ACTA MICROBIOLOGICA BULGARICA Volume 35 / 4 (2019)

Spread of African Swine Fever Virus Epizooty

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Abstract

African swine fever (ASF) is a highly contagious and devastating disease in feral and domestic swine. The infection spreads rapidly through swine farms with clinical signs of high fever 42°C 4-5 days after initiation of the infection followed by dullness, coughing, ocular and nasal discharge, breathing difficulty, cyanosis of the extremities, vomiting, and abortions in pregnant sows. The causative agent of ASF is a large, double-stranded DNA virus of the *Asfarviridae* family named African swine fever virus (ASFV). ASFV causes haemorrhagic fever in domestic pigs and wild boar, resulting in mortality rates of up to 100%.

The disease spreads over a short period of time in many regions of the country. In Bulgaria, the first outbreak of the disease in domestic pigs (backyard, village of Tutrakantsi, Provadiya, Varna district) appeared in August 2018. Later in the same year, 20 new outbreaks in wild boars were recorded in Silistra and Dobrich regions. In 2019, fourteen new cases of ASF in wild boars (WBs) were found, with the last event recorded at the beginning of June 2019 in Alfatar, region Silistra. Later in that year, in July, 16 new outbreaks were found in the Northern part of the country – Pleven, Burgas, V. Tarnovo, Vratca, Russe, Vidin. The situation in Russe region was the worst. The disease was detected in three industrial pig farms – Nikolovo, Brashlen and G. Vranovo. In order to stop the spread of the disease in industrial pig farms, large numbers of pigs were destroyed.

The clinical symptoms observed in diseased pigs were typical of ASF infection. Samples investigated by real time PCR were strongly positive (Ct < 30), which was evidence of high quantity of ASFV DNA.

Currently, there is no vaccine against ASF and biosecurity measures are the key to prevent the spread of ASF within and among domestic pig farms.

Keywords: African swine fever (ASF), African swine fever virus (ASFV), pathology, real time PCR

Резюме

Африканската чума по свинете (АЧС) е силно заразна и фатална болест при диви и домашни свине. Инфекцията се разпространява бързо с клинични признаци - висока температура 42°C 4-5 дни след започване на инфекцията, последвана от залежаване, кашлица, очни и носни секрети, затруднено дишане, цианоза на крайниците, повръщане и аборти при бременни свине. Причинителят на ASF е голям, двуверижен ДНК вирус в семейство *Asfarviridae*, наречен вирус на африканската чума по свинете (ASFV). ASFV причинява хеморагична треска при домашните диви свине, която води до 100% смъртност.

Заболяването се разпространи за кратък период от време в много региони на страната. В България първото огнище на болестта при домашните свине (заден двор, с. Тутраканци, Провадия, област Варна) се появи през август 2018 г. По-късно през същата година бяха регистрирани 20 нови огнища на заболели диви свине в Силистренска и Добричка области. През 2019 г. бяха открити четиринадесет нови случая на АЧС при диви свине, като последното избухване беше в началото на юни 2019 г. в Алфатар, област Силистра. По-късно през юли бяха открити 16 нови огнища в северната част на страната - Плевен, Бургас, В.Търново, Враца, Русе, Видин. Положението в регион Русе беше най-лошо. Заболяването беше установено в три промишлени свинеферми - Николово, Бръшлен и Г. Враново. За да се спре разпространението на болестта в промишлените свинеферми, бяха унищожени голям брой свине.

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Клиничните симптоми, наблюдавани при болни прасета, бяха типични за инфекция с ASF. Изследваните проби чрез PCR в реално време бяха силно положителни (Ct <30), което е доказателство за високото количество ASFV ДНК.

Понастоящем няма ваксина срещу АЧС и мерките за биологична сигурност са основният ключ за предотвратяване на разпространението на АЧС в и между домашните свинеферми.

Introduction

African swine fever (ASF) is a highly contagious viral disease in swine with acute to chronic course, characterized by high fever, hyperemic skin and hemorrhages in the internal organs.

ASF was first discovered in Kenya in 1921 (Montgomery, 1921). Today, the disease is endemic in most sub-Saharan African countries, including the island of Madagascar. The first occurrence of the disease outside Africa was in Portugal in 1957, later in 1960 it spread across Portugal and Spain, in 1970-1980 in the Netherlands, Italy, France and Belgium. In 1990, the disease was eradicated from the European countries and remained endemic only on the island of Sardinia (Mur *et al*., 2016). In 1971, the disease spread in Cuba, the Dominican Republic, Haiti, Brazil, causing great economic losses, but later was also eradicated.

In 2007, African swine fever virus (ASFV) was introduced into Georgia by ship waste which was disposed around the port of Poti and subsequently the virus quickly spread throughout the whole country with fifty-eight outbreaks notified to the OIE (Rowlands *et al*., 2008). After Georgia and Armenia, the disease swept across Russia (2007), Azerbaijan (2008), Ukraine (2012) (OIE, 2012), Belarus (2013) and Moldova (2016) (OIE 2019). Within the EU, nine countries were affected between 2014 - 2018 (Lithuania, Poland, Latvia, Estonia, Czech Republic, Romania, Hungary, Bulgaria and Belgium) (OIE report). In 2019, the disease was also reported in Slovakia and Serbia (OIE, 2019). From January 2014 to May 2019, a total of 16101 wild boar (WBs) and 1673 domestic pig outbreaks (ADNS DATA, 2019) were detected. The disease affected 68% of domestic pigs and 32% of wild boar. In Asian countries the disease has spread from August 2018 to date, and in China after outbreaks more than 1020000 pigs were culled. In Mongolia the first ASF outbreak was at the beginning of 2019, and 3115 pigs accounting for more than 10% of the pig population, died/ were destroyed due to ASF outbreaks. In Vietnam the first ASF outbreaks were in February 2019, and more than 89600 pigs were culled. In Cambodia the first ASF outbreak was in April 2019, and more than 2400 pigs died or were culled (FAO's Emergency Prevention System for Animal Health, OIE World Animal Health Information Database WAHIS reports, 2019).

African swine fever virus

The causative agent of ASF is a unique, enveloped, cytoplasmic, double-stranded DNA arbovirus, which is the sole member of the family *Asfarviridae*, genus *Asfivirus* (Dixon *et al*., 2005). Genetic characterization of all the ASF viruses isolated so far has demonstrated 23 geographically related genotypes with numerous subgroups, illustrating the complexity of ASF epidemiology. The genotypes are used mainly for phylogenetic and molecular epidemiological purposes (e.g. to identify the source of outbreaks). The capsid protein p 72 (VP 72) is responsible for the creation of neutralization antibodies and is antigenically stable and has epitopes which are conservative in most strains (Dixon *et al*., 2013). Small variation of gene coding p 72 can be used for genotyping of ASFV (Bastos *et al*., 2003, Nix *et al*., 2006).

The virus infects monocytes and macrophages. ASF viruses can vary in virulence, the highly virulent cause up to 100% mortality and those of low virulence from 0 to 60% mortality. The low-virulence viruses cause seroconversion. Recent studies based on a hemadsorption inhibition assay have reported the classification of 32 ASFV isolates in eight different serogroups (Malogolovkin *et al*., 2015).

ASFV has spherical virions or integral viral particles with a diameter of 175 to 215 nm and include a genomic center consisting of a nucleoprotein structure with a size of 70 to 100 nm in diameter. This central structure is protected by an icosahedral protein capsid (T=189–217) ranging between 172 and 191 nm in diameter (Salas and Andres, 2013). Each capsid includes 1892 to 2172 capsomeres, the matrix protein, and an outer lipid envelope. The virus is with 41% G+C content and molecular weight of 102 MD.

The ASF virus has icosahedral morphology and contains four concentric layers: the central nucleoid, the core shell, the inner envelope and the icosahedral capsid. The extracellular viruses acquire its external envelope by budding through the plasma membrane. ASFV morphogenesis occurs in specialized areas in the cytoplasm named viral factories, which are proximal to the microtubule

organization center near the nucleus Salas and Andres (2013).

The ASF virus has a large genome, with half proteins of unknown functions. Unique transmission cycles exist among domestic pigs, wild boar and soft ticks and high in vitro survivability (Alejo *et al*., 2018). Intracellular viral morphogenesis occurs in the perinuclear regions, in which a cluster of particles named "virus factory" forms from the endothelial reticulum (Rouiller *et al*., 1998). The main capsid protein p72 accounts for 35% of the viral mass. Structural proteins, including p150, p37, p34 and p14, derived from polyprotein p220 represent 25% of the virus mass (Andres *et al*., 1997). There exist two infectious forms of ASFV virions: intracellular virions (IV) and extracellular virions (EV) (Galindo and Alonso, 2017).

Epizootology of ASF disease

The ASF virus persists in distinct cycles: the sylvatic cycle, domestic pig-pig cycle, and wild boar-domestic pig cycle, the tick-pig cycle (Beltran-Alcrudo *et al*., 2017).

Sylvatic cycle: It involves warthogs, the natural host of ASF, and soft ticks *Ornithodoros moubata*, which act as biologic vectors in Africa. The virus is maintained by tick to warthogs transmission.

Domestic cycle: In this cycle most commonly infected are domestic pigs and the virus is maintained in pigs in the absence of ticks and wild boars. The virus is spread by direct contact through the oro-nasal route, after contact with the excretion of the infected pig or ingestion of products contaminated by the virus, or indirectly through fomities.

Wild boar-domestic pig cycle: In Eastern Europe, the Caucasus and Sardinia the population of wild boar play an important role for the circulation and maintenance of the virus, especially in regions with free-ranging or scavenging pigs, infected feed, fences allowing close contacts as well as in transportation of wild boar by hunters.

Tick-pig cycle: In Southern and Eastern Africa, the sylvatic cycle of disease is predominant, in which warthogs and ticks maintain the virus. In West African countries, virus transmission occurs through direct contact, pig movement, contaminated fomites or infected meat. Soft ticks do not participate in the maintenance of the disease. On the Iberian Peninsula (1957-1995) domestic pigs and wild boars were affected and one of the main routes of transmission was by direct contact between animals and ingestion of infected meat. The soft tick *O. erraticus* also contributed to disease transmission in outdoor pig production systems, and served as a long-term reservoir of ASFV in affected areas. In Sardinia, the Caucasus and Eastern Europe, ASF occurred in domestic pigs and wild boar, and ticks were excepted as a factor for the disease transmission. In the Baltic States and Poland, only a small part of outbreaks involved domestic pigs while in the great majority the main factor were wild boars. Therefore, currently, the possibility of wild boar populations maintaining ASFV without its reintroduction by domestic pigs is being considered.

When the affected territory is naive, acute ASF is observed. Because of the infection circulation around the area, between 2-10 % of the infected animals could survive, keeping the virus in different organs and lymph nodes. These animals are a source of infection for the environment and the healthy animals (wild boar and domestic pigs). Virus excretion can begin two days prior to clinical signs and virus shedding depends on the virulence of the ASF strain. Pigs infected with less virulent strains are persistently infectious for more than 70 days. The virus is shed by the nasal secretion, saliva, tears, feces, urine, and genital secretion. Blood contains a large amount of the virus. Infected pigs and materials can be transported over long distances by vehicles and people.

The introduction of new pigs often causes them to bite each other and if there exist viral carriers among the pigs the disease can spread. In free-ranging or scavenging pigs the infection can spread after contacts with roaming pigs, wild boars, their carcasses or food leftovers. The virus can be transmited by using the same needle for the treatment or vaccination of the swine. Transmission via artificial insemination has not been proven, but may be possible. Blood-sucking insects such as *Stomoxys calcitrans* can transmit the disease within herds (Melor *et al*., 1987). Contamination of large bodies of water such as lakes or rivers is unlikely, as the virus rapidly becomes diluted and will not have enough infectious titer.

Clinical symptoms of ASF

The incubation period is between 4 and 19 days and depends on the virulence of the virus, the host and the route of infection. Two to three days after infection, the clinical signs are visible, even with a quite low dose of the virus. The typical ASF clinical symptoms include high fever (>41°C), dullness and loss of appetite, red-purple skin discoloration especially of extremities (e.g. ears) and ventral body and sometimes haemorrhages,

however, pigs may die without external signs of haemorrhages. The following symptoms are more variable and not present in all diseased pigs or in all groups of diseased pigs: gastrointestinal-vomiting and diarrhoea/dysentery (bloody diarrhoea) and nervous signs – ataxia. Other signs as oculonasal discharges, epistaxis, dyspnoea, coughing and reproductive effects (in breeding herds) with abortion are observed.

The course of disease is peracute, acute, subacute, chronic and asymptomatic, and depends on the virulence of the virus.

The highly virulent strains cause peracute and acute form of the disease with high fatality rate between 90-100%. The acute disease is characterised by high fever, anorexia, weakness, recumbence, lethargy, erythema, cyanosis, hemorrhages on the skin and snout, diarrhea, abortion, respiratory symptoms, nasal discharges, dyspnea and death after 7-10 days.

The middle virulent strains cause subacute form of disease with approximately 60% mortality rate. In that form the symptoms observed are abortions, fever, erythema, cyanosis, hemorrhages, thrombocytopenia, leukopenia, and death or recovery within 3-4 weeks.

The low-virulence strains are the reason for chronicor asymptomatic course of the disease with mortality rate 2-20%. In chronic disease intermittent low fever, anorexia, depression, emaciation, stunting, respiratory signs – coughing, diarrhea, joint swelling, occasional vomiting, skin lesions may be observed.

If the infection is detected in one or two pigs in the group, they die before the ASF virus has spread to infect larger numbers of pigs, which can take one to two weeks, after which increased mortality is seen. This slower spread is associated with minimal transmission of the infection by aerosol. There are very high amounts of virus in the blood and tissues of affected pigs but relatively low amounts of virus in excretions and secretions from infected pigs. Thus, contact or consumption of carcasses of pigs or wild boars or their products is a possible method of transmission.

Pathology of ASF

In white-skinned pigs affected by ASF, the ears, tail, legs and underside of the body appear deeply flushed and may develop a bluish tinge (cyanosis). Widespread haemorrhages may be visible on the skin. Blood-tinged fluid often accumulates in the body cavities. Haemorrhages may be visible in multiple organs and on the serosal linings of the body cavities. The spleen is often markedly enlarged and dark red in color and friable. The lymph nodes, especially the gastrohepatic, are enlarged and haemorrhagic. At later stages of disease the haemorrhagic area increases and the lymph nodes become almost black. Tracheo-bronchial lymph nodes are often nearly black and look like coagulum. Pinpoint to larger haemorrhages are visible on the kidney capsule. The liver is hemorrhagic with enlarged gallbladderand hemorrhagic lymph nodes. The wall of the intestine is congested, hemorrhagic with bloody fluid contents and multiple point hemorrhages. In the fundus of the stomach hemorrhagic gastritis with multiple haemorrhages is visible. Hemorrhagic cistitis with multiple hemorrhages and suffusion is visible in the urinary bladder. In the heart, abundant straw-colored pericardial fluid (hydropericardium), and multifocal hemorrhages in the epicard are visible. The lung is noncollapsed and edematous and shows dorsal hemorrhage and ventral tan consolidation.

Diagnosis and differential diagnosis of ASF

Tissue samples for investigation need to be submitted on ice and should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease. The most appropriate organs for investigation are the spleen, lymph nodes, tonsils, lungs, kidneys, and blood.

Diagnostic tests for antigen detection are the isolation of the virus in primary porcine monocytes and macrophage cells and confirmation of ASFV by a haemadsorption test (HAd). Conventional and real time polymerase chain reaction (PCR) is used for genome detection. PCR has an advantage over other diagnostic test as HAd, ELISA/fluorescence antibody test (FAT), direct imunoperoxidase monolayer assay (DIPMA).

For antibodies detection, the most appropriate diagnostic tests are ELISA, immunoblotting, indirect immunoperoxidase monolayer assay (IIPMA), indirect fluorescence antibody test (IFAT).

The differential diagnosis for ASF has to be made with different diseases as hog cholera (more commonly known as classical swine fever), which is clinically indistinguishable from ASF. Other diseases with similar signs are acute porcine reproductive and respiratory syndrome (PRRS), erysipelas, salmonellosis, eperythrozoonosis, actinobacillosis, Glasser's disease (Haemophilus parasuis infection), Aujeszky's disease (pseudorabies), thrombocytopenic purpura, warfarin poisoning, heavy metal toxicity, and other generalized septicemic or hemorrhagic conditions.

Prevention and control

For ASF there is no vaccine or drugs available to prevent or treat itunlike other transboundary animal diseases (TADs). Preventing the entry of ASFV into both domestic and wild swine populations, and controlling and eradicating the disease as soon as it is detected, are the best ways of minimizing its impact. Prevention starts with stringent measures at the borders and raising awareness among all stakeholders involved. Early detection, diagnosis, response, and good communication are critical for minimizing the spread of the disease. The most effective measures against the transmission of ASF are the ban on the movement of contaminated pig and animal products, direct contact between live animals, including wild swine, and the prevention of *Ornithodorus* tick bites

Measures taken to limit and eradicate the disease in domestic pigs at primary outbreak in settlements located in the 3 km-zone around the epidemiological focus are: enforced humane killing and disposal of all infected and contact domestic pigs in the affected establishment, as well as pigs reared in livestock establishments for personal needs and establishments not registered under Art. 137 of the Veterinary Law; mechanical cleaning and repeated disinfection of pig premises, equipment, vehicles, yards and streets in and around the breeding establishments. Owners of identified killed animals from registered sites are compensated under the Veterinary Law.

In settlements within the 10 km-area around the epidemiological focus the entry and exit of people, motor vehicles and vehicles powered by animal traction, including agricultural machinery, is prohibited. The measures are: counting and updating the number of animals; collection of double blood samples from domestic pigs from each locality; weekly clinical examinations for ASF; sampling of each dead pig for virological examination. In the territory of primary outbreak the trade, movement and transport of domestic pigs are prohibited, with the exception of those from industrial pig holdings and Type A farms located outside the settlements within the 10 kmzone around the epidemiological focus, for immediate slaughter. Checks on the unauthorized movement and/or trade of domestic pigs are mandatory.

Measures taken in the event of a primary wild boar disease are: determination of the geographical coordinates of the primarily infected wild boars and designation of the infected area of 200 km^2 around the case found, prohibition of wild boar hunting. Access of people to the infected area is restricted and dogs are not allowed for hunting but only for detection of carcasses of dead feral pigs; disinfection of work clothes and vehicles.

The measures taken for wild boar carcasses include: detection of dead wild boar carcasses and notification to the official veterinarian, sampling for ASF and sending for laboratory testing, on-site burial or transportation in waterproof bags to a grave/pit for disposal of specific animal products (SAP) and disinfection of the grave site and the tools used.

Measures outside the infected areas in feral pigs are: after shooting a wild boar, sampling for ASF and burial of the ASFV-specific animal organs. When traps are used for catching wild boars, the animals should be destroyed, sampling for ASF and on-site processing of the specific for ASFV animal organs. In the Northwest, North Central and North Eastern and private enterprises in the 20 km-areas around the established ASF outbreaks wild boar density below 0.3 pigs for 100 ha must be be maintained, for other hunting areas up to 0.5 units/100 ha.

ASF in Bulgaria

Epizootological investigation

In August 2018, in the village of Tutrakanci, Provadia, Varna region, an increase in the number of diseased and dead pigs was observed in domestic pigs from backyard farms. Samples for laboratory investigation were taken from the diseased pigs. Later in 2018, twenty wild boars (WBs) (3 in Silistra and 17 in Dobrich regions were investigated.

In 2019, fourteen WBs (Silistra region - 3 in Dulovo and 5 in Alfatar municipality; Dobrich region - 5 in Tervel municipality; Varna region - 1 in Devnya municipality) were found dead. The last dead WBs were found in June 2019 inAlfatar municipality, Silistra region, and samples were obtained for virological and molecular biological investigation. On 2.7.2019, in the village of Zhernov, Pleven region, in backyard farms 14 pigs were detected, 2 of which were dead and 2 diseased. Samples for laboratory investigation were taken from dead and alive animals. Later in same month, a second outbreak in domestic swine was found in the village of Novachene, Pleven region, in a backyard farm with 2 pigs (1 dead, 1 sick). The situation in Russe region was the worst. The disease was detected in three industrial pig farms - Nikolovo, Brashlen and G.Vranovo.

Clinical and pathological studies in diseased swine

Clinical and pathological investigation was performned in the zones with increasing numbers of diseased and dead pigs. The diseased pigs were observed for changes in the general condition of the animals, body temperature and superficial changes of the skin and the duration of clinical signs. Pathological studies were performed after the necropsy of the dead pigs.

Molecular biological investigation

Pig samples from the spleen, lymph nodes and whole blood were tested by real time PCR according to the Manual of diagnostic standard for terrestrial animals (OIE, 2019, Section 3.8.1) for detection of ASFV. The results were read after automatic determination of cycle threshold (Ct), which is the point where the fluorescence measurement is above the background signal and reaches the detectable level. For positive samples a sigmoid-shaped amplification curve will be obtained where the Ct value is less than 40. Strongly positive samples have a Ct value less than 30. Samples with Ct values more than or equal to 38 should be suspected for ASFV and need to be repeated for confirmatory purposes. Samples maintaining the fluorescence profile under the background fluorescence level and when the equipment will not report any Ct value, or Ct value more than 40 can be considered as negative.

Measures for prevention of ASF spread

Due to clinical features observed in sick pigs similar to ASF infection, measures have been taken in accordance with the contingency plan to prevent the spread of the disease (Council directive 2002/60/EC).

Results

Epizootological investigation

The disease was first established in August 2018 in the village of Tutrakanci, Provadia, Varna region, in domestic pigs from backyard farm.

In 2018, twenty WBs (3 in Silistra and 17 in Dobrich regions, and in 2019 fourteen WBs (Silistra region - 3 in Dulovo and 5 in Alfatar municipality; Dobrich region - 5 in Tervel municipality; Varna region - 1 in Devnya municipality) were found with changes typical of ASF infection. The last confirmed outbreak was in June 2019 - Alfatar municipality, Silistra region (Fig. 1).

In domestic pigs the first outbreak was diagnosed in the village of Zhernov, Pleven region, on 2.7.2019 in a backyard farm with 14 pigs, 2 of which were dead and 2 diseased.

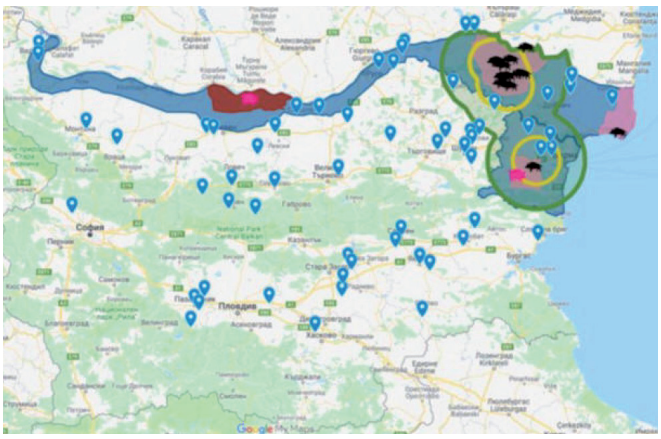

Fig. 1. The spread of ASF in Bulgaria during summer of 2019. Black colored pigs are the proven WBs with ASF infection. Pink colored areas are outbreaks with domestic pigs and with blue balloons are shown the industrial farms. Regionalization is in accordance to the Annex of CID 2014/709 (Part I – with blue, II – with pink and III – with red color). In 2-20 km2 zone (noted with yellow curve) is performed the active searching for WBs with ASF infection and in 20-40 km2 zone (noted with green curve) around the proven diseased WBs

Following epizootological studies it was determined that the probable infection was mechanically transmitted by contaminated vehicles/people because of the Gernov village location (transit road with Romania with passing trucks or vehicles) or due to poor biosecurity at the farm entrance (equipment of the owner or visitors, including private vet and hunters). The other possibility is by direct contact with WBs, because the farm has well-fenced premises but no external fence at the back of the yard, which borders a field. The owner did not use swill-feeding, and no new animals have been introduced in the past months in the farm. The second outbreak in domestic swine was found in the village of Novachene, Pleven region, in a backyard farm with 2 pigs (1 dead and 1 sick). Later in the same month, the disease was confirmed in three industrial farms - Nikolovo, Brashlen and G.Vranovo, where all pigs were destroyed.

Measures for prevention of ASF spread

To fight ASF, two zones were created around the outbreak of infection - 3 km for protection and 10 km for surveillance. The measures in the 3 km-zone were culling of all domestic pigs and cleansing and disinfection of premises. In this protective zone a total of 51 pigs were culled.

In the 10 km-zone clinical examinations were performed and sampling of swine and creation of

disinfection posts and vehicle checks. Later, other measures were enforced in Pleven region, such as clinical examinations of pigs in the entire Pleven region, checks for illegal movement of animals, ban of animal markets and exhibitions of pigs and the implementation of the measures set by the epizootic commission in all high-risk regions. The movement regime of swine in Pleven region was set out with ordinance by the Bulgarian food safety agency (BFSA) and was in line with community decision (EU) 2014/709 (i.e. pre-movement testing of each batch of pigs) and ban for pigs movement at this stage in the 3- and 10-km zones (in the 10-km zone there is one type A family farm).

Clinical and pathological studies in diseased swine

The disease was running acutely with an elevated temperature of 41.5 - 42°C. Abortion with expulsion of the fetuses was observed in one sow prior to the onset of the characteristic clinical signs. Hemorrhagic diathesis and different degrees of bleeding on the internal organs were visible. After a high fever, the diseased sows refused to feed and lay down. Serous hemorrhagic conjunctivitis with closing of the eyelids and redness of the skin around the eyes were established. Animals were breathing fast, reluctant to move and consumed large amounts of water. After refusing feed, they died after 4-6 days.

In diseased swines redness on the skin of the body, ears, throat, limbs and lower body parts was observed. At autopsy, the mucous membranes of the oral and nasal cavities were red and streaked with multiple haemorrhages. When opening the chest and abdominal cavity, a large amount of serous fluid with red color was found. Multiple petechial hemorrhages in the larynx, trachea and bronchi with flowing blood red foamy exudate was established. On the endocardium of the heart petechial hemorrhages were visible. The lungs were enlarged with rounded edges and numerous petechial hemorrhages and ecchymoses (Fig. 2 A).

The spleen was with a dark black-red color, with rounded edges, a stretched capsule, and a soft consistency when compressed and was enlarged 3-4 times (Fig. 2 B). Dark red color on the cut surface was visible after section. The liver was slightly enlarged with rounded edges and hyperemic. The gallbladder was with a thick wall, enlarged and its fluid was gelatinous and mixed with blood. The most significant changes were observed in the gastrohepatic, mesenteric, renal lymph nodes, which were enlarged 2-3 times and look like a blood coagulum after the incision (Fig. 2 C). The digestive tract was affected, with the strongest changes observed in the stomach and colon. In them, symptoms of hemorrhagic gastritis with extensive hemorrhages, suffusions, and ecchymoses were established. The mucosa of the intestine was littered with petechial hemorrhages and hemorrhagically inflamed.

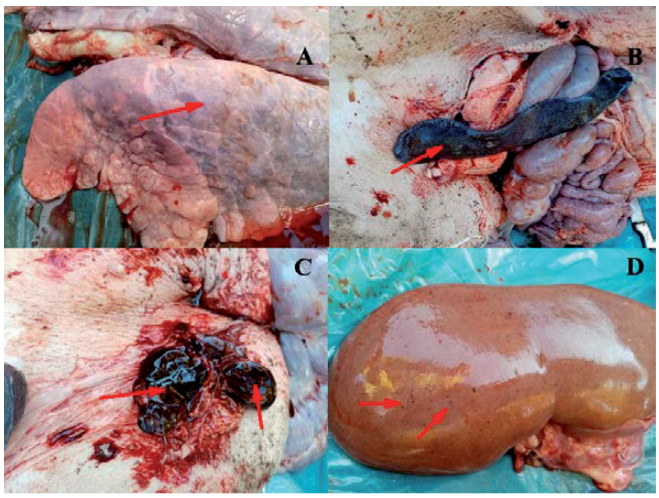

Fig. 2. Pathological changes in dead pigs from the ASF. A - lungs- noncollapsed and edematous with hemorrhages, B - spleen-markedly enlarged and dark red in color, C - lymph nodes-enlarged and haemorrhagic and look like coagulum, D - kidneys-pinpoint to larger haemorrhages

Multiple petechial hemorrhages in the capsule and in the parenchyma of the kidney were visible (Fig. 2 D). In the urinary bladder, hemorrhages of different sizes were found.

Molecular biological investigation

After obtainig the virus DNA from the internal organs (spleen, lymph nodes) and whole blood of diseased and dead pigs, real time PCR for detection of DNA amplification was performed. Five of the investigated samples were strongly positive (Ct < 30), which is evidence of high quantity of ASFV DNA (Fig. 3).

Conclusions

There is no cure for the ASF disease. No vaccine has been developed so far. People do not become infected or ill from ASF.

To prevent the disease, it is essential to increase the biosecurity measures in the breeding establishments, which are reduced to: control of movement of persons, animals and vehicles; use of special work clothing; avoiding consumption of kitchen waste; quarantining newly arrived animals and separation of different groups of animals, tick control, awareness campaign for owners, veterinarians and hunters.

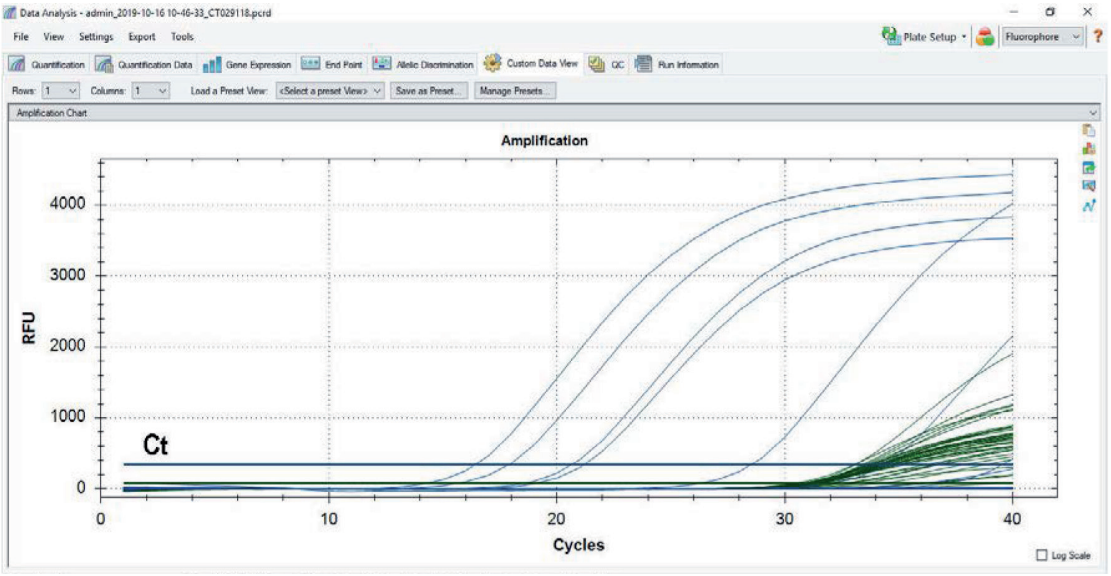

Fig. 3. Results from real time PCR of different samples (spleen, lymph nodes and wholl blood) obtained from diseased and dead pigs. Five of investigated samples are strong positive with cycle threshold Ct < 30.

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