Aujeszky's Disease

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10.1 History

The history of PRV goes back to the nineteenth century, but at that time, the disease was not linked with swine. In 1813, Dr. Hildreth, a physician from Marietta, Ohio, reported the first case of what later known as Aujeszky's disease (AD). He described a case of "mad itch" in one of his client's cows. The clinical symptoms of the disease included rubbing of the head, twitching neck muscles, scratching and mutilating itself. The cow died in agony 12–14 h from the onset of clinical signs. Due to similarities of the clinical pattern to rabies, the name "pseudorabies" was given to the disease observed in cattle.

In the first decades of the twentieth century, AD was mainly observed in cattle and dogs in Hungary, Romania, France, Russia, Brazil and the USA (Wittmann and Rziha 1989). In pigs, a first description of AD was made in 1902 by the Hungarian veterinary surgeon Aladár Aujeszky. He also revealed that the disease has a viral background. The first enzootic occurrence was reported in 1931 in the Netherlands (Wittmann and Rziha 1989). In general, at that time AD was observed rarely in pigs and was associated with central nervous system disorders. The strong intensification of pig production occurred during 1960–1970 probably contributed to an increase in case numbers and change in disease patterns (respiratory disorder, fever, anorexia, high mortality in piglets, abortion in sows) (Glorieux 2009). Similar changes of disease patterns have been observed in the USA, Europe and in Northern Ireland (Pensaert et al. 1987; Pol 1990; Wittmann and Rziha 1989; Nauwynck et al. 2007).

The rapid spread of infection and huge losses in swine sector forced the introduction of eradication strategy focused on culling of infected herds and vaccination programme. Thereafter, to distinguish infected herds vs. vaccinated herds, marker

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vaccine (deletion of *gE* gene) and appropriate ELISA test were developed. Introduction of this marker vaccine contributed to the eradication of AD in the USA and significant part of Europe (Mettenleiter 2000). AD is a notifiable disease by the World Organisation for Animal Health (OIE) (list B).

10.2 Aetiology of AD

Aujeszky's disease virus (ADV), the causative agent of AD, is a DNA virus of 150–180 nm in diameter belonging to the subfamily *Alphaherpesvirinae* within the family *Herpesviridae*, genus *Varicellovirus*.

The virion of ADV consists of four morphologically distinguishable structures: the core containing the linear double-stranded DNA of approximately 142 kilo base pairs (kbp), capsid, tegument and envelope. The capsid consists of 162 capsomers and 150 hexons that surround and protect the genome. Together, genome and capsid form nucleocapsid. Between the icosahedral capsid and envelope, a proteinaceous layer is situated – called as a tegument. The tegument protein plays an important role in viral life cycle (Luxton et al. 2006; Guo et al. 2010). The outer layer of ADV is a bilayered phospholipid membrane containing 11 glycoproteins (gB, gC, gD, gE, gG, gH, gI, gK, gL, gM and gN) and three nonglycosylated proteins (UL20, UL43, US9). The envelope proteins play important roles in viral entry, egress, cell-to-cell spread, induction of protective immunity and immune evasion (Mettenleiter 2000; Pomeranz et al. 2005; Nauwynck et al. 2007).

Herpesvirus genomes are split into six classes, A to F, and the ADV genome belongs to D class (Roizman and Pellet 2001). The genome of ADV contains 72 open reading frames (ORFs), which encode 70 different proteins. The characteristic feature of D class is that the genome is composed of two unique regions, a long (unique long (UL)) and a short one (unique short (US)). These two regions are bracketed by inverted repeat sequences, an internal repeat sequence (IRS) and a terminal repeat sequence (TRS) (Nauwynck et al. 2007). The whole genome of ADV has been sequenced and published by Klupp et al. (2004).

10.3 Distributions of AD

Due to trade restrictions imposed by AD-free countries, national AD eradication programmes have been conducted in many countries. Thereafter, ADV has been eliminated from domestic pig population in Denmark, Finland, France, Germany, Hungary, Slovakia, Luxembourg, the Netherlands, Sweden, Switzerland, Great Britain (England, Scotland and Wales), Canada, the USA and New Zealand. Till date, ADV has never been detected in Australia. In ADV-free countries, vaccination is forbidden. Nowadays, AD is endemic in commercial pigs in Eastern and Southeastern Europe, Latin America, Africa and Asia. Despite AD has been eradicated from many countries, the ADV continues to circulate among feral swine in the USA and wild boars in many European countries (Müller et al. 2011; Pedersen et al.

2013). In the USA, 18 % of feral swines were seropositive in 25 of 35 states where samples were collected (Pedersen et al. 2013). In Europe, serosurveys revealed the occurrence of ADV infections in wild boar in several European countries, including Belgium, Croatia, the Czech Republic, the former Yugoslavia, France, Germany, Italy, Poland, Romania, Russia, Slovenia, Spain and Switzerland. The average seroprevalence in these countries for the investigated periods was ranging from 0.57 to more than 60 % at national or regional level (Szweda et al. 1998; Zupancic et al. 2002; Lari et al. 2006; Vengust et al. 2006; Köppel et al. 2007; Sedlak et al. 2008; Kukushkin et al. 2009; Montagnaro et al. 2010; Boadella et al. 2012; Pannwitz et al. 2012; Meier et al. 2015). Wild swine are known reservoirs of ADV and may serve as a potential source of virus reintroduction to domestic swine and the other susceptible mammals. Thus, due to the risk of spill over infections to domestic pigs, attention should be paid to control AD in populations of wild swine.

10.4 Replication Cycle

Replication cycles are almost identical among alphaherpesviruses. First step of ADV infection is attachment to the host cell membrane (Fig. 10.1). This process consists of labile binding of the viral envelope glycoprotein gC (and less gB) to cellular heparan sulphate proteoglycans found at the cell surface (Mettenleiter et al. 1990). Afterwards, glycoprotein gD interacts with cellular receptors, herpesvirus entry mediator B (HveB), nectin-2, poliovirus receptor-related protein 2 (PRR2), HveC (nectin-1, PRR1) and HveD (poliovirus receptor (PVR), CD155), which leads to stable binding of virus to the host cell (Nixdorf et al. 1999; Spear 2000; Mettenleiter 2002). Attachment is followed by the viral envelope fusion with host plasma membrane. The glycoproteins gB, gD and gH/gL are involved in this process (Rauh and Mettenleiter 1991; Mettenleiter 1994; Klupp et al. 1997). After penetration to the host cell, capsid is transported to the nucleus via interaction with microtubules (Guo et al. 2010). Upon released into the nucleus, the ADV genome circularises and is transcribed in a tightly regulated cascade-like manner in the order immediate-early (IE), early (E) and late (L) gene expression. ADV encodes only one IE protein termed as IE180. The ADV IE180 gene codes immediate-early transcriptional activator of viral genes required for DNA replication and RNA transcription (Pomeranz et al. 2005). Subsequently, E genes are activated, which are mainly involved in replication of the viral DNA (Huang and Wu 2004). Finally, the L genes are transcribed, encoding mainly structural proteins (capsid, tegument and envelope (glyco)proteins) involved in virion assembly. Capsid assembly occurs inside the nucleus. Nucelocapsid is formed by pulling monomeric units of DNA through a cylindric entry pore to new capsid. Nucleocapsid leaving to the cytosol consists of two steps: primary envelopment (budding of capsids at the inner leaflet of the nuclear membrane) and de-envelopment (primary envelope fusion with the outer leaflet of the nuclear membrane). The primary envelopment is mediated by two viral proteins encoded by UL34 and UL31 (Mettenleiter et al. 2009). Thus, US3 protein is involved in de-envelopment

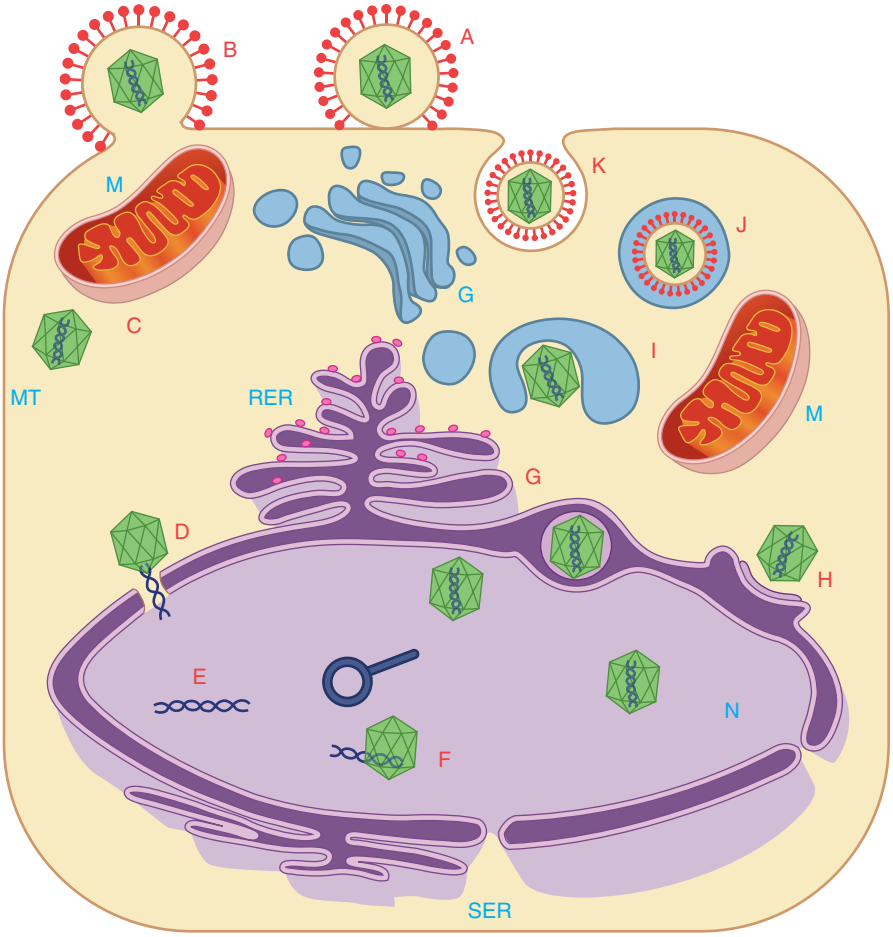

Fig. 10.1 Schematic replication cycle of the ADV in the host cell. ADV attachment (*A*) and penetration (*B*) to the host cell, capsid transport (*C*) along microtubules (*MT*) to the nucleus (*N*), release of the viral genome into the nucleus (*D*), DNA transcription and replication (*E*), encapsidation of viral genome (*F*), primary envelopment (*G*), de-envelopment (*H*), secondary envelopment (*J*) and egress of mature and enveloped virion (*K*). *G* Golgi apparatus, *M* mitochondrion, *MT* microtubules, *N* nucleus, *RER* rough endoplasmic reticulum, *SER* smooth endoplasmic reticulum (Adapted from Mettenleiter 2000 with own modification)

at the outer leaflet of the nuclear membrane (Granzow et al. 2004). Thereafter, the nucleocapsids are transported to the trans-Golgi area in which different viral glycoproteins are expressed. The acquisition of final tegument and envelope is done by budding of nucleocapsids into trans-Golgi-derived vesicles. During this process, different tegument and envelope proteins are assembled into the mature virion. The ADV proteins such as UL11, glycoprotein E (gE) and gM are involved in secondary envelopment of tegumented nucleocapsids in the cytoplasm (Kopp et al. 2004). Release of newly formed virion into the extracellular space occurs by

fusion of the vesicle with the plasma membrane. Spread of ADV infection may occur via free virions and by cell-associated way of spreading. Several different mechanisms of the cell-associated spread have been described (for details, see Nauwynck et al. 2007).

10.5 Pathogenesis

The nasal mucosa acts as a main entry gate of ADV. After intranasal/peroral exposure, ADV primary replicates in the respiratory tract including the nasal cavity, tonsils, pharynx and lungs (Sabo et al. 1969; Baskerville 1973; Miry and Pensaert 1989). Replication in lower parts of the respiratory tract is restricted, except when the virus is directly administrated in these locations (intratracheal or aerosol inoculation) (Baskerville 1973; Miry and Pensaert 1989). Within 24 h, ADV crosses the basement membrane barrier in the nasal respiratory epithelium and infects all sorts of cells including fibrocytes, endothelial cells and mononuclear cells in the lamina propria (Kritas et al. 1994; Glorieux 2009).

In ADV invasion through the basement towards the lamina propria, trypsin-like serine protease activity is involved (Glorieux et al. 2011). After reaching blood vessels, nerves and lymphatics localised in lamina propria, ADV spreads to internal organs wherein the secondary replication takes place. Viraemia can be observed at 24 h after challenge and for up to 9 days, whereby monocytes are the most susceptible mononuclear cells to ADV infection (Nauwynck and Pensart 1995). Replication in secondary site can be observed starting from 48 h postinoculation (Sabo et al. 1969; Wittmann et al. 1980).

One of the important secondary sites of ADV replication is the central nervous system (CNS). ADV travels from the nasal mucosa to the CNS via some of the cranial nerves such as the olfactory and trigeminal nerves (McFerran and Dow 1965; Sabo et al. 1969; Wittmann et al. 1980). Like the other alphaherpesviruses, ADV is capable of moving efficiently within axons to distant cell body (retrograde) and away from cell bodies (anterograde) (Smith et al. 2001). After axonal entry, virus particle moves along microtubules, which are oriented with the plus end at the cell periphery or axon terminal and the minus end towards the cell body. The kinesin family of motor proteins generally mediates plus-end-directed anterograde transport, and dynein motor complexes (recruited by virus U36 protein) generally mediate minus-end-directed retrograde transport (Kapitein and Hoogenraad 2011; Zaichick et al. 2013). A recent study indicated that for efficient ADV retrograde transport and infection of cell bodies, local translation of host proteins including proteins involved in cytoskeletal remodelling, intracellular trafficking, signalling and metabolism is required (Koyuncu et al. 2013b). Another important site of secondary replication is the genital tract. It is suggested that ADV reaches genital organs via blood or nerves (McFerran and Dow 1965) and via ADV-contaminated semen (Bolin et al. 1985). Replication of ADV has been observed in the ovaries and the uterus of sows (Hsu et al. 1980) and in the mesorchium of boars (Miry and Pensaert 1989).

10.6 Virulence Factor

Individual ADV strains show various biological features concerning their virulence as expressed by the different severities of disease in pigs, the quantity and duration of virus shedding. For example, one of the recent studies comparing the differences in pathogenicity between ADV JS-2012 strain (emerging ADV strain isolated in Bartha-K61-vaccinated piglets in 2012, China) and classical virulent ADV SC strain indicated higher pathogenicity of JS-2012 (the earlier onset of clinical signs, higher mortality and more severe pathological lesions) compared with SC strain (Tong et al. 2015).

ADV virulence is determined by viral membrane glycoproteins, virus-encoded enzymes and non-essential capsid-associated proteins (Mettenleiter 2000). Among ADV membrane glycoproteins, five (gB, gD, gH, gK, gL) are essential for viral replication in cell culture. Thus, gC, gE, gG, gI, gM and gN are non-essential but have been involved in important viral functions such as target cell recognition and virulence (Mettenleiter 1996; Pomeranz et al. 2005). From the non-essential glycoprotein, gE is important for the expression of ADV virulence. There is evidence that deletion of gE can induce a considerable impairment in the neuroinvasiveness of ADV, either directly or through its association with gI (Babic et al. 1996). Another study indicated that deletion of two amino acids (valine (125) and cysteine (126)) in gI strongly decreases virulence and neurotropism of ADV for pigs (Jacobs et al. 1993). Besides glycoproteins, thymidine kinase (TK) enzyme (non-essential for virus replication in cell culture) is also an important determinant of ADV virulence. Removal of the TK function leads to attenuation of ADV (McGregor et al. 1985; Prieto et al. 1991; Pan et al. 2001). Studies on viral components affecting the ADV virulence have important significance for the development of efficient engineered vaccines.

10.7 Latency

The hallmark of the herpesviruses infection is its ability to establish a latent state within specific tissues and its characteristic feature for each virus: *Alphaherpesvirinae* (PNS neurons), *Betaherpesvirinae* (monocytes and macrophage precursors) and *Gammaherpesvirinae* (B and T cells)) (Koyuncu et al. 2013a). The viral latency is the ability of the virus to remain dormant (latent) inside the neurons, which allow evading the host's immune system. In the domestic swine, ADV mainly hides in the trigeminal ganglia and other sites, such as the olfactory bulb, medulla oblongata and less frequently the brainstem and spinal cord (Rziha et al. 1986). While in feral swine, ADV typically shows latent infection of the sacral ganglia (Romero et al. 2003). During latency, viral DNA persists, but infectious virus is not produced. Under this state, expression of ADV gene is limited to a small region of the viral genome termed latency-associated transcript (LAT) and to several microRNAs (miRNAs) (Cheung 1989; Farrell et al. 1991; Umbach et al. 2008). There is evidence that miRNAs are involved in blocking apoptosis of infected neurons and in

the control of reactivation from latency (Koyuncu et al. 2013a). Moreover, it is speculated that IFN alpha (IFN-α) represents a key immune component involved in the efficient establishment of *Alphaherpesvirus* latency in sensory neurons (De Regge et al. 2010). Reactivation from latency takes place after stress stimuli (pregnancy, parturition, fever, extreme temperatures, transportation) and is associated with virus production, excretion and spread in the environment (Davies and Beran 1980; Tanaka and Mannen 2003, 2008).

10.8 Co-infections with Other Pathogens

ADV is one of the primary viral aetiologic agents of porcine respiratory disease complex (PRDC). Co-infection of ADV with other swine pathogens exacerbates clinical signs and pathological lesions in the respiratory tract. For example, co-infections of specific pathogen-free (SPF) pigs with porcine reproductive and respiratory syndrome virus (PRRSV) and ADV increase and prolong the clinical signs and pathologic lesions in the lungs (Shibata et al. 2003). Other studies also indicated that pigs dually infected with ADV and *Mycoplasma hyopneumoniae* or ADV and *Actinobacillus pleuropneumoniae* show very severe clinical symptoms and pneumonia and lung lesions as compared to pigs infected only with one agent (Sakano et al. 1993; Shibata et al. 1998).

10.9 Other Animals

Most mammals and birds including dogs, cats, cattle, sheep, goats, foxes, hedgehogs, polecats, opossums, jackals, some nonhuman primates (rhesus macaques, marmosets), chickens, pigeons, geese, ducks, buzzards, sparrow, hawks, rabbits, guinea pigs, rats and mice are susceptible for ADV infection. However, only members of the family *Suidae* (true pigs) are known to survive an acute infection and in addition have subclinical and latent infections. Therefore, swine represents natural reservoir of ADV and the source of virus transmission (Mettenleiter 1996). In non-porcine species, ADV typically causes fatal neurologic disease (respiratory distress, salivation, muscular stiffness), often associated with localised pruritus resulting from self-trauma (Glass et al. 1994; Zanin et al. 1997; Cramer et al. 2011; Verpoest et al. 2014). There are two possible explanations with regard to the fatal infection in nonnatural host: (a) the immune system fails to control the infection and viral replication destroys cells and tissues, and (b) the immune system responds too strongly and a systemic inflammatory response overwhelms the host (Brittle et al. 2004).

Among non-porcine species, deviation from hallmark clinical patterns is presented in minks. In this species, pruritus is usually not observed, and the inflammatory reaction in the mink nervous system is minimal or even absent, and the most prevalent lesions are haemorrhages and ischaemia associated with a systemic vasculopathy, which may be the consequence of an increased endotheliotropism of ADV in this species (Marccacini et al. 2008).

10.10 Viral Transmission

ADV is transmitted most efficiently by direct contact between pigs. The virus is not very contagious. The high quantities of virus are usually necessary to infect animals (>$1 \times 10^{4\text{-}5}$ $TCID_{50}$), except piglets (1×10^{2} $TCID_{50}$) (Wittmann 1991). In vaccinated pigs, a dose needed to infect pigs has been reported to be 10–1000 times higher (Visser 1997; Wiśniewski and Siemionek 1988).

Virus can also spread by oral route through contact with contaminated materials (including water, bedding, meat products, other contaminated feedstuffs and contaminated carcasses of rats and raccoons) (Donaldson et al. 1983). Transmission of virus by the aerosol route over short and long distances may occur (Allepuz et al. 2009; Kluge et al. 1999). Also, venereal transmission by contaminated vaginal mucosa or semen is possible. Virus can be transmitted vertically (transplacental), and foetuses may be infected in utero in the last third of gestation. ADV can also spread via colostrum and milk from an infected sow to suckling piglets (Beran 1991). Other animals such as dogs, cats, raccoons, skunks and rats are considered potential carriers within an endemic area (Kirkpatrick et al. 1980). Common sources of viral transmission into herds are animals with long-term or recrudescent shedding.

Typically, ADV enters into a susceptible swine via the nasal mucosa (when they inhale the virus) or via the tonsils or oral/digestive tract mucosa (when they ingest the virus) (Donaldson et al. 1983).

10.11 Duration and Routes of Shedding

Infected swine can shed ADV in high concentrations from almost all body secretions, excretions and aerosol. At the height of the disease, pigs can excrete $10^{5.8}$–$10^{8.3}$ $TCID_{50}$ ml^{-1} nasally (Pensaert and Kluge 1989). Virus shedding starts 1–2 days after infection, with nasal and oral excretion, and in adult swine vaginal, preputial and/or milk secretion prior to the onset of viraemia and clinical signs. Peak of shedding occurs at 2–5 days of infection and it will last up to 17 days (Müller et al. 2001; Wittmann 1991). Persistence of ADV in infected swine has been observed from 6 weeks to 13 months (Beran et al. 1980). Transplacental transmission leads to considerable virus shedding during abortion and birth (Beran 1991; Blaha 1989).

Lifelong latent infection commonly follows clinical recovery or inapparent infection, with virus remaining in trigeminal ganglia and tonsils (White et al. 1994; Balasch et al. 1998). Stress such as subsequent disease, farrowing, crowding, mingling with unfamiliar animals or transport can lead to recrudescence of shedding for 3–4 days (Schoenbaum et al. 1990a).

10.12 Persistence of Virus in the Environment

ADV is one of the most environmentally persistent animal viruses. However, virus survival depends on many environmental factors, such as temperature, pH and humidity.

Virus inactivation rates increase with increasing temperature (Turner et al., 2000). The virus is inactivated at 5 and 20 °C in 15 weeks and 2 weeks, respectively. At 35 °C (mesophilic conditions), the virus is inactivated in 5 h and at 55 °C (thermophilic conditions); no virus could be detected after 10 min (Bøtner 1991). In another study, persistence of ADV was determined in liquid cattle manure stored at temperatures of 4 and 20 °C for up to 26 weeks (Biermann et al. 1990). Infectivity of ADV in an aerated slurry at pH 9.6 and 44 °C declines in 1–3 weeks (Wittman 1991). Temperatures during composting process effectively and quickly kill ADV (Morrow et al. 1995; Garcia-Siera et al. 2001; Paluszak et al. 2012). In food waste, the virus is inactivated within 24 h at 20 and 30 °C but remains infectious at 10 °C for 48 h and at 5 °C for 96 h (Donaldson et al. 1983). Maturation of pig meat at 4 °C does not inactivate the virus. ADV in pig muscle and bone marrow is killed by freezing (−18 °C) for 35 days (Durham et al. 1980) and after heat treatment of meat at 80 °C (Donaldson et al. 1983).

ADV is stable at pH 4–12 and even at extreme pH values of 2.0 and 13.5; complete inactivation can take 2–4 h (Benndorf and Hantschel 1963).

The best survival of ADV was shown to occur at intermediate (50 %) relative humidity (half-life of 36.1 min) (Schoenbaum et al. 1990b). Survival times of this virus were lower and similar at high and low humidity, with half-lives of 17.4 and 18.8 min, respectively.

Davies and Beran (1981) have shown that at 37 °C and pH 6–8, ADV outside the living host is inactivated at a rate of 0.6 $\log_{10}$ per day. ADV becomes inactive at pH of 4.3 and temperature of 39 °C between 1 and 7 days (Kluge et al. 1999).

ADV has been shown to be persistent in well water for up to 7 days and in chlorinated water for less than 1 day. Studies examining the survival of ADV in buffered solutions found that the virus was stable for at least 10 days in PBS and saline solution. On polypropylene, vinyl, loam soil and meat and bone meal, virus inactivation rate was more rapid with 7 $\log_{10}$ reduction within 7 days. For straw, concrete and pelleted feed, virus dropped below detectable levels within 4 days. For wood, faeces, green grass, alfalfa and denim fabric, virus dropped below detectable levels in 1–2 days (Schoenbaum et al. 1990b).

Susceptibility to disinfectant ADV like other enveloped viruses can be inactivated by many disinfectants, including orthophenylphenate compounds, formalin, quaternary ammonium compound, iodine compound, sodium hydroxide, hypochlorides and chlorine solutions (Beran 1991; Dvorakova et al. 2008).

10.13 Clinical Signs

Clinical picture of AD depends on the virulence of the ADV isolate, the dose and route of infection and the age of the affected swine. Infection can cause neonatal mortality due to neurological disease, respiratory disease in growing and adult pigs and reproductive disorders in breeding animals. The most sensitive age groups to virus infection are newborn pigs. In piglets less than 3 weeks old, sudden death can occur with few, if any, clinical signs. More often, fever, lethargy, loss of appetite, weakness, lack of coordination and convulsions precede death.

Vomiting and diarrhoea 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 2 days, occasionally having manifested violent shaking and shivering (shaker pig syndrome). Piglets infected immediately after birth show clinical signs within the first 2 days and usually die before they are 5 days old. In older pigs, the symptoms start with fever followed by loss of appetite, listlessness, agony, somnolence, laboured breathing, vomiting, rambling and, in some animals, lack of coordination and weakness occurring in the hindquarters. 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 impairs with rising age. Hence, in adult pigs, the disease is usually not severe. Fever is always present, and nasal discharge, coughing, agony and somnolence frequently occur, whereas typical nervous symptoms can be observed only occasionally. Usually, no marked pruritus develops in pigs of any age (Pensaert et al. 1982; Wittmann 1986; Kluge et al. 1999; Mettenleiter et al. 2012).

In sows infected in the first trimester of pregnancy, resorption of foetuses can occur. ADV infection in the second or third trimester results in abortion or stillborn pigs (Kluge et al. 1999). In boars, virus replication occurs in the mesorchium, which leads to exudative periorchitis and increased scrotal fluid, resulting in a severely swollen scrotal region. Moreover, morphologic alteration and lowered sperm cell concentration can be observed, leading to infertility (Miry et al. 1987; Miry and Pensaert 1989; Kluge et al. 1999; Mettenleiter et al. 2012).

10.14 Pathological Examination

Gross pathological changes in necropsied pigs are often subtle, absent or difficult to find. Many pigs have serous or fibrinonecrotic rhinitis, but this may be visible only if the head is split and the nasal cavity opened. Pulmonary oedema, congestion or consolidation is sometimes found, and secondary bacterial pneumonia can result in more severe gross lesions. In the lymph nodes, small haemorrhages may be observed. Affected pigs may also have necrotic tonsillitis or pharyngitis, congested meninges or necrotic placentitis. Necrotic foci can occur in the liver; this is particularly common in very young piglets.

Microscopic examination of the white and grey matter typically reveals non-suppurative meningoencephalitis with relatively mild myelitis. Mononuclear perivascular cuffing and neuronal necrosis may be seen, and the meninges are usually thickened from mononuclear cell infiltration. Additional microscopic findings may include necrotic tonsillitis, bronchitis, bronchiolitis and alveolitis. Very often, in the liver, spleen, adrenal glands and lymph nodes of affected foetuses, focal necrosis is present (Narita et al. 1985; Mettenleiter et al. 2012; Wang et al. 2015).

10.15 Immunity

The viral envelope glycoproteins (gB, gC, gE) are reported as major immunogenic components involved in the induction of protective immunity against ADV. Among the mentioned glycoproteins, gB is important for the induction of cell-mediated immunity (CMI), including cytotoxic T-lymphocyte (CTL) responses, but not for inducing virus-neutralising antibody. The gD is important for inducing the virus-neutralising antibody, but not for inducing CMI. Thus, gC elicits only a weak CMI response and virus-neutralising antibody (van Rooij et al. 2000). In contrast, other reports have shown that gC offers complete protection against lethal ADV challenge (Gerdts et al. 1997). Another finding indicated that gB produces the strongest ADV-specific IgG response in the sera. gB and gD induce contrast pattern of immunity with a bias to Th1 and Th2 types, respectively. Moreover, it has been shown that gC is a potent inducer of $CD8^+$ T-cell-mediated CTL activity against ADV (Yoon et al. 2006). In-depth knowledge on the immunological parameters relevant for protection is a pivotal step for improving the effectiveness of vaccines against ADV.

Infection of pigs with ADV elicits specific antibody and T-cell response against ADV. CMI may be detected from day 4 after infection. In immune pigs, secondary lymphoproliferation responses were detectable from day 2 post-challenge, and both $CD4^+$ and $CD8^+$ cells contribute to lymphoproliferation responses. Humoral immunity appears few days later than CMI. Neutralising antibodies may be detected at day 7, reaching optimal titres at day 14. Interestingly, challenge of previously infected pigs does not result in a secondary B-lymphocyte response. In contrast, the proliferative T-lymphocyte response is enhanced after challenge, which suggests that humoral immune response may be of minor importance in the control of ADV infection (Wittman et al. 1976; Kimman et al. 1995; De Bruin et al. 1998).

10.16 Colostral Immunity

Passively acquired maternally derived antibodies (MDA) prevent newborn pigs against transmission and invasion of ADV in the nervous system and decrease clinical manifestation of the disease (McFerran et al. 1979; Wittmann and Jakubik 1979; Bouma et al. 1997). However, MDA did not provide protection against establishment of latent infection in piglets after exposure to low doses of virulent ADV (McCaw et al. 1997). The amount of passive immunity depends on antibody titre in sow, colostrum intake by the newborn piglets and the number of suckling piglets (Andries et al. 1978). MDA can be detected in ELISA as long as 27 weeks postpartum, while in the serum neutralisation test, up to 15 weeks postpartum. The half-life of maternal neutralising antibodies was determined to be 11.3 and 21 days in domestic swine and wild boars, respectively (Tenhagen et al. 1995; Müller et al. 2005).

The disadvantage of MDA is that they may reduce or abolish antibody development after vaccination (Andries et al. 1978; Bouma et al. 1997; Pomorska-Mol et al. 2010b).

Although early vaccination (at 7 days) against AD in the presence of high levels of MDA did not impair T-cell responses, the intensity and duration of recall proliferative response were dependent on the time of booster vaccination (Bouma et al. 1998; Pomorska-Mól and Markowska-Daniel 2010a).

10.17 Evasion Strategy

ADV has developed several strategies to counter and evade host's antiviral responses. One of the strategies is evasion of antibody-dependent cell lysis. It has been reported that gE-gI expressed on the viral envelope acts as receptor for Fc, and by binding to the constant portion of IgGs (Fc) at the surface of virus-infected cells, they inhibit downstream immune functions of IgG (Walle 2003). Moreover, gE-gI can participate in creating antibody bipolar bridging (ABB) by simultaneous binding of the Fab sides of an antibody to viral proteins in the viral envelope or on the surface of infected cells and of the Fc side of the same antibody to viral Fc receptors (Ndjamen et al. 2014). Another ability of the ADV is evasion of major histocompatibility complex (MHC) class I-dependent cell lysis. A downregulation of the MHC I antigen presentation pathway allows ADV to avoid the CTL response. The downregulation process is associated with the ability of ADV to interfere with peptide transport activity (transporter associated with antigen presentation), due to interactions of viral gN protein encoded by the UL49.5 gene (Ambagala et al. 2000; Flori et al. 2008). Furthermore, it has been shown that the US3 of ADV is required but not sufficient for MHC I downregulation process. However, the mechanisms of ADV-mediated MHC I cell surface downregulation are highly cell type dependent, with variable roles for US3, UL49.5 and, additionally, unidentified early viral proteins (Deruelle et al. 2009). Immune circumvent is also associated with anti-apoptotic activity. Apoptosis, known as programmed cell death, is a fundamental cellular process required for embryonic development, organogenesis and the elimination of damaged or aged cells during maintenance of cellular homoeostasis (Fuentes-González et al. 2013). Many cells undergo apoptosis in response to viral infection, which results in reduction in the release of progeny virus (Thomson 2001). It is known that ADV possesses the ability to block apoptosis of infected cells, which allow to complete the replication cycle in host cell (Aleman et al. 2001). In relation to ADV, there is evidence that virus US3 protein kinase possesses anti-apoptotic activity that protects cells from undergoing apoptosis during infection (Geenen et al. 2005). The next immune evasion mechanism is suppression of expression of the most IFN-β-stimulated genes that have potent antiviral effects (Brukman and Enquist 2006). Also, gG inhibits chemokine responses that mediate the migration of immune cells to sites of injury and infection (Viejo-Borbolla et al. 2010). Moreover, it has been reported that after infection of ADV, the interleukin-18 (IL-18) protein expression in the tracheobronchial lymph node is impaired. Importantly, IL-18 is involved in the induction of strong Th1-type immune response (IL-2 and IFN-γ) (Miller et al. 2010). Furthermore, one of the latest studies pointed out the role of ADV in impairing natural killer (NK) cell functions. It was reported that expression

of gD protein leads to degradation of CD112/nectin-2, a ligand for the NK-activating receptor DNAX accessory molecule 1 (DNAM-1). This impairs binding of DNAM-1 to the cell surface, thereby suppressing NK-mediated killing of virus-infected (or gD-transfected) cells (Grauwet et al. 2014).

Identification of immune evasion mechanism helps in the design and/or improved herpesvirus vaccines and herpesvirus-based therapeutic vectors.

10.18 Diagnosis

The clinical manifestation of ADV can resemble other infectious and noninfectious diseases including rabies, polioencephalomyelitis (teschovirus), Nipah virus infection, classical and African swine fever, Japanese encephalitis, haemagglutinating encephalomyelitis, *Streptococcus suis* infection, salt poisoning and hypoglycaemia, which should be excluded before the start of ADV laboratory confirmatory test. In sow, diseases which are able to cause reproductive disorders such as porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), swine influenza virus (SIV) and leptospirosis should be taken into account for the differential diagnosis.

In non-porcine species, other diseases causing neurological symptoms (rabies, scrapie (sheep), bovine spongiform encephalopathy (BSE), hepatic encephalopathy (dogs, cats)) and itching need to be considered.

Various methods are used for the laboratory diagnosis of ADV infections. AD can be confirmed by virus isolation, DNA detection by polymerase chain reaction (PCR) and indirectly by serological methods. The oropharyngeal fluid, nasal fluid (swabs) or tonsil swabs are appropriate specimens for the virus isolation from living pigs. From dead pigs, the preferred specimens are brain, tonsil and lung samples. In latently infected pigs, the trigeminal ganglia is the most consistent site for virus isolation, although latent virus is usually non-infective unless reactivated, making it difficult to recover in culture. In non-porcine species, the section of the spinal cord that innervates the pruritic area should be collected. The pruritic area of the skin, together with subcutaneous tissues, should also be submitted (The Center for Food Security and Public Health 2006; OIE Terrestial Manual 2012).

The collected samples are homogenised in normal saline or cell culture medium with antibiotics. The resulting suspension is next clarified by low-speed centrifugation. The virus particle-containing supernatant is used to inoculate sensitive cell culture system for virus passage and cytopathic effect (CPE) examination. The typical CPE is usually observed 24–72 h after inoculation and is characterised as the infected cells detached from each other, became round and form an approximate circular or oval plaque. In addition, large syncytia can also be formed (Ren et al. 2012). However, virus identity should be confirmed by immunofluorescence, immunoperoxidase or neutralisation using a specific antiserum (OIE Terrestial Manual 2012).

Serological tests such as VN, a variety of ELISA tests, latex agglutination test (LAT) and agar gel immunodiffusion test (AGID) can be used to diagnose AD (Corn et al. 2004; Yong et al. 2005; OIE Terrestial Manual 2012; Serena et al. 2015). It can

be carried out only in pigs (feral swine, wild boars), as other susceptible animals (herbivores and carnivores) die before mounting the humoral response.

Among serology methods, VN has been recognised as the reference method and is considered as a gold standard test for ADV infection. In general, VN has been widely replaced by ELISA assay because of its suitability for large-scale testing. Moreover, VN is very expensive and time-consuming and both require expensive equipment and trained technicians. Due to these conditions, VN is not used in routine laboratory diagnostics. Moreover, some ELISA tests (i.e. gE differential ELISA test) are able to distinguish naturally infected and the glycoprotein E (gE)-deleted modified live virus vaccine-immunised pigs. This step is a pivotal part of successful eradication of AD in domestic swine.

Any serological technique used in AD diagnosis should be sensitive enough to give a positive result with the OIE international standard reference serum. This serum can be obtained from the OIE reference laboratory for AD in France. For international trade purposes, the test should be sensitive enough to detect the standard serum diluted one half. To authorise pig movement from an area where deleted gE vaccines are used to a free area, serological assays should be able to detect gE of the OIE reference standard serum at one eighth dilution as prescribed by the European Commission (2008) (OIE Terrestial Manual 2012).

At present, real-time PCR, a high-throughput test system is widely used in AD diagnosis in domestic and feral swine, as well as non-porcine species. The advantages of real-time PCR are the rapid and specific ADV detection (acute phase of infection – before seroconversion) and differentiation of gE-deleted vaccine viruses from field ADV (Ma et al. 2008; Zanella et al. 2012).

10.19 Vaccination

In 1977, Norden Laboratories (Lincoln, Nebraska) licensed the first conventional, commercial modified live vaccine (MLV) and inactivated ADV vaccine. The first gene-deleted PRV vaccine (gG deletion) with a companion differential serological diagnostic test was licensed in 1988. In 1990, a new vaccine based on a virus with a gE glycoprotein deletion and a companion differential ELISA diagnostic serology test has been introduced to the market (USDA 2008).

In several studies, it has been documented that both live and inactivated vaccines induce protective immunity after wild-type ADV challenge (Alva-Valdes 1981; Cook et al. 1990; Vannier and Cariolet 1991; Vilnis et al. 1998; Mikulska-Skupień et al. 2005). Inactivated vaccines are capable of mounting equal or higher titre of VN antibodies such as MLV, but it is less effective in inducing virus-specific IFN-γ-producing cells (Zuckermann et al. 1998; Zuckermann 2000). It has been reported that CMI response is a major component of early protection against ADV infection and that ADV-induced IFN-γ responses may serve as a suitable indicator for assessing the immune status of vaccinated pigs (Hoegen et al. 2004; van Rooij et al. 2004). An additional important issue associated with MLV ADV vaccine is the ability of vaccine virus to prevent or reduce the establishment of latent infection by the

wild-type virus. Moreover, it has been reported that efficacy of MLV depends on the MLV strain and route of administration (Vilnis et al. 1998).

The important findings reported recently indicated that simultaneous administration of therapeutic doses of antibiotics (doxycycline, enrofloxacin, ceftiofur) and vaccination (MLV) against ADV might affect the development of post-vaccinal humoral and cellular immune responses (Pomorska-Mól et al. 2014, 2015a, 2015b). Whereas, development of humoral and CMI response after vaccination against ADV in pigs with subclinical infection, e.g. PCV2, was altered (Díaz et al. 2012).

10.20 Prevention and Control

Severe economic consequences in pig husbandry are related to AD, mainly due to international trade restrictions, as well as the costs of vaccination and/or slaughtering of infected pigs. For example, an AD epidemic in West Germany in 1980–1982 caused indirect losses of 61 million Deutsche Marks in compensation to farmers for slaughtered animals (Vicente-Rubiano et al. 2014). In response to huge economic losses, most countries have launched strict AD control and eradication programmes mainly based on vaccination and elimination of infected herds.

Nowadays, due to rigorous AD control and eradication programmes, several countries reach the AD-free status. However, ADV still circulates globally in non-domestic swine population and might reintroduce the disease to commercial swine as well as other domestic animals and wildlife. To prevent the reintroduction, proper surveillance and strict biosecurity measures are compulsory especially in areas where wild swine population may have a direct contact with commercial swine (free-roaming pig husbandry). In Spain where open-air pig production coexists with a large wild boar population (ADV seropositive), a new approach for rapidly assessing the risk of AD reintroduction into a disease-free Spanish territory by analysing the movement of live pigs and potential contacts with wild boar has been proposed. This new approach may help in designing risk-based surveillance and control strategies to rapidly eradicate the disease from endemic areas and to prevent further reintroduction of the virus into free territories (Vicente-Rubiano et al. 2014).

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