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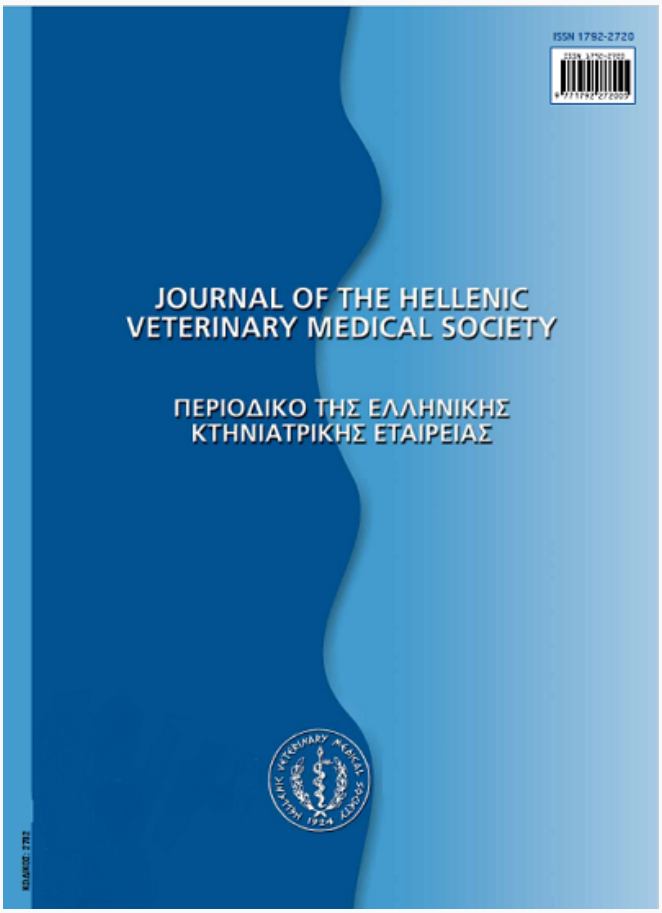


# Aujeszky's Disease (Pseudorabies). An old threat in current pig industry? Part I. Pathogenetic information and implications

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Review article
Ανασκόπηση

# Aujeszky's Disease (Pseudorabies). An old threat in current pig industry? Part I. Pathogenetic information and implications

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# Νόσος του Aujeszky (Ψευδολύσσα). Μια παλιά απειλή για τη σύγχρονη χοιροτροφία; Μέρος Ι. Παθογένεια και επιπτώσεις.

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**ABSTRACT.** Aujeszky's disease (AD) (or pseudorabies) is an important viral disease of swine causing neurological signs in neonatal pigs, respiratory problems in fatteners and reproductive disorders in breeding stock. Glycoproteins and enzymes of the AD virus (ADV) and their interactions with the cellular components majorly determine its pathogenesis. After primary replication in the epithelia of the upper respiratory tract, ADV travels via the nerves to the central nervous system and via the blood to secondary replication sites (lymph nodes, lungs, uterus etc). Depending on the age of the affected swine, it can cause neonatal mortality due to neurological disease, respiratory disease in growing and adult pigs and reproductive disorders in breeding animals. One of the characteristic features of ADV is latency. In this paper, its major points on etiology, pathogenesis, clinical and post mortem findings and diagnosis are presented.

***Keywords:*** Aujeszky's Disease, glycoproteins, pathogenesis

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**ΠΕΡΙΛΗΨΗ.** Η νόσος του Aujeszky (NA) ή ψευδολύσσα αποτελεί ένα σοβαρό ιογενές νόσημα του χοίρου, που χαρακτηρίζεται από νευρικά συμπτώματα στα νεογέννητα χοιρίδια, αναπνευστικά προβλήματα στους παχυνόμενους χοίρους και αναπαραγωγικές διαταραχές στα ζώα αναπαραγωγής. Ο Aladar Aujezsky, ο Ούγγρος κτηνιάτρος από τον οποίο πήρε το όνομά του το νόσημα, για πρώτη φορά περιέγραψε τη νόσο το 1902. Η ΝΑ προκαλείται από έναν DNA ιό, ο οποίος ανήκει στην υποοικογένεια Alphaherpesvirinae της οικογένειας Herpesviridae. Ο χοίρος αποτελεί το μοναδικό φυσικό ξενιστή του ιού, καθώς και ένα από τα ελάχιστα είδη ζώων που μπορεί να επιβιώσει της μόλυνσης από αυτόν. Σημαντικοί παράγοντες που καθορίζουν την παθογόνο δράση του ιού της ΝΑ είναι οι ικές γλυκοπρωτεΐνες και ένζυμα, αφού αυτά αλληλεπιδρούν άμεσα με τα κύτταρα-στόχος. Το αρχικό σημείο πολλαπλασιασμού του ιού κατά την είσοδό του στον οργανισμό είναι το επιθήλιο του ανώτερου αναπνευστικού συστήματος, απ' όπου στη συνέχεια ο ιός μπορεί να μεταφερθεί σε δευτερογενή σημεία πολλαπλασιασμού (λεμφογάγγλια, πνεύμονες, μήτρα κ.λπ.) μέσω της κυκλοφορίας του αίματος, καθώς και στο κεντρικό νευρικό σύστημα μέσω της οσφρητικής οδού και της οδού του τρίδυμου νεύρου. Τα συμπτώματα της ΝΑ στο χοίρο εξαρτώνται κυρίως από την ηλικία των μολυσμένων ζώων. Η νόσος στα νεαρά χοιρίδια οδηγεί τις περισσότερες φορές στο θάνατο με χαρακτηριστικά νευρικά συμπτώματα, ενώ σε ζώα μεγαλύτερης ηλικίας εμφανίζεται λιγότερο σοβαρή με χαρακτηριστικό τα συμπτώματα από το αναπνευστικό σύστημα. Στις σύες, η μόλυνση από τον ιό της νόσου του Aujeszky μπορεί να προκαλέσει αποβολές, καθώς και γενικότερα αναπαραγωγικά προβλήματα. Ένα σημαντικό χαρακτηριστικό του ιού αυτού είναι η δυνατότητα παραμονής του στο ζώο-ξενιστή σε λανθάνουσα κατάσταση. Σε αυτήν την περίπτωση, ενώ το γενετικό υλικό του ιού υπάρχει μέσα στον οργανισμό του ζώου, δεν πολλαπλασιάζεται και δεν παράγονται ικά σωματίδια. Ωστόσο, κάτω από ορισμένες συνθήκες ο ιός είναι δυνατό να ενεργοποιηθεί και να αρχίσει να πολλαπλασιάζεται και να απεκκρίνεται από τον οργανισμό. Τα παθολογο-ανατομικά ευρήματα είναι δυνατό να απουσιάζουν στην περίπτωση της μόλυνσης από τον ιό της ΝΑ. Στο κεντρικό νευρικό σύστημα ιστολογικά μπορεί να παρατηρηθεί μη πυώδης μηνιγγοεγκεφαλίτιδα και γαγγλιονευρίτιδα, ενώ είναι δυνατή και η παρατήρηση εστιακών νεκρώσεων σε διάφορα όργανα (ήπαρ, σπλήνα, αμυγδαλές κ.λπ.). Για τη διάγνωση της ΝΑ είναι αναγκαία η συνεκτίμηση διαφόρων παραμέτρων. Η αρχική διάγνωση βασίζεται στο ιστορικό της χοιροτροφικής μονάδας, σε συνδυασμό με τα κλινικά συμπτώματα στις διάφορες ηλικιακές ομάδες και τις αλλοιώσεις, ενώ οριστική αιτιολογική διάγνωση επιτυγχάνεται με εξειδικευμένες εργαστηριακές δοκιμές.

***Λέξεις ευρετηρίασης:*** Νόσος του Aujeszky, γλυκοπρωτεΐνες, παθογένεια

## INTRODUCTION

Aujeszky's Disease (AD) is an acute, frequently fatal disease, that primarily affects pigs and incidentally other domestic and wild animals. The disease was first described in 1813 in cattle showing extreme pruritus and, hence, it was first called "mad itch". The term "pseudorabies" was used as a result of its clinical resemblance to rabies. Aladar Aujeszky, the Hungarian veterinarian after whom the disease was named, had first described and reproduced the disease in 1902 (Lee and Wilson 1979), providing evidence that the etiologic agent was filterable (e.g. not a bacterium). He was convinced that this disease was different from rabies, based on the shorter time of incubation, the faster development of the disease and the infectivity of blood. Shope, in 1931, has shown that AD and "mad itch" were serologically identical (Shope 1931) and the virus was later called Suid Herpes Virus 1 (SHV1) or pseudorabies virus (PRV) (Pejsak and Truszczynski 2006). As pig production has grown since the 1960's, Aujeszky's Disease Virus (ADV) has emerged as an important global pathogen.

The purpose of this review paper is to provide some data of etiology, pathogenesis, clinical and pathological diagnosis of a potentially re-emerging old infectious disease.

## ETIOLOGY

ADV has been classified in the genus *Varicellovirus*, subfamily *Alphaherpesvirinae*, family *Herpesviridae*. Equine herpesvirus 1 (EHV-1) and varicellazoster virus have close homology with ADV and they, also, belong to the same genus (Mettenleiter 2000). Pseudorabies virus particles have the typical architecture of a herpes virion (Figure 1). Their shape is quasispherical, with an overall diameter of 150-180 nm, composed of a central core surrounded by three layers. The core contains the genome, a linear, double-stranded DNA molecule bound to proteins. The core is surrounded by an icosahedral capsid of 162 capsomers. The nucleocapsid is enclosed by the envelope, a bilayer of phospholipids in which glycoproteins form projections at the surface of the virus particle (Nauwynck 1997, Mettenleiter 2000). According to their role on viral replication in cell cultures, glycoproteins have been categorized as essential glycoproteins (gB, gD, gH, gK and gL) and

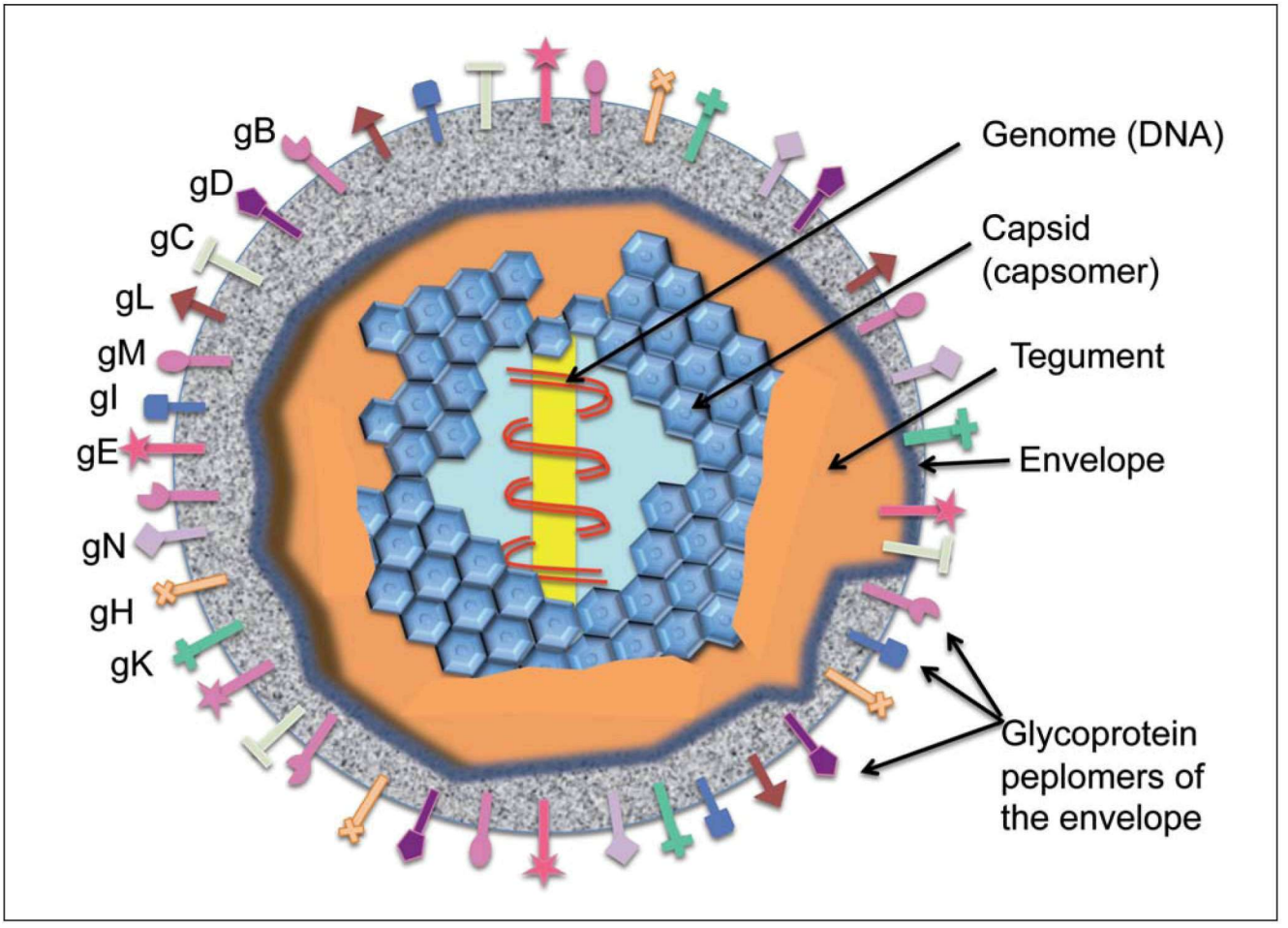


**Figure 1.** Structure of a PRV virion.

non-essential glycoproteins (gC, gE, gG, gI and gN). The envelope of ADV originates from intracellular membranes of vesicles in the Golgi area. In between the capsid and the envelope, there is a structure called tegument, an amorphous material in which several proteins have been identified. Tegument proteins are important for the entrance of the virus in the cell, the preparation of the cell for virus replication and the primary and secondary envelopment, at the inner nuclear membrane and at trans-Golgi vesicles, respectively (Mettenleiter 2000, Nauwynck 2007).

The various strains of ADV differ in their infectivity and virulence in pigs as well as in their ability to be shed (quantity and duration) during infection. Those differences are associated to identified differences in their genome (Nauwynck 2007).

## PATHOGENESIS

The pathogenesis of ADV is dependent on the age of the pig, the dose and the route of the inoculation, as well as the virus strain.

The development and severity of clinical signs diminish with increasing age. Neurological signs are severe in pigs prior the stage of weaning while older animals are relatively resistant to nervous disease.

Infection of pigs has been established by several experimental routes of inoculation such as intra-muscular, -venous, -cerebral, -gastric, -nasal etc. (Pensaert and Kluge 1989, Kritas 1994). Inoculation via oronasal route resulted in clinical signs and in distribution of the virus to the various organs similar to those observed in field cases (Sabo et al. 1968, 1969, Baskerville 1971, 1972b, Kritas et al. 1998).

The dose of virus needed to infect suckling piglets by intranasal administration was estimated to be 1-3 log10$TCID_{50}$ while 6-week-old animals can be infected with doses equal or higher than 3 log10$TCID_{50}$. Larger

doses of virus are needed for the introduction of the disease by oral than by nasal route (Baskerville 1972c, Jakubik 1977). The infecting dose for vaccinated pigs is 100 to 1000 fold higher than in the susceptible pigs (Wittmann 1982).

Field isolates of ADV have been reported to vary in their virulence for pigs (Kritas et al. 1999a). During an outbreak in Northern Ireland, the $NIA_3$ strain killed 13% of 14 to 20-week-old pigs, while other field isolates, such as $NIA_1$ and $NIA_2$, did not show virulence for pigs of this age (McFerran and Dow 1975).

After natural infection, the primary site of viral replication is the epithelia of the upper respiratory tract, including nasal, pharyngeal and tonsillar epithelium (Kritas 1994). Thereafter, the virus can spread from the primary sites to distant secondary sites of replication in free form or via infected leucocytes. Infected monocytes and lymphocytes must be considered as the possible carrier cells for ADV (Nauwyck and Pensaert 1995). After the infection of alveolar macrophages, the virus is transmitted to alveolar epithelium and the underlined connective tissue. The transmission occurs via a direct cell-to-cell contact followed by a cell-associated viremia. This allows ADV to infect inner organs (spleen, liver, uterus, etc). Once the virus reaches pregnant uterus, it will spread cell-to-cell over its contents, causing abortion (Nauwyck and Pensaert 1992).

From the primary site of replication, ADV may, also, spread towards the central nervous system (CNS) via trigeminal and olfactory nerves (Kritas et al. 1994a, 1994b), where it, also, replicates causing nervous disorders, as a result of a non-suppurative meningo-encephalitis. Thus, the olfactory nerves transport the virus from the olfactory mucosa towards the olfactory bulb, the lateral olfactory gyrus, rostral perforated substance and piriform lobe; the trigeminal nerve from the nasopharyngeal mucosa to the pons and medulla oblongata, the cerebellum and thalamus (Kritas et al. 1994a, 1994b). There, it appears that ADV is retrogradely transported towards the nerve bodies in the ganglia in a non-infectious form (nucleocapsids) and that, after replication, infectious enveloped viral particles are anterogradely transported from the ganglia towards the periphery (Kritas et al. 1995).

The role of viral glycoproteins and enzymes in ADV infection mechanism is crucial. The interaction between virus glycoproteins (gC and gD) and cellular surface components is critical for the attachment of virus to target cells (Mettenleiter 2000). First of all, there is an interaction of gC with heparan sulfate proteoglycans at the surface of target cell. The gC – heparan sulfate interaction is thought to be enhanced by gB (Nauwynck 1997). This attachment turns into a strong binding by the presence of gD, which interacts with its cellular receptors. The tight contact of the cell membrane with the virion envelope is a key factor for the development of fusion process and the consequent entry of the virion in the cell. For this process, which is an essential step for the replication of ADV in the cell, at least four glycoproteins are involved (gB, gH/gL and gD) (Mettenleiter 2000). Glycoprotein E (gE) is one of the key glycoproteins that play an important role in neural invasion and spread of ADV, while thymidine kinase (TK) enzyme is necessary for the replication of ADV in non-divided cells, such as the neurons (Jamieson et al. 1974, Kit et al. 1985, McGrecor et al. 1985, Kritas et al. 1999b). Kritas and co-workers showed that gI and gG glycoproteins play a less important role than gE in neuroinvasion and that gC does not influence the viral neuropathogenesis (Kritas et al. 1994a, 1994b, 1994c, 1999c, 1999d). Although the absence of gE results in a reduction of virulence of ADV, following a reduced invasion and spread in the CNS, it does not affect the replication of virus in the nasal epithelium after intranasal infection (Kritas et al. 1994a, 1994b). A role of gE and gI in the anterograde transport of ADV in the nervous system of the pig is possible (Kritas et al. 1995). It is obvious that the importance of these two glycoproteins, particularly that of gE, in the neural invasion of AD, makes them excellent deletions to be used for safe vaccine strains nowadays.

Viral excretion begins 2 to 5 days after infection and virus can be recovered from the primary sites of replication for more than 2 weeks. (Gutekunst and Pirtle 1979, Kritas 1994).

ADV can become latent in its host. Latency, a characteristic of Herpesviruses, is specified as a condition in which infectious virus is not produced, although viral DNA persists (Mettenleiter 2000). Nervous tissue appears to be important in the latency of ADV. During a primary ADV infection, the virus moves along the axons of the cranial sensory nerves to the corresponding ganglia, where it may become

latent. For an unknown reason, most probably associated with stress factors (transport, crowding, corticosteroid injections or farrowing), the virus is reactivated and, subsequently, moves down the sensory nerves, until it reaches the nasopharyngeal mucosa. After replication in epithelial cells, virus is shed (recrudescence) and, consequently, spread to susceptible individuals. Pigs infected with latent ADV may be a serious risk in a population and that should always be considered in eradication programs. TK was suggested to play a role in the latency of ADV in neural tissues, since this enzyme is necessary for the establishment of the neuronal infection (Jamieson et al. 1974, Tenser 1991). However, mutants deleted in TK gene can establish a reactivable, latent infection in pigs (Mengeling 1991). The molecular basis for alphaherpesvirus latency has not been fully understood. Important sites of ADV latency are the trigeminal ganglion, the olfactory bulb and brain stem (Yoon et al. 2006). Latent virus has, also, been reported in the tonsils, although it is not determined whether the virus is truly latent at this site or the tonsils are infected at low levels (The Center for Food Security & Public Health 2006). Recently, certain molecular techniques have been proven to be suitable for the detection of ADV latent infections. Nested and real-time polymerase chain reaction (PCR) can be used to determine the prevalence and quantity of latent ADV DNA (Yoon et al. 2005). Latency detection in live pigs can be experimentally achieved by the administration of large doses of corticosteroids, resulting in virus reactivation followed by shedding.

## CLINICAL SIGNS

The manifestation of the disease varies depending on the age of infected pigs, the virulence of the infecting strain and the previous exposure. The incubation period for AD ranges from 1 to 11 days, although, in most of the cases, the onset of clinical signs takes place after 2 – 6 days.

**Suckling Piglets.** In suckling piglets, the incubation period ranges from 1 to 5 days and the primary clinical signs consist of fever (41°C), anorexia, depression and listlessness, followed by typical neurological signs, such as trembling, hypersalivation, incoordination, nystagmus, opisthotonus, seizures and loss of voice. Some pigs may press the head on the bottom or the walls of the cage, while some others may be observed sitting on the hind limps (dog sitting) because of respiratory distress or as a neurological disorder. Pruritus rarely occurs in pigs, although it is a quite common symptom in other animals infected with ADV. Vomiting and diarrhea, also, occur. In piglets with characteristic CNS signs, death occurs usually within 1 – 2 days after the onset of these symptoms. Mortality in suckling piglets often approaches 100% and gradually declines with increasing age (Kritas 1994, Kritas et al. 1994a, 1994b, Pejsak and Truszczynski 2006). Maternally derived antibodies against ADV are efficient to protect neonatal piglets against neural invasion of the virus. Kritas et al. (1997, 1999a) had established the correlation between the level of maternal immunity, as well as that of virus strain, and the protection against invasion of ADV in the nervous system of neonatal pigs.

**Weaners.** In weaners (3 – 9 weeks of age), the clinical signs are similar to the symptoms observed in suckling pigs but less severe. In this group of age, AD is mainly a respiratory illness, characterized by fever, anorexia, weight loss, coughing, sneezing, conjunctivitis and dyspnea. It is not uncommon for the respiratory disease to be complicated by secondary bacterial infections caused by *Pasteurella multocida, Actinobacillus pleuropneumoniae, Streptococcus suis, Haemophillus* spp or other bacteria (Pejsak and Truszczynski 2006). Signs from the CNS may be seen occasionally. Mortality in pigs between 3 and 4 week of age may approach 50%. In pigs between 5 and 9 week of age, the mortality is less than 10%, but the infection results in a remarkable loss of body weight (1 – 8 week delay in reaching the expected weight). Most pigs in this age-group recover in 5 – 10 days with the exception of the animals that develop symptoms from CNS, where the disease is fatal (Pejsak and Truszczynski 2006).

**Growers and Fatteners (2-6 months).** In growers and fatteners, ADV infection is usually mild and it is characterized mainly by the development of respiratory symptoms. The morbidity in a herd often reaches 100%, due to grouping of pigs in large groups, but the mortality ranges from 1 – 2%. In cases complicated by secondary bacterial infections, the losses increase dramatically. The onset of clinical signs, following ADV infection, usually occurs after 3 – 6 days. Clinical signs are characterized by fever, depression, anorexia, sneezing, nasal discharge,

coughing and dyspnea. ADV causes a delay of at least 1 week in the production cycle of growers and fatteners, although the symptoms decrease within 6 – 10 days. CNS symptoms are usually absent in pigs of that age (Pejsak and Truszczynski 2006). Some exceptions may occur as reported with the NIA3 strain, which killed 13 to 25% of pigs up to 20 weeks old (Baskerville et al. 1973, McFerran and Dow 1975).

**Boars and Sows.** In boars and sows, AD infection is characterized mainly by respiratory symptoms. In pregnant sows, abortion might be the first sign of the disease. Abortion usually occurs almost 10 days after the infection (Papatsas et al. 1995, Nauwynck and Pensaert 1992). If the sow is infected during the first trimester of pregnancy, there is resorption of the fetuses and return to estrus. Furthermore, if the infection occurs during the second or third trimester of pregnancy, it causes abortion of mummified fetuses and the birth of weak pigs (infection close to the birth). Affected litters can contain a mixture of normal piglets, stillborn piglets and weak piglets. In boars, clinical signs may include orchitis and bad semen quality caused by the fever leading to a reversible infertility. Other clinical signs concerning boars and sows include anorexia, dullness, respiratory disorders and fever (41˚C) (Pejsak and Truszczynski 2006).

In species other than swine, the disease is almost always fatal within a few days (1 – 2 days). The first symptom is intense pruritus, which is usually manifested as severe licking or rubbing. The affected animals become weaker and eventually recumbent, while the subsequent neurological signs, the pharyngeal paralysis and the production of saliva may resemble rabies (Pensaert and Kluge 1989).

## LESIONS

Gross lesions in the nasopharynx can be observed in 15-20% of the infected pigs and in the tonsils in 50-60% of the pigs (Csontos and Szeky 1966). Serous to fibrinonecrotic rhinitis, necrotic tonsilitis and hemorrhagic pulmonary lymph nodes may be seen (Csontos and Szeky 1966). Lesions in the lower respiratory tract range from pulmonary congestion, edema or consolidation to more severe findings in the case of secondary bacterial infection (The Center for Food Security & Public Health 2006). Gross changes are seen mostly in young pigs. There may be necrosis of the tonsils and sometimes of the trachea and the esophagus. Small necrotic foci (2 – 3 mm) may be scattered throughout the liver and the spleen (Grant Maxie and Youssef 2007). Similar findings, most of the times, are typically found in aborted fetuses and neonatal piglets (<7 days old). These findings may, also, be observed in fatteners in case of infection with high virulence strains of ADV. In aborted sows, the lesions may consist of endometritis and inflammation of the placenta. Aborted fetuses may be macerated or, occasionally, mummified (Papatsas et al. 1995).

It has been reported that strains with different predilection for the lungs produce variable pulmonary lesions varying from localised edema to massive and widespread necrosis (Baskerville 1972a, 1972b, Baskerville et al. 1973). The apical and cardiac lobes are most commonly involved. In the case of complications with bacteria, variability in the pattern of pulmonary findings is likely (Pejsak and Truszczynski 2006).

Histologic lesions are more commonly seen in young, aborted or stillbirth piglets, where the brain lesions are more common. These include non-suppurative meningoencephalitis and a ganglioneuritis in grey and white matter. Perivascular cuffing consists predominantly of mononuclear cells, with the presence of few granulocytes. The meninges are infiltrated by mononuclear cells, so they become thickened. Regarding the neurons, there might be a focal neuronal necrosis and it is common for the infected neurons to be diffusely scattered. Similar lesions can be found in the spinal cord, especially in cervical and thoracic regions. In addition, microscopic findings may include necrotic tonsilitis, bronchitis, bronchiolitis, alveolitis. Intranuclear inclusion bodies are observed in neurons, astrocytes and oligodendroglia (Dow and McFerran 1962, Csontos and Szeky 1966, Olander et al. 1966, Baskerville 1972a, b, 1973). Focal necrotic areas may, also, be found in the liver, spleen, lymph nodes and adrenal glands of macerated fetuses. In boars, there might be findings in the reproductive system concerning the degeneration of seminiferous tubules and the presence of necrotic foci in the tunica albuginea of the testicles. Furthermore, the denaturation of the semen consisting of abnormal spermatozoa may, also, be observed (Olander et al. 1966, Pejsak and Truszczynski 2006).

## DIAGNOSIS

The diagnosis of AD can be established by case

history of the herd, clinical signs, post mortem lesions and laboratory tests. Neurological symptoms with high mortality in suckling piglets, respiratory symptoms (nasal discharge and coughing) that may be observed in nursery and fattening pigs may be strong indications of AD. Reproductive disorders in breeding animals (boars and sows) can, also, provide clinical evidence of AD, as well. The accidental appearance of dead domestic animals, such as dog and cats, around the farm may corroborate the diagnosis of AD in the herd. Histopathological findings are not characteristic of the disease (Pejsak and Truszczynski 2006).

The virus can be isolated from the tonsils and the lungs of affected pigs, especially if they develop respiratory signs. AD is difficult to clinically diagnose if only fatteners or adult pigs are involved. In these age groups, the disease may be misdiagnosed as swine influenza or Porcine Reproductive and Respiratory Syndrome (PRRS). Similar respiratory symptoms may be caused by bacteria, such as *Pasteurella multocida, Actinobacillus pleuropneumoniae* and *Streptococcus suis* (Pejsak and Truszczynski 2006).

In pigs with neurological disorders, the virus can be isolated from the nervous tissue. Nervous signs can sometimes occur in pigs infected with classical swine fever (CSF) virus. Neurological signs may, also, be observed during porcine teschovirus infection, where the respiratory symptoms are missing, *Streptococcus suis* infection, Glasser's disease, salt poisoning, hypoglycemia and poisoning by organic arsenic or mercury. Moreover, stillbirths and abortions can be induced by parvovirus, *Leptospira spp, Brucella spp, Circovirus* and PRRS virus infection. In case of dual infection with ADV and PRRSV, the clinical disease is much more severe than that produced by either virus alone (Narita and Ishi 2006).

Virological methods are indispensable for the accurate diagnosis of AD. Brain, spleen, tonsils and lungs are the organs of choice for virus isolation from dead pigs, while nasal and pharyngeal swabs or tonsil biopsies can be used for the isolation of ADV from living individuals. Diagnostic methods for antibody detection include serologic tests, such as serum neutralization, latex agglutination and enzyme linked immunosorbent assay (ELISA) (The Center for Food Security & Public Health 2006). Maternal immunity in young pigs may become a "disadvantage" for the detection of ADV infection. Maternal antibodies can be present up to 4 months of age, so if pigs from immunized sows are tested too early, they may be identified as infected, although the antibodies are of maternal origin. Therefore, the examination of paired samples may be necessary in order to determine the decreasing levels of maternal antibodies and to differentiate them from the antibodies produced against the field virus strain. ELISA and virus neutralization are the reference tests for international trade (The Center for Food Security & Public Health 2006). The sensitivity of ELISA is generally superior to that of virus neutralization test and the development of commercially available ELISA kits enables the rapid processing of a large number of samples. ELISA is, also, used to differentiate antibodies produced against vaccine strains from those produced as a result of natural infection. Pigs vaccinated with a gene-deleted mutant vaccine do not develop an antibody response to the glycoprotein coded by the deleted gene (Van Oirschot et al. 1986).

Another method that may be used for ADV identification in secretions or organ samples is the PCR. The main advantage of PCR compared to other methods is its speed as with modern equipment the whole process may be completed in less than 1 day. Although ADV is difficult to be detected in latently infected pigs, a nested PCR assay is regarded as a powerful tool for detection of ADV latent infection (Yoon et al. 2006). The virus isolation in latent infections is most likely to be successful from the trigeminal ganglion. The development of a multiplex PCR method provided the capability of rapid and simultaneous detection and differentiation of four important swine DNA viruses including pseudorabies virus, porcine circovirus type 2, porcine parvovirus and PRRS virus in clinical specimens (Yue et al. 2009, Huang et al. 2004).

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