Acta Veterinaria-Beograd 2021, 71 (2), 219-229
UDK: 636.4.09:[616.98:578.8(497.11)
DOI: 10.2478/acve-2021-0019

Short communication

AFRICAN SWINE FEVER OUTBREAK INVESTIGATION ON LARGE COMMERCIAL PIG FARM IN SERBIA

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(Received 17 May, Accepted 31 May 2021)

The first confirmed case of African Swine Fever (ASF) in Serbia occurred in 2019. Since then, numerous outbreaks in domestic pigs and wild boars have been reported. Until April 2021, all the detected ASF cases were in backyard pigs. Beside backyard and smallholders farming systems as a dominant pig production system in Serbia, large commercial pig farms can also be found, located mostly in villages. In the beginning of April 2021, a large commercial farrow-to-finish pig farm in Serbia with almost 19,000 animals was affected with the ASF virus. The pig farm analysed in this paper is located in an area where ASF was confirmed earlier in both backyard and wild boars. In this study, we describe the overall epidemiological course of the ASF outbreak. Epidemiological investigation using a hypothesis-based approach was conducted in order to reconstruct the disease course until the official notification. All the available data from the farm were analysed with the aim to determine the high-risk period (HRP). Some of the key points to consider when it comes to the sources of infection and entry route of ASF are the following: contamination of the area in the immediate vicinity of the farm, risky human activities, irregularities and some omissions in the external farm biosecurity and immediate proximity of the city waste-yard where the communal waste is disposed of. It was concluded that when commercial pig farms are surrounded by villages with a large number of backyards, hence the anthropogenic factor is the key risk factor for ASF spreading.

Key words: African swine fever, outbreak investigation, biosecurity, pigs, Serbia

INTRODUCTION

The first case of ASF in Serbia was recorded in July, 2019 in the village Rabrovac, Mladenovac municipality in domestic backyard pigs [1]. Although the Veterinary Directorate of the Republic of Serbia issued a set of control and preventive measures to be implemented in the country and at the borders with Romania and Bulgaria as high risk areas in 2017, the first case was detected in the central region of Serbia.

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Since the first outbreak, this disease has been (sporadically) recorded in the population of domestic pigs, predominantly in backyard pigs and in the wild boar population. Contrary to this, in most of the European countries, the epidemic is present in wild boars, but domestic pigs are not primarily affected [2,3]. However, in the region of South-East Europe strong association of ASF with the domestic pig population on small holdings, where the animals are kept at low-biosecurity like backyards and even under free-range management was noted [4,5]. In the domestic pig production cycle, and in certain aspects of the wild boar-habitat cycle, the driver of ASF transmission is human activity [3,6]. ASF transmission risks in the domestic pig production cycle are the highest when pig production is dominated by small holdings or backyards, with low levels of biosecurity [5,7]. Likewise, the risk contact and probability for disease transmission between domestic pigs and wild boars are higher in such systems compared to industrialised (commercial) pig farming systems [8,9]. Despite higher biosecurity measures, the presence of the ASF virus in backyard pig populations is a constant threat to domestic industrial pig production [9,10]. Backyards are a common practice in villages in Serbia and quite a high percentage of pigs are bred in this way. Besides backyards, a large number of small holdings i.e. family type pig farms can be found. Biosecurity measures are not officially required by veterinary regulations; they are only given as a recommendation to the farmers [10]. Consequently, it is quite difficult to protect the domestic pigs from ASF when large commercial farms are surrounded by a number of smallholdings and backyards [11-13].

In this study, we present the results of an epidemiological investigation conducted in one large commercial farrow-to-finish pig farm, affected by ASF. In the beginning of 2021, a larger number of ASF cases were registered in wild boars and in backyards that were in the proximity of this farm, in the East of Serbia. Generally, the backyard pig production in this region of the country is characterized with a very low level of biosecurity measures.

MATERIALS AND METHODS

Farm description

The investigated commercial pig farm is located in the East of Serbia, only 4 km away from the Bulgarian border. From the production point od view, it represents a large farrow-to-finish farm with almost 19,000 pigs, including around 900 sows and all other categories (sucklings, weaned pigs, fatteners, gilts and boars) on one-site. The main production characteristics are similar to most commercial pig farms in Serbia: piglets are weaned at the age of 28-35 days, and they are transferred to a weaning and later growing unit. Finishing pigs are slaughtered at the weight of approximately 110 kg or when they are about 25 weeks old [10]. Based on the production stage, all animals are kept in separate barns with a common yard.

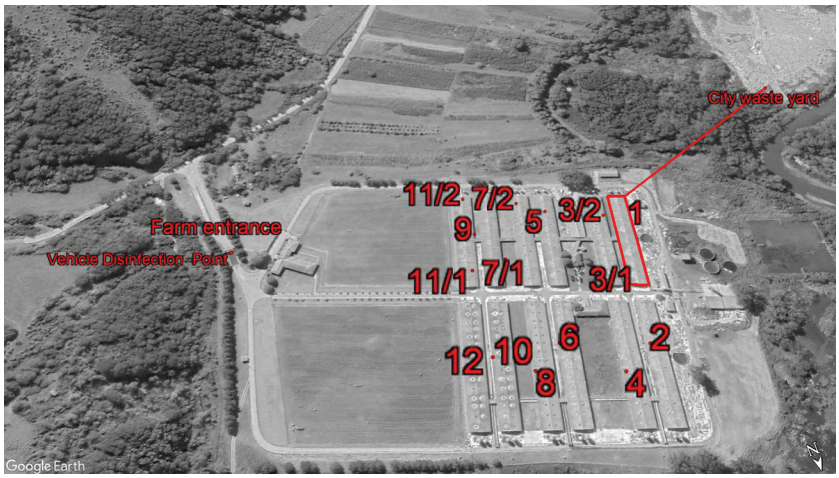

Figure 1. Geospatial farm setup
Legend: No.1, 9 – gestating stable; No. 3/2, 7/2, 11/2 – farrowing room; No.5 – insemination stable; No. 3/1, 7/1, 11/1 – weaning stable; No. 2,4,6,8,10,12 – fattening stable

Epidemiological investigation

Epidemiological investigation included on-site inspections, interviews with relevant staff from the farm's management, veterinarians and workers directly involved in daily care of the farm animals [11]. The outbreak investigation was performed by a local epidemiologist and veterinary authorities, as required by Serbian legislation. The main points to check were the farm organizational structure and location, and all points related to external biosecurity: entrance of all transportation vehicles, employee structure, the record of persons who have recently entered farm premises, animal movements from the beginning of 2021 and feed purchase. Also, the percentage of mortality in all production categories (analyzed separately) in the last three months, timeline of the detected disease, as well as clinical and postmortem findings were analyzed.

RESULTS AND DISCUSSION

Chronology of events

There were cases of dead sows (animals previously excluded from the further production cycle), between 25th and 31st March, 2021. During this period, a total 7 sows died, five of which were from facility No. 1 (the barn for gestating sows), the facility which is the closest to the city waste-yard. Following this, in accordance with the farm production strategy, a few sows were transferred from facility No. 1: on 5th April 2021 sows were relocated to the farrowing stable No. 3/2- and on the next day, 6th April, two more pregnant sows were transferred to another barn for gestating sows

(No. 9). After this, in the period between 1st and 6th April 2021, another 2 sows from the same facility No. 1 died (on the 1st and 5th April).

The official documents issued on 7th and 8th April showed that there were additional two cases of sow deaths and one abortion in facility No. 1 on 7th April, while the report from 8th April marks the deaths of two sows from the facility No. 1 and No. 7 and two abortions from facility No. 1. The alarming number of deaths in the category of gestating sows and abortions on the commercial farm was reported to the veterinary authority on 9th April 2021, when a total 11 sows died: 7 sows from facility No. 1, one sow from facility No. 9 and three sows from the farrowing stable (No. 3/2). The first abortion was detected on 7th April, following another two abortions on 8th April. Considering the heath issues of sows, the control showed that there were no written records on pig abortions before 7th April 2021.

Clinical and gross pathological findings

About 15 sows in the facility No. 1 (gestating sows) and 3/2 (farrowing stable) had the following clinical symptoms: high fever (ranging between 41 °C and 42 °C), general weakness, loss of appetite and anorexia, severe depression, rapid breathing. Further, the animals showed discoloration (redness) of the skin, especially on the region of the chest and abdomen, perineum, distinct cyanotic spots and skin colour changes on the ears, even haemorrhages of the skin. Also, increased foam discharge from the mouth and nostrils, and conjunctivitis with ocular discharge were notified. Two pigs also had extensive yellow foaming saliva and they were vomiting. Clinically, mass abortions in all pregnant sows, regardless of gestation stage were detected. In the other production units (sucklers, weaners, fattening pigs, gilts, boars) no clinical signs of acute infective disease were notified.

After the urgent necropsy and gross pathological examination, the following changes on the organs and tissues were determined in dead pigs from facility 1 and facility 3/2: splenomegaly (the spleen was enlarged, connective tissue in the spleen was too tight, which makes the spleen feeble and it breaks very easily; the pulp is softened and squashy); the lymph nodes (epigastric, mesenterial, portal lymph nodes) were very enlarged, dark red and bloody on cross section. The serosa and mucosa of small and large intestines were extremely hyperemic. After removal of the renal capsule, rare petechial haemorrhages were detected on the kidneys. There were also petechial, spotty bleedings on the pericardium. The clinical picture and gross pathology changes in diseased/dead sows correspond to the acute course of ASF described by other authors [14,15].

Immediately after the report on a large number of dead sows and massive abortions on the farm, the blood swab samples and swabs of organ (spleen) were taken from the facilities No. 1 and 3/2. At the same day, by laboratory examination (molecular diagnostics, real time PCR) the positive result on the presence of ASF virus genome was confirmed at the National Referent Laboratory for ASF in Serbia. In total nine

out of twelve samples were ASF positive. Following disease confirmation, all control measures according to Serbian law were ordered (animal movement restrictions, stamping out of all farm animals, safe disposal).

Estimation of the high-risk period

It is very important to detect infected farms as early as possible after ASF virus entry in order to reduce the further spread of the disease and to minimize the losses in the pig sector and costs associated with outbreak eradication [12]. Control of farm records disclosed that the mortality rates by production categories were not higher than the technologically accepted normative for this type of commercial pig production in Serbia. The date that was noted as the first clinical sign of acute infective disease, i.e., the first clinical manifestation was the abortion, i.e., index case was 7th April. The deaths of sows and abortions are typical for ASF on pig farms and it can be considered as a direct clinical manifestation of ASF [14,15]. High Risk Period (HRP) is the likely length of time that ASF has been present on the farm before notification [11].

a. HRP "Scenario A"

The date when this disease was first reported on the farm was 9th April (the date when the investigation was conducted). Clinical picture of the acute course of the disease in sows whose abortion was recorded on 7th April, 2021 started on 6th April, 2021 (when abortion theoretically began). If we take into account the fact that the shortest period of ASF incubation is 4 days and the longest is 19 days, then the date when the virus most likely entered the farm was 18th -19th March (19 days of incubation), while the latest date when the virus entered the farm could be 2nd-3rd April (four-day incubation). Therefore, we can consider that HRP is from 7 to 21 days (Figure 2).

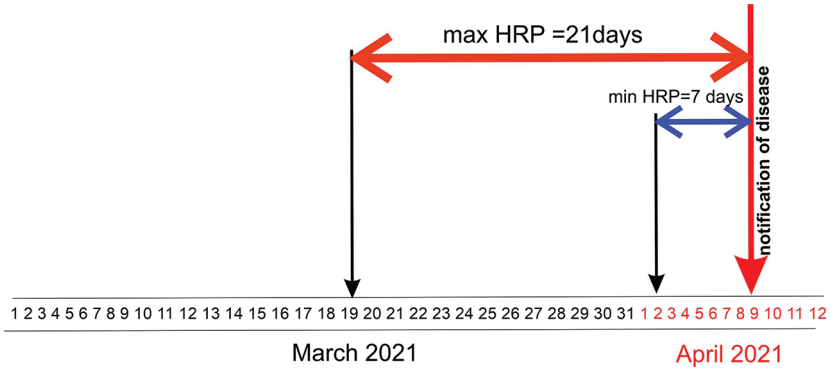

Figure 2. Time line with HRP estimation for "Scenario A"

b. HRP "Scenario B"

The farm records show that there was an increased number of sow deaths in the period from the 25th to 31th of March. with a total of 7 dead sows, five of which were from the facility No. 1. It is also stated that the sows that died had a different etiology

before death: two sows died due to leg injury, one due to pneumonia, another one due to stomach ulcer, while the cause of death of one of the sows was uterine torsion and one had a potential heart condition. These death cases were not sent to laboratory examination for the ASF virus. In this period, the ASF virus was probably already in the facility No. 1, so it is very likely that the first record of sow deaths in this period was related to ASF virus. If the deaths of sows between 25th and 31st March 2021 are potentially directly related to ASF entry, then it means that the virus had entered the farm between 5th and 6th March at the earliest (19-day incubation) or between 20th and 21st March (4-day incubation period). In this case, HPR period can be estimated to range from 19 to 34 days (Figure 3).

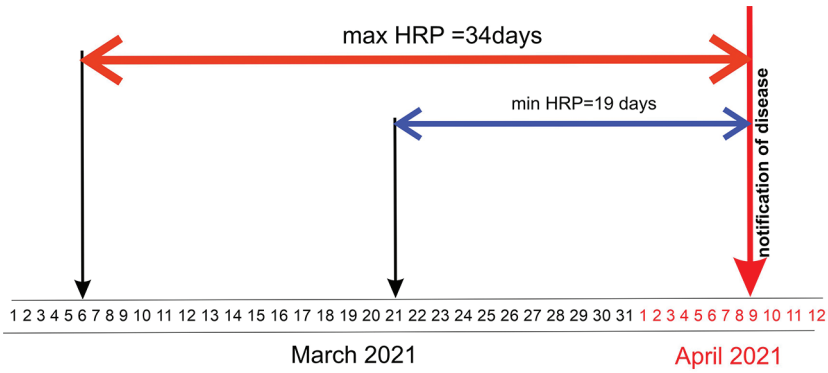

Figure 3. Time line with HRP estimation for "Scenario B"

Potential ASF spreading scenario

Additional laboratory testing of the swab samples (RT-PCR) conducted on 12th April 2021 showed a positive result to ASF in only one sample of a diseased gestating sow (barn No. 9), while all the other samples from other production phases (weaning and finishing barn) taken on the same day were negative (1 out of 178 samples). This shows that the virus was spreading slowly on the farm, affecting only the sows. This has also been confirmed by some other researchers so far [5,11,14]. The sick sow was transferred from stable No. 1 to stable No. 9 and No. 3/2 on 5th and 6th April. This is another piece of evidence to confirm our presumption that the disease first appeared in stable No. 1 and then it spread to the farrowing barn (No. 3/2) and to the gestation room (stable No. 9). Regardless of disease speeding, early ASF diagnosis and implementation of control measures necessary to control and eradicate the disease are imperative [16].

External biosecurity farm control

The investigated commercial farm is located in the East of Serbia, only 4 km away from the Bulgarian border, which is another risk factor, having in mind that the current unfavourable epidemiological ASF situation in the neighbouring Bulgaria [4,17]. Indeed, during the 2018-2020 period, ASF virus was diagnosed in neighbouring countries Romania and Bulgaria in both domestic pigs and wild boars [8,16]. Romania

was particularly affected by the ASF virus, with more than 1,000 outbreaks reported during the second half of 2018 and the situation is still not under control [4,13].

Considering the external biosecurity measures, all farm facilities are fenced with double fence. There is a disinfection barrier on the farm entrance for all transportation vehicles and automatic sprayer with nozzles. According to the research of Bellini et al. [18] and De Lorenzi [19], high levels of farm biosecurity including paying special attention to the disinfection process is considered as the most important tool for preventing ASF virus entering pig farms.

The production system requires the vehicles to come very close to the production area in order to transfer feed to the bin, after they have performed the disinfection process. This is a major biosecurity issue for most of commercial pig farms in Serbia. However, another problem is the farm production technology which is organized in such way that gilts are brought from two different locations every 6-7 weeks, from the North of the country (ASF free region). Animals are quarantined for 6 weeks on the farm and are physically separated from other production units. The last delivery of gilts was from ASF free region (the North of the country) in mid-February.

Regarding the assessment of external biosecurity, it is important to point out that the commercial farm is located on a very unfavorable location: in the vicinity of the city waste yard (about 200 m from the farm facility (facility marked No.1). The waste yard has a large population of rodents, and they can easily migrate to the pig facilities. The city waste yard is also a place where the owners of backyards pigs may dispose of potentially infected biological material after home slaughtering and/or the carcasses of dead animals.

It is also significant to emphasize that this commercial farm is in the immediate vicinity of a village (about 6 km away), where ASF was confirmed in the backyards in March 2021. During 2020 and 2021, ASF was detected in wild boars in the hunting areas in this district. It is especially important that a certain number of employees on the farm live in the village where ASF was diagnosed. The farm has taken appropriate biosecurity measures regarding employee control: the employment contract prohibits employees from keeping backyard pigs and from having contacts with other domestic pigs in the village after working hours. However, indirect (mechanical) ASF transmission of virus is realistic and possible to happen by people (there is a chance that clean and potentially dirty routes that people use can intersect) [11]. Another potential way to transmit the virus is through frequent feed unloading, where vehicles can contract the ASF virus when passing through the infected area near the farm [18].

Considering biosecurity measures targeted to humans, employees are banned from bringing any food to the farm. The staff working on the farm is provided meals by the farm (at a staff canteen). It is mandatory for farm workers to perform hand disinfection and take a shower before entering the farm. They also have to change clothes, shoes and put on their uniforms that are kept on the farm. Before entering pig barns, they change their shoes again and go through disinfection barriers placed on the entrance of each unit. From the organizational point, workers in fattening units do not perform any other activities in other facilities, but they all use the same toilets

and canteen. However, the workers from maintenance department (e.g., electricians, repairmen, etc.) are allowed to enter all the facilities on the farm. However, it is essential to point out that a couple of employees on the industrial farm live in the village where the cases of ASF had been recorded. Pig farming in this region of Serbia has a number of traditional and cultural characteristics like pig slaughtering in backyards, natural mating, swill feeding, etc. These activities are highly risky in terms of disease transmission [3,8], but unfortunately are frequently underestimated by local farmers, the backyard owners.

Table 1. Hypothetical introduction routes and their estimated probability

Hypothetical Introduction Route	Risk Factors	Description	Probability of Introduction Pathway
Link to infected wild boar	Set up of the farm	All animal facilities are located inside the farm perimeter. The farm area is enclosed by a double fence, with a buffer zone between. There is no direct contact with wild boars.	Negligible
	Indirect contact with wild boar environment	The ASF in wild boar population was confirmed last year.	Moderate
	Anthropogenic factor	Workers are banned from breeding backyards. Contacts with domestic pigs from the village cannot be ruled out.	High
Contact to contaminated fomites/ food	Feeding regime	Pigs are fed with complete feed, which originates from ASF free area.	Negligible
	Anthropogenic factor	Farm workers are banned from bringing food to the farm.	Negligible
Trade/animal movement	Introduction of infected animals	The last gilts delivery was from ASF free area at the beginning of the year.	Low
	Animal movement	Contact are restricted to ASF free area	Low
Contact to contaminated surrounding area	Close to the farm	Farm is in the vicinity of a city waste-yard. The waste from the whole region is disposed of at this waste-yard.	High
	Distant from the farm	It is close to the village where ASF was confirmed in domestic pigs.	High
Transport vehicles	Feed transport	Vehicles come close to the fence in order to unload feed.	High
	Transport of fatteners	Vehicles come close to the facility in order to load fatteners.	High
	Rendering plant	Vehicles come relatively close to carcass containers.	Moderate

CONCLUSION

Although the appropriate farm authorities have urgently undertaken all well-known external biosecurity measures, they were not able to prevent numerous high- risk human activities in the surrounded infected area (indirect contacts with infected backyards, wild boars). Our investigation showed that anthropogenic activities have most likely contributed to ASF virus entering the commercial pig farm, together with the proximity to the backyards with ASF infected pigs and contaminated surrounding area.

Acknowledgement

This study was funded by Ministry of Education, Science and Technological development of Republic of Serbia by the Contract of implementation and financing of scientific research No 451-03-9/2021-14/200031.

Authors' contributions

NM and RB carried out the epidemiological and clinical investigation and gross pathology. GS and DR participated in surveillance and disease control measures. PT, PRJ and PV participated in the design of the epidemiological study and coordination, and helped to draft 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.

REFERENCES

1. Miličević V, Kureljušić B, Maksimović Zorić J, Savic B, Stanojević S, Milakara E: First occurence of African swine fever in Serbia, Acta Veterinaria-Beograd 2019, 69(4):443-449.
2. Chenais E, Fischer K: Increasing the local relevance of epidemiological research: situated knowledge of cattle disease among Basongora Pastoralists in Uganda. Front. Vet. Sci. 2018, 5:119.
3. Chenais E, Depner K, Guberti V, Dietze K, Viltrop A, Stahl K: Epidemiological considerations on African swine fever in Europe 2014-2018. Porcine Health Manag. 2019, 5:6.
4. EFSA Panel on Animal Health and Welfare: Risk assessment of African swine fever in the south-eastern countries of Europe. EFSA Journal 2019, 17(11): p. e05861.
5. Zani L, Dietze K, Dimova Z, Forth JH, Denev D, Depner K, Alexandrov T: African Swine Fever in a Bulgarian Backyard Farm-A Case Report. Vet. Sci. 2019, 6, 94.

6. Penrith ML, Bastos AD, Etter EMC, Beltran-Alcrudo D: Epidemiology of African swine fever in Africa today: Sylvatic cycle versus socio-economic imperatives. Transbound Emerg Dis. 2019, 66(2):672-686.
7. Penrith, ML, Bastos A, Chenais E: With or without a vaccine—a review of complementary and alternative approaches to managing african swine fever in resource-constrained smallholder settings. Vaccines 2021, 9(2):116.
8. Boklund A, Dhollander S, Chesnoiu VT, Abrahantes JC, Bother A, Gogin A, Gonzales VLC, Gortazar C, More SJ, Papanikolaou A, Roberts H, Stegeman A, Stahl K, Thulke HH, Viltrop A, Van der Stede Y, Mortensen S: Risk factors for African swine fever incursion in Romania domestic farms during 2019. Sci Rep 2019, 10:10215.
9. Bellini S, Rutili D, Guberti V: Preventive measures aimed at minimizing the risk of African swine fever virus spread in pig farming systems. Acta Vet Scand 2016, 58:82.
10. Prodanov-Radulović J, Vučićević I, Polaček V, Aleksić-Kovačević S: Current swine respiratory diseases morphology in intensive swine production in Serbia. Acta Veterinaria-Beograd 2020, 70 (1):1-36.
11. Lamberga K, Olševskis E, Seržants M, Berzinš A, Viltrop A, Depner K :African Swine Fever in two large commercial pig farms in Latvia-estimation of the high risk period and virus spread within the farm, Vet Sci 2020, 7:105
12. Arias M, Jurado C, Gallardo C, Fernandez-Pinero J, Sanchez-Vizcaino JM: Gaps in African swine fever: analysis and priorities. Transbound. Emerg. Dis. 2017, 1 (65):235–247.
13. Dixon LK, Stahl K, Jori F, Vial L, Pfeiffer DU: African Swine Fever epidemiology and control. Annu. Rev. Anim. Biosci. 2020, 8:221-46.
14. Sanchez-Vizcaino JM, Mur L, Gomez-Villamandos JG, Carrasco L: An update on the epidemiology and pathology of African Swine Fever. J.Comp. Path. 2015, 152: 9-21.
15. Sanchez-Cordon PJ, Montoya M, Reis AL, Dixon LK: African swine fever: are-emerging viral disease threatening the global pig industry. Vet J 2018, 233:41-48.
16. Cwynar P, Stojkov J, Wlazlak K: African Swine Fever status in Europe.Viruses 2019, 11:310.
17. Olesen AS, Belsham GJ, Rasmussen TB, Lohse L, Bodker R, Halasa T, Boklund A, Bother A: Potential routes for indirect transmission of African swine fever virus into domestic pig herd. Transbound Emerg Dis. 2020, 67:1472-1484.
18. Bellini S,, Casadei G,, De Lorenzi G,Tamba M: A review of risk factors of African Swine Fever incursion in pig farming within the European Union scenario. Pathogens 2021, 10:84.
19. De Lorenzi G, Borella L, Alborali GL, Prodanov- Radulovic J, Štukelj M, Bellini S: African Swine Fever: a review of cleaning and disinfection procedures in commercial pig holdings. Res Vet Sci. 2020, 132:262-267.

ISTRAŽIVANJE POJAVE AFRIČKE KUGE SVINJA NA VELIKOJ KOMERCIJALNOJ FARMI SVINJA U SRBIJI

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Prvi slučaj afričke kuge svinja (AKS) u Srbiji je dijagnostikovan 2019. godine. Od tada, beleži se pojava bolesti u populaciji domaćih i divljih svinja. Sve do aprila 2021. godine žarišta AKS su bila lokalizovana u populaciji domaćih svinja u seoskim dvorištima. Pored seoskih gazdinstava i porodičnih farmi kao dominantnog načina proizvodnje, u Srbiji se nalaze i velike komercijalne farme svinja, uglavnom oko seoskih naselja. Početkom aprila 2021. godine, u komercijalnoj farma svinja, proizvodnog kapaciteta oko 19000 jedinki, potvrdjena je infekcija virusom AKS. U radu je sa epizootiološkog aspekta, hronološki opisan tok infekcije AKS. Epizootiološko istraživanje je zasnovano na hipotezama u cilju utvrdjivanja potencijalnih puteva unošenja AKS. Analizirani su dostupni farmski podaci kako bi se utvrdio vremenski period visokog rizika (HRP). Farma svinja se nalazi u području gde je u prethodnom periodu potvrđena AKS u seoskim gazdinstvima, kao i u populaciji divljih svinja. Kontaminacija ambijenta u neposrednoj blizini farme, visoko rizične aktivnosti humane populacije u okruženju (brojna seoska gazdinstva), postojanje pojedinih aktivnosti visokog rizika u realizaciji eksterne biosigurnosti i neposredna blizina gradske deponije gde se odlaže komunalni otpad, su svakako ključni momenti kada se razmatraju izvori infekcije i put unošenja AKS. Zaključeno je da u uslovima kada su komercijalne farme okružene selima, u kojima su dominira uzgoj domaćih svinja u seoskim dvorištima, ljudske aktivnosti predstavljaju ključni faktor rizika za širenje AKS.