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Short communication

FIRST OCCURENCE OF AFRICAN SWINE FEVER IN SERBIA

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Until July 30th, 2019 when the first case of African swine fever (ASF) was confirmed, Serbia was a country free from ASF. After the owner reported atypical illness and death of a sow, the local veterinarian submitted the organ samples to the National Reference Laboratory for Classical Swine Fever (CSF) and African Swine Fever within the Institute of Veterinary Medicine of Serbia in Belgrade. Observed gross lesions included splenomegaly, serous edema of the wall of the gallbladder and hemorrhages in the enlarged portal lymph nodes, petechial hemorrhages on the kidney and epicardium, and petechial and echymotic hemorrhages on the mucosa of the urinary bladder. Results of real-time PCR confirmed that the cause of illness and death of the swine was African swine fever virus. The samples were sent for confirmation to the EU Reference Laboratory where it was confirmed that Serbian domestic pig virus isolates based on p72 belong to genotype II. In total, 270 pigs from 18 affected holdings were killed in the infected zones. According to the on-record data, mortality was 6.89%, whereas lethality reached 64.5%. Currently, an extensive surveillance program is being conducted, aiming to force passive surveillance. ASF in wild boar has not been confirmed so far.

Key words: African swine fever, backyard, domestic pigs, Serbia

INTRODUCTION

African swine fever (ASF) is a severe, contagious viral disease of domestic pigs and wild boars. Clinical manifestation of the disease depends on the virulence of the virus, the infectious dose, and the infection route. However, in European both domestic and wild pigs, ASF usually has an acute course with mortality up to 100% [1]. African swine fever virus (ASFV) is very complex by itself, belonging to the family *Asfaviridae* and genus *Asfivirus*. So far, 22 genotypes and 8 serogroups have been described [2]. ASFV is very resistant but there are efficient disinfectants that can inactivate the virus, as well as temperature above 70 °C [3]. After the occurrence in 2007 in Georgia, the disease has progressively been spreading toward the west affecting Ukraine (2012), Belorussia

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(2013), EU countries – Lithuania, Poland, Latvia and Estonia (2014), Moldova (2016), Czech Republic (2017), Romania (2017), Bulgaria (2018), Belgium (2018), Hungary (2018) and Slovakia (2019). Except for Hungary, Belgium and the Czech Republic where the disease occurred only in wild boar, in the rest of countries both domestic and wild pigs are affected [4]. Considering the devastating effects on pig production, and no vaccine available, the early diagnosis is considered as the key factor of successful control and eradication of ASF. Therefore, the passive surveillance and exclusion of ASF in the case of any hemorrhagic fever symptoms that are shared between several other infectious diseases is the imperative. By resolving ASF, The Czech Republic demonstrated that early detection of the first cases followed by immediate response to stop the spreading can lead to the elimination of the virus from swine populations [5].

MATERIAL AND METHODS

On the 28th of July 2019, the pig owner contacted a local veterinarian and reported health disorders in one sow such as anorexia and ataxia. The next day, the veterinarian visited the farm, but the sow had already died. According to the owner, the first signs of illness had been observed a few days ago. The farm of backyard type, housing 3 sows and 21 weaned piglets, was located in the village Rabrovac-Šume, in the municipality Mladenovac. Additionally, the owner reported that recently, in the neighborly yard, 2 sows aborted, followed by death of six sows.

Clinical signs and sampling

According to the local veterinarian observation, an atypical illness was noted. Clinical signs such as fever (40.2 to 40.8 ˚C), anorexia, ataxia, vomiting, multiple sharply demarcated foci of cutaneous hemorrhage and necrosis of the skin predominantly on the distal parts of the legs were observed (Fig. 1A). The veterinarian performed partial necropsy of the dead sow, taking the liver, spleen, hearth, kidney and urinary bladder for further laboratory investigation. The organs were submitted to the Department of Pathology within the Institute of Veterinary Medicine of Serbia which is the National Reference Laboratory for Classical Swine Fever and African Swine Fever, on the 30th of July.

Laboratory diagnosis

For laboratory testing, samples of spleen and kidney were used. The pool of tissues was prepared as a 10% homogenate in PBS, whereas the supernatant obtained after centrifugation was used for DNA/RNA extraction by a commercial kit (Jena Bioscience Viral RNA+DNA Preparation, Jena Bioscience, Germany). For the ASFV genome detection, real-time PCR, carried out as a 25 μl reaction using primers described by King et al. (2003) [6], was applied. The presence of CSFV genome was investigated according to the protocol published by Hoffmann et al. (2008) [7].

RESULTS AND DISCUSSION

Until July 30th, 2019, Serbia was a country free from African swine fever. Since the disease was confirmed in the neighboring countries, the Ministry of Agriculture, Forestry and Water Management has been implementing numerous measures to prevent the introduction of the virus in the country. The measures included: ban on both live animals, animal products import from the affected countries, confiscation of any pork products at borders, building- and lifting-up biosecurity on the farms, intense passive surveillance in domestic pigs and wild boars, prevention of domestic and wild boars contact, swill feeding ban, awareness campaigns etc. Even more, the active surveillance of wild boars was established earlier, in November 2017. Considering the bordering area towards Bulgaria and Romania as at the highest risk, the most intensive activities were undertaken right in these regions, particularly to prevent ASF occurrence in wild boars. Within this set of preventive measures, the reduction of wild boar populations to a biological minimum was conducted in the high-risk areas, which apparently, to some extent, contributed to the absence of ASF in the wild boar so far. Nevertheless,

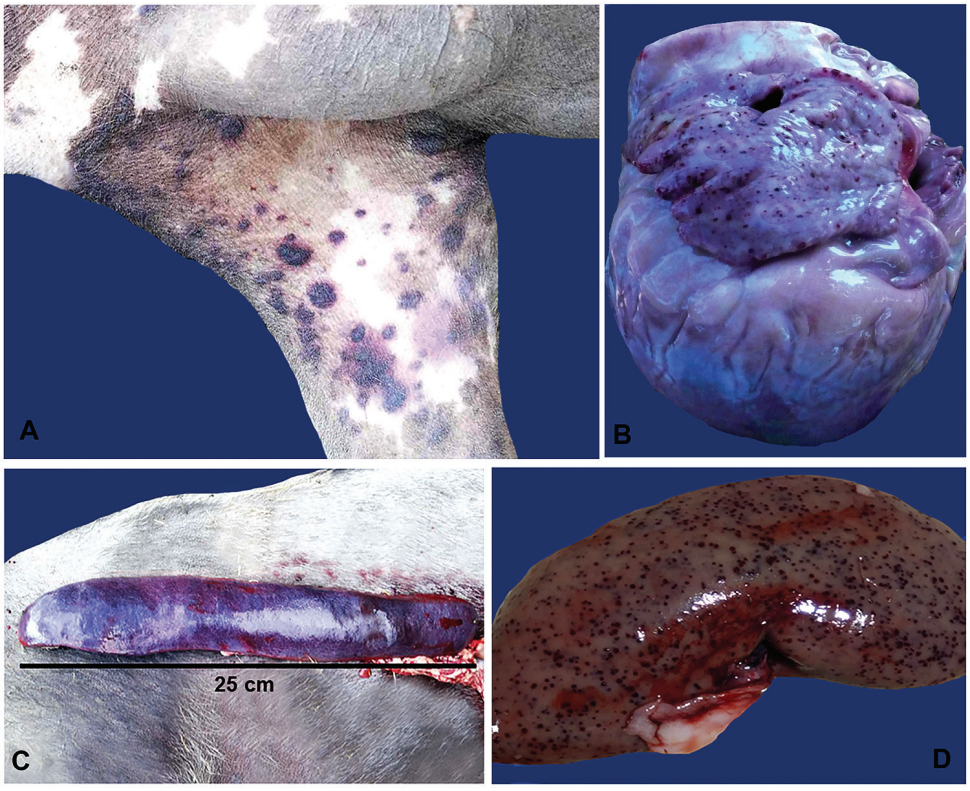

Figure 1. Macroscopic findings in ASFV positive pig. **A** – Multiple sharply demarcated foci of cutaneous hemorrhage and necrosis, **B** – Petechial hemorrhages on the epicardium, **C** – Severe splenomegaly, **D** – Petechial hemorrhages on the kidney cortex

the first ASF suspicion was established in central Serbia in a domestic backyard farm, based on gross lesions, as well as clinical findings. The observed gross lesions included: splenomegaly, serous edema of the wall of the gallbladder and hemorrhages in the enlarged portal lymph nodes, petechial hemorrhages on the kidney and epicardium, and petechial and echymotic hemorrhages on the mucosa of the urinary bladder (Fig. 1B, 1C, 1D). The spleen was severely enlarged and moderately hyperemic while the distinction of red and white pulp was not possible. Serous edema of the wall of the gallbladder was particularly expressed on the wall which connected to the liver parenchyma. Hemorrhages on the kidneys, predominantly located on the renal cortex, were also confirmed on the cut section of the kidney medulla. Results of real-time PCR demonstrated that the cause of death of swine was the African swine fever virus. As this was the first case of ASF in Serbia, the samples were sent for confirmation to the EU Reference Laboratory (CISA-INIA, Madrid, Spain) where it was confirmed

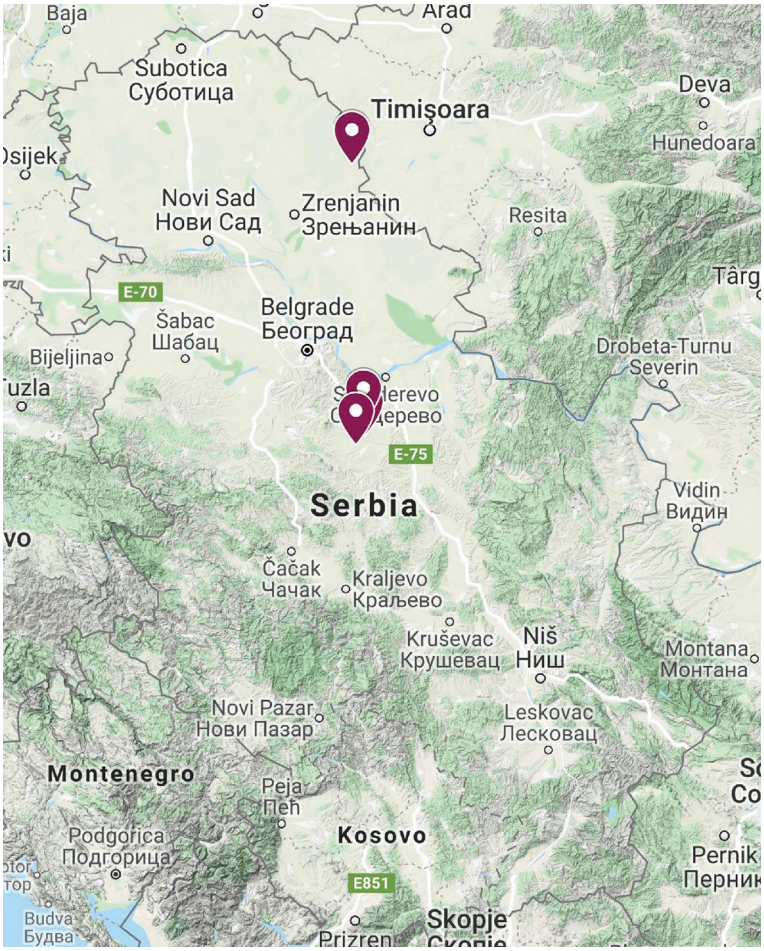

Figure 2. Locations of ASF outbreaks

that Serbian domestic pig viruses based on p72 belong to genotype II. Further on, the viruses clustered within the CVR-1 variant and IGR I73R-I329L variant 2 (IGR-2). The MGF 505 subtyping placed the Serbian domestic pig viruses within the MGF variant 1 (MFG-1). However, antibodies were not detected. Based on genotyping, the origin of the virus could not be determined nor the way of its introduction, due to the very stable ASFV genome. However, as swill feeding is the common practice in backyard farms in Serbia, along with the low level of biosecurity, it has been supposed that the virus was brought in with the meat products from the affected countries. After the confirmation, the Veterinary Directorate established the infected and protection zones, applyed the stamping out procedure in infected as well as in contact yards, as an urgent measure to stop the virus spreading. After the proper disposal of carcasses, thorough cleaning and disinfection were conducted. Further on, other measures such as pig holdings census, animal identification, holding registration, movement control, thorough disinfection of vehicles, official disposal of carcasses, by-products, and waste, ban on the semen and embryo trade, reporting and testing of any sick and dead animals, surveillance of wild boars etc. were undertaken in the infected and protection zone. Following the first, the second outbreak was confirmed on the 1st of August 2019. in Velika Krsna village, located, also, in the Mladenovac municipality. The third outbreak occurred on the 6th of August 2019, in the same village, in a backyard farm with 23 pigs. The next outbreak was registered in the village Kusadak, Smederevska Palanka municipality, neighboring village to the Rabrovac on the 7th of August 2019. The last outbreak was confirmed in Vojvodina province, village Srpski Itebej, municipality Žitište, at the border with Romania (Figure 2). As a consequence, 270 pigs from 18 affected holdings were killed in the infected zones. Additionally, and 339 animals were euthanized in the protection zones. According to the on-record data, mortality was 6.89%, whereas lethality reached 64.5%. Currently, an extensive surveillance program is being conducted, aiming to force passive surveillance. The program envisages ASF exclusion in all cases of abortions, deaths of sows and boars, as well as testing of 2 dead animals older than 2 months per production unit weekly. ASF in the wild boar has not been confirmed so far.

Authors' contributions

MV drafted the manuscript and helped in carrying out the laboratory tests. KB performed necropsy and helped to draft the manuscript. MZJ carried out the laboratory tests. BS performed necropsy and helped in epidemiological investigations. SS performed epidemiological investigations. ME coordinated the field work and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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PRVI SLUČAJ AFRIČKE KUGE SVINJA U SRBIJI

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Do 30. jula 2019. kada je potvrđen prvi slučaj afričke kuge svinja, Srbija je bila zemlja slobodna od ove bolesti. Nakon prijave vlasnika o netipičnoj bolesti i uginuću jedne krmače, lokalni veterinar je uzorkovao unutrašnje organe i prosledio na dalja ispitivanja Nacionalnoj referentnoj laboratoriji za klasičnu (KKS) i afričku (AKS) kugu svinja pri Naučnom institutu za veterinarstvo Srbije u Beogradu. Uočene patomorfološke promene su obuhvatale splenomegaliju, edem zida žučne kese i krvarenja u uvećanim portalnim limfnim čvorovima, petehijalna krvarenja na bubregu i epikardu, kao i petehijalna i ekhimozna krvarenja na sluznici mokraćne bešike. Rezultati real time PCR-a su potvrdili da je uzrok bolesti i smrti svinja bio virus afričke kuge svinja. Uzorci su poslati na potvrdu u referentnu laboratoriju EU gde je potvrđeno da izolati virusa afričke

kuge iz domaćih svinja, na osnovu p72, pripadaju genotipu II. Ukupno je ubijeno 270 svinja sa 18 pogođenih gazdinstava u zaraženim zonama. Prema osnovnim podacima mortalitet je iznosio 6,89%, a letalitet 64,5%. Trenutno se provodi obiman program nadzora koji ima za cilj jačanje pasivnog nadzora. Afrička kuga kod divljih svinja do sada nije potvrđena.