

A REVIEW OF SOME IMPORTANT VIRAL DISEASES OF WILD BOARS

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Abstract: Wild boars are one of the widest-ranging mammals worldwide and represent reservoirs for many important viruses. Disease outbreaks in domestic swine are often described as a consequence of contact with wild boars, and traditional rearing conditions are a particular risk factor. Examples of such diseases include classical swine fever (CSF), African swine fever (ASF), Aujeszky's disease (AD), and diseases caused by porcine circoviruses and parvoviruses. Some viral infections causing high mortality rates are easily noticeable and thus reported, though many viruses infecting wildlife are insidious impacting survival rates and reproduction in wild animals. Samples from wild boars for laboratory testing are usually collected postmortem and include various tissues or blood sera. The recovery of viable viruses during virus isolation depends on the virus species and the condition of the sample. Since this method does not yield timely results, most diagnostic procedures are based on PCR or antigen detection methods. Serological surveys are inexpensive and appropriate for prevalence studies. When interpreting the results of diagnostic tests, both virus and host characteristics, and the epizootiological situation must be accounted for. Disease control techniques such as fencing or feeding wild boars cause animal aggregation and give rise to population density which favors pathogen maintenance in the environment. Hunting reduces the number of susceptible animals and is helpful as an additional control measure and for sampling. Available data on infectious disease dynamics in wild boars is scarce, and constant knowledge improvement on pathogenesis, clinical symptoms, risk factors, and adequate control measures are required.

Key words: CSFV, ASFV, PCV, PPV, SuHV1, wild boar

Introduction

Wild boars (*Sus scrofa*) are one of the widest-ranging mammalian species worldwide, and this species gradually spreads, occupying new areas (Ruiz-Fons et al., 2008). The behavioral and ecological characteristics of the species favor its abundance, while the high reproduction rate is further aided by climate change that prolongs the mating season (Acevedo et al., 2007; Massei et al., 2015). Human-driven factors also play a major role in wild boar expansion, namely abandoning rural areas, as well as the intensification of wild boar farming and supplementary feeding in line with the expansion in commercial hunting (Acevedo et al., 2007). Moreover, recreational hunting has little effect on reducing wild boar population sizes (Massei et al., 2015). Wild boars represent practically perfect reservoirs for many important infectious diseases of veterinary importance owing to their natural susceptibility to various pathogens, worldwide distribution, biological and ecological traits (Ruiz-Fons et al., 2008; Meng et al., 2009). Wild boars tend to roam large distances, and host movement is known as an essential component of the dynamics of infectious diseases depending on the mobility of the animals, as well as the biological characteristics of infectious agents (Podgorski and Smietanka, 2018; Nišavić et al., 2021a,b). For example, in extreme conditions such as hunting pressure, wild boars can cross distances of up to 250 km (Milićević, 2016). Many disease outbreaks in domestic swine, especially animals reared in traditional conditions, have been described as a consequence of contact with wild boars, and human activities in rural areas have only further increased the possibilities of such events (Gibbs, 1997; Turcitu et al., 2011; Franzo et al., 2020; Petrović et al., 2021). The estimated density of the wild boar population in Serbia is 0.2 - 1.38 animals per km², while the country has the highest pig density in the Western Balkans with around 2.7 million domestic pigs, and traditional farming with low or without any biosecurity measures is still very common (Milićević et al., 2016; Petrović et al., 2021). Wild boars abandon densely populated areas, and households with traditional pig rearing systems become ideal food sources for these wild animals (Milićević, 2016). The constantly growing wild boar population in Europe, along with rising animal density enables pathogen persistence in nature, thus leading to health risks for domestic pigs along with the potential economic losses to the livestock industry. This review concerns several viral diseases of swine that also implicate wild boars as significant factors for pathogen tenacity and transmission.

Classical swine fever (CSF)

Classical swine fever is the cause of major economic losses around the world, particularly in countries with widespread pig farming (Zhou, 2019). The

CSF virus (CSFV) is an enveloped positive-stranded RNA virus categorized in the genus Pestivirus within the *Flaviviridae* family (ICTV, 2021). A single CSFV serotype has been described to date, however, serological cross-reactions with other viruses from the same genus such as bovine viral diarrhea virus (BVDV) or border disease virus (BDV) can impair the results of serological diagnostics (Beer *et al.*, 2015; Moennig, 2015; OIE, 2019a). The virus is sensitive to commonly used disinfectants and detergents, but it can survive for months in various pork meat products and moist conditions (Moennig, 2015). Domestic pigs have been the subject of extensive studies concerning the clinical course of CSF, however, wild boars are just as susceptible to CSFV infection (Artois *et al.*, 2002; Milićević *et al.*, 2013; Moennig, 2015; Zhou, 2019). Classical swine fever occurs throughout the world and is endemic in areas such as Asia, South, and Central America, parts of Europe, and Africa (Brown and Bevins, 2018). The presence of the virus in wild boar populations poses a constant threat of CSFV introduction into domestic pig populations and even reintroduction of the virus in certain countries (Beer *et al.*, 2015; Zhou, 2019). The virus was found to be readily transmitted between domestic and wild pig populations in regions with extensive pig farming. Wild boars are viewed as important reservoirs of CSFV in nature (Artois *et al.*, 2002; Kaden *et al.*, 2005; Moennig, 2015; Zhou, 2019). The clinical course of the disease can be acute, chronic, or late-onset, which occurs as a consequence of prenatal infection of piglets (Moennig, 2015). After crossing the placental barrier in pregnant sows, CSFV infection of fetuses most commonly results in stillbirth or abortion. However, if the infection occurs in late gestation, persistently infected piglets are born and represent a major source of the virus since these animals shed the virus for weeks until they die. These late-onset infections may pass unnoticed, with wasting as the most common symptom. This clinical form of the disease has not been observed in wild boars since it is difficult to track such occurrences in the wild, however, it has been demonstrated experimentally (Kaden *et al.*, 2005; Moennig, 2015). It is thought that adult animals are less important as virus shedders in wild boar populations and that the persistent infection of piglets is the main route for the spread of CSF in these animals (Artois *et al.*, 2002). The acute course of CSF lasts a few weeks (usually 1 to 3, or up to 4 weeks) and passes with complete recovery or death of affected animals. The outcome of the acute disease form depends on various factors, such as the virulence of the infecting viral strain or the immune status of the animal (Moennig, 2015; Zhou, 2019). Clinical manifestation of CSF is milder in older animals with less specific symptoms and frequent recovery. The main clinical symptoms include fever, inappetence, conjunctivitis, nasal discharge, diarrhea, convulsions, and loss of coordination (Zhou, 2019). Characteristic signs also include the appearance of petechial and ecchymotic bleeding on the skin and internal organs on postmortem examination (Artois *et al.*, 2002; Zhou, 2019). Chronically infected animals are mostly adult pigs and this disease course lasts more than 4 weeks during which the specific

disease signs seen in the acute form are replaced by less specific and include intermittent fever, chronic enteritis with consequential wasting (Artois *et al.*, 2002). This disease form has not been fully described in wild boars, and it can be questioned whether these chronically infected animals could survive for a long period in a natural setting (Kaden *et al.*, 2005; Moennig, 2015). The oro-nasal route is the most common infection pathway, and the virus primarily replicates in the tonsils wherefrom it is transmitted to corresponding lymph nodes, while the onset of viremia enables the infection of internal organs (Artois *et al.*, 2002). CSFV is transmitted in susceptible animal populations through direct contact or indirectly through fomites (Zhou, 2019). Contaminated meat products are also an important source of the virus and can lead to the introduction of CSFV in areas free of infection (Moennig, 2015). Wild boars get infected through direct contact with domestic pigs or by feeding in contaminated areas (Artois *et al.*, 2002; Ito *et al.*, 2019; Zhou, 2019). The virus persists and becomes endemic, especially in large and dense populations of wild boars where it is transmitted within a group of animals as well as between different groups, both by direct or indirect contact. This occurs through contaminated excretions, carcasses, during rutting, or after hunting seasons which drives the formation of new groups of animals (Moennig, 2015). Adult wild boars that survive the infection become immune, however, piglets lacking passive immunity to CSFV act as reservoirs (Artois *et al.*, 2002; Moennig, 2015). Maternal antibodies against CSFV protect newborn animals during the first several weeks however, they do not prevent the shedding of the virus (Artois *et al.*, 2002). The outbreak of CSF in wild boar populations is suspected in cases of high mortality detected within a certain population, along with noticing atypical behavior in live animals. Pathological findings include the appearance of widespread hemorrhages, particularly in lymph nodes that are marbled red, tonsils, larynx, and other internal organs (Artois *et al.*, 2002; OIE, 2019a). Splenic infarctions and necrotic ulcerations in the gastrointestinal tract are also frequently observed (OIE, 2019a). Suspicion of CSFV infection must be confirmed using laboratory tests, and tonsils, lymph nodes, spleen, ileum, and kidneys are usually sent for analysis (OIE, 2019a). The virus can be isolated in cell cultures of porcine origin (e.g. PK-15 cell line), however, CSFV is not cytopathic, and its presence must be indirectly identified using immunofluorescence or immunoperoxidase staining (Moennig, 2015; Petrović *et al.*, 2019; OIE, 2019a). Since virus isolation is often time-consuming, reverse transcription polymerase chain reaction (RT-PCR) is the method of choice due to its sensitivity, speed, and the potential for analysis of multiple samples (Milićević *et al.*, 2013; Nišavić *et al.*, 2016). Serology is performed in sera collected from shot wild boars and recommended tests include the virus neutralization test (VN) and the ELISA using monoclonal antibodies that discriminate between CSF and BVD/BD antibodies (Artois *et al.*, 2002; OIE, 2019a). Until now, three CSFV genotypes containing various subgenotypes have been discovered, and the occurrence of novel genotypes is being constantly

monitored (Beer *et al.*, 2015). Studies conducted in Serbia show that all examined isolates belonged to the 2.3 CSFV subgroup (Milićević *et al.*, 2013; Petrović *et al.*, 2019). In December 2019, Serbia has stopped vaccination against CSF which brings the country one step closer to being declared as free of CSF (Službeni Glasnik RS, Br. 87, 2019).

African swine fever (ASF)

African swine fever is one of the most important viral diseases of pigs, and the lack of vaccine combined with the presence of infected wild boars further complicates the implementation of effective control measures (Podgorski and Smietanka, 2018; OIE, 2019b). The causative agent is the African swine fever virus (ASFV), a double-stranded DNA virus from the *Asfarviridae* family (ICTV, 2021). Unlike most DNA viruses, ASFV replicates in the cytoplasm of infected cells, and its main target cells are macrophages which aid virus dissemination throughout the body of the infected animal (Blome *et al.*, 2020). This virus is very stable, especially in moist conditions (blood, manure, etc.) as well as in raw pork products where it survives for months, however, proper cooking results in virus inactivation (Dixon *et al.*, 2019). Outbreaks of this disease must be reported to the World Organisation for Animal Health (OIE), and trade restrictions are imposed on countries with reported cases of ASF (OIE, 2019b; Blome *et al.*, 2020). African swine fever is endemic to sub-Saharan Africa, Sardinia, and parts of the Caucasus and Eastern Europe (Beltran-Alcrudo *et al.*, 2017). ASFV infects domestic pigs and wild suids (warthogs and bushpigs in Africa; wild boars in Europe and Asia) (Beltran-Alcrudo *et al.*, 2017; Milićević *et al.*, 2019; Petrović *et al.*, 2021). The virus circulates between *Ornithodoros moubata* soft tick species and warthogs in Africa in which it causes no clinical symptoms (Blome *et al.*, 2020). This sylvatic cycle is maintained since warthogs are naturally resistant to ASFV, and the virus remains in tick populations due to transovarial, transstadial, and transsexual transmission (Dixon *et al.*, 2019). Conversely, the Eurasian wild boars manifest clinical symptoms similar to domestic pigs and excrete high levels of virus (Blome *et al.*, 2020; Dixon *et al.*, 2019). Wild boar populations in Sardinia, Eastern Europe, and the Caucasus are important for maintaining the cycle of ASFV infection (Beltran-Alcrudo *et al.*, 2017; Dixon *et al.*, 2019). The virus is transmitted through scavenging, as well as by human factors such as supplementary feeding, fencing, or hunting (Beltran-Alcrudo *et al.*, 2017). In Europe and Asia, domestic pigs can be infected by wild boars both through direct and indirect routes (Podgorski and Smietanka, 2018; Blome *et al.*, 2020). Wild boars can move across borders and therefore represent important means of virus transmission and a reservoir for domestic pigs, especially in areas with low biosecurity measures on extensive pig farms (Podgorski and Smietanka, 2018; Chenais *et al.*, 2019). In areas with low wild boar population densities, the infection of these animals

originated from domestic pigs, however, in regions with dense wildlife populations, wild boars are reservoirs of ASFV for domestic pigs (Beltran-Alcrudo et al., 2017; Petrović et al., 2021). The clinical presentation of ASF is variable and dependent on virulence of the virus, infectious dose, exposure route, as well as the affected breed of swine (Dixon et al., 2019; OIE, 2019b). Peracute and acute disease forms are caused by highly virulent strains and often result in the death of affected animals. Acute ASF is characterized by inappetence, high fever, lethargy, ocular and nasal discharge, vomiting, melaena, purple areas and hemorrhages on the ears, abdomen, and legs (Dixon et al., 2019; Milićević et al., 2019; Blome et al., 2020). These characteristic hemorrhages are difficult to observe in wild boars due to dark skin and thick hair (Beltran-Alcrudo et al., 2017). Infection with viruses of moderate virulence leads to acute and subacute forms of the disease characterized by somewhat lower mortality rates of 30-70% (Blome et al., 2020). Clinical signs are usually less severe than in acute cases, however, hemorrhages and edemas are more evident, as well as impaired movement due to joint swelling as a result of fluid and fibrin accumulation (Beltran-Alcrudo et al., 2017; Blome et al., 2020). Virus isolates of low virulence are present in endemic areas, and the infection is more dependent on the exposure route and infectious dose. These strains cause chronic disease manifested by mild fever, wasting, arthritis, skin ulcers, and respiratory symptoms (Dixon et al., 2019). Characteristic pathological findings include enlarged hemorrhagic lymph nodes, splenomegaly (the spleen is dark red to black with round edges), petechiae in the kidneys and other organs (Beltran-Alcrudo et al., 2017; OIE, 2019b). Clinical manifestations of ASF may not be easily distinguished from other viral and bacterial diseases of pigs, and a definitive diagnosis is established based on laboratory tests (Beltran-Alcrudo et al., 2017). Laboratory diagnostic methods are based on the identification of viral DNA, antigens, or specific antibodies, and the choice of proper methods is based on the disease course and epizootiological situation (Nišavić et al., 2016). Samples for testing include blood in anticoagulant, serum, spleen, lymph nodes, bone marrow, lung, tonsil, and kidney (Milićević et al., 2019; OIE, 2019b). Virus isolation is performed by inoculation of pig leukocyte or bone marrow cultures with sampled material and the replication of ASFV produces a cytopathic effect (CPE) in the infected cells (Beltran-Alcrudo et al., 2017; OIE, 2019b). The haemadsorption (HAD) test is often performed since a positive result in the HAD test is definitive for ASF diagnosis. Pig erythrocytes adhere to the surface of pig monocyte or macrophage cells cultured *in vitro* and infected with ASFV which is a unique trait for this virus (Beltran-Alcrudo et al., 2017). The presence of the virus can also be confirmed in cell cultures by immunofluorescence or PCR (OIE, 2019b). The viral antigen can also be directly detected in sampled tissues by antigen ELISA test or immunofluorescence, however, these methods are most sensitive for diagnosing acute ASF (Beltran-Alcrudo et al., 2017). Conventional and real-time PCR is most often used for the detection of the ASFV genome in samples from both pigs and

ticks (Milićević *et al.*, 2019; Blome *et al.*, 2020). This method is equally applicable unrelated to the disease course in suspected infections (Beltran-Alcrudo *et al.*, 2017). The presence of anti-ASFV antibodies is mostly detected using ELISA and immunofluorescence and is indicative of current infection or past exposure since there is no vaccine available (OIE, 2019b). However, serological diagnostic methods are not applicable in acute and peracute cases of infection (Beltran-Alcrudo *et al.*, 2017). ASFV is a genetically stable virus with low rates of mutation. Today, the molecular epizootiology of ASF is mostly based on whole-genome sequencing that enables a more detailed analysis of potential genetic changes and an understanding of virulence factors (Blome *et al.*, 2020). The first reported case of ASF in Serbia was detected on July 30, 2019, in a domestic pig population in Mladenovac municipality (Milićević *et al.*, 2019). The epizootiological situation in Romania, Bulgaria, and Hungary during 2017 and 2018 lead to the establishment of programs of surveillance of wild boar populations in bordering areas with Serbia (Petrović *et al.*, 2021). However, the virus was introduced due to human activities, most probably including the illegal trade of pork products (Milićević *et al.*, 2019). Since then, numerous outbreaks in both domestic pigs and wild boars have been reported, and the epidemiological pattern shows that ASFV circulates among small farms, wild boars, but also affects large farms with intensive rearing systems (Petrović *et al.*, 2021).

Aujeszky's disease (AD)

Aujeszky's disease (AD) is an economically important viral disease primarily associated with pigs or wild boars as natural hosts (OIE, 2018; Tan *et al.*, 2021). The disease is caused by Suid Herpesvirus 1 (SuHV1), a double-stranded DNA virus from the genus *Varicellovirus*, subfamily *Alphaherpesvirinae* of the *Herpesviridae* family (ICTV, 2021). The virus exists in a single serotype and four genotypes circulating worldwide (Milićević *et al.*, 2016; Sehl and Teifke, 2020). SuHV1 causes the infection of multiple organs, dominantly the central nervous system of diverse mammalian species (carnivores, rabbits, cattle, etc.), however, it is not zoonotic (Sehl and Teifke, 2020). The pigs are the only species that survive the infection and remain latently infected after recovery, making them ideal reservoir hosts (OIE, 2018). Clinical manifestations of AD in pigs depend primarily on the age of the animal and its immunological status as well as the virulence of the infecting SuHV1 strain (Helke *et al.*, 2015). Piglets in the first two weeks of life are highly susceptible to infection and show severe neurological symptoms followed by death (Sehl and Teifke, 2020). Older categories of pigs mostly have respiratory disease symptoms that are frequently complicated by secondary bacterial infections, and pregnant sows abort due to the ability of the virus to cross the placental barrier (Helke *et al.*, 2015). The disease in other animal species is fatal and also known as pseudorabies (similar to rabies) or "mad-itch"

since it leads to behavioral and nervous disorders often manifested by scratching due to intense pruritus that in turn causes severe tissue damage (Sehl and Teifke, 2020). SuHV1 is shed in large quantities by infected pigs and is spread in the population by direct and indirect contact (Nišavić and Milić, 2017a). Cattle, sheep, and goats mostly acquire the infection through direct contact with infected pigs, whilst carnivores get infected through unprocessed pig meat (Helke et al., 2015; Sehl and Teifke, 2020). After replication in the respiratory tract of the pig, the virus is transported to tonsils and regional lymph nodes, and it spreads throughout the body during the viraemic phase of infection (Nišavić and Milić, 2017a). SuHV1 demonstrates tropism towards various tissues including the endothelium, lymphocytes, macrophages, and epithelial cells (Sehl and Teifke, 2020). Similar to other herpesviruses, latency is established in the trigeminal and sacral ganglia as well as in the tonsils (Helke et al., 2015; Nišavić et al., 2018; Radalj et al., 2021). Herpesviruses are successfully maintained in animal populations through constant cycles of latency and reactivation of the virus which results in shedding and infection of other susceptible animals (Radalj et al., 2021). Clinical symptoms of AD are rarely observed in wild boars, and disease outbreaks may pass unnoticed, while some studies demonstrate that younger animals are mostly affected (OIE, 2018; Sehl and Teifke, 2020). The occurrence of AD in wild boar populations can be triggered by stress-induced by various factors including environmental conditions or human activities (Meier et al., 2015). In extensive pig farming systems, domestic pigs are often exposed to contact with wild boars, and on the other hand, human factors can influence the distribution of wild boars that potentially induce interactions with domestic pigs (Charrier et al., 2018). On pathological examination, notable changes can be seen in younger animals and include congestion of the brain and lymph nodes, and necrotic alterations in tonsillar tissue and parenchymatous organs (Sehl and Teifke, 2020). The appearance of white spots on the liver indicates AD infection in very young piglets (OIE, 2018). Laboratory diagnosis of AD is based on virus isolation, PCR, and serology (Nišavić and Milić, 2017a). Suitable samples for analysis include oral fluid and nasopharyngeal swabs from living animals (OIE, 2018). In the case of wild boars, tissues taken on post-mortem examination are usually sampled and often include samples of brain, spleen, tonsils, lungs, kidneys, or blood (Milićević et al., 2016; OIE, 2018). Neural tissue is the sample of choice for attempting virus isolation in latently infected animals (Radalj et al., 2021). SuHV1 can be successfully isolated in various cell lines, with PK-15 being the most preferred, and virus replication induces the development of CPE within 24 to 72h of specimen inoculation. Following isolation, SuHV1 is further identified by virus neutralization, immunofluorescence, or PCR (OIE, 2018). Conventional or real-time PCR is the method of choice since it is reliable in detecting both active infection and latently present virus and enables the examination of multiple samples in a relatively short time (Nišavić et al., 2016). ELISA is most frequently used for serological diagnosis

and is especially suitable for large-scale studies on wild boar populations (Boadella *et al.*, 2012; Charrier *et al.*, 2018). Seroprevalences in wild boars are highly variable from country to country, with the highest being in Spain, Italy, and neighboring Croatia and Romania (Meier *et al.*, 2015). Even though AD occurs worldwide, some countries have managed to eradicate the disease in domestic pigs (Canada, USA, New Zealand, and some EU states) (OIE, 2018). However, wild boar populations still pose a threat since these animals are risk factors for introducing the virus into previously free areas (Boadella *et al.*, 2012). For example, in Spain, where AD has been eradicated in domestic pigs, occasional spillovers from wild boars to extensively reared pigs still occur and this is controlled by mandatory vaccination (Muller *et al.*, 2021). Conversely, in Serbia, there are no eradication or vaccination programs, and domestic pigs are mostly vaccinated on large commercial farms due to economic reasons. In Serbia, SuHV1 was successfully isolated from wild boars demonstrating respiratory symptoms during the winter of 2014/2015. The isolates were genetically analyzed, and the results showed little difference from domestic pig SuHV1 isolates, again emphasizing the importance of establishing adequate biosecurity measures in some areas of the country where pigs are extensively farmed (Milićević *et al.*, 2016).

Porcine circovirus infections

Porcine circoviruses (PCVs) are known etiological agents of disease in both domestic pigs and wild boars, however, due to the widespread use of advanced molecular techniques, novel PCVs are still being detected (Nišavić and Milić, 2017b; Zhang *et al.*, 2020; Nišavić *et al.*, 2021a). These viruses are one of the smallest DNA viruses with a circular single-stranded genome and are members of the *Circoviridae* family (ICTV, 2021). To date, PCVs have been divided into four species, i.e., PCV1-4 (Opriessnig *et al.*, 2020). PCV1 does not cause diseases in pigs and was discovered as a contaminant of porcine cell lines. However, PCV2 is listed as one of the most important viruses of swine and causes various disease syndromes in these susceptible animals also known as a porcine-circovirus-associated disease (PCVAD) (Segales *et al.*, 2012; Zhai *et al.*, 2019). PCVAD includes the postweaning multisystemic wasting syndrome (PMWS), pneumonia, reproductive problems, and porcine dermatitis and nephropathy syndrome (PDNS). Available literature information dominantly concerns PCV2 since it was the most studied of all circoviruses. It is well-known that aside from infection with PCV2, other factors are required to induce severe disease (Segales *et al.*, 2019). PCV3 has been identified recently in domestic pigs and wild boars and is also connected with similar disorders as PCV2, however, this virus was also detected in healthy animals, and it is suggested that PCV3-associated disease is most often subclinical and also dependent on the influence of other factors (Prinz *et al.* 2019; Saporiti *et*

al. 2021). PCV4 was identified very recently in China, however, its global distribution and potential disease association are still largely unknown (Zhang *et al.*, 2020). PCV2 easily spreads in the susceptible population, mostly through direct contact, and is shed for a long time, thus exposing susceptible pigs to contaminated respiratory, digestive, and urinary secretions (Rose *et al.*, 2012). Definitive diagnosis of infectious diseases includes laboratory confirmation of the etiological agent after clinical sign assessment. This may be difficult in the wild, and thus most studies concern the analysis of wild boar samples collected postmortem (Prinz *et al.* 2019; Nišavić *et al.* 2021a). The most suitable samples for conventional or real-time PCR analysis include the spleen, liver, tonsils, lymph nodes, and sera (Amoroso *et al.*, 2021; Nišavić *et al.*, 2021a). PCV2 affects the immune system in wild boars leading to the aggravation of other diseases present in these animals (Rose *et al.*, 2012). Some wild boar hunting grounds are fenced and correspond to domestic pig extensive breeding farms that can also deteriorate PCVAD (Ellis *et al.*, 2003). Overall, it is considered that wild boars are PCV2 reservoirs and represent a substantial risk for domestic pigs (Amoroso *et al.*, 2021). A study conducted in Italy emphasizes the limited efficacy of biosecurity control measures considering high PCV2 infection prevalence in both domestic pig and wild boar populations (Franzo *et al.*, 2020). PCV2 prevalence in wild boars is variable from one country to another, and often, PCV2-positive domestic pigs are detected in areas with high densities of wild boar populations and where pigs are traditionally farmed (Turcitu *et al.*, 2011; Franco *et al.*, 2020). Traditional pig production with irregular vaccination strategies is common in Serbia, indicating the possible role of domestic pigs as an infection source for the wild boar population (Nišavić *et al.*, 2021a). PCV2 isolates are genetically heterogeneous and are currently divided into eight genotypes, i.e. (PCV2a-PCV2h) (Franzo and Segales, 2018). In a recent study by Nišavić *et al.* (2021a), the presence of PCV2 was confirmed in 40.32% of organ samples from 124 wild boars hunted in areas with widespread traditional pig farms. The sampled animals were clinically healthy, showing the importance of cofactors that support disease development. The most prevalent genotype in wild boars in Serbia was PCV2d which corresponds to the current global PCV2 genotype shift (Song *et al.*, 2020; Nišavić *et al.*, 2021a). Nevertheless, the emergence of new PCV2 strains and genotypes is probably driven by vaccine immunity and shows the necessity for more effective preventive measures that also include limiting possible contact of wild boars and domestic pigs, creating the flux of PCV2 strains in both directions (Franzo and Segales, 2018; Franco *et al.*, 2020). Accumulating results from different studies demonstrate that PCV3 is also highly prevalent in wild boar populations around worldwide, however, the presence of this virus was not determined in wild boars in Serbia (Prinz *et al.*, 2019; Amoroso *et al.*, 2021; Nišavić *et al.*, 2021a).

Porcine parvovirus infections

Porcine parvoviruses (PPVs) are small single-stranded DNA viruses classified within the *Parvoviridae* family (ICTV, 2021). New PPVs are being successively discovered in recent years, and currently, PPV1-7 have been detected in samples from both domestic pigs and wild boars around the world (Xiao *et al.*, 2013; Nišavić *et al.*, 2021b; Park *et al.*, 2021). One of the main characteristics of parvoviruses is replication in cells with a high mitotic index, and therefore fetal tissues provide an ideal environment (Nišavić and Milić, 2017c; Truyen and Streck, 2019). It is known that PPV1 causes infection in pigs and wild boars manifested by stillbirths, mummification, embryonic death, and infertility (SMEDI), while the exact role of other parvoviruses in causing disease remains to be determined (Streck *et al.*, 2015; Truyen and Streck, 2019). The outcome of infection in pregnant sows depends on the gestation stage, namely, early infections lead to reproductive failure and fetal mummification, while fetuses infected in the second half of gestation may survive the infection. Moreover, the virulence of the infecting strain plays a major role, and more virulent PPV1 strains cross the placental barrier more efficiently (Truyen and Streck, 2019). PPV is highly resistant in the environment facilitating its indirect transmission, and it is shed through feces and other secretions (Helke *et al.*, 2015). The transmission of PPV between wild boars and domestic pigs is very probable due to the prolonged survival of this virus in the environment (Nišavić and Milić, 2017c; Malmsten *et al.*, 2018). The virus primarily replicates in the tonsils, after which it reaches regional lymph nodes, causes viremia, and reaches the placenta causing clinical manifestations of the disease (Helke *et al.*, 2015). PPV1 is highly prevalent in wild boars across Europe and recent studies also concern the presence of other newly discovered parvoviruses (Malmsten *et al.*, 2018; Nišavić *et al.*, 2021b; Park *et al.*, 2021). PPV3 is related to human parvovirus 4, and a recent study from Italy speculates that it has an immunosuppressive effect on wild boars, however, this virus is mostly detected in healthy animals (Amoroso *et al.*, 2019; ICTV, 2021; Nišavić *et al.*, 2021b). PPV3 was the most common parvovirus in wild boars in Serbia with a detection rate of 69.6% amongst all positive samples, and different PPV3 strains were found to be circulating amongst Serbian domestic pigs and wild boars (Nišavić *et al.*, 2021b). All novel PPVs except for PPV2 have been described in the organs of Korean wild boars, with frequent findings of co-infection (Park *et al.*, 2021). The study conducted by Nišavić *et al.* (2021b) in Serbia also aimed to investigate the presence of different PPVs in wild boars for the first time. Similar to the results of other studies, co-infections were a frequent finding, and PPV1, PPV2, and PPV3 were determined in the samples of lymph nodes, spleen, and tonsils of wild boars. Laboratory submissions for the confirmation of PPV1 usually include mummified fetuses and fetal tissues, however, when analyzing the presence of PPV1 or other related PPVs in wild boar populations, samples of lymphatic

tissue and parenchymatous organs are usually taken from animals shot on hunting grounds (Helke et al., 2015; Nišavić et al., 2021b). Isolation of PPV1 can be attempted, however, it is seldom successful if samples are not processed on time, i.e. the recovery of viable viruses depends on the condition of the sample. The virus is further confirmed by immunofluorescence, hemagglutination, or PCR (Truyen and Streck, 2019). Routinely, all PPVs are detected using molecular methods that are generally more sensitive, specific, and suitable for the analysis of autolyzed tissue samples (Nišavić et al., 2016; Nišavić et al., 2021b; Park et al., 2021). Serological methods such as ELISA or hemagglutination-inhibition test are performed to determine the previous exposition of certain wild boar populations to PPV (Malmsten et al., 2018; Truyen and Streck, 2019). High seroprevalence is detected in wild boar populations from regions with more developed extensive domestic pig production (Roić et al., 2005).

Control of viral diseases in wild boars

Wild boars represent an exceptional species for investigating the epidemiology of wildlife diseases since the species is indigenous around the world, adapts easily to new habitats, has a high reproductive rate, and shares common infectious agents with domestic pigs (Ruiz-Fons et al., 2008; Massei et al., 2015). These animals are reservoirs for some viral pathogens of domestic pigs thus, disease control programs must address the issue of contact prevention between wild and domestic swine (Moennig, 2015). However, it must be noted that the spillover events can occur both ways, and that wild boar can be infected by domestic pigs thus, becoming pathogen reservoirs in nature (Nišavić et al., 2021a; Petrović et al., 2021). Pathogen eradication is arduous in wild animals, and control measures should not be limited to wildlife only and should begin with the domestic pig population (Meier and Ryser-Degiorgis, 2018). Disease prevention in domestic swine includes surveillance programs, implementation of adequate biosecurity measures, and vaccination if applicable (Ruiz-Fons et al., 2008; Meier and Ryser-Degiorgis, 2018). Some control techniques such as the use of fencing or feeding of wild boars cause the aggregation of these animals in certain areas and give rise to population density which favors efficient disease transmission and pathogen maintenance in the environment (Ruiz-Fons et al., 2008; Moennig, 2015; Beltran-Alcrudo et al., 2017). High population densities are found to be connected to high prevalences of ADV, CSF, PCV2, and are important for maintaining and spreading ASF, thus ideal measures would include population control without animal aggregation (Meier and Ryser-Degiorgis, 2018). Hunting is another method that reduces the number of susceptible animals in the wild, however, available data show that regular hunting did not lead to the decrease of the wild boar population over time (Massei et al., 2015). Therefore, this is useful as an additional control measure, also valuable in terms of sample collection for laboratory diagnosis

(Moennig, 2015; Milićević *et al.*, 2016; Nišavić *et al.*, 2021a; Nišavić *et al.*, 2021b). Serological surveys are appropriate for epizootiological studies and are inexpensive, practical, and demonstrate the previous contact with certain viruses, while virological assays require more samples to correctly determine prevalence rates (Boadella *et al.*, 2012; Charrier *et al.*, 2018; OIE, 2019b; Tryen and Streck, 2019; Nišavić *et al.*, 2021b). Attention must be paid to the interpretation of results of diagnostic tests since the detection of a virus does not automatically determine its excretion (Beltran-Alcrudo *et al.*, 2017; Radalj *et al.*, 2021). Therefore, virus and host characteristics, as well as the epizootiological situation on-site must be taken into account when choosing the appropriate diagnostic approach.

Conclusion

Wild boars are similarly affected by viral diseases as domestic pigs and have great reservoir potential for certain pathogens. Some viral diseases characterized by high mortality rates can significantly influence wild boar populations in the wild, and are more easily noticeable and thus reported. On the other hand, many viruses infecting wildlife are more insidious and impact survival rates and reproduction in wild animals. In some cases, wild boars might be less susceptible to disease than domestic pigs, particularly due to the absence of factors that aid disease development present in farmed animals. There is still no sufficient data available on all aspects of infectious disease dynamics in these animals in the wild, and many available studies give opposing conclusions on the same subjects of investigation. This seeks the constant improvement of knowledge on pathogenesis, clinical aspects of the disease, risk factors for disease development, epizootiology, and adequate control measures of viral diseases of these animals.

Pregled značajnih virusnih oboljenja divljih svinja

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Rezime

Divlje svinje su jedna od najrasprostranjenijih vrsta sisara na planeti, a ujedno predstavljaju i rezervoare mnogih značajnih virusa. Pojava oboljenja u populacijama domaćih svinja se javlja kao posledica kontakta sa divljim svinjama pri čemu tradicionalan način uzgoja životinja predstavlja faktor rizika. Primeri takvih oboljenja su: klasična kuga svinja, afrička kuga svinja, Aujeckijeva bolest i

oboljenja izazvana svinjskim cirkovirusima i parvovirusima. Određene virusne infekcije sa visokom stopom mortaliteta se mogu lako detektovati, a samim tim i prijaviti, međutim neki virusi divljih svinja ne dovode do vidljivih promena što otežava njihovo otkrivanje. Uzorci za laboratorijska ispitivanja poreklom od divljih svinja se najčešće prikupljaju postmortalno i uključuju različita tkiva ili krvni serum. Uspešnost izolacije virusa u kulturi ćelija zavisi od vrste virusa kao i od stanja dostavljenog uzorka. S obzirom da primena navedene metode oduzima vreme, većina procedura se zasniva na PCR ili metodama detekcije antigena. Pored toga, serološke metode su ekonomski isplative i pogodne za izvođenje studija prevalencije. Prilikom interpretacije rezultata laboratorijskih analiza je izuzetno značajno uzeti u obzir više parametara uključujući osobine virusa i domaćina kao i epizootiološku situaciju na terenu. Metode kontrole zaraznih bolesti divljih svinja poput ograđivanja ili dohranjivanja životinja dovode do povećanja gustine populacije što pogoduje transmisiji patogena. Lov dovodi do smanjenja broja osetljivih životinja u određenoj sredini, međutim koristan je kao dodatna mera kontrole i omogućuje prikupljanje uzoraka. Dostupni podaci o dinamici infektivnih oboljenja divljih svinja su ograničeni i neophodno je konstantno izučavanje njihove patogeneze, kliničkih osobenosti, faktora rizika kao i procena primene određenih mera kontrole.

Ključne reči: CSFV, ASFV, PCV, PPV, SuHV1, divlje svinje

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Author Contributions

JN and AR conceptualized the paper, which was developed further in discussion with NM, AS, AŽ, and DB, JN, AR, and IP collated articles for review, wrote and critically reviewed various drafts. NM, AŽ, AS, DB, and IP contributed to the preparation of the final version and provided consent for submission.

Conflicts of Interest

The authors declare no conflicts of interest.

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