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MAIN RISK FACTORS OF AMERICAN FOULBROOD SPREADING IN HONEY BEES IN SERBIA

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Abstract

American foulbrood (AFB) is one of the most important contagious honey bee diseases. In Serbia, it is mandatory to report AFB, and this disease is registered in our country every year. Starting from 2018, active surveillance of the presence of the AFB has been conducted throughout the country. The paper analyses the data on the occurrence of AFB during the period between 2019 and 2021 from official disease reports in the National Animal Disease Notification System "VetUp". Results of this research indicate that AFB appears every year in the Republic of Serbia, despite the measures that are being applied. The results indicate that in 36 settlements in the country, this disease has reoccurred in the same localities in the observed period. Namely, in 17 localities AFB occurred consecutively in the 2019 - 2020 period, while in 21 localities the disease was re-registered consecutively in the period between 2020 and 2021 (until November 6th, 2021), and it reoccurred in 2021 in 9 localities, compared to the registered cases of AFB in 2019. It was found that the disease has consecutively been reoccurring in 5 location between 2019 and 2021 in the same locations. The fact that the disease has been occurring for several years in the same places speaks in favour of the fact that the control measures applied in the control of this disease are not effective enough. Continuing education of beekeepers, veterinarians and veterinary inspectors in the field of diagnosis and effective decontamination and neutralization of all potential sources of AFB reinfections, revision of current legislation, as well as raising awareness of the importance of early diagnosis of as many cases of this disease as possible are the key factors in successful AFB control.

Key words: American foulbrood, *Paenibacillus larvae*, honey bees, bee diseases, Serbia

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GLAVNI RIZICI ŠIRENJA AMERIČKE KUGE PČELINJEG LEGLA U REPUBLICI SRBIJI

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Kratak sadržaj

Američka kuga pčelinjeg legla (AKPL) je jedna od najznačajnijih infektivnih bolesti medonosne pčele. Ova bolest je obavezna za prijavljivanje i svake godine se javlja u Srbiji. Počev od 2018. godine, na teritoriji čitave zemlje počinje da se primenjuje aktivni nadzor na prisustvo AKPL. Ovaj rad analizira zvanične podatke Uprave za veterinu - sistema „Vet-up“ o pojavi AKPL u periodu od 2019 do 2021. Rezultati ovog istraživanja ukazuju na to da se AKPL pojavljuje u Srbiji svake godine, bez obzira na mere koje se primenjuju u kontroli ove bolesti. Takođe, rezultati pokazuju da se u 36 naselja AKPL ponovno javlja, u posmatranom periodu. Na 17 lokaliteta se ponovno javlja u periodu 2019 -2020., dok se na 21 lokacije AKP je ponovno registrovana u periodu 2020 - 2021. (posmatrano do 6.11.2021.). Na 9 lokaliteta bolest je ponovno registrovana 2021. godine u odnosu na registrovane slučaje AKPL u 2019. godini, dok se na 5 lokaliteta u Srbiji AKPL pojavljuje svake godine, počev od 2019. do 2021. godine. Činjenica da se bolest pojavljuje više godina na istim lokalitetima govori u prilog da mere koje se primenjuju u kontroli ove bolesti nisu dovoljno efikasne. Nprekidna edukacija pčelara, veterinara i veterinarskih inspektora na polju dijagnostike ove bolesti, efikasne dekontaminacije i neutralizacije svih potencijalnih izvora infekcije AKPL, revizija važeće legislative koja reguliše ovu oblast, kao i podizanje svesti o značaju rane dijagnostike što većeg broja slučajeva bolesti su ključni faktori u uspešnoj kontroli ove bolesti.

Ključne reči: Američka kuga pčelinjeg legla, *Paenibacillus larvae*, medonosna pčela, bolesti pčela, Srbija

INTRODUCTION

American foulbrood is one of the most severe infectious diseases of the honey bees (Beims et al., 2020; Djukic et al., 2014; Genersch, 2010). It is caused

by the spore-forming, Gram-positive rod-shaped bacterium *Paenibacillus larvae* (De Graaf et al., 2013; Forsgren et al., 2018). The spores are the only transmissible stage of the bacteria. They are highly resistant and can remain infectious for more than 35 years (Dobbelaere et al., 2001). Long-lived endospores are an infectious form and only bee larvae younger than 36 h are susceptible. Oral uptake of about ten spores is sufficient to initiate a fatal intestinal infection in bee larvae (Genersch, 2010). After germination, *P. larvae* massively proliferate the larval midgut. The vegetative cells breach the epithelium and invade the haemocoel of bee larvae. This invasion coincides with the death of infected larvae, which are subsequently decomposed into a brown glue-like liquid. The emerging rosy mass dries and develops into a highly contagious scale, which contains a vast number of *P. larvae* spores (Beims et al., 2020; Forsgren et al., 2018; Genersch, 2010). Burning colonies and contaminated hive material are widely considered to be the only workable control measure for diseased colonies. Thus, AFB is a serious problem in apiculture and causes considerable economic loss to beekeepers all over the world (Genersch, 2008; Genersch, 2010). Nowadays, the existence of five different genotypes (ERIC I-V) of *P. larvae* has been determined (Beims et al., 2020; Žugelj et al., 2021). Genotypes of *P. larvae* are differed by the level of virulence. ERIC I is a slow killer, ERIC III a medium fast killer, while ERIC II and IV represent fast killers. While larvae infected with genotypes ERIC II to ERIC IV were killed within only 6 to 7 days, it took *P. larvae* ERIC I around 12 to 14 days to kill all the infected individuals. It has been proposed that the fast-killing phenotype allows nurse bees to remove infected larvae more efficiently. Larvae infected with slow killing *P. larvae* die in cells which are already capped. This apparently reduces the effect of hygienic cleaning by nurse bees. As a result, the infected larvae remain in the cell, convert into infectious spores and thus contribute to disease progression within and beyond the colony (Rauch et al., 2009). EPIC V genotype has recently been discovered in honey samples originating from Spain (Beims et al., 2020).

According Statistical Office of Republic of Serbia, 980,000 beehives were registered in whole country in 2020 (Anon, 2021), but according Association of Beekeeping Organizations of Serbia (SPOS) the number of registered beehives in 2021 is more than 1,500,000 and therefore is a significant branch of agriculture. Reporting AFB in Serbia is mandatory. Since 2005, AFB has been the second most frequently reported disease, according to National Animal Diseases Notification System –“Vetup”, with 1,252 total number (average annual number 86) of registered AFB cases in the 2005 to 2018 period, (Polaček et al., 2019.) Starting from 2018, active monitoring of all beehives has been car-

ried out in the Republic of Serbia in the diameter of 3 km from the bee yards where AFB was confirmed by laboratory diagnostics in the previous year. The plan involves clinical examinations of all beehives within a three-kilometre diameter, sampling of enclosed honey combs from bee colonies susceptible to AFB and sending samples to laboratory analysis (Anon, 2021). The rulebook regulating this issue has not changed since 1988 (Anon, 1988). The measures applied in bee yards where this disease is found include destruction of the diseased colony, disinfection, quarantine and compensation of the market value of the bee colony and hive. The treatment of diseased colonies or the application of shook swarm method are not allowed. Although active monitoring of the presence of ABF in Serbia is being carried out, there is an impression that, apart from registering cases, the number of cases of this disease has not decreased in recent years. On the contrary, it is increasing. The aim of this paper is to analyse the data on the occurrence of AFB in Serbia, and analyse the most important factors in the spread of this disease in the Republic of Serbia.

MATERIAL AND METHODS

The paper analyses the data on the occurrence of AFB during the 2019 - 2021 period from the official disease reports in the National Animal Disease Notification System "VetUp". The data were analysed using ESRI ArcGIS Map 10.8, ESRI ArcGIS Online, ESRI ArcGIS Pro 2.8, Microsoft Excel, and Microsoft Access 365.

RESULTS

The results show the occurrence of ASF between 2019 and 2021. This disease was recorded each year during the observed period. During 2019, 86 cases of ASF were found in 69 locations in the Republic of Serbia, while there were 113 cases of ASF in 83 locations during 2020. Finally, by November 6th, in 2021, there were 107 cases of ASF at 69 locations. Figure 1 shows the spatial distribution of the cases for the observed period. Namely, AFB appeared consecutively in the 2019 - 2020 period in 17 localities and in 21 localities the disease was re-registered consecutively in the period between 2020 - 2021 (until November 6th, 2021), while in 9 localities this disease reappeared in 2021, compared to the registered AFB cases in 2019. Table 2 shows that at 5 localities in the Republic of Serbia (Bukovik, Trnava, Šabac, Azbresnica, Novi Pazar) it occurred consecutively every year in the 2019 - 2021 period.

Table 1. Number of registered AFB cases in Serbia from 2019 to 2021 (until November 6th, 2021)

Year	Number of places with AFB outbreaks	Number of AFB registered cases
2019	69	86
2020	83	113
2021	69	107

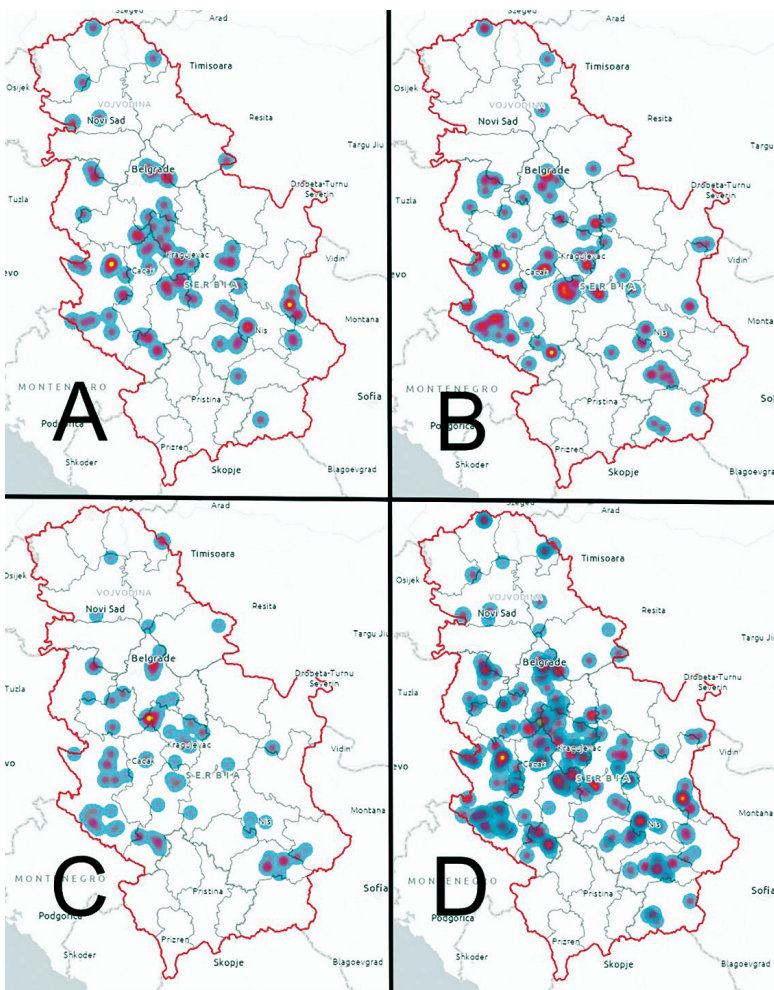


Figure 1. A - AFB outbreaks in 2019; B - AFB outbreaks in 2020; C - AFB outbreaks in 2021; D - Total AFB outbreaks from 2019 to 2021 (until November 6th, 2021)

Table 2. The number of repeated places with AFB outbreaks in at last two years in Serbia from 2019 to 2021 (until November 6th, 2021)

Municipality	Place	Repeated AFB outbreaks 2019-2020	Repeated AFB outbreaks 2020-2021	Repeated AFB outbreaks 2019-2021
Arandelovac	Bukovik	1	1	2
Arandelovac	Garašani	1	1	2
Bajina Bašta	Perućac	1	1	2
Beograd	Beograd	1	1	2
Čukarica	Čukarica	1	1	2
Beograd-Grocka	Zaklopača	1	1	2
Beograd-Rakovica	Beograd Rakovica	1	1	2
Beograd-Voždovac	Beograd Voždovac	1	1	2
Čačak	Čačak	1	1	2
Čačak	Preljina	1	1	2
Čačak	Trnava	1	1	2
Čajetina	Drenova	1	1	2
Leskovac	Strojkovce	1	1	2
Kikinda	Kikinda	1	1	2
Knjaževac	Knjaževac	1	1	2
Kragujevac-city	Donja Sabatna	1	1	2
Kragujevac-city	Kragujevac	1	1	2
Kragujevac-city	Poskurice	1	1	2
Kraljevo	Dragosinjci	1	1	2
Kraljevo	Kraljevo	1	1	2
Kraljevo	Jarčujak	1	1	2
Kraljevo	Žiča	1	1	2
Merošina	Azbresnica	1	1	2
Merošina	Dudolajce	1	1	2
Nova Varoš	Bukovik	1	1	2

Municipality	Place	Repeated AFB outbreaks 2019-2020	Repeated AFB outbreaks 2020-2021	Repeated AFB outbreaks 2019-2021
Nova Varoš	Vilovi			
Novi Pazar	Novi Pazar			
Paraćin	Paraćin			
Prijepolje	Divci			
Prijepolje	Kosatice			
Rača	Rača			
Rača	Viševac			
Šabac	Šabac			
Subotica	Subotica			
Sjenica	Sjenica			
Ub	Pambukovica			
Užice	Trnava			
Total number of repeated places with AFB outbreaks		17	21	9

DISCUSSION

Results of this research indicate that AFB reoccurs in the Republic of Serbia every year, despite the measures that are being applied. The fact that the disease has been occurring in the same places for several years speaks in favour of the fact that the control measures applied in the control of this disease are not effective enough.

It has been pointed out that the endospores of the pathogen itself are very resistant in the external environment. The choice of disinfectants for disinfecting the apiaries and accessories is therefore very important. The data about effective disinfectant against *P. larvae* endospores in literature are scarce. Dobbelaere et al. (2001) examined the efficiency of various disinfectants on the efficiency of wood disinfection, and the material commonly used for building hives. They found that disinfectants based on sodium hypochlorite and the products based on the combination of glutaraldehyde and formaldehyde destroy 100% of AFB spores for 30 min only if used in concentrations above 50% in working solutions. The use of disinfected compounds based on amphoteric

compounds (quateral ammonium compounds) did not result in a decrease in the number of viable AFB spores. The use of warm, dry air at the temperature of 160 - 180 °C destroys 100% of spores but only for 2 hours. The use of liquid paraffin at a temperature of 170 °C destroys AFB spores for 10 minutes (Dobbelaere et al., 2001). Earlier research by Japanese researchers found much lower concentrations of disinfectants that effectively neutralize AFB spores. This research was conducted in laboratory conditions and on culture media, without the presence of organic matter (Okayama et al., 1997).

These facts speak in favour of the importance of the method of decontamination of equipment and accessories in infected beehives. In Serbia, as a measure of disease control, the destruction of diseased colonies is used, where laboratory diagnostics is used to determine a positive finding for the presence of the causative AFB agent. The diseased colonies are closed, and the method of suffocation with the sulphur strips is most commonly used. After that, complete beehives with honeycomb are burned, on the very location of the bee yard or in the immediate vicinity. The choice of the method of destroying the causative agent, the choice of disinfectant, as well as the concentration for the preparation of working solutions has a crucial role in the control of this disease. If it is known that the spores are extremely resistant to the commonly used concentrations of disinfectants, it is a question whether after the disinfection of the entire apiary the spores of the causative agent are destroyed and to what level. The fact that bee yards and hives are most often found on the ground, the effect of disinfectants on the surface of the earth is extremely limited to the surface layer and if used in recommended concentrations that definitely destroy spores, so the economic aspect of disinfection is questionable as well as the problems of environmental pollution by various chemical compounds.

To our knowledge, there is no published data about AFB genotypes in Serbia. Based on research conducted in Slovenia, in the period from 2017 to 2019, it was determined that 70.2% of the causative agents of AFB belong to the EPIC II genotype, and that 29.8% belong to the EPIC genotype EPIC I (Žugelj et al., 2021). It has been proposed that the fast killing phenotype allows nurse bees to remove infected larvae more efficiently (Rauch et al., 2009). In the colonies with good hygienic instinct, dead bee larvae will be expelled, and therefore that the common clinical symptoms of the disease in colony will be missing, as is the case with slow-killing genotypes. We can hypothesize that this finding could be connected with the reports of several veterinary inspectors, who reported difficulties in finding AFB clinical symptoms on larvae, because of spotty brood and missing dead or infected larvae under capes. Thus, the ERIC

II major prevalence and faster death of larvae in combination with the hygienic behaviour of bees can result in problematic recognition of clinical symptoms which, as a result, causes a delayed confirmation of AFB disease on the clinical level (Rauch et al., 2009). The moment when a beekeeper starts suspecting that there are diseased colonies in his bee hive is often crucial, because during this period, a beekeeper unintentionally spreads the pathogen over the apiary. In the case of migrating beehives, this poses an additional risk. One of the main reasons why many cases of AFB remain undiagnosed is that a large number of beekeepers do not recognize the disease or do not want to report it, out of the fear from the consequences, and because they try to disguise or solve it the problem on their own.

A crucial time for each of these variants is when the causative agent of AFB spreads to wider areas through contaminated bees from the diseased bee colony. Another important factor in the spread of AFB is the method of beekeeping, i.e. whether it is a stationary or mobile method. In Serbia, a large number of beekeepers is involved in mobile beekeeping. In early spring, some of the beekeepers decide to move their hives to oilseed rape crops in Vojvodina Province. The largest number of beekeepers move their hives from April to July to the Cer Mountain for acacia foraging and after that to the linden foraging on Fruška Gora Mountain. This is followed by sunflower foraging on the territory of Vojvodina Province. This migration of a large number of beekeepers and the concentration of a large number of bee colonies in the same locations is one of the extremely important factors in the spread of the pathogen throughout the country. Researchers in Slovenia used molecular epidemiology methods to determine the connection between certain genotypes of AFB pathogens in different localities in the country caused by migration of bee colonies (Žugelj et al., 2021).

Transmission of spores within or between bee colonies occurs by contaminated adult bees and honey or by interventions of the beekeeper (Genersch, 2010). AFB is not only horizontally transmitted between colonies, e.g. through diseased, weakened colonies being robbed out by other colonies and (Lindström et al., 2008) but also vertically through swarming of strong although infected colonies. One question, however, is still open in this context: Some researchers still address the following question is swarming really a vertical transmission route for *P. larvae* on colony level or is it a cure? (Fries et al., 2006).

In Serbia, after establishing a laboratory diagnosis of the presence of the causative agent of AFB in a beehive, the measures applied are closing and destroying the infected bee colonies. In order to calculate the compensation, a commission that assesses the level of damage needs to be formed, so the period

since the initial suspicion, laboratory diagnostics, and then the destruction of the colony and disinfection of apiaries can be quite long. During this period, a beekeeper may inadvertently transmit the causative agents within the apiary.

In Serbia, the treatment of bee colonies with antibiotics is not allowed, but since samples of antibiotics can be found in honey, it can be assumed that they are still used illegally (Kartalović et al., 2020). Antibiotics are not effective against the infectious spores, hence, they only suppress clinical symptoms and mask the disease but cannot cure AFB; chemical residues can persist in honey affecting its quality and safety for human consumption (Kartalović et al., 2020; Lodesani and Costa, 2005).

The AFB control measures that are applied, as mentioned, include the destruction of only bee colonies at infected bee yard when AFB has been confirmed by laboratory diagnostics. Other bee colonies are not destroyed, but quarantine is introduced, there is a ban on the relocation of bee colonies, the sale of queens, swarms, etc. (Anon, 1988). One of the greatest risks for the spread of AFB pathogens in Serbia is this measure, because a beekeeper will certainly spread the pathogens to other bee colonies when he works in the bee yard. There is a high risk of spreading the pathogen during this period.

In the chain of detecting clinical suspicion to AFB, the training of veterinarians for the tasks of this diagnostics is also an important factor, together with the readiness to cooperate with the veterinary inspection. Very few veterinarians are trained to inspect bee colonies, except for those veterinarians who are beekeepers themselves. Clinical examinations of bee colonies require a lot of physical effort and a long time. Veterinarians are not motivated to do this because the Veterinary Directorate has prescribed the price of a whole bee yard inspection, within the AFB active surveillance annual plan regardless of the number of hives at 2,000 Serbian dinars (approximately 16 €) and arriving at these places needs extra time. At the same time, it is much more profitable for a veterinarian to perform several other interventions that bring more money. If we add the fear of bees to this, inadequate protective equipment and problems with allergic reactions, it is clear why veterinarians hesitate to perform clinical examinations of bee colonies.

In Republic of Serbia, we can freely say that the largest number of beekeepers are those who do it as a hobby or because they need an additional source of income. Many beekeepers are pensioners. In the case of a beekeeper's illness or death, beehives are often left unattended and they perish. Such beehives are often the target for robber bees especially when the risk of spreading AFB, and other diseases is high. Old and abandoned apiaries and beekeeping equipment that can be found in various locations where people use it for various purposes

and can also be a source of AFB if they come from infected beehives, and if adequate decontamination was not performed.

CONCLUSION

The AFB occurs in Serbia every year and causes significant economic damage to beekeepers. In the period from 2019 to 2021, the disease has been occurring consecutively in several locations, which proves the fact that the undertaken measures are not effective enough in the control of this disease. The regulations are being applied have not changed since 1988 and they need to be revised, especially those regarding destruction of exclusively infected colonies, and not the entire apiary. Also, very precise instructions/procedures should be introduced regarding the decontamination of the equipment and tools used in infected beehives, which effectively destroy the cause of this disease. In addition to this, timely detection of diseased bee colonies is an important step in prevention of the risk of disease spreading. Continuous education of beekeepers, veterinarians and veterinary inspection in the field of diagnosis of this disease and raising awareness of the importance of early diagnosis of as many cases of this disease as possible are some of the key factors in successful AFB control. Further research on the determination of AFB genotypes and its spatial distribution in Serbia is needed.

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Author's Contribution:

PV - writing manuscript and maps production, BÐ - data collection and preparations for analysis, JPR and JP - data analyses, RR - writing manuscript, MŽB and SJ - critical revision of the manuscript.

Competing interest

The authors declare that they have no competing interests.

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