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Epizoothological, clinical features and control measures of American foulbrood in the world (literature review)

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Abstract. *The article summarizes and analyzes modern scientific data on the epizootological and clinical features of American foulbrood (AFB) of bees, the causative agent of which is the spore-forming bacterium Paenibacillus larvae. The historical aspects of the study of the disease, the biological properties of the pathogen, it's high resistance in the environment and the ability to maintain infectivity for a long time are highlighted. The ERIC I–V genotypes, their prevalence and differences in virulence are characterized. Based on WOAH (WAHIS) and ProMED data, the modern epizootic situation in the countries of Europe, Asia, Africa and America is analyzed, which indicates the global spread of the disease and its significant economic impact on the beekeeping industry. Typical clinical signs of AFB are described, in particular, variegation of brood, sunken cell lids, the presence of a viscous putrefactive mass and the formation of dense scales. Modern approaches to laboratory diagnostics are considered, including microscopic, bacteriological and molecular genetic methods with an emphasis on the high sensitivity of PCR for early detection of the pathogen. The main preventive measures are summarized, which include compliance with veterinary and sanitary standards, regular monitoring of apiaries, control of bee movements and timely elimination of dysfunctional colonies, which are key to preventing the spread of American foulbrood.*

Keywords: American foulbrood, honey bee, epizootic situation, factors of pathogen spread, diagnostics, risk assessment.

Foulbrood diseases of bees are a group of infectious diseases of bee colonies, characterized by the defeat of open and sealed brood and pupae. The most serious of them is considered to be *American foulbrood (AFB)*, which affects the larval stage of the honey bee *Apis mellifera* and other species of bees. The causative agent of the disease is the spore-forming bacterium *Paenibacillus larvae*. AFB disease is included in the list of transboundary infectious diseases subject to mandatory notification to the World Organization for Animal Health (WOAH) (Zabrodski, 2022; WOAH, 2025). Due to the seriousness and potential risk of the disease, WOAH does not exclude the possibility of its rapid global spread and identifies AFB as the most economically significant disease of bees worldwide (WOAH, 2025; Matović, 2023).

The economic importance of American foulbrood is associated not only with the direct death of bee brood but also with significant losses in honey production, reduced pollination efficiency, and the necessity of implementing costly veterinary and sanitary measures. In affected apiaries, the disease can persist for many years due to the exceptional resistance of P. larvae spores, which remain viable in contaminated hive materials, honey, wax, and beekeeping equipment. As a result, AFB continues to represent a major challenge for both commercial and small-scale beekeeping operations worldwide (Genersch, 2010; Djukic, 2018).

The epidemiology of AFB is closely linked to the biological characteristics of *P. larvae*. Infection occurs when young larvae ingest food contaminated with bacterial spores. Following

germination in the larval midgut, vegetative cells proliferate rapidly, invade host tissues, and eventually cause larval death. During decomposition of infected larvae, billions of new spores are formed, serving as a persistent source of infection within and between colonies. The spread of the pathogen is facilitated by robbing behavior, drifting bees, contaminated beekeeping equipment, movement of colonies, and the trade of infected bee products (Ebeling, 2016; Alvarado, 2013).

Despite the implementation of preventive and control measures in many countries, American foulbrood remains widely distributed and continues to be reported on all inhabited continents. The emergence of antimicrobial resistance among bacterial pathogens, restrictions on antibiotic use in apiculture, and the need for environmentally safe disease-control strategies have stimulated research into alternative approaches for AFB prevention and treatment. Therefore, studies aimed at improving diagnostic methods, understanding pathogen biology, and evaluating effective antimicrobial agents remain highly relevant for modern apiculture and veterinary medicine (Djukic, 2018).

The aim of study was to systematize and analytically investigate information data from the scientific literature on outbreaks of *American foulbrood* in the world. Based on the data obtained, to characterize the etiology of the disease, determine epizootological features, clinical signs, and present modern diagnostic methods.

Material and methods. The study was carried out by studying and analyzing information data from scientific literature on American foulbrood. The material for the article was statistical data from WOAH (WAHIS), ProMED (WOAH, 2025; International Society for Infectious Diseases, 2025).

Results. Typical symptoms of bee blight were mentioned in works written before the Christian era and by Aristotle (384–322 BC), where lethargy of bees and an unpleasant smell from the hive were noted (Genersch, 2010; Yost, 2016). Schirach used the term “foulbrood” to encompass the collective concept of bee brood pathology, and in 1771 he established that there were two types of “foulbrood” (University of Arkansas Division of Agriculture, 2025) (WOAH, 2025). In 1885, Cheshire and Cheyne published data containing the results of studies on bee brood pathology, including a description of *Bacillus alvei* (Cheshire, 1885). For more than a decade after the appearance of this work, it was generally accepted that there was only one pathogen, *Bacillus alvei*, capable of causing bee brood pathology. During the decade from 1890 to 1900, many American beekeepers, especially in New York State, noticed differences in the clinical presentation and course of the disease, which in turn may indicate two different diseases. Soon, in the United States, *American foulbrood (AFB)* and *European foulbrood (EFB)* were distinguished on the basis of course and clinical presentation (Shimanuki, 2000).

Considering the characteristics of the disease and its prevalence on five continents, during the 19th century, the following names were found in literary sources: in Switzerland – “nichtstinkende Faulbrut” (Burri, 1904), in Austria and Australia – «Faulbrut» (Muck, 1915), in Germany, England and Ireland – «Brutpest» (Zander, 1928).

Initially, the AFB pathogen was classified as a *Bacillus* genus, but in 1993, it was reclassified and separated into a separate genus, *Paenibacillus larvae*, based on a number of characteristics (Amšiejute, 2022). The gram-positive bacterium *Paenibacillus larvae* is the etiological agent of *AFB* in honey bees, a disease that is notifiable in many countries. The pathogen forms spores that are resistant to the external environment, as well as to physical and chemical factors. In pathological material, remains of dried larvae, scales, old honeycomb, endospores remain infectious for up to 35 years, and endospores released from wax, propolis, honey and other beekeeping products retain their infectious ability for more than 70 years (Pernal, 2008; Khezri, 2013; Splitt, 2022; Binney, 2023; Erban, 2024).

Paenibacillus larvae spores are infective only to worker bee larvae; drone and queen larvae, and adult bees are not infected by ingesting spores. Larvae are most susceptible to infection in the early stages, i.e. 12–36 h after egg hatching. The minimum infectious dose is considered to be the ingestion of about ten spores through contaminated larval food. The pathogen can accumulate in an amount of more than 1 billion spores on an infected larva. In sealed cells, the pathogen penetrates the larval intestinal wall into the hemolymph and spreads throughout the body within two days (Truong, 2023). The larvae die, and the pathogen continues to multiply for another 10–12 h; sporulation occurs after 7–10 days. Within the colony, the

pathogen is spread by nurse bees and cell cleaner bees. Transmission factors are honey, beehives, combs, hives, wax, beeswax, apiary equipment and equipment. Spread between colonies occurs through drones and thief bees. American foulbrood appears in the apiary in the second half of June - August (Alburaki, 2024).

Clinically, the loss of body segmentation is observed in diseased larvae. They become grayish and die after the cell is sealed (before the larva turns into a pupa). The wax caps over the dead larvae sink and have a hole. The dead larvae rot, turning into a sticky putrid mass of milky-coffee color with the smell of boiled carpenter's glue, which is a characteristic sign of this disease. The corpses of the larvae dry out and turn into dark brown scales that stick to the side walls of the cell and cannot be cleaned by the bees. The brood has a motley appearance. Depending on the number of sick and dead larvae, a weak, medium and strong degree of damage to the brood is distinguished. With a weak infestation of the brood, up to 10 sick and dead larvae are found in the nest, with an average infestation, from 10 to 50 larvae on each honeycomb frame with brood, and with a strong infestation, more than 50 sick and dead larvae on each honeycomb frame (Obshta, 2023).

AFB is distributed worldwide and cases have been reported in almost all regions where *Apis mellifera* or other bee species are kept and bred (Fig. 1). According to the ERIC (enterobacterial repetitive intergenic consensus) classification, five genotypes are distinguished, i.e. I, II, III, IV and V, which differ in virulence and prevalence in colonies. Genotypes ERIC I and ERIC II have been the most frequently isolated worldwide (Zabrodski, 2020). Genotypes ERIC III and ERIC IV have not been isolated for a long time (Papić, 2024), but according to Beims et al., 2020, genotype ERIC V was recently detected in Spanish honey samples (Beims, 2020). All five genotypes have different endospore shapes. Morphologically, endospores of genotypes ERIC I and II have a smooth surface, while endospores of genotypes ERIC III–V have longitudinal membranes. Although all genotypes cause the same clinical manifestations in bee larvae—a sticky, stretchy mass that eventually dries into scales—there are bacteriological differences between them in terms of colony appearance, pigmentation, hemolysis, carbohydrate fermentation, and degree of virulence. Genotype ERIC I causes 100% mortality of infected larvae after 10–12 days, genotypes ERIC II–IV kill infected larvae after 6–7 days, and genotype ERIC V kills larvae after 3 days (Beims, 2020). In the case of infection of *P. larvae* of genotype ERIC II, clinical manifestations may be mild because larvae die earlier and bees clean their cells earlier, and therefore testing may result in false-negative results for AFB (Jończyk-Matysiak, 2020).

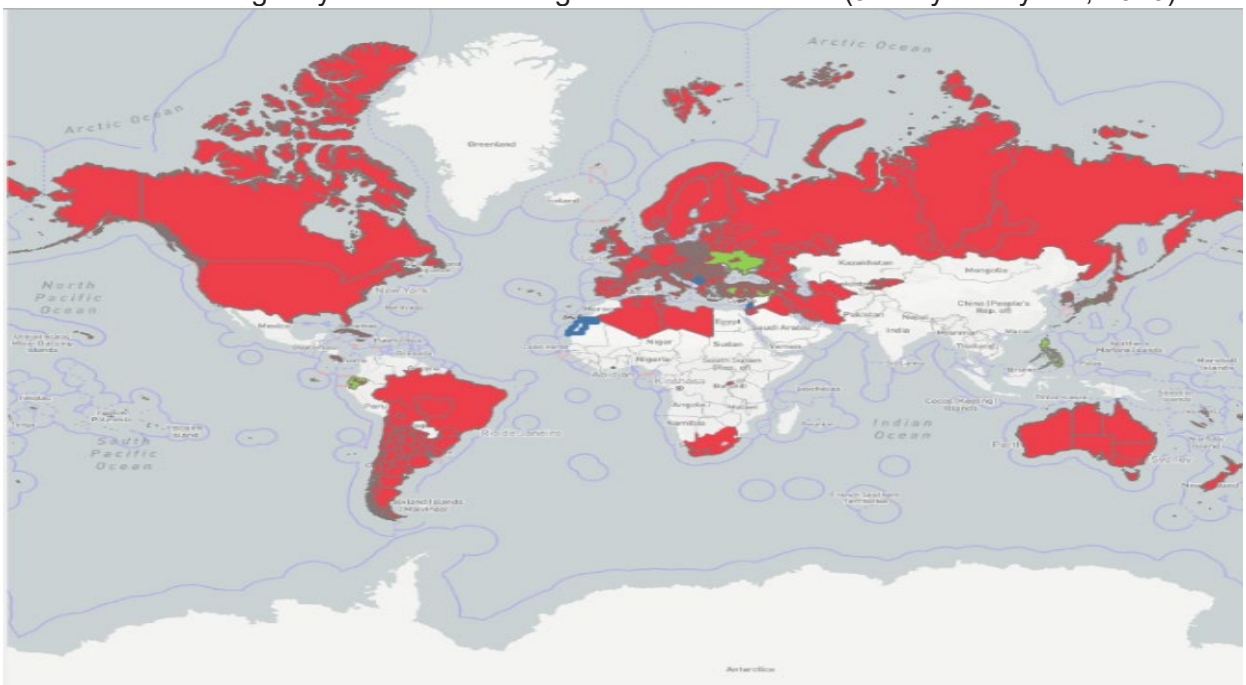

Fig. 1. Registration of American foulbrood in the world during 2005–2025 (WOAH, 2025)

The prevalence of ERIC genotypes varies between countries and continents. Genersch et al. (Genersch, 2010), studying P. larvae strains from Germany, Finland and Sweden, found only ERIC I and II genotypes. In Austria and Italy, only ERIC I and II genotypes were confirmed. In a

study of South and North America, ERIC I genotypes were found among Argentine and Uruguayan strains (Castelli, 2019). In the USA, ERIC I genotype was confirmed (Krongdang, 2019). ERIC V genotype was found in a honey sample from Spain (Nowotnik, 2024).

AFB was suspected in South Africa after the Zimbabwean authorities received reports in July 1996 that bee colonies imported from Johannesburg were possibly infected with *P. larvae* (Beekeeping Association, 1996). However, laboratory tests of 57 honey samples from 57 apiaries failed to confirm the presence of P. larvae spores. This was not the case until 2009, when the South African Department of Agriculture inspected bee colonies and the honey retail chain, which confirmed the presence of AFB in the Western Cape. According to the World Organisation for Animal Health (WOAH), AFB outbreaks have been reported intermittently in North and East Africa since 2005 (Fig. 2). The low prevalence of American foulbrood in Africa and sub-Saharan Africa is likely due to primitive beekeeping, hygienic behavior, and the physiological susceptibility of African bees. However, in temperate continental countries, where the disease is widespread, the situation is somewhat different.

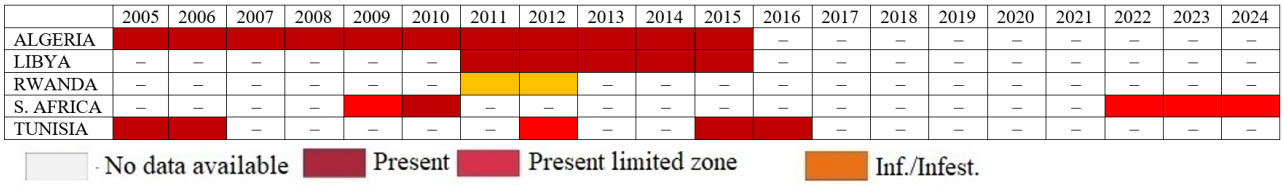

Fig. 2. Outbreaks of American foulbrood on the African continent (WOAH, 2025)

AFB was first officially recorded in South America in Argentina in 1989, and it was assumed that the pathogen had entered the country through bees imported from the USA (Alippi, 2004; Antúnez, 2007; Alburaki, 2025).

AFB quickly spread to most of the important beekeeping centers of the country, with incidences of up to 30,0% in some geographical regions of the continent. In total, from 30,0 to 45,0% of bee colonies were lost to AFB during 1989–1996. The spread of the disease was stopped in 2002 after its detection in Chile. However, despite all efforts, new outbreaks of the disease were detected in 2005 in different regions of the American continent.

In south-central Canada, in Saskatchewan, AFB cases are detected through a combination of random inspections and investigations of suspected beekeepers. In 2018, four AFB outbreaks were reported, mostly among small-scale and amateur beekeepers. In 2019, the number of cases increased to 8, but mostly in commercial operations (Fig. 3). In a study of 4790 bee brood samples collected from 2015 to 2022 by state inspectors in the United States of America from 49 states, AFB was detected in 31 states. Infection rates varied between states, ranging from 0,0 % до 54,8 % (Alburaki, 2024).

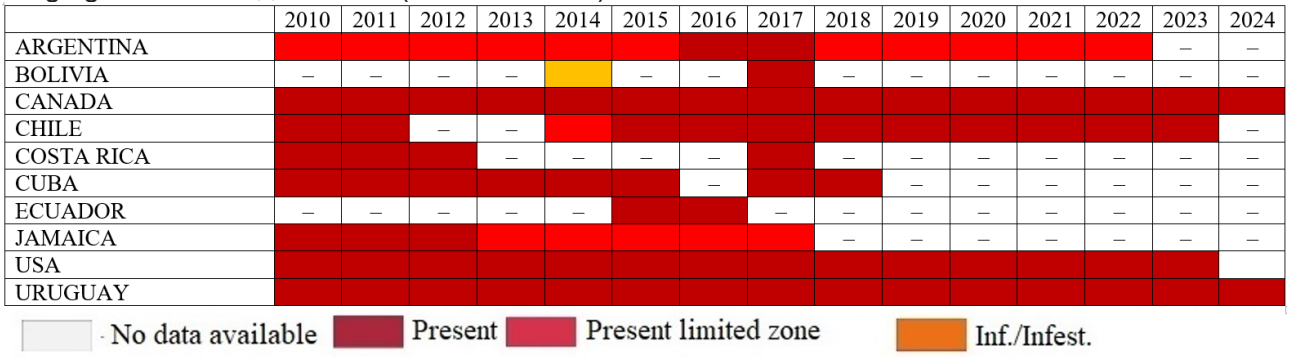

Fig. 3. AFB outbreaks in the Americas during 2010–2024 (WOAH, 2025)

In an assessment of AFB prevalence in Asia using PCR, two out of 100 samples from bee apiaries in Kurdistan province were found to be positive, indicating a relatively low prevalence of AFB in the province's apiaries. Studies conducted in Hatay and Adana provinces in Turkey showed a prevalence of AFB of 29,0% (Fig. 4). The prevalence of AFB in northwestern Pakistan was 37,3%. A total of 57 samples from different regions of Jordan were found to contain *Paenibacillus larvae* spores, accounting for 35,0% of the total samples tested (WOAH, 2025). In a study of honey samples from Tehran province, 25,6% of samples were found to contain AFB, indicating a relatively high prevalence of the disease in apiaries (Khezri, 2018). In 2010,

Yusefkhani M. and Lotfi A. reported that the level of AFB infection in bee colonies in East Azerbaijan province was 5,8%. By studying bee brood in Lorestan province by PCR method, it was possible to establish that the level of hive contamination was 13,0% (Binney, 2023). According to WOAH data, during 2010–2024, Cyprus, Iran, Israel, Japan, Jordan, Korea, Turkey can be considered as permanently unfavorable for AFB, where AFB is registered every year.

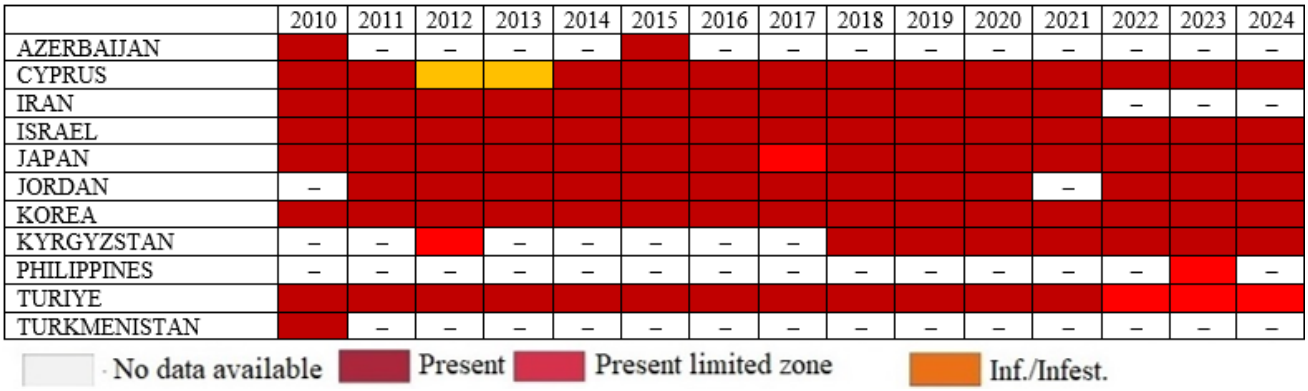

Fig. 4. AFB outbreaks on the Asian continent during 2010–2024 (WOAH, 2025)

During 2010–2024 AFB became quite widespread on the European continent. It was reported in 34 countries (Fig. 5). According to WOAH, in Western Europe, in Austria, the number of registered outbreaks varies significantly from year to year, and the largest number of registered outbreaks was observed in 1998 – 211 and in 1999 – 383. In the period from 2010 to 2024, there is a positive trend towards a decrease in the number of outbreaks and, accordingly, as of 2024, 19 cases were registered. In order to eliminate the disease in Austria, 1,181 bee colonies were destroyed in the period from 2010 to 2024. In Belgium, only 23 cases were recorded from 1997 to 1999 among approximately 10,000 beekeeping farms, but after the detection of Paenibacillus larvae spores in honey samples from colonies that did not show clinical signs of the disease in 1999, the low prevalence of AFB in Belgium was questioned (De Graaf, 2001). During the period 2010–2024, 101 new outbreaks of the disease were recorded in this country and 301 colonies were destroyed.

In Germany, the number of outbreaks varied from 100 to 400 affected apiaries per year between 1950 and 2001. According to Otten et al., the lowest number of outbreaks was found in Baden-Württemberg, Bavaria and Saxony-Anhalt between 1980 and 2001 (0,2 affected apiaries per 1000 families), while, for example, in North Rhine-Westphalia, it was 0,8 apiaries per 1000 families (Otten, 2003). The studies concluded that colony density did not significantly affect the spread of the disease, as Baden-Württemberg and Bavaria had the highest colony densities of 5,1 and 4,9 colonies per square kilometer, respectively, but the lowest number of outbreaks was recorded there. On the other hand, in North Rhine-Westphalia, with its low colony density, many more outbreaks were observed, suggesting that climatic conditions, bee migrations and different Paenibacillus strains may be responsible for these regional differences. The difficult situation with AFB outbreaks in Germany remained from 2010 to 2024, with 2,913 cases recorded during this period. In Switzerland, only 660 outbreaks were observed between 2010 and 2024; 1,424 colonies were destroyed to eliminate the disease. The situation is also quite difficult in France, where AFB is detected every year. In Eastern Europe, in Bulgaria, 350 outbreaks were recorded in 1989, during which 4,800 colonies had to be destroyed. During 271 outbreaks in 1992, 1910 colonies were destroyed. The following years, 1993–2008, were characterized by a significant decrease in the number of outbreaks – within 32–76, with an increase to 102 in 2013. Since 2019, the situation has become more controlled, however, the pathogen is detected from time to time, but the disease is not of a mass nature (Antúnez, 2007; Alippi, 2005). In Moldova, from 2010 to 2024, 1823 new outbreaks were registered and 49178 bee colonies were destroyed, while in Poland, 142638 new outbreaks were registered during this period, in Romania – only 225 outbreaks, during which 4025 bee colonies were destroyed, in Slovakia – 958 outbreaks and 3054 bee colonies were destroyed. Rudenko E.V. with colleagues (Rudenko, 2023) in 2023, studying 18 samples of commercial honey obtained in Ukraine, revealed negative trends in general microbial contamination, in particular by the pathogens of rot Paenibac. larvae and Paenibac. alvei. The difficult situation with AFB is also observed in the territory of Southern (Bosnia and Herzegovina, Greece, Italy, North Macedonia, Portugal, Serbia, Slovenia, Montenegro) and

Northern Europe (Great Britain, Denmark, Estonia, Ireland, Iceland, Latvia, Norway, Finland, Sweden) (Elzen, 2002; Crane, 1999; Brady, 2017).

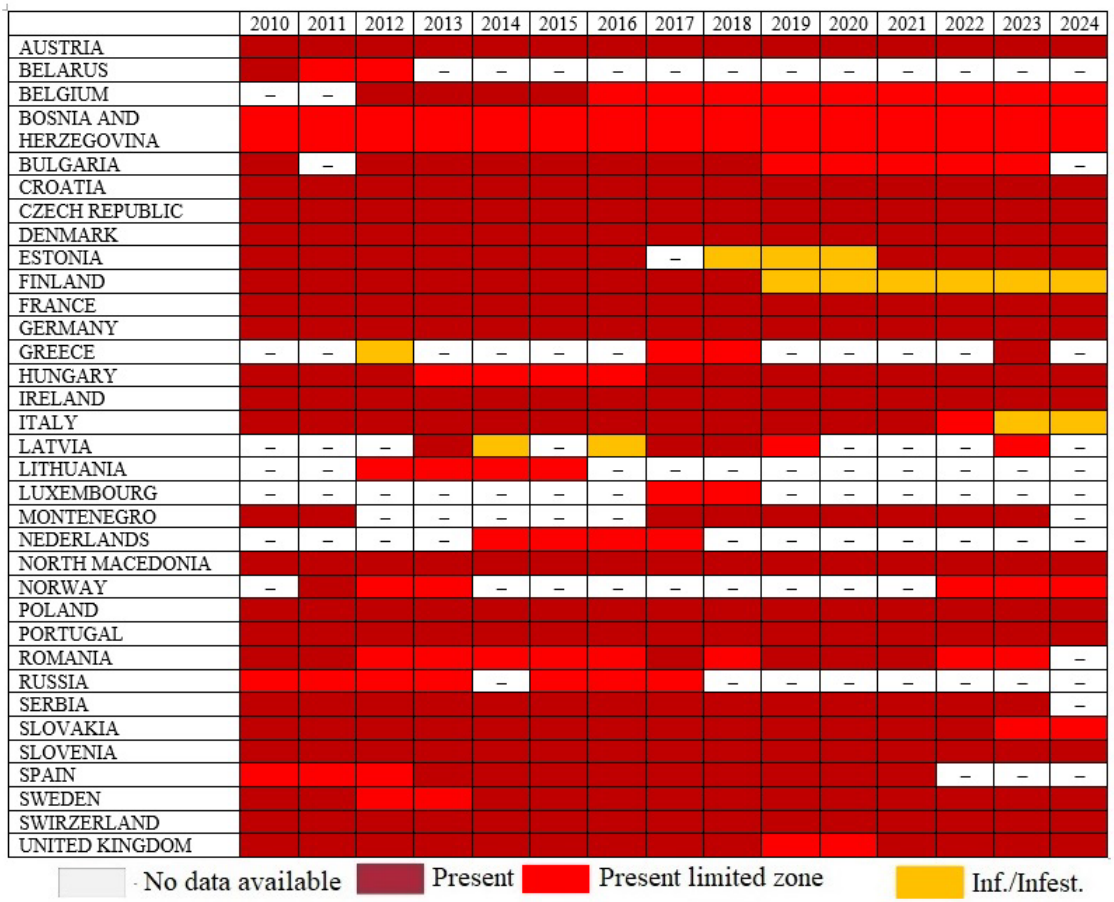

Fig. 5. AFB outbreaks on the European continent during 2010–2024 (WOAH, 2025)

AFB diagnosis is established comprehensively, taking into account clinical signs, laboratory studies and epizootological data. Laboratory diagnostics of AFB is based on the identification of the pathogen (Matiašovic, 2023). In practical terms, the selection of pathological material samples depends on the purpose of the laboratory study (diagnosis or monitoring). Laboratory studies are carried out in an accredited and authorized laboratory. Microscopic, bacteriological, serological and molecular genetic research methods are used to identify AFB (Hansen, 1999). The priority research methods are bacteriological and PCR methods, which are highly sensitive and specific. These methods are also able to detect the pathogen in bee colonies that do not have visible clinical signs, which makes it possible to predict the outbreak of the disease and prevent it in time (Govan, 1999; Kušar, 2021).

According to the WOAH recommendations, a whole comb or a 20 cm² sample containing the affected brood is taken from a sick or suspected bee colony (El-Meihy, 2024). In order to facilitate packaging and transportation, it is customary to collect the remains of larvae or pupae from the cells using a sterile applicator. For microscopic examination, thin smears of putrefactive mass or scales are prepared, which are pre-soaked in sterile warm (30–40 °C) saline for 20–30 min (de Graaf, 2015; Dobbelaere, 2001; Stephan, 2020). After air drying, the smears are packaged for shipment to the laboratory for microscopic examination. Every colony in the vicinity of such a clinical case of AFB should be considered suspect, and a wide range of samples should be taken to confirm (or refute) the suspicion. Colonies located adjacent to clinically affected ones are considered suspect. For confirmation, it is recommended to take samples of adult bees, honey, hive debris and larvae for complex analysis (bacteriological and PCR). It is recommended to conduct early diagnostics of neighboring colonies (without clinical signs) by selecting a wide range of samples for PCR and predicting the possible development of AFB. Combined diagnostics should be used: clinical examinations, microscopy and molecular methods (Beekeeping Association, 1996; Liu, 2017; Daisley, 2020). In addition to brood samples, honey, pollen and royal jelly, adult bees and crumbs from the bottom of the hive can be taken to detect the presence

of P. larvae spores. Thus, the detection of spores is possible not only in brood, but also in honey, pollen, adult bees and debris on the bottom of the hive; this allows predicting the development of AFB in the colony. To obtain the most reliable actual situation, bees from the brood nest (and not from honeycombs) should be analyzed. Honey samples can be used to detect AFB in colonies where no clinical signs are observed and for a rapid detection monitoring program (Morrissey, 2015).

Preventive measures for AFB include compliance with veterinary and sanitary norms and rules, namely: use only disinfected equipment (frames, hives, knives, etc.); avoid transplanting brood from other apiaries; regular disinfection of hives (heat treatment, burning, chemicals – 2,0% caustic soda solution, chlorine-containing preparations, etc.); control over the origin of queen bees and packages; purchase queen bees and packages only from safe apiaries with appropriate veterinary documents; biological and organizational methods; replacement of old frames with new ones (change at least 30,0% of frames each year); periodic transplanting of bees into clean hives; destruction or removal of weak families that may be a source of infection (Genersch, 2010; Dobbelaere, 2001). Therapeutic and prophylactic feeding, use of permitted antibiotics (e.g. terramycin) should only be carried out under the supervision of a veterinarian in compliance with the requirements of national and international legislation. Constant monitoring of the condition of the brood, regular inspections of hives for the presence of characteristic signs of the disease (musty smell, rotting brood, sticky cell contents). At the slightest suspicion - notification of the veterinary service and isolation of affected families.

Conclusions. The spread of AFB in the world remains a pressing problem for beekeeping. However, through preventive measures, proper control and improvement of conditions for bees, it is possible to reduce the risk of outbreaks and protect bee colonies from this disease.

For effective detection of AFB, it is necessary to use a combination of microscopic, bacteriological, serological and molecular genetic methods. Bacteriological and PCR methods are especially important, which have high sensitivity and specificity, allowing to detect the pathogen even in colonies without clinical signs of the disease. This makes it possible to take timely measures to prevent and prevent the spread of the disease.

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Епізоотологічні, клінічні особливості та заходи контролю американського гнильця у світі (огляд літератури)

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Резюме. *У статті узагальнено та проаналізовано сучасні наукові дані щодо епізоотологічних і клінічних особливостей американського гнильця (AFB) бджіл, збудником якого є спороутворююча бактерія Paenibacillus larvae. Висвітлено історичні аспекти вивчення хвороби, біологічні властивості збудника, його високу стійкість у навколишньому середовищі та здатність зберігати інфекційність тривалий час. Охарактеризовано генотипи ERIC I–V, їх поширеність і відмінності за вірулентністю. На підставі даних WOAH (WAHIS) і ProMED проаналізовано сучасну епізоотичну ситуацію у*

країнах Європи, Азії, Африки та Америки, що свідчить про глобальне поширення захворювання та його значний економічний вплив на галузь бджільництва. Описано типові клінічні ознаки AFB, зокрема строкатість розплоду, запалі кришечки комірок, наявність тягучої гнильної маси та утворення щільних лусочок. Розглянуто сучасні підходи до лабораторної діагностики, включаючи мікроскопічні, бактеріологічні та молекулярно-генетичні методи з акцентом на високу чутливість ПЛР для раннього виявлення збудника. Узагальнено основні профілактичні заходи, що передбачають дотримання ветеринарно-санітарних норм, регулярний моніторинг пасік, контроль переміщення бджіл та своєчасну ліквідацію неблагополучних сімей, що є ключовими для запобігання поширенню американського гнильця.

Ключові слова: американський гнилець, медоносна бджола, епізоотична ситуація, фактори поширення збудника, діагностика, оцінка ризику.

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