Aujeszky’s disease

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Summary: This review presents data concerning economic losses due to Aujeszky’s disease (AD), followed by a description of the clinical signs of the disease in pigs, cattle, small ruminants, dogs and cats.

The geographic distribution of AD, its evolution, the sources of the virus, the susceptibility of animal hosts to the virus and means of virus transmission are described.

Advantages and disadvantages of clinical and laboratory diagnosis are discussed, with regard to practicability. Special emphasis is placed on histopathological changes, methods of virus isolation in laboratory animals and cell cultures, detection of viral antigens by immunofluorescence and immunoperoxidase techniques, and the most important serological assays (neutralization, ELISA, immunodiffusion). In addition, other less important serological tests and the skin test are critically reviewed.

Possible legislative measures for the control of AD are listed. Vaccination is widely used in this context. Even though latently infected animals can remain in the vaccinated herds, they are not liable to restrictive measures. The problems which result are discussed. Precautionary measures to prevent the introduction of AD into AD-free countries are mentioned. The author’s view on the perspective for eradication of AD is presented.


INTRODUCTION

Aujeszky’s disease (AD), which is caused by a virus of the herpesvirus group, has gained increasingly in significance all over the world since its identification by the Hungarian veterinarian, Aujeszky, in 1902 (11). The intensification of pig production has favoured the spread of the disease, causing great economic loss. In the Federal Republic of Germany (FRG) DM 61 million were paid in compensation for animals slaughtered from 1980 to 1982. Costs of £ 22.8 million were incurred by the eradication programme in the United Kingdom (10), which started in 1983 and is still running. In a French study (35), direct and indirect loss caused by AD in two farms, each with eighty sows, was calculated. The loss was estimated at FF 167,750 though only three sows and five fattening pigs died and five abortions occurred.

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CLINICAL SIGNS

Pigs

The clinical picture of AD in pigs varies greatly according to the age of the animal. The younger the animals, the more serious the symptoms and the higher the mortality. The incubation period ranges from 1 to 11 days, most frequently being 3 to 6 days. The mortality rate is up to 100% in piglets of less than 2 weeks, about 50% in 3-week old piglets and decreases to less than 5% in mature pigs. However, not only age, but also other factors are influential, such as the amount and virulence of the virus, individual condition of the animal and stress situations (60). Accordingly, mortality rates can be higher at any age.

In piglets less than 3 weeks old, sudden death can occur with few, if any, clinical signs. More often death is preceded by fever, lethargy, loss of appetite, weakness, lack of coordination and convulsions. Vomiting and diarrhea can be present. Pigs less than 2 weeks old usually die. Suckling piglets can be infected before birth in the uterus. After birth they die within two days, occasionally having manifested violent shaking and shivering (shaker pig syndrome). Piglets infected immediately after birth show clinical signs within the first two days, and usually die before they are 5 days old. In older pigs the symptoms start with fever followed by loss of appetite, listlessness, aphonia, somnolence, laboured breathing, vomiting, rambling and, in some animals, lack of coordination and weakness occurring in the hind-quarters. Death is usually preceded by convulsions. Involvement of the respiratory tract becomes apparent with sneezing, coughing and nasal discharge. Recovered pigs have significant loss of weight. The intensity of the clinical signs mitigates with rising age. Hence, in adult pigs, the disease is usually not severe. Fever is always present, and nasal discharge, coughing, aphonia and somnolence frequently occur, whereas typical nervous symptoms can be observed only occasionally. Usually no marked pruritus develops in pigs of any age, but aggressiveness may occur.

ADV infection of sows in the early stage of pregnancy results in death and resorption of their foetuses. Infection in middle pregnancy causes abortion of mummified foetuses, whereas infection in late pregnancy brings on abortion, stillbirth or birth of weak piglets which die within a few days.

Cattle and other ruminants

The incubation period varies from 3 to 6 days. Nasal discharge can be the first sign, followed by serious symptoms 2 or 3 days later, namely restlessness, dyspnoea, salivation, foaming and tympanicity. Loss of appetite does not generally occur, but the animals drink excessively. Muscle tremor is often seen. The animals paddle with their legs when lying in a lateral position and spasms of head, neck and abdominal muscles occur. Intense pruritus is the most characteristic sign of AD, but it is not present in every case. The animals lick their shoulders and their foreand hind-legs almost continuously; they scratch their heads with the hind-legs and rub the irritated parts and the perineum against a surface, inflicting open wounds. The animals groan and bellow and may become aggressive. High fever can occur. Quite suddenly the animals will fall down and die, usually within 2 to 3 days of the onset of the serious symptoms. In calves, death can occur so fast that no typical symptoms of AD are able to develop (90). Recovery of cows from the disease is extremely rare (39, 84).
In sheep and goats AD follows a pattern similar to that in cattle. Restlessness, dyspnoea, fever, lack of coordination and pruritus are observed. The animals lick and scratch themselves, tearing out their wool, with resultant skin lesions. They lie down and die within a few hours. The disease often takes such a rapid course that no typical symptoms occur.

**Dogs and cats**

The incubation period in dogs and cats varies from 2 to 4 days. The animals present loss of appetite, salivation, vomiting and dyspnoea, but usually no fever. Periods of apathy alternate with periods of excitement. The dogs bite at the air without attacking man. They become cowed and drink water excessively. Severe pruritus accompanied by self-mutilation occurs in most cases. Completely exhausted, the dogs die within 24 hours following the onset of the symptoms.

Infected cats are apathetic and prefer dark places. The head is frequently distorted. The animals mew loadly and hoarsely and salivate. Alternating periods of exhaustion and excitement occur and the animals attack other cats but not man. Pruritus occurs relatively seldom. The animals die within 24 hours.

**EPIDEMIOLOGY**

**Geographic distribution**

According to the FAO/WHO/OIE Animal Health Yearbook, 1984, Aujeszky's disease is endemic in Ireland and Northern Ireland, the Netherlands, Belgium, France, the Federal Republic of Germany and Spain. Sporadic outbreaks occur in the United Kingdom, Denmark, Sweden, the German Democratic Republic, Poland, USSR, Austria, Czechoslovakia, Hungary, Romania, Bulgaria, Yugoslavia, Albania, Greece, Italy and Portugal. AD is widespread in the USA and Mexico and occurs in Cuba, Guatemala, Venezuela, Brazil and Argentina. AD is reported in Togo and in Syria. Thailand is strongly infected, followed by Laos, Vietnam, the Philippines, Malaysia, Korea (Democratic People's Republic) and Japan. AD also occurs in New Zealand and Samoa.

The real incidence of AD in the world may be much greater, since the willingness to accept diagnosis and control increases with the economic loss caused by the disease. AD has no chance of developing in Islamic countries where pig breeding is not practised and therefore the basis for the development of the disease is absent.

**Evolution in infected countries**

As an example of the evolution of AD in a country, the data for the Federal Republic of Germany (FRG) are presented in Fig. 1. Until 1976 AD was no problem. Afterwards a gradual increase set in, which exploded in 1980 and has continued despite legislative measures and increasing vaccination. AD is endemic in areas with a dense pig population and intensive, specialized farming management which involves much animal movement between farms. This means that the distribution of AD in the FRG and within the Federal States differs somewhat. AD is not endemic in districts where small farms predominate which frequently produce their pigs themselves. If single outbreaks occur they are usually caused by pigs imported from infected areas. The heavily infected districts are situated in Lower
Saxony, North Rhineland-Westphalia and Schleswig-Holstein, where 64 per cent of the fattening pigs and 75 per cent of the breeding pigs of the FRG are produced. In southern Germany sporadic outbreaks occur, but there is a rising tendency in Bavaria.

The epidemiological course of AD is subject to seasonal cycles. During the warm season the number of outbreaks drops and reaches a minimum from June to September. During the cold season the number of outbreaks rises showing a peak from December to April. The reason for this is unknown, but the survival conditions of the virus are certainly better in winter than in summer. Interestingly, the seasonal course of classical swine fever is completely the opposite.

Concerning the type of farm involved, AD occurs most frequently in farms which buy pigs from different sources. Thus fattening farms are predominantly affected.

**Sources of the virus**

ADV-infected pigs are the main source of virus spread. Other species are less important since they usually die and virus spread is interrupted.
Large amounts of virus can be isolated from nasal and oropharyngeal swabs of infected pigs (26, 38, 52, 54). Virus is found in vaginal and foreskin (ejaculate) secretions (5, 55, 64), in milk (48) and irregularly in urine, but is never isolated from faeces (5, 48), although it has been detected in rectal swabs (26).

Virus is also spread by vaccinated, ADV-infected pigs (38, 89), and by latently infected pigs after reactivation of the virus genome. It is important to realize that not only unvaccinated but also vaccinated and passively immune pigs can develop virus latency after infection (57, 65, 68, 92).

The virus was isolated in nasal secretions of experimentally nasally-infected cattle (90). In dogs and cats virus is excreted in the saliva (87). In rats the virus is found in the nasal and oral mucus (51).

The virus is eliminated into the air (26, 50), into liquid manure and dung (50), into soil and onto various other objects and materials. Because of the high stability of the virus it survives for a long period (20, 48, 67, 79, 95). Virus dried on objects and virus in soil can survive for 2 to 3 weeks in summer and 5 to 6 weeks in winter. In packed-down dung, ADV survives for up to 3 weeks, and in liquid manure, for 27 weeks at 4°C and 15 weeks at 23°C (Strauch, pers. comm.).

ADV in meat (88), lymph nodes, bone marrow and offal of slaughtered pigs is a source of oral infection in carnivores. Pigs can be orally infected by garbage (95). The virus is not killed in the course of meat maturation, but it becomes inactivated after freezing of the meat or the organs at about –18°C within 35 to 40 days (26, 30). Additional virus sources for carnivores are ADV-infected rats and mice.

**Susceptibility of the animal host**

ADV has a very broad host range. Natural infection of domestic animals occurs in pigs, cattle, sheep, goats, dogs and cats. In fur farms, mink, polar foxes and silver foxes are susceptible. Amongst wild animals, AD has been reported in hares, wild rabbits, foxes, badgers, polecats, martens, wild pigs, ferrets, wild deer and stags, porcupines, hedgehogs, coatis, raccoons, polar bears, jackals, leopards, otters, rats, as well as hedgehogs and field mice. The list of susceptible wild animals may be considerably longer. Laboratory rabbits, mice and guinea-pigs are highly susceptible to ADV infection and are used as experimental animals.

Natural infection of horses, chickens, turkeys, geese, ducks and pigeons does not occur; however, experimental infection is possible when large virus doses are injected IC, SC or IM. In this connection a report (61) is of interest in that a batch of 49,000 day-old chickens was vaccinated against Marek’s disease and 10,000 of these animals died of AD after 2 or 3 days. It was concluded that the vaccine had been contaminated with ADV. Rhesus monkeys, macaques and Grivet monkeys can be infected experimentally, but not baboons and chimpanzees. Man is not considered to be susceptible to ADV, since ADV infection has been proved neither virologically nor serologically in suspected cases.

Susceptibility to infection is dependent on several factors: virulence of the virus strain, amount of infecting virus, route of infection, species, age and condition of the animal (e.g. exposure to stress) (5, 13, 14, 26, 41, 51, 60, 69, 71, 90, 92). For example, larger quantities of virus are necessary for oral infection than for nasal infection, piglets need less virus than adult pigs, and cattle need more virus than pigs.
Means of transmission

Natural infection of pigs takes place by the oro-nasal and the genital route, ruminants are infected by the nasal route and carnivores by the oral route. ADV is not highly contagious, since relatively high amounts of virus (about $10^5 \text{TCID}_{50}$) are necessary to infect adult animals. Usually not all the pigs or cattle within a building become infected. In pigs the percentage of animals infected fluctuates between 50% and 100% and, in cattle, between 3% and 60%. The spread of infection within a herd depends primarily on close contact. Spread of infection, therefore, is highest within a pen but lower between pens.

AD is predominantly transmitted through trade with ADV-infected, vaccinated or unvaccinated pigs while the disease is in the incubation period or in a subclinical phase, or else the animals are latently infected. In the latter case, the stress of transportation may evoke reactivation of the viral genome, followed by virus multiplication and virus excretion.

Genital virus transmission can be induced by artificial insemination with ADV-contaminated semen or when sows are serviced in ADV-infected boar stations (5, 55, 64). ADV may be transmitted by embryo transfer (21); in practice, however, this seems rather unlikely (43).

Virus can be transmitted by contaminated implements, vehicles, food and by man, but the likelihood of these routes must not be overestimated. This is also applicable to virus transmission by cats, dogs and rats, since virus excretion is very low in these animals (17, 51, 87). Experimentally, no contact transmission was found between sick cats and pigs (87). Insects are apparently not involved in virus spread. Virus transmission to dogs, cats and other carnivores as well as rats occurs via ADV-infected meat, offal or carcasses and, in pigs, via ADV-contaminated garbage. Suckling pigs can acquire ADV from the milk of infected sows, or infection may already have taken place in the uterus.

Investigations in recent years have demonstrated that air-borne transmission of ADV is possible. An infected pig excretes up to $10^{4.8} \text{TCID}_{50}$ of ADV during a 24-hour period (26). In the field, ADV has been isolated in air samples from ADV-infected farms (50). Further experiments showed that virus transmission can be effected by means of the air stream from a ventilator between premises 10 to 20 m apart (17, 26). In all likelihood air-borne transmission may occur over distances ranging from 500 m up to 2000 m (17; 11th Conference of the OIE Regional Commission for Europe, Vienna, 1984). A further source of air-borne virus may be ADV aerosols appearing when pig liquid manure is sprayed on land (50).

**DIAGNOSIS**

**CLINICAL DIAGNOSIS**

Clinical diagnosis of AD in individual pigs is difficult, but AD can be suspected if the situation of the whole herd is taken into consideration. Such herd symptoms are: numerous deaths of suckling pigs during the first 3 weeks of life, nasal discharge, coughing, dullness, somnolence and nervous disorders in older pigs, high frequency of abortions and still-births. It is characteristic that morbidity and mortality decrease with rising age of pigs. Furthermore, AD-suggestive signs in dogs and cats on a farm, or the discovery of dead animals of these species, are further hints pointing to AD.
In cattle and sheep, diagnosis is easy when pruritus is present and the animals are kept in or near pig-houses, but pigs need not be ill (inapparent infection). Pruritus is also a typical sign of AD in dogs and cats. However, diagnosis is difficult when pruritus is absent, but any information that raw pig meat or offal have been fed would support diagnosis.

A post-mortem does not reveal any pathological alterations typical of AD.

DIFFERENTIAL DIAGNOSIS

AD resembles transmissible gastroenteritis or *E. coli* enterotoxosis in newborn pigs when diarrhoea is present. Respiratory signs can be caused by bacteria, especially *Pasteurella*, and by swine influenza virus but, in the latter case, all pigs in all age groups become severely ill without dying. Nervous signs can occasionally occur in classical swine fever (CSF) and, when no gross pathological changes of CSF are present, it is difficult to differentiate CSF from AD. Nervous disturbances evoked by Teschen disease are not accompanied by infection of the respiratory tract. NaCl poisoning causes excitement, and arsanilic acid and mercurial poisoning causes lethargy of the animals, but these events occur suddenly without fever. Still-births and abortion can be evoked by parvovirus infection.

In cattle AD is difficult to differentiate from real colic and, when no pruritus is present, from lead poisoning and rabies, but the presence of pruritus can also be a sign of ectoparasites. On the other hand, licking may be due to a mineral salt deficiency. In dogs and cats AD can be confused with rabies, but aggressiveness against man is lacking with AD.

In most cases laboratory diagnosis is necessary to confirm AD.

LABORATORY DIAGNOSIS

Histological changes

Histological changes in pigs, indicative of AD, are only present in the CNS, mainly restricted to the brain. They display the picture of a non-suppurative meningoencephalitis with relatively mild myelitis (27). The predilection sites are the cerebral and cerebellar cortices while lesions of the brain stem are less marked. Characteristic lesions are diffuse and focal microglial infiltrations, occasionally with necrosis of neurons, and perivascular and meningeal infiltrations by lymphocytes, neutrophilic granulocytes and macrophages. Sometimes, intranuclear inclusions of type A are found. Lesions of the spinal cord are frequently mild and they decrease from the cranial to the caudal area.

It can be difficult to differentiate the neural changes in AD from those in Teschen disease and classical swine fever (CSF). In Teschen disease lesions are prominent in the base layer of the cerebellum and in the lumbar region of the spinal cord and the dorsal root ganglia while, in AD, changes in the caudal spinal cord are less marked and usually no reactions occur in the dorsal root ganglia. In CSF the brain stem is most severely involved, while cerebrum and cerebellum are relatively less affected by way of contrast with AD. In addition, the perivascular infiltrates in CSF are associated with marked endothelial damage. No inclusions have been observed in Teschen disease and CSF.
The histological lesions in cattle are similar to those in pigs; however, their distribution in the CNS is somewhat different (27, 90). The changes are very prominent in the spinal cord, especially in the cervical, lumbar and sacral regions, with ganglia also being affected. In the brain region the olfactory bulb, the adjacent cerebral cortex, the brain stem, and the medulla oblongata are predominantly involved.

Histological examination of slides is less favoured now than immunofluorescence, which is easier to perform and gives positive results more frequently.

**Detection of virus and virus antigen**

Tonsils, brain (especially olfactory bulb, quadrigeminal body, hippocampus, pons, cerebellum, medulla oblongata), cervical and lumbar regions of the spinal cord, lung and spleen (especially in suckling pigs) are the most appropriate tissues in pigs for detecting virus. Selected tissues in cattle are the brain (cerebral cortex and the parts mentioned above) and the whole spinal cord. Virus detection in cats and dogs is most successful in the tonsils and brain.

The distribution of the virus in the tissues, especially in the nervous tissues, can vary. Thus tissue sections from several parts of the organ should be made for immunofluorescence, and pieces from several parts of the organ should be mixed and homogenized for inoculation into animals or cell cultures.

**Virus isolation**

For detecting ADV, tissue homogenates are inoculated into cell cultures and into small laboratory animals. Rabbits are the most susceptible, followed by mice and rats (6, 42, 53). Nowadays, tests on animals have been almost completely replaced by tests in cell cultures. The virus multiplies in a great number of cell cultures of different species. The most used cell lines are the porcine cell lines PK-15, SK and SK-6, the hamster cell line BHK-21, the rabbit cell lines RK-13 and NRK, the bovine cell line MDBK, the cat cell line CRFK, the dog cell line MDCK and the monkey cell line Vero. Primary cell cultures from kidneys of pigs, calves, lambs, rabbits and dogs, from testes of pigs and calves, and from chick embryos are used too.

The virus induces two types of cytopathic effect (CPE): syncytial formation and rounding of the cells. Both types result in cell lysis. Syncytia are mainly found with highly virulent virus isolates, cell rounding with isolates of lower virulence (16). This difference is especially marked in primary pig kidney cells, whereas, in other cell cultures, mixed forms appear in line with the prevalence of one of the two CPE types.

**Immunofluorescence (IF)**

IF is the principal technique for detecting ADV antigen in tissues sections (56, 63, 72) or in impression smears (7). IF usually appears in the cytoplasm and only exceptionally in the nucleus.

Comparing the sensitivity of virus isolation in cell cultures (CC) and of IF, Neumann and Beckmann (63) detected that 14% of the samples were positive for CC but negative for IF; Hirschert (40) found 15.6% of the samples positive only for CC, and 21.3% positive only for IF. A similar distribution was also described by Akkermans *et al.* (6). With samples from cattle and carnivores the sensitivity of
virus isolation for CC was significantly better (40). Fifty per cent of the samples of cattle were CC+ and IF−, and 16.5% were CC− and IF+. All the samples from dogs and cats were positive for CC, but only a small percentage reacted in the case of IF.

If virus isolation in CC is negative in respect of IF-positive samples, the presence of antibody which had neutralized the virus, or virus inactivation in old samples, may be responsible for this. In the opposite case, virus concentration may be too small to be detected in IF.

**Immunoperoxidase technique (IPT)**

Another tool for detecting ADV antigen is the IPT (29). The field of application is the same as with IF, but positive reactions involve both nucleus and cytoplasm. However, IPT in paraffin-embedded sections is more complicated and time-consuming than IF. The IP-labelling of impression smears, described by Allan *et al.* (8), may be of more practical interest. In brain smears the IPT was as sensitive as IF and more sensitive than virus isolation.

**Serological diagnosis**

**Serum-neutralization test (SNT)**

The SNT in cell cultures is widely used for detecting ADV antibodies (12, 15, 34, 37, 78, 85, 86). Usually it is performed as a microtest on plastic plates. The sensitivity of the test depends on several factors: type of cell culture, macro- or microtest, number of cells used, amount of virus used, preincubation period of the virus/serum mixture, addition of complement, time of reading the test, type of diluent, quality of the plates, etc. (93). However, the most drastic influence on the sensitivity of the SNT is produced by the incubation period of the virus/serum mixture. This effect was first described by Bitsch and Eskildsen (15), who used incubation at 37°C for 24 h. We were able to confirm this, finding a 5.6-fold to 10-fold rise of ND_{50} values in strongly positive sera and a 8.9- to 17-fold rise in weakly positive sera after 24 h incubation in comparison to 60 min (93). It was shown that the virus is either not at all or only insignificantly inactivated during this time.

Further enhancement of neutralization can be achieved by adding guinea-pig or rabbit complement (C) to the virus/serum mixture during the preincubation period. This is important for detecting IgM antibodies in 'early' sera, which need C for virus neutralization. Thus it is possible to detect neutralizing antibodies as early as 3 or 4 days after infection or vaccination (15, 42). An enhancement also occurs in 'late' sera, since some IgG subclasses are dependent on C.

Whatever the test method used, ND_{50} titres of 1:2 are generally considered as positive, and some laboratories even do this at titres of 1:1. However, false positive results may occur with undiluted sera. Occasionally virus neutralization is masked by the cytolytic properties of low serum dilutions.

Since ADV shares common antigenic components with other herpesviruses, serological cross-reactions occur. The most important one is that with IBR virus. It is predominantly a one-way relationship, which means that IBR-virus antibodies react with ADV but ADV antibodies do not react with IBR virus. Thus, positive ADV neutralization in cattle serum is usually caused by IBR antibodies when the cattle have not been vaccinated against ADV, because cattle usually do not survive ADV infection (3, 4, 14, 80).
Enzyme-linked immunosorbent assay (ELISA)

Antibody detection by ELISA (12, 22, 23, 31, 58, 59) is easy and cheap to perform, yields results within a few hours, is highly sensitive and has a good correlation with SNT. It can be readily automated; it is not disturbed by cytolitic properties of the serum; and it is not dependent on a continuous supply of cell cultures. Every laboratory can employ it without great effort, since several commercial test kits are available; however, some kits do not detect IgM antibodies.

Because of these advantages ELISA is replacing SNT more and more, but it is evident that ELISA detects a broader spectrum of antibodies than SNT, including those which are not involved in immunity. On the other hand, ELISA may recognize non-neutralizing, opsonizing antibodies which act in virus clearance and thus indirectly in immunity.

ELISA can also be used to detect antibodies in the exudates of organs, such as liver, kidney and muscle (32).

For routine tests with commercial kits, serum dilutions of 1:20 and 1:40, or 1:40 only are recommended. With the Behring kit a positive reaction is revealed if the difference in absorbance between test serum and ADV and test serum and control antigen is equal or more than 0.2 OD. The kits of Flow and of IFFA-Mérieux include a negative and a weakly positive control serum, which must differ in their optical density by 0.2 at least. Otherwise, the plate has to be discarded. The absorbance of the test serum is positive if the value is at least 0.2 OD above that of the negative reference serum.

Positive reactions in serum dilutions of 1:40 or above are considered as ADV-specific. Reactions in serum dilutions <1:20 are negative. Positive reactions in a serum dilution of 1:20 can be either ADV-specific or non-specific. In this case, the test should be repeated with a later serum of the same animal to check whether the titre was increased.

ELISA is thought to be more sensitive than SNT; however, this is only true if SNT techniques of low sensitivity are used. For example, we have found that 11 out of 20 ELISA-positive sera (≥ 1:40) were false-negative with SNT when the incubation of the virus/serum mixture was carried out at 37°C for 1 hour. However, after 24 h incubation only one serum was false-negative with SNT, but 3 sera were false-negative (1:20) with ELISA. Serum samples taken one week later were positive in both tests.

Differences in sensitivity may occur between different commercial kits and between different batches (86). Hence the quality of new batches should be tested before use using a weakly positive reference serum. Moreover, it appears that hyperlipaemic or bacterially contaminated sera can evoke unspecific colour reactions. Substrate indicator solutions exposed to sunlight can become unstable and discoloured, therefore the test must be done in a dark place.

Immunodiffusion test (IDT)

ADV antibody can be detected by IDT, done either as double or radial ID (12, 37, 38, 44). The test is dependent both upon the method used for antigen preparation and upon the specificity of the antiserum (66). IDT is less sensitive than SNT when the SN-titres are below 1:16. A very bad correlation between the two tests was found in pigs having maternally derived antibodies, even at SN-titres above 1:16.
The low sensitivity of the IDT may be the reason why antibody cannot be detected before day post-infection (DPI) 10 and only irregularly before DPI 14, although IgM as well as IgG and IgA antibody react with IDT.

On account of its low sensitivity, IDT is advantageous for the screening of pig herds only, but not for testing individual animals.

In order to improve sensitivity a radial immunodiffusion enzyme assay (RIDEA) was developed which combines the principle of radial immunodiffusion with ELISA (45). In a field trial (83) RIDEA and SNT were equally sensitive in detecting antibodies resulting from infection with a field strain of the virus, but the RIDEA had a slightly reduced sensitivity with sera from vaccinated sows, and a significantly lower sensitivity in detecting maternally transmitted antibodies. In all instances RIDEA was as sensitive as SNT at SN-titres of $\geq 1:16$.

Other tests

**Complement fixation test (CFT):** CFT can be used to detect ADV antibody in pig serum (15, 76). There is a good correlation between CF- and SN-titres. CF antibodies can be detected from DPI 7 onwards and disappear 5 to 8 weeks after infection. However, the potential use of the CFT is impaired since most of the pig sera show haemolytic activity in the presence of complement up to dilutions of 1:32.

**The radioimmunoassay (RIA)** is a highly sensitive method for detecting ADV antibody (25, 46); however, it is restricted to laboratories with special equipment, since the use of radioactive iodine is necessary. Accordingly, the test has no practical importance and no advantage over ELISA.

**Countercurrent electrophoresis (CIE)** for antibody detection (12) is of no practical interest, because its sensitivity is between those of IDT and SNT.

**Skin test:** The delayed hypersensitivity reaction to ADV antigens is used in the USA to detect ADV-infected pig herds (24, 74, 77). The skin test is much less sensitive than the SNT. Thus, only ADV-infected herds with infection rates of at least 46.7% can be identified by skin testing adult pigs. In younger animals, particularly suckling and weanling pigs, the test is unsatisfactory. The overall correlation between the skin test and SNT fluctuates between 10.5% and 80%, but it is not correlated with the SN-titres. On the other hand, pigs vaccinated with inactivated ADV vaccines showed specific skin reactions, while the SN-titres were below 1:4 (36, 42). These divergences are not surprising since the skin test detects cell-mediated immunity but not antibody-mediated immunity. Hence, pigs with maternal antibody do not react to the skin test. On the other hand, the skin test may be positive early after infection when antibodies are not yet detectable, or at late stages of infection when antibodies have disappeared. Serological surveys by the SNT are not impaired by the skin test, since the antigens used in the latter do not evoke seroconversion.

**METHODS OF CONTROL**

An essential precondition for the control of AD is the duty of notification. Possible measures on the infected farm are: removal of pigs only for slaughter; killing of diseased pigs or of all pigs of the herd; heat treatment of meat and offal; destruc-
tion of dead animals, aborted foetuses, stillborn piglets and afterbirths; refrain of
the use of semen for artificial insemination; disinfestation of rats; decontamination
of dung, liquid manure and waste material; disinfection (92) of implements, vehicles
and other objects, and of thoroughfares, including the entrance and exit of the pig-
shed. Unauthorized persons, cats and dogs should be kept from the pig-shed, and
authorized persons should effect hand disinfection and decontamination of clo-
thing and shoes when leaving the pig-shed. Vaccination of the infected herd as well
as of neighbouring herds should be carried out.

The regulations for infected farms can also be applied to pig markets, pig exhi-
bitions, and to the transport of pigs when an AD outbreak occurs, or when other
animals than pigs contract AD on a farm.

The main problem is when to lift the ban. No difficulties arise when all the pigs
have been removed. But in severely affected districts the infected herd is usually
vaccinated, whether or not the sick animals have been removed. Despite such herds
remaining latently infected, the restrictions must be cancelled to avoid overpopula-
tion. Depending on the period of virus excretion and the incubation period, a mini-
imum safety period of 35 days should be allowed to elapse after the last case of AD
on a vaccinated farm before lifting the ban. From fattening farms the animals can
be sold for slaughter and new animals can be bought. It is recommended that the
new animals be vaccinated. The breeding stock must be vaccinated regularly on
breeding or on piglet-producing farms. The offspring may either be vaccinated or
remain unvaccinated. In the latter case, unvaccinated animals which stay on the
farm should be examined serologically to check for virus spread. In any case, all
these farms remain latently chronically infected and can be sources of virus spread.

Prophylactic vaccination is widely used for protecting non-infected pig herds in
endemic areas. However, vaccination does not completely cover the totality of the
infected area when it is voluntary. Hence, sufficient unvaccinated herds are present
to allow virus spread. Besides, vaccination does not prevent subclinical ADV infec-
tion which converts into latent infection (9, 89, 91). These animals can shed virus
when under stress, despite being vaccinated; then the virus circulates within the
herd (73, 81, 96) and can be transmitted to other herds. For this reason, each vacci-
nated pig should be marked regardless of whether it originates from an infected
herd or an apparently healthy herd, so that a buyer can avoid any risk.

In general one can say that vaccination prevents or reduces clinical disease and
therefore economic loss, but it does not prevent spread of ADV. Thus the disease
will never be eradicated in this way. Furthermore, serological surveys are complica-
ted by the impossibility of distinguishing between vaccinated and infected animals.
If prophylactic vaccination is practised, then all the fattening herds and all the breed-
ing stock should be vaccinated in the infected area.

The final aim of control of AD is its eradication. From the foregoing comments
it is evident that eradication of AD can only be achieved by the slaughtering of all
the seropositive animals and by strict control of pig movement. Such an eradication
programme is very expensive and time-consuming. The UK started such a pro-
gramme in 1983 (49) which is not yet finished. Despite the low incidence of the
disease (127 outbreaks from 1979 until 1982), 400,000 pigs had to be slaughtered by
1985, entailing costs of £ 22.8 million.

What about the possibility of eradicating AD by sanitation of the infected
herds? Several programmes exist (1, 2, 75, 82, 97). It is most convenient to vacci-
nate the infected herd and rear unvaccinated, seronegative offspring. However, sanitation is also very costly and time-consuming. It takes 2 to 3 years for a herd to be cleared, provided that good isolation facilities are present and that any introduction of ADV can be prevented. The large-scale eradication of AD by the herd sanitation method is either completely impossible or else it takes ten years or longer (2).

What measures should be taken to prevent the introduction of ADV into an AD-free country? First of all, the trade in pigs must be carefully controlled. According to the OIE International Zoo-Sanitary Code (article 3.1.2.1.), Veterinary Administrations of importing countries should require, for breeding pigs from unvaccinated herds, the presentation of an international zoo-sanitary certificate attesting that the animals (1) come from a herd in which no clinical sign of Aujeszky’s disease (AD) was officially reported during the twelve months prior to shipment; (2) were isolated in the establishment of origin for thirty days before entry into a quarantine station, showed negative response to the serum-neutralization (SN) or enzyme-linked immunosorbent assay (ELISA) test for AD and were clinically healthy; (3) were kept in a quarantine station for the thirty days prior to shipment and, not less than twenty-one days following the test referred to in paragraph 2) above, showed negative response to the SN or ELISA test for AD. Furthermore it should be attested that all pigs in the quarantine station satisfy all the requirements of the paragraphs above.

Similar recommendations should be made by the OIE for the import of boar semen.

According to article 3.1.2.2. of the Code, Veterinary Administrations of importing countries should require for fresh meat and meat products of pigs, the presentation of an international sanitary certificate attesting that the entire consignment of meat comes from animals slaughtered in an abattoir and found to be healthy both before and after slaughter.

This only excludes pigs with clinical AD but not latently infected animals. Since the presence of ADV in meat — but not in offal, bone marrow, etc. — is apparently rather rare (19, 26, 70, 71), this proposal may be sufficient, especially when meat has been frozen at a temperature of about \(-18^\circ\text{C}\) for at least 40 days (26, 30). However, one has to keep in mind that ADV may be in fresh raw meat products such as sausages (47), and nothing is known about the persistence of the virus in the bone marrow at temperatures around \(-20^\circ\text{C}\).

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