds were similar before and after the vaccination. This is reasonable to assume, as the time span was relatively short between the end of the serological study and the start of the vaccination programme (10-32 months). There were no known changes in management apart from the selective culling of seropositive animals at some stage during the vaccination programme. In herd A most of the seropositive sows were already culled within the first year of the programme, whereas in herd B and the farrow-to-finish herd, culling was not based on serostatus until the second year of the programme.

A high efficacy of vaccination is supported by experimental data presented by De Jong and Kimman (1994), who compared transmission of a mildly virulent virus within vaccinated and unvaccinated groups of SPF pigs. In the unvaccinated groups, all 10 contact-exposed animals became infected, and in the vaccinated groups, 3 of 10 became infected, which implies an efficacy of 70%.

Implications and speculations

No escalation of the infection

The present results and results from subsequent vaccination/eradication programmes indicate that in a breeding herd where a proper vaccination programme is implemented, it is unlikely that an infectious dose emanating from a single pig (whether introduced acutely infected or following reactivation) would be multiplied within the herd to cause widespread seroconversion. The infection is likely to be limited to within the pen and then die out, irrespectively of herd size. Thus, reactivation of latent virus should not be a major concern in the vaccinated herd.

The suggestion that transmission is independent of population size among vaccinated pigs, is supported by experimental data from Bouma and others (1995) who found no significant difference in transmission of ADV in groups of 10 and 40 vaccinated pigs respectively. However, this probably does not hold true for unvaccinated populations, where airborne transmission within the herd must also be taken into account. The larger the herd, the more likely it is that the critical mass for airborne transmission within the herd is achieved.

Reintroduction by aerosol

It is reasonable to assume that for widespread seroconversion to occur in a properly vaccinated breeding herd the challenge should be very large. Thus, it is likely that such an infectious dose has first been built up elsewhere, in a susceptible unvaccinated population and introduced by aerosol. This could be the unvaccinated fatteners in a farrow-to-finish herd or a neighbour herd that is not vaccinated. Reintroduction of virus to breeding pigs from the unvaccinated fattening pigs in the same herd has been reported (Van Nes et al. 1996).

Inadequate vaccination

A second explanation for outbreaks or widespread seroconversion to occur in vaccinated herds is an inadequate vaccination procedure with regard to, for example, vaccination intervals, handling of vaccine and injection technique. In an inventory of setbacks of the vaccination/eradication programme in the Netherlands, Bol and others (1998) found that outbreaks or high seroprevalence were associated with the lack of use of ”gold standard vaccine” or an incorrect vaccination strategy.

Risk behaviour

One aspect of vaccination against ADV that has not been sufficiently elucidated, is the possibility that vaccination may bring about a changed attitude in herd managers towards eradication. It has been observed in France that it is much more difficult to obtain eradication or control of AD when vaccination is applied than when only sanitary measures are applied (Vannier et al. 1997).

Since vaccination protects against clinical symptoms, the herd owner may not feel it is urgent to eradicate the virus, and the incentive to take any further measures may be reduced. As a consequence, if the herd owner regards vaccination as a safeguard against further problems, basic principles of biosecurity may be neglected. An example of this is the observation in study IV that two herd owners showed a relaxed attitude towards following the plan for eradication once vaccination had been carried out. Also, in one of these herds, newly arrived gilts were placed among unvaccinated fatteners. This implies that a vaccination programme could actually *promote* risk behaviour among herd managers.

Final remark

The development of marker tests and marker vaccines has been a very important breakthrough in the control and eradication of ADV. The fact that infected and vaccinated pigs can be distinguished from one another has eliminated most hesitation towards using vaccines. However, it is important that the vaccination procedures are correctly applied and that basic principles of biosecurity are not neglected during the programme. The possibility that vaccination could promote risk behaviour needs further attention in future studies.

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