FACTORS INFLUENCING THE OCCURRENCE OF DISEASES AND ANOMALIES IN THE QUEEN HONEY BEE, WITH FOCUS ON QUEEN SELECTION

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Abstract. Among the numerous factors that affect the health of the bee colony, diseases, as well as congenital, developmental anomalies of the queen, occupy a significant place. Understanding the occurrence and course of diseases, their spread, control and treatment is a key factor in preserving the health and reproduction of the queen bee, as well as the entire bee colony. *Varroa destructor*, viruses and nosema are the most important factors that lead to the loss of bee colonies, as well as the queen bee itself. However, there are also diseases that only queen bees suffer from, as melanosis, carrying immature eggs, carrying unfertilized eggs, blockage of the oviduct with sperm cells etc. In addition to the aforementioned diseases, various anomalies can also occur in the queen during its development. In addition to pathogenic microorganisms, other biotic and abiotic factors, as genetic factors also affect the health and quality of the queen. Genetic selection of queens can greatly reduce the incidence of infectious diseases in bee colonies. That is why the health of the queen bee, as well as the ability of the bee colony to prevent the occurrence of certain diseases, is of increasing importance in selection and breeding programs.
Keywords: *Apis mellifera, breeding, stress factors, pathogens, Montenegro*

Abbreviations: BLUP animal model - best linear unbiased prediction animal model; DWV - deformed wing virus; CBPV - chronic bee paralysis; ABPV - acute bee paralysis virus; IAPV - Israeli acute bee paralysis virus; SBV - Sacbrood virus; BQCV - black queen cell virus; *Varroa destructor - V. destructor; Apis mellifera - A. mellifera; Apis mellifera carnica - A. mellifera carnica; Nosema apis - N. apis, Nosema ceranae – N. ceranae*

Introduction

The quality of a bee colony is crucially affected by the health and quality of the queen (Porporato et al., 2015; Zhong et al., 2024). A healthy and high-quality queen is essential for the survival of a bee colony because of her ability to lay a large number of eggs, but also because the queen's pheromones affect the coherence of the colony (Amiri et al., 2017; Facchini et al., 2021; Holmes et al., 2023; Copeland et al., 2024). The success of egg laying depends on numerous internal and external factors, such as: genetic traits, availability of space for egg laying, age of the queen bee, presence of diseases and/or anomalies, climatic conditions, air humidity and temperature, availability of food, toxicity of substances from agricultural activities or pollution, stress during transport of the queen, presence of predators, unsuccessful mating (Czeńkoska, 2000; Anderson and Trueman, 2000; Vidau et al., 2011; Porporato et al., 2015; Amiri et al., 2020b; Rehman et al., 2024. The health of a bee colony is significantly affected by diseases, as well as congenital, developmental anomalies, i.e. defects of the queen. The queen, like other castes of bees, can suffer from varroosis, nosemosis, acarosis, amoebic disease, "May" disease, septicemic diseases, viral diseases—such as deformed wing virus disease, acute paralysis, chronic paralysis, black

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queen cell disease etc. (Lolin, 1991; Plavša and Pavlović, 2017; Bojanić Rašović, 2024). Of the viruses, deformed wing virus and black queen cell disease virus are particularly important for the health of the queen bee (Zhang et al., 2012; Plavša and Pavlović, 2017). The parasitic varroa mite, viruses and nosema are the most important factors leading to the loss of bee colonies, as well as the queens themselves (Plavša and Pavlović, 2017; Rakić, 2020). These factors contribute to the emergence of collony collapse disorder. Diseases such as varroosis are a major stress factor for bee colonies. The mite *V. destructor*, in addition to feeding on the hemolymph and fat body of the bee, also serves as a vector of viruses. *V. destructor* viruses 2, 3, 4, 5 and 9 are only present in bees infested with *V. destructor* (Kim et al., 2026). The health condition of the bee colony is worse if it is simultaneously attacked by parasites, bacteria and viruses, due to the weakening of the immune response (Lannutti et al., 2022., Mazur et al., 2025). Working together with viruses *Varroa* has a very harmful effect on bees, leading to their premature death (Dahle et al., 2010; Hristov et al., 2020; Nekoei et al., 2023; Spurny et al., 2017; Chagas et al., 2020). Varroa infestation can lead to a reduction in the body weight of worker bees from 6.3% to 25% (de Jong et al., 1982; Hilsmann et al., 2025). Queen bees infected with nosema stop laying eggs, especially in the spring, and die a few weeks after infection, usually outside the hive (Galajda et al., 2021). Nosemosis was found in all 40 examined provinces in Turkey, in a total of 1194 examined bee colonies. The average prevalence ratio was 64.3%, the maximum prevalence value 95%, and the minimum 40.5%. *N. ceranae* was dominant, it was detected in all positive hives, while co-infection of *N. ceranae* and *N. apis* was found only in four colonies (Akpınar et al., 2024). Infection with *N. ceranae* can cause a sudden collapse of bee colonies. *N. ceranae* was found in bee samples collected from all 21 examined districts and all three climatic areas—Mediterranean, mountainous and continental part of Croatia (Tlak Gajger et al., 2010).

However, there are also diseases that only queens suffer from. These are: melanosis, carrying immature eggs, carrying unfertilized eggs, blockage of the oviducts with sperm cells, blockage of the genital tract with excrement, catalepsy. In addition to the aforementioned diseases, various anomalies can occur in the queen during its development. The most common and most significant developmental anomalies of the queen are: queen stunting, deformed wings, ovarian hypoplasia, underdevelopment of the oviducts, spermatheca anomalies etc. (Porporato et al., 2015; Plavša and Pavlović, 2017; Rakić, 2020; Santrač et al., 2021). The aim of the paper was to point out the basic characteristics of queen bee diseases and anomalies, factors that influence their occurrence, as well as measures for their control. Special attention was paid to the importance of queen selection and breeding for her health and the health of the bee colony.

Basic characteristics of the queen honey bee

The queen bee is the only reproductively capable individual of the female sex in the bee society. It lives much longer than bee workers and drones, an average of 2-5 years, and it has its highest productive activity during the first two years of life (Stanimirović et al., 2000; Porporato et al., 2015; Amiri et al., 2017; Copeland et al., 2024). Already at the end of the second year, due to the reduced amount of sperm, her ability to lay eggs drops significantly. After the first year of the queen's life, only 38% of spermatozoa are found in her spermatheca, and after the second year only 21% compared to the number of

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spermatozoa in a newly mated queen (Al-Lawati et al., 2009; Porporato et al., 2015). That is why in modern beekeeping it is recommended to replace the queen every year or every other year. A good queen bee in good grazing conditions can lay up to 2000 eggs per day, the total weight of which is greater than the queen's weight. The weight of bee queen *A. mellifera* is about 0.2 g (186.5–209.0 mg). Queen bees produce eggs throughout their entire lives, but mostly during the first two years (Stanimirović et al., 2000; Kahya et al., 2008; Avni et al., 2014; Amiri et al., 2017; Aamidor et al., 2022). Bee workers and queens hatch from fertilized eggs, and drones from unfertilized eggs. To lay eggs, the queen consumes a lot of energy and therefore it is necessary to feed on royal jelly. The reproductive system of the queen consists of two ovaries, two fallopian tubes that flow into one common fallopian tube, a seminal vesicle and a vagina (Stanimirović et al., 2000; Porporato et al., 2015; Kozii et al., 2021a). Each of the ovaries contains 233–438 ovarioles–oviducts (Jackson et al., 2011). There are usually 5-6 million spermatozoa in the spermatheca of a fertilized queen, which is enough for her reproduction for the rest of her life. The length of life of the queen directly depends on the number and vitality of the stored spermatozoa in the spermatheca. The sperm unites with the egg in the vagina and fertilization of the egg occurs. Mating with 15 or more drones ensures genetic diversity within the bee colony, and therefore a greater chance for the colony to survive (Porporato et al., 2015; Phokasem et al., 2021). Queen cells are the largest cells in the honeycomb. The period from egg laying to the formation of a young queen lasts 16 days. After 3-6 days of hatching, the queen enters the heat, and mating occurs at the age of 8-10 days, rarely after 3-4 weeks. Mating of the queen and drones takes place outside the hive, during the flight. A quality queen bee should have the required weight, be healthy, have a large spermatheca and a large number of spermatozoa, as well as a large number of ovarioles, have high egg-laying ability, high brood production, good hygienic behavior, good honey yield, calmness of the bees on the honeycomb and a low tendency to swarm (Hatjina et al., 2014; Porporato et al., 2015).

The body weight of the queen bee at hatching is most often used as an indicator of queen bee quality. However, it should be borne in mind that the body weight of the queen bee varies drastically depending on the period of her life as an adult. The body mass of the queen bee gradually decreases from the moment the queen bee is hatched until mating, with the fastest loss during the first 36 h. During this time, queens lose about 40 mg of their body weight (Skowronek et al., 2004). Queen bees with a larger body mass lose more body mass during hatching than lighter queen bees. This is explained by the fact that flying during mating requires a lighter body of the queen bee due to easier lifting and a longer duration of flight, all with the aim of as successful mating as possible. After mating, queen bees begin to increase, i.e. that they return their body mass to the level after laying down. Restoring the body weight of the queen bee has a positive effect on her better adoption into the new bee colony (Yadava et al., 1971; Skowronek et al., 2004; Kahya et al., 2008; Amiri et al., 2017). In addition to the influence of natural variations and environmental conditions, hereditary factors also influence the weight of the queen at hatching (Moritz et al., 2005).

Queen bee diseases

Diseases that occur in queens can be common diseases with other castes of bees or they are diseases specific to the queen, primarily defects and anomalies of the reproductive tract of the queen. Queens are susceptible to most infectious and parasitic

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diseases that attack worker bees, including varroosis, nosemosis, acarosis, viral diseases, bacterial septicemia, "May" disease, amoebic disease, etc. (Plavša and Pavlović, 2017; Bojanić Rašović, 2025a). Queens are considered to be less susceptible to infections than bee workers—because they are continuously monitored and fed by young worker bees that provide them with physical and social support, and thus protection from infection. In addition, queens have a unique immune defense system. The queen bee is protected from pathogens by several social immune mechanisms, such as nurturing by nursing companion bees, secretion of antimicrobial substances in royal jelly, and isolation within the hive, but she is not completely immune to infections. Gut microbiota plays a significant role in nutrient absorption and overall immune function in honey bees. The composition of the mother's gut microbiota changes with age, potentially affecting her health and susceptibility to disease (Copeland et al., 2022). Intestinal microflora has also been found to influence the ovarian metabolism and reproductive abilities of the queen bee (Li et al., 2023). The health and immunity of the queen are related to the expression of vitellogenin. Vitellogenin is an egg yolk protein that plays a role in immunity, oxidative stress, nutrition and longevity (Copeland et al., 2024). Due to the modern way of beekeeping, queens are an important factor in the spread of diseases between colonies, even to distant destinations. Beekeepers usually replace queens due to their poor egg-laying ability, aggressive behavior of the colony, etc., but rarely because of their illness. This is because queen bee diseases are rarely recorded and reported, and knowledge about infectious and other queen diseases is insufficient. A healthy, high-quality queen can mitigate the effects of disease on the bee colony and make it more resistant to disease (Lolin, 1991; Amiri et al., 2017; Plavša and Pavlović, 2017; Santrač, 2021; Copeland et al., 2024). The most important queen diseases common to other bee castes are shown in *Table 1*.

Viral diseases

The largest number of viruses that cause bee diseases is classified in the order *Picornavirales*. So far, over 30 viruses that infect honey bees have been registered (McMenamin and Flenniken, 2018; Brutscher et al., 2015; DeGrandi-Hoffman and Chen, 2015; Amiri et al., 2020a). A bee colony can be infected with viruses without showing clinical symptoms of the disease. However, if a strong viral infection occurs, the manifestation of the disease can be very severe and the bees may die. Many bee viruses damage the brain of bees, which leads to a weakening of their sensory organs, disorientation, impaired memory and learning, difficulties in collecting nectar, inability to fly, and death (Santrač, 2013; Cirkovic et al., 2018). The occurrence and intensity of viral diseases depend on a large number of stress factors, which weaken the resistance of the colony (Amiri et al., 2017; Bojanić Rašović, 2019a, b; Santrač, 2021). These factors affect the bee's immunity, i.e. the success of its defense against virus infection (Norton et al., 2025). Stress factors that contribute to the occurrence of viral diseases of bees are: low nectar yield, migration of bee colonies, queen rearing, excessive feeding of bees with sugar, cold winters, cold, temperature fluctuations, overcrowding of apiaries, humidity, hunger, poisoning, infection of bees with other pathogenic microorganisms of bees, infestation with parasites, etc. These negative factors stimulate the multiplication of viruses, whereby the disease can pass from a latent form to a lethal form—which ends with the death of bees. Together with other pathogenic microorganisms, viruses act synergistically and have a stronger negative impact on the manifestation of the disease (Nazzi and Pennacchio, 2018; Harwood and Dolezal, 2020;

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Parekh et al., 2021; Tlak Gajger et al., 2025; Lamas et al., 2026). Unlike worker bees, queen bees rarely show symptoms of viral diseases. The occurrence of viral diseases in queen bees directly depends on her contact with infected worker bees. Therefore, the queen does not have to be infected in hives where the virus is present. Queen bees are less exposed to viruses, because queen bees are not attractive to varroa mites. It is believed that queen bees are most often infected with viruses through nutrition and vectors—varroa mites (Ullah et al., 2021; Bojanić Rašović, 2019a, 2025b; Kevill et al., 2020; Nekoei et al., 2023). Several viruses cause serious health problems in queen bees, and some of them can be transmitted by the queen to subsequent generations (Ravoet et al., 2015). Studies have shown that 93% of queen bees are infected with multiple viruses. The viruses found are black queen bee virus in 85%, chronic bee paralysis virus in 14%, deformed wing virus in 100%, Kashmir bee virus in 21% and sacbrood virus in 62% of the tested queens (Francis et al., 2013). Gregorc and Bakonyi (2012) found DWV, BQCV, SBV and ABPV in 58%, 24%, 11% and 10% of queen bees, respectively. Viral infections inhibit egg laying in honeybee queens and lead to their premature cessation of growth (Chapman et al., 2024).

Deformed wing virus

Deformed Wing Virus has been found in various parts of the world and is the most common virus in bee colonies. It causes deformed wings and reduced size of bees. This virus has been found in all developmental stages of bees – in eggs, larvae, pupae, adult bees of all castes (queen, worker, drone) (Francis et al., 2013; Lang et al., 2024; Copeland et al., 2024). It is most often transmitted during feeding from nurse bees to larvae. Depending on the stage of bee development at which the infection occurs, brood may fail due to larval death, and the life of worker bees is shortened. Deformed wings appear in bees that have just emerged from the cells. Queens are infected during mating – fertilization (Fievet et al., 2006; Amiri et al., 2016). DWV virus has been found in drone samples and sperm collected from the spermatheca of the queen, all of which indicate the possibility of sexual transmission of this virus. It has also been found that the virus can be transmitted vertically from an infected queen to her offspring. *V. destructor* is a biological vector for the transmission of DWV virus and enhances its pathogenicity, as it directly inoculates it into the hemolymph of larvae and adult honey bees (Sircoulomb et al., 2025; Norton et al., 2025). This virus is now ubiquitous in all regions where varroa is present. The virus has been detected in both older and younger queens. Young queens usually have lower levels of virus than older queens, indicating that the virus replicates in the queen throughout her life. In queens, deformed wings rarely occur as a result of infection with deformed wing virus. However, deformed wing virus has been found to attack the head, fat tissue, intestines, and ovaries of queens (Fievet et al., 2006; Bouuaert et al., 2021). High virus titers in reproductive tissue can lead to ovarian degeneration and affect the viability and viability of stored sperm. Since queens mate with more than 15 drones, each new mating increases the risk of queen infection. Queens have been found to have mechanisms to reduce the consequences of DWV infection (Amiri et al., 2017., Yue et al., 2006; Brutscher et al., 2015; Kevill et al., 2020). If varroa is not present, DWV is found in low titers within the colony and there are no visible symptoms (Copeland et al., 2024). DWV-B, BQCV and Lake Sinai Virus were the most common viruses – detected in > 68% of colonies tested. BQCV and DWV-B had the highest prevalence in mated queens, 95.19% and 75.85%, respectively. This confirms that mating is critical point for viral infection of queens (Bhandari et al., 2025).

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Table 1. *Queen bee diseases common to other bee castes*

Diseases	Causative agent	Get sick	Transmission	Clinical signs	Disease prevention measures	Disease control measures	Literature source
Viral							
Deformed wing virus (DWV)	Genus *Iflavirus,* fam. *Iflaviridae*	Eggs, larvae, pupae, adult bees of all castes	Through food, during fertilization, through eggs, through the mite *V. destructor*, the small hive beetle *Aethina tumida*	Larval death, wing deformities, shortened life span and death of worker bees	Timely treatment of bee colonies against varroa, strengthening the immunity of bee colonies, good beekeeping practice	There is no specific therapy for viral diseases. Timely treatment of bee colonies against varroa, strengthening the immunity of bee colonies, good beekeeping and hygiene practice, queen bee replacement	Francis et al., 2013; Lang et al., 2024; Copeland et al., 2024., Sircoulomb et al., 2025; Norton et al., 2025; Copeland et al., 2024., Ferreira et al., 2026
Chronic bee paralysis virus (CBPV)	(Unclassified) With the influence of stress factors (pesticides, nosema infection, etc.), genetic factors	All castes of adult bees	Orally through pollen, by contact	Trembling of wings and abdomen, enlarged abdomen, diarrhea, inability to fly, appearance of black bees, loss of hair, death	Strengthening the immunity of bee colonies, good beekeeping and hygiene practices	Supplementary feeding with sugar syrup with vitamins, good beekeeping practice, hygiene measures, selection of virus-resistant queens	Srebočan and Gomerčić, 1989; Lolin, 1991; Amiri et al., 2014, 2017; Diao et al., 2018; Giovanni and Nanetti, 2024. Lolin, 1991; Ribière et al., 2007
Acute bee paralysis virus (ABPV)	Genus *Aparavirus,* fam. *Dicistroviridae*	Bee brood, all castes of adult bees	Orally—most often with contaminated pollen, *V. destructor* tick bite	Young bees crawl in front of the hive, stagger, gather in groups, cannot fly, and die quickly	Regulation of varroa infestation, good beekeeping practice measures, elimination of stress factors, regular queen replacement, bee supplementary feeding	Regulation of varroa infestation, good beekeeping practice measures, elimination of stress factors, regular queen replacement, bee supplementary feeding	Amiri et al., 2014; Ferrufino et al., 2025; Grubić, 2018; Rakić, 2018
Israeli acute bee paralysis virus (IAPV)	Genus *Aparavirus,* fam. *Dicistroviridae*	Eggs, bee brood, all castes of adult bees	Through the bite of the mite *V. destructor*, through food, vertically, sexually	Trembling of the wings, dark color of the abdomen and chest, paralysis and death	Varroa control, strengthening bee colonies, queen replacement, supplementary feeding, good beekeeping practice measures	Varroa control, strengthening bee colonies, queen replacement, supplementary feeding, good beekeeping practice measures	Amiri et al., 2017, 2019, 2020a; Bakonyi et al., 2002; Chen et al., 2014.
Sacbrood virus (SBV)	Genus *Iflavirus,* fam. *Iflaviridae*	Larvae up to 2 days old and young bees	Through contaminated honey, royal jelly, predation, swarming, moving frames from hive to hive, beekeeping equipment, through *V. destructor*	The larvae change color to yellow and brown, turn into a closed fluid-filled sac, fail to progress to the pupal stage, and the young bees die	Provide enough food, especially in spring, and carry out stimulating feeding with sugar syrup, especially in May, hygiene measures	Provide enough food, especially in spring, and carry out stimulating feeding with sugar syrup, especially in May, hygiene measures	Amiri et al., 2017; Wei et al., 2022
Black queen cell virus (BQCV)	Genus *Triatovirus,* fam. *Dicistroviridae*	Queen bee larvae and pupae	Oral, vertical—from mother to offspring	Sick larvae first turn light yellow, then brown and black. The appearance of a black coating on the wall of the queen chamber, in which you can see a dead pre-pupa or queen pupa	Responsible management of the apiary, implementation of adequate hygiene measures when breeding queen bees	Implementation of adequate hygiene measures when breeding queen bees	Spurny et al., 2017; Chagas et al., 2020; Moritz et al., 2010; Amiri et al., 2017; Bojanić Rašović, 2019a; Grubić, 2018.

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Diseases	Causative agent	Get sick	Transmission	Clinical signs	Disease prevention measures	Disease control measures	Literature source
Fungal							
Nosemosis	*N. apis, N. ceranae*	All castes of adult bees	Through the excrement of infected bees, which contains a large number of spores	The bees cannot fly, they crawl along the flight board, fall to the ground and jump around anxiously. Their wings are spread, they gather on the grass in cluster-like piles and die in a short time	Good beekeeping practice measures, regulation of the number of bee colonies at a specific location, regular replacement of combs, disinfection of combs, frames, accessories, strengthening of bee colonies, regular replacement of combs, hygiene of drinking troughs	Burning of extremely weak bee colonies, treatment of bee colonies, transfer of bees from infected to disinfected hives	Gajda et al., 2021; Naudi et al., 2022; Czekońska, 2000; Amiri et al., 2017, 2019; Galajda et al., 2021; Grubić, 2018; Rašić, 2018
Stone brood disease	*Aspergillus flavus*	Bee brood and adult bees	Through food, water, by contact	The larvae first turn into yellowish-white hard mummies, which then turn yellow-green	The bee colony should be overwintered in good, undamaged hives that are leak-proof and well ventilated; the apiary should be located in an elevated, dry, sunny location; the strength of the colony is crucial for protection against aspergillosis; good hygiene and beekeeping practices, equipment disinfection	Burning of infected bee colonies, cleaning and burning of beehives, destruction of honey from infected beehives, equipment disinfection, good hygiene and beekeeping practices, strengthening the immunity of bee colonies	Amiri et al., 2017; Czekońska, 2000; Nekoei et al., 2023; Grubić, 2018; Rašić, 2018
Chalky brood disease	*Ascosphaera apis*	Covered brood	Through food, water, contact	Loss of bee brood, larvae are first yellowish in color, later they mummify, become brittle and as if sprinkled with lime	Strengthening of bee colonies, hygiene measures, good ventilation, regular replacement of queens, do not keep bee colonies in thick shade	Wintering in undamaged hives, transfer of diseased colonies to a new hive, addition of a frame with litter, better feeding, addition of a young queen, good beekeeping and good veterinary practice, beekeeper education	Amiri et al., 2017; Czekońska, 2000; Nekoei et al., 2023; Grubić, 2018
Bacterial							
American foulbrood	*Paenibacillus larvae* White	Bee brood	Orally, by ingesting spores of *P. larvae*	The larva turns into a sticky, stretchy, amorphous brown mass with a sour smell. It is stretched in the form of a thin thread and then dried	Hygienic measures, prevention of predation, precautions when moving bees, strengthening bee colonies	Burning of sick colonies, ban on moving and selling bee colonies, mandatory clinical examination of all bee colonies in the apiary, Bacteriological examination of a sample of honeycomb with changes litter	Stephan et al., 2020; Fries et al., 2006; Genersch, 2010; Chemurot et al., 2016; Locke et al., 2019; Matović et al., 2023; Nilsson et al., 2024; Grubić, 2018
European brood plague	*Melissococcus plutonius,* with influence non-specific factors (insufficient amount of water, weak bee colony, bad weather conditions)	Bee brood disease	Orally	The larva changes its proper curled position, straightens, becomes spindly, brown in color and dies	Removal of non-specific factors, implementation of good beekeeping practice measures	Bacteriological examination of a sample of honeycomb with changes, removal of non-specific factors, disinfection of the hive litter	Forsgren, 2010; Forsgen et al., 2013; Raymann et al., 2018; Fünfhaus et al., 2018; Grubić, 2018

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Diseases	Causative agent	Get sick	Transmission	Clinical signs	Disease prevention measures	Disease control measures	Literature source
Septicaemia of adult bees	*Pseudomonas apiseptica, Serratia marcescens, Hafnia alvei,* with the influence of stress factors (intensive supplementary feeding, unfavorable climatic conditions	All castes of adult bees	By the vector mite *V. destructor;* orally	Change in the color of the hemolymph of adult bees, intense muscle degradation, do not take food, they are agitated, they cannot fly, Diseased bees usually die in 24 h, and the longest in 36 h, death, bees move slowly	Queen bee replacement, varroa control, good beekeeping and hygiene practice measures	Queen bee replacement, varroa control, good beekeeping and hygiene practice measures	Raymann et al., 2018; Fünfhaus et al., 2018; Van Herzele et al., 2024
"May" bee disease	*Spiroplasma apis* and nonspecific factors	Through the bite of *V. destructor,* orally	Adult young bees	Constipation, trembling, abdominal cramps, inability to fly, death	Provide clean drinking water, good nutrition, avoiding stressful factors, control of varroosis, hygiene measures, strengthening bee colonies	Provide clean drinking water, feed the bees with a thin syrup with the addition of 0.5-1% sodium chloride, strengthening bee colonies	Mouches et al., 1982; Mouches et al., 1983; Grubić, 2018
Parasitic							
Varroosis	*V. destructor*	Through the bite of *V. destructor*	Vector for a large number of bee viruses, it feeds on hemolymph and adipose tissue of bees	Viral diseases, immunosuppression, weight loss, reduced lifespan of bees and the death of the bee colony, decline of the bee colony, death	Regular inspection of bee colonies, strengthening of the bee colonies	Insertion of the frame of the builder, treatment with natural preparation, swarming	Brutscher et al., 2015; Tlak Gajger, 2017); Bouuaert et al., 2021; Nekoei et al., 2023; Morfin et al., 2024; Santrač, 2021; Grubić, 2018
Acarosis	*Acarapis woodi*	Lives in the respiratory tract of bees, feeds on hemolymph, damages tracheal epithelium	Through the stigmatic openings of the first pair of tracheae	Mechanical injuries, damage to the tracheal system, reduced airflow in the trachea, and paralysis of the flight muscle, bees cannot fly, die	Regular inspection of bee colonies, control of imported bee colonies, good beekeeping and good hygiene practices	Treatment, closing the infected apiary until cured	Plavša and Pavlović, 2017; Amiri et al., 2017; Santrač, 2021
Amoebic disease	Protozoa, *Malpighamoeba mellificae*	By ingesting food contaminated with amoeba cysts or by licking the feces of infected bees	All castes of bees, worker bees are most susceptible	Parasitizes in Malpighi tubules, diarrhea, weakness and death of bees and bee colonies	Regular inspection of bee colonies, strengthening of the colony, good beekeeping and good hygiene practices	There is no specific therapy. Good beekeeping and hygiene practices should be applied, strengthening of the colony	Iredale et al., 2023; Schäfer et al., 2022; Grubić, 2018
Non-infectious							
Poisonings	Pesticides, heavy metals	Larvae, pupae, adult bees	Through water, air, food, by contact	Aggressive to lethargic behavior, the guard bees do not let go trips to the hive, dying bees in front of the hive, bee colonies are left without bees-trippers	Monitoring of bee poisoning, environmental protection, good beekeeping practice	Taking bee samples for laboratory examination, timely information about spraying dates and pesticide toxicity, adequate nutrition	Grubić, 2018; Williams et al., 2015; Gajger et al., 2017; Amiri et al., 2025; Pineaux et al., 2023; Bojanić Rašović, 2022b

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Chronic bee paralysis virus

The occurrence of chronic bee paralysis can lead to high worker bee mortality and colony collapse during a single foraging season. Often, the queen and a few workers remain as the only individuals in the attacked colonies. Studies have shown a low prevalence of chronic bee paralysis virus in queens. This may be related to the queen avoiding contact with infected worker bees. However, it should be noted that experimentally infected queens show the same symptoms of chronic bee paralysis as worker bees, such as trembling legs, spread and separated wings, a bloated abdomen filled with hemolymph and an enlarged honey stomach. This means that queens are also susceptible to CBPV infection as workers. Chronic bee paralysis virus replicates successfully in queens and the disease develops in almost the same way as in worker bees. The number of colonies affected by chronic bee paralysis increased exponentially between 2007 and 2017 (Amiri et al., 2014, 2017; Diao et al., 2018; Budge et al., 2020; Giovanni and Nanetti, 2024). The disease was spatially highly clustered within most years, indicating local spread, but not between years, indicating that the disease had disappeared with periodic reintroduction.

Acute bee paralysis virus and israeli acute bee paralysis virus

Acute Bee Paralysis Virus, Israeli Acute Paralysis Virus are related viruses, which usually cause latent infections with low virus levels in the hemolymph of bees. Together with *Varroa* mites, they are additional factors that cause loss, collapse of honey bee colonies. ABPV and IAPV cause paralysis, tremors, and rapid death of worker bees 1-2 days after infection. These viruses have been found in different castes and developmental stages of bees, but they are rarely found in high titers in queens (de Miranda et al., 2010; Amiri et al., 2014). It should be taken into account that this virus is widespread and usually does not cause visible clinical symptoms (Ferrufino et al., 2020; Ferrufino et al., 2025). However, when the virus is present in high concentrations, high mortality rates of all castes of bees occur. ABPV was detected with high prevalence percentage in Argentinean apiaries (21.5%) and in 62.5% of queen pupae samples in queen-rearing apiaries. This leads to significant losses in queen rearing apiaries, which is why the control of this virus in queen honey bee production is very important (Ferrufino et al., 2020). IAPV is the third most frequently isolated virus in viral infections in bee colonies—after DWV and black queen bee virus (BQCV). IAPV has been detected in eggs, intestines, ovaries and spermatheca of infected queens (Bakonyi et al., 2002; Chen et al., 2014; Amiri et al., 2017, 2019, 2020a). The virus is transmitted horizontally and vertically. It was discovered in pollen and bee droppings, and is also transmitted by Varroa mites. IAPV can be detected in all developmental stages of drones and workers, including eggs. The frequency of infection with IAPV in weak colonies is significantly higher than in strong colonies. Experiments showed that queens fed both infected and uninfected bee workers with IAPV had a lower infection rate than those fed only infected bee workers. Queens are better protected by individual and social immunity, but this protection is not sufficient to completely prevent IAPV infections (Amiri et al., 2019). A significant correlation was found between colonies affected by colony collapse disorder and infection with IAPV virus (Maori et al., 2007; Chen et al., 2014).

Sacbrood virus

Sacbrood virus causes bee brood disease, but it has also been found in adult bees, although it does not cause visible signs of the disease. SBV virus is mainly transmitted

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orally from adult worker bees to larvae, and visible symptoms appear a few days after the brood is closed. Infected larvae do not develop into pupae, they turn into a closed mass filled with liquid, change color from pearly white to pale yellow and die quickly. SBV virus was also found in queens, mainly in ovaries and non-specific tissue (Amiri et al., 2017; Wei et al., 2022).

Black queen cell virus

BQCV infection often occurs in apiaries where queens are reared and attacks queen larvae and pupae. Black queen cell disease is widespread worldwide, and its occurrence is closely related to the presence of *Nosema* and *Varroa* mites in bee colonies. It is one of the most frequently isolated viruses in adult bees worldwide (Spurny et al., 2017; Chagas et al., 2020). Symptoms of the disease are most often manifested in spring and early summer. The disease causes great losses if, most often due to the failure to implement adequate hygiene measures, it occurs in mass production of queen cells. Black queen cell virus was first isolated from dead larvae and pupae of closed queen cells, which turned dark brown to black. Black queen cell virus was found in high titers in weakened and dead bee colonies. Co-infection of worker bees with BQCV virus and *N. apis* leads to increased mortality. In queen honey bees, it is found mainly in the intestines, feces and ovaries (Moritz et al., 2010; Amiri et al., 2017). The disease is transmitted to queen larvae through food given to them by infected worker bees. The virus causes the death of queen larvae or pupae. The disease is manifested by the appearance of a black coating on the wall of the queen chamber, in which a dead pre-pupa or queen pupa can be observed. Diseased larvae first acquire a light yellow color, they resemble larvae infected with mixed litter, and then they begin to darken—they acquire a brown and then black color. A weak society is more sensitive to the black queen cell disease, as well as to other infectious and parasitic diseases. Black queen cell disease tends to occur during cooler seasons—especially during fall, winter and spring. The heat acts as a preventive measure against the appearance of the disease of black bees. Responsible management of the apiary will prevent the occurrence of black brood disease (Plavša and Pavlović, 2017; Bojanić Rašović, 2019a, b).

Fungal diseases

Nosemosis

The queen bee is most commonly affected by nosema. *N. apis* and *N. ceranae* are pathogenic fungi that attack and damage the epithelial cells of the midgut and cause nosema in European honey bees (Gajda et al., 2021). Queens, as well as other members of the colony, can be infected with both *N. apis* and *N. ceranae*. *Nosema* spp. spores are transmitted horizontally, by the fecal-oral route (*Fig. 1.*) Feeding queens royal jelly contaminated with large numbers of *N. apis* spores can lead to queen death (Naudi et al., 2022). *Nosem*a can be transmitted sexually from drones to queens, but not vertically—from queens to offspring. Both types of nosema are limited to reproduction in the midgut, although *N. ceranae* has also been found in the fat tissue and ovaries of the queen. Symptoms caused by *N. apis* include diarrhea and the finding of large numbers of dead bees at the entrance to the hive in early spring. Symptoms of disease caused by *N. ceranae* are increased foraging time, reduced flight frequency and general stress in worker bees—reducing their longevity and leading to collapse. A diseased queen is a danger to the entire bee colony, because by throwing out excrement on the hive, it leads to the spread of the disease. Nosemosis in young queens can lead to oviduct blockage immediately after

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mating. Queens, due to their long lifespan, can represent a permanent reservoir of *Nosema*. Young queens are not able to survive high levels of *Nosema* infection (Czekońska, 2000; Amiri et al., 2017, 2019; Bojanić Rašović, 2018a). Nosemosis in queens causes physiological disorders, intestinal damage, later egg laying compared to healthy queens, disruption of pheromone production, degeneration of egg cells – leading to infertility. *N. apis* infection can seriously reduce the lifespan of a queen by an average of almost 50 days, which consequently leads to queen replacement by the colony. In queens infected with *Nosema,* a compensatory increase in the levels of vitellogenin and other antioxidant enzymes occurs. However, these changes cannot protect the queen from death in the long term (Czekońska, 2000; Amiri et al., 2017, 2019; Bojanić Rašović, 2018a; Galajda et al., 2021).

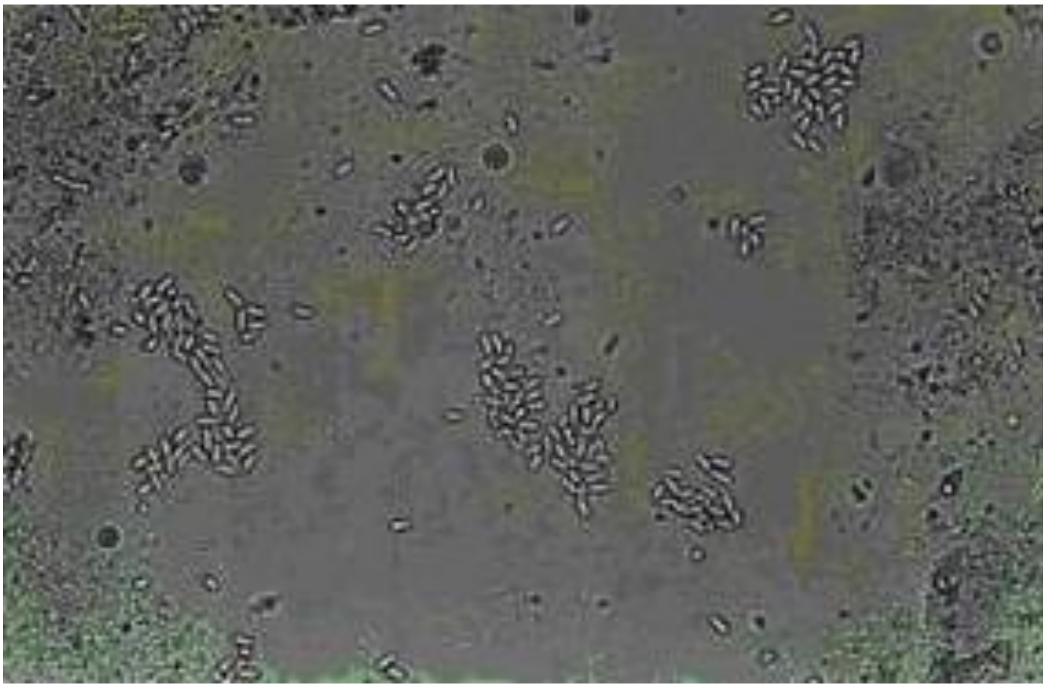

Figure 1. *N. apis spores, resembling a grain of rice (native preparation, 400 x), found in bee feces (Bojanić Rašović)*

Stone brood disease and chalky brood disease

The pathogenic mold *Aspergillus flavus* causes stone brood disease, and the pathogenic mold *Ascosphaera apis* causes chalky bee brood. Larvae of all castes of bees are sensitive. Chalky bee brood and stone brood are very similar in terms of initial symptoms. In both diseases, the larvae first turn into yellowish-white hard mummies. Only after the formation of breeding bodies on the surface of the mummified larvae do the differences become visible—the calcareous brood becomes brownish-gray, and the stone litter usually turns yellow-green. The causative agent of chalky bee brood does not cause disease in adult bees, while the causative agent of stone brood also causes disease in adult bees. Covered brood in most cases dies from chalky bee brood disease, and uncovered brood more often dies from stone brood. More often than not, a stone brood leads to the collapse of the entire bee society (Amiri et al., 2017; Czekońska, 2000; Bojanić Rašović, 2019e, f; Nekoei et al., 2023).

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Bacterial diseases

American foulbrood

American foulbrood is the most dangerous infectious disease of uncapped and capped bee brood. It affects bee larvae and pupae. The larvae from which bee worker bees develop are particularly susceptible. Queen and drone larvae rarely become ill in natural conditions. The causative agent of American foulbrood is the bacterium *Paenibacillus larvae* (Bojanić Rašović, 2018d; Stephan et al., 2020). When found in favorable conditions, the bacterial spores germinates into a vegetative form. The spores can survive for a very long time in unfavorable environmental conditions. When food contaminated with spores is ingested into the digestive tract of bee larvae, the spores germinate into a vegetative form, which leads to infection and death. The disease is less commonly transmitted vertically (Fries et al., 2006; Genersch, 2010; Chemurot et al., 2016). The disease spreads rapidly and persists in apiaries for a long time (Locke et al., 2019; Matović et al., 2023; Nilsson et al., 2024).

European brood plague

European brood plague is an infectious disease of uncovered brood, sparsely covered brood caused by the bacterium *Melissococcus plutonius* (White, 1912; Budge et al., 2025). Bee workers and drone brood are susceptible to the disease, but larvae from which queens develop can also be affected (Forsgren, 2010; Forsgen et al., 2013). Young cleaner bees, which become infected through food, participate in the spread of European brood plague. The degree of larval mortality is influenced by the number of bacteria introduced, the number of bees feeding the brood, and the amount of food obtained (Lolin, 1991; Plavša and Pavlović, 2017). Larvae usually die at the age of 4-5 days. Unlike American plague, where symptoms most often occur in covered brood, in European plague, symptoms of the disease most often occur in open brood. A significant difference is that in European plague, the content of dead larvae is not as distended as in American plague of bee brood (Matović et al., 2007; Bojanic Rasovic et al., 2022).

Septicaemia of adult bees

Bee septicaemia occurs as a result of the multiplication of pathogenic bacteria in the hemolymph of bees. This disease is most often caused by the bacterium *Pseudomonas apiseptica* (syn. *Pseudomonas aeruginosa*). Septicaemia can also be caused by other bacteria, such as *Serratia marcescens* and *Hafnia alvei*. The causative agents of septicaemia are transmitted by the mite *V. destructor*, in which these bacteria multiply. Worker bees, drones and queens can suffer from septicaemia. The bee brood does not suffer from this disease. It is considered that infection of bees in natural conditions most often occurs through the tracheal system. Stressful factors such as inadequate feeding, unfavorable climatic conditions during brood rearing, swarming, active construction of combs and others, influence the occurrence of the disease. The disease usually occurs between May and October (Rayman et al., 2018; Fünfhaus et al., 2018; Bojanić Rašović, 2019c). The disease is manifested by a change in the color of the hemolymph of adult bees and intense muscle degradation. Sick bees do not take food, they are agitated, they cannot fly, they fall from the flight to the ground with a slightly bent abdomen and crawl with spread wings. Diseased bees usually die in 24 h, and the longest in 36 h (Lolin, 1991; Fünfhaus et al., 2018; Bojanić Rašović, 2019c).

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"May" bee disease

"May" bee disease occurs in the month of May, hence its name. It is a disease that manifests itself in constipation and death of young bees. The intestinal contents of diseased bees are thick, hard and dark brown. The disease most often ends in the death of bees, with dead bees being observed on the flight path and in front of the hive. In the month of May, there is the most brood that young bees need to feed, so if due to poor conditions they cannot fly out and take the necessary amount of water and pollen, constipation occurs (Lolin, 1991; Mouches et al., 1984; Plavša and Pavlović, 2017). Diseased bees leave the hive suddenly and in large numbers, showing restlessness, trembling while moving in front of the hive, cannot fly and eventually die with convulsive movements. Dead bees can be observed on the flight path and in front of the hive. Diseased bees very often die unnoticed, far from the hive (Mouches et al., 1982, 1983). In addition to non-specific factors—inadequate nutrition and others, the disease is also caused by the bacterium *Spiroplasma apis*, which reproduces in the hemolymph of bees. Spiroplasmas have also been isolated from the mite *V. destructor*, which indicates the possibility of their transmission to bees through the bite of this mite (Fünfhaus et al., 2018; Bojanić Rašović, 2019c; Van Herzele et al., 2024).

Parasitic diseases

Varroosis

V. destructor is an ectoparasite of honey bees and is considered the most serious threat to bee colonies. The impact of *Varroa* on bee health is particularly serious because it is a vector for a large number of bee viruses (Brutscher et al., 2015; Bouuaert et al., 2021; Nekoei et al., 2023). This parasite, when feeding on the hemolymph of bees, directly injects a large number of viruses, thus leading to viral diseases, immunosuppression, weight loss, reduced lifespan of bees and the death of the bee colony (Bojanić-Rašović et al., 2018; Morfin et al., 2024). The level of Varroa infestation in honey bee colonies in the autumn is related to the extent of colony mortality in the spring. Colonies with mite infestation rates of 1%, 2%, or 3% in the fall had mortality rates of 58%, 66%, and 66%, respectively. The loss of bee colonies caused by varroa mites in Western Canada was 55%, and in some regions (Okanagan and Kootenay) up to 86% (Morfin et al., 2024). The mite parasitizes more often in drone brood than in worker and queen brood, because it takes longer for drones to develop, 24 days. Due to the short development period after the closure of the queen cell, *Varroa* cannot successfully complete its reproductive cycle in the queen cell. In addition, queens are under constant protection from worker bees. Queen bee infestation with *Varroa* can only occur when the colony is extremely invaded by these mites and in the almost complete absence of drone or worker brood (Harizanis, 1991; Amiri et al., 2017; Bojanić Rašović et al., 2018; Santrač, 2021).

Acarosis

The tracheal mite, *Acarapis woodi*, is a microscopic parasite that lives in the respiratory tract of bees and causes acarosis in adult bees. The pathogenic effects of *A. woodi* on individual bees depend on the number of parasites in the trachea, and are manifested by mechanical injuries, damage to the tracheal system, reduced airflow in the trachea, and paralysis of the flight muscles. Bee societies with high infestations of these mites survive the winter poorly. Whether the queen will be infected depends on the level

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of infection of the workers in the vicinity of the queen (Lolin, 1991; Plavša and Pavlović, 2017; Amiri et al., 2017; Santrač, 2021).

Amoebic disease

Amoebic disease—amoebiasis is a parasitic disease of adult bees caused by the protozoan—the amoeba *Malpighamoeba mellificae* Prell (1926). It parasitizes in the western honey bee, *A. mellifera.* The protozoan was named after the Malpighi tubules in which it parasitizes—*Malpighamoeba mellificae* (Iredale et al., 2023). These tubules represent the excretory organ of the bee, i.e. they have the function of a kidney in bees. The causative agent occurs in vegetative form and in the form of a cyst (Schäfer et al., 2022). It forms cysts only under adverse conditions, i.e. when there is a lack of food or water. All castes of bees are susceptible, but worker bees are most susceptible. Drones and queens are rarely infected. The disease is manifested by diarrhea, weakness and death of bees and bee colonies. Bees are infected by ingesting food contaminated with amoeba cysts or by licking the feces of infected bees (Lolin, 1991; Liu, 1985; Bojanić Rašović, 2019d).

Specific diseases of the queen bee

In addition to diseases common to other castes of bees, queens also suffer from specific diseases related to the reproductive organs, which lead to a slow or complete cessation of egg laying. These are: melanosis, carrying immature eggs, carrying unfertilized eggs, blockage of the oviducts with sperm cells, blockage of the genital tract with excrement, catalepsy. However, these diseases are not sufficiently studied and more thorough and extensive research is needed in this area. Specific diseases of the queen bee are shown in *Table 2*.

Melanosis is a disease of the ovaries and young and older queens that leads to sterility. As a result of this disease, the ovaries change color from normal white-yellow to yellow-brown and brown-black. Melanosis manifests itself in three forms: black egg disease (black eggs), melanosis H and melanosis B (Lolin, 1991; Plavša and Pavlović, 2017; Rakić, 2020). Melanosis, which is characterized by the appearance of black eggs, is most often a consequence of cold. Cold leads to a disorder in the metabolism of the albumin protein, which leads to its decomposition in the ovaries and the formation of melanin. Melanin and degenerated tissue accumulate in the ovaries in the form of a granular mass of yellow to brown and black color, which causes the ovaries to change color. The diseased queen loses her ability to lay eggs, so she needs to be replaced as soon as possible (Lolin, 1991; Plavša and Pavlović, 2017). It is considered that this degeneration may occur also due to long-term intoxication (Porporato et al., 2015). Yeasts have been isolated from this granular mass, which also play a role in the occurrence of this disease. When this disease occurs, bees try to silently replace the queen. In melanosis H, the causative agent is the yeast *Melanosella mors-apis*. In the diseased ovarian tissue, this imperfect fungus can be found in the form of yeast-like cells that reproduce by budding and can form pseudohyphae or in the form of hyphae that form mycelium. Young hyphae are light and become dark and black with age. The cross-section of the hyphae is 1.5–6 µm. In old cultures, they form dark chlamydospores with dimensions greater than 10 × 13 µm. Chlamydospores develop in the epithelial cells of the ovaries, fallopian tubes, but also in other organs (Mukminov, 2018). This microorganism creates damage in the ovaries in the form of nodules, which are hard, granular in structure and surrounded by a brownish-black or black crust. Ovarian necrosis occurs in queens. In addition to the ovaries, these changes can be found in the vagina, the

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rectal wall, as well as the queen's venom gland. Infection occurs by ingesting the aforementioned fungus during pollen feeding. Sick queens become lethargic and fall to the bottom of the hive. Their abdomen is enlarged, and a fecal plug is partially visible from the anus. In sick drones, the excretory ducts of the genital organs turn outward, which leads to death. The disease most often occurs in the second half of summer (Mukminov, 2018). Melanosis B is a fairly common disease of the genital organs, most often in young queens. The causative agent is the bacterium *Enterobacter cloacae* (Jordan, 1890) (Former name *Aerobacter cloacae* (Jordan, 1890). When this disease occurs, lesions—black lesions—are formed in the ovaries of the queen bee, which are not nodular in structure, unlike melanosis H. Queens that initially lay healthy eggs become sterile. The occurrence of melanosis is associated with increased humidity and sudden cooling, feeding the bees with inadequate honey, and inadequate hygiene. After penetrating the hemolymph, the bacteria spreads throughout the body, causing sepsis, which results in necrosis in various tissues and organs, including the ovaries. The disease usually occurs in the second half of summer, when the queen reduces egg laying due to the occurrence of sudden unfavorable weather conditions. When the intestines are also affected, the feces become thick and the anus becomes blocked. It is believed that workers can also get sick and die from this disease during the summer on pasture. Nests of sick colonies do not have eggs and young larvae. It is believed that melanosis can also occur after artificial insemination of queens, if the rules of asepsis are not followed (Lolin, 1991; Plavša and Pavlović, 2017; Rakić, 2020; Santrač et al., 2021; Bojanić Rašović, 2025c).

Carrying immature eggs results in the larval development being arrested at the very beginning. The fertilized queen lays worker eggs, which develop into an embryo that dies very quickly. Eggs are usually flattened, dry quickly and are easily removed by bees. This disorder is thought to occur due to genetic changes, anomalies of the genital organs, such as ovarian hypoplasia, the existence of two spermathecae, etc. (Plavša and Pavlović, 2017; Rakić, 2020; Santrač et al., 2021).

Laying of unfertilized eggs usually occurs in older queens whose sperm supply has been depleted. In young queens, unfertilized eggs often occur due to the death of sperm in the spermatheca. This is most often due to a dysfunction of the spermatheca. Since this condition cannot be improved, the old queen is replaced with a new one (Lolin, 1991; Plavša and Pavlović, 2017; Rakić, 2020).

Ovarian tube blockage with sperm leads to their impassability and prevention of egg laying. Due to this blockage, the posterior part of the queen's abdomen is swollen and bent downwards. The oviducts are dilated and filled with a dense, partially hardened mass (Lolin, 1991; Plavša and Pavlović, 2017; Rakić, 2020).

Obstruction of the genital organs with excrement is manifested by the appearance of hardened feces in the form of a white or brown plug protruding from the abdomen. This disease can lead to the prevention of egg laying (Lolin, 1991; Plavša and Pavlović, 2017; Rakić, 2020).

Changes that occur due to the aging of the queen are degenerative processes occur in the advanced age of the queen bee. As a result of aging, degeneration of organs such as the toxic gland, Malpighian tubes, etc. occurs. The poison gland acquires a brown to black color, and the Malpighian tubes a yellowish, yellow-green, green or dark green color—due to the accumulation of pigment in the epithelial cells. Due to degenerative changes, old queens lay fewer and fewer eggs, there is a sudden cessation of egg-laying, and the larvae that hatch are mostly drone-like. If the beekeeper does not replace the queen, the bees will (Lolin, 1991; Porporato et al., 2015; Plavša and Pavlović, 2017; Rakić, 2020).

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Table 2. *Specific queen bee diseases*

Disease	Cause	Clinical signs	Measures for prevention and control	Literature source
Melanosis				
Black eggs	Cold, long-term intoxication, infection with yeasts, viral infection	Disorder in the metabolism of the albumin protein. Melanin and degenerated tissue accumulate in the ovaries in the form of a granular mass of yellow to brown and black color, loss of ability to lay eggs	Proper selection and breeding of queen bees, strengthening bee colonies, applying good beekeeping practices, eliminating stress factors	Plavša and Pavlović, 2017; Rakić, 2020; Porporato et al., 2015
Melanosis H	*Melanosella mors apis*, ingesting the fungus during pollen feeding	Damage in the ovaries in the form of nodules, which are hard, granular in structure and surrounded by a brownish-black or black crust. Ovarian necrosis	Proper selection of queen bees, regular replacement of queen bees, strengthening the colony, application of good beekeeping and good hygiene practices	Plavša and Pavlović, 2017; Rakić, 2020
Melanosis B	*Enterobacter cloacae,* with increased humidity and sudden cooling, feeding the bees with inadequate honey, and inadequate hygiene, after artificial insemination of queens	Black lesions in the ovaries, which are not nodular in structure, sterility	Proper selection of queen bees, strengthening the bee colony, applying good beekeeping and good hygienic practices	Plavša and Pavlović, 2017; Rakić, 2020; Porporato et al., 2015; Mukminov, 2018
Carrying immature eggs	Genetic factors, anomalies of the genital organs, such as ovarian hypoplasia, existence of two spermathecae, sperm damage, influence of abiotic and biotic factors on the development and health of the queen	Larval development is stopped at the very beginning; Eggs are usually flattened, dry quickly and are easily removed by bees	Proper selection and breeding of queen bees, queen bee replacement, application of good beekeeping practices	Plavša and Pavlović, 2017; Rakić, 2020; Santrač et al., 2021
Laying of unfertilized eggs	Usually occurs in older queens whose sperm supply is depleted; in young queens whose spermatozoa have died in the spermatheca; spermatheca dysfunction, genetic factors, influence of abiotic and biotic factors on the development and health of the queen	The queen lays unfertilized eggs	Regular replacement of the queen bee, proper selection and breeding of queen bees, application of good beekeeping practices, removing stress factors	Lolin, 1991; Plavša and Pavlović, 2017; Rakić, 2020
Ovarian tube blockage with sperm	Due to the blockage of the fallopian tube by sperm, the passage of the egg to the vagina is prevented; genetic factors, the influence of abiotic and biotic factors on the development and health of the queen bee	The posterior part of the abdomen of the queen bee is swollen and bent downwards. The fallopian tubes are dilated and filled with a dense, partially hardened mass	Proper selection and breeding of queen bees, regular replacement of queens, good beekeeping practice measures, removing stress factors	Lolin, 1991; Plavša and Pavlović, 2017; Rakić, 2020
Obstruction of the genital organs with excrement	Fallopian tube obstruction due to pressure from feces from the digestive tract	appearance of hardened feces in the form of a white or brown plug protruding from the abdomen, inability to lay eggs, sterility	Proper selection and breeding of queen bees, regular replacement of queens, good beekeeping practice measures	Lolin, 1991; Plavša and Pavlović, 2017; Rakić, 2020
Changes that occur due to the aging of the queen	Queen bee age	degeneration of organs such as the toxic gland, Malpighian tubes, reduced egg laying, hatching of drone larvae, cessation of egg laying	Regular queen bee replacement	Lolin, 1991; Porporato et al., 2015; Plavša and Pavlović, 2017; Rakić, 2020
Formation of enteroliths	impermeability of the genital opening due to pressure of enteroliths on the genital organs, the cause may be bacterial infections	Cessation of egg laying, sterility	Regular queen bee replacement, proper queen bee breeding, good beekeeping and hygiene practices	Fyg, 1964; Porporato et al., 2015; Plavša and Pavlović, 2017; Rakić, 2020; Santrač, 2021
Catalepsy	Stress factors adding, marking, transporting, etc., and especially after rough handling	State of numbness, shock, fainting, paralysis	Good beekeeping practice measures, avoidance of stress factors, proper transportation	Plavša and Pavlović, 2017; Rakić, 2020; Santrač, 2021
Tumors of the ovarioles, ovaries, spermatheca	Genetic factors, stress factors, infectious agents	Queen bee sterility	Proper selection and breeding of queen bees, measures of good beekeeping and good hygiene practices	Plavša and Pavlović, 2017; Rakić, 2020; Santrač, 2021

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One of the queen bee diseases is also the *formation of enteroliths.* Enteroliths are stones that form in the intestines from accumulated feces and lead to a significant expansion of the posterior part of the queen's intestine. They are mainly composed of uric acid salts. Larger stones also put pressure on the genital canals located below the rectum and prevent egg laying. Due to the pressure of enteroliths on the genital organs, queen bees become sterile (Fyg, 1964; Porporato et al., 2015; Plavša and Pavlović, 2017; Rakić, 2020; Santrač, 2021).

Catalepsy is a disease that occurs if the queen bee is disturbed, and is manifested by paralysis—deprivation and loss of mobility. Catalepsy is a state of numbness, shock, fainting, paralysis. This condition occurs during procedures with the queen bee, such as adding, marking, transporting, etc., and especially after rough handling. After these procedures, it happens that the queen bee suddenly calms down and becomes stiff. Catalepsy can also occur due to the effect of static electricity, which is why you should not work with equipment made of materials that easily generate static electricity. This condition can last from a few minutes to an hour, after which the queen behaves normally again, but it can also die (Plavša and Pavlović, 2017; Rakić, 2020; Santrač, 2021). Other diseases that can occur in the queen are *tumors of the ovarioles, ovaries, spermatheca*, etc. (Fyg, 1964; Porporato et al., 2015). Tumors lead to damage and blockage of the genital organs and sterility of the queen bee.

Developmental anomalies of the queen bee

Developmental anomalies—malformations represent morphological deviations from the normal structure of the body or its parts with permanent consequences. The development of anomalies can begin at various stages of the queen's development. The organogenesis phase is particularly susceptible to the development of malformations. In addition to hereditary factors, environmental factors also play a role in the development of deformities, such as mechanical, chemical, metabolic, actinic and infectious factors (Lolin, 1991; Plavša and Pavlović, 2018; Bojanić Rašović, 2018b). However, developmental anomalies of the queen bee have also not been sufficiently studied, which is why further and more comprehensive research is needed. The queen rearing technique has a major influence on the occurrence of developmental anomalies (Porporato et al., 2015). The most common and most significant developmental anomalies of the queen are: queen stunting, deformed wings, ovarian hypoplasia, underdevelopment of the oviducts, spermatheca anomalies etc. (Porporato et al., 2015; Plavša and Pavlović, 2017; Rakić, 2020; Santrač et al., 2021). The most common developmental anomalies of the queen bee are shown in *Table 3.*

Stunted - dwarf queen is an anomaly that occurs due to insufficient nutrition of the larva of the future queen bee. Stunted queen bees barely reach the size of a worker bee and are sterile (Lolin, 1991). *Deformations of the queen's wings* occur even in the pupal stage and are thought to be caused by mutations. The deformities are manifested by the appearance of front and rear stumps - rudiments. Such queens cannot fly and cannot be fertilized naturally (Lolin, 1991). *Ovarian hypoplasia* is anomaly is manifested by a reduced size of the ovaries, which leads to permanent sterility. The cause of this disease is not yet known, but it is considered that viruses may play a role (Fyg, 1964; Lolin, 1991; Plavša and Pavlović, 2017; Porporato et al., 2015). *Fallopian tube underdevelopment* is an anomaly that occurs more often than ovarian hypoplasia. The two paired fallopian tubes may be stunted, so that there is no connection between the ovary and the vagina. In some cases, one fallopian tube may be underdeveloped (Lolin, 1991; Porporato et al.,

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2015). Various *anomalies can occur in the spermatheca*, such as: lack of spermatheca, small spermatheca, change in spermatheca color, abnormal shape of spermatheca. Irregularly shaped spermatheca, such as the number "8", are common. White spermatheca do not contain sperm and the bee colony immediately replaces such queen bees. Transparent spermatheca also do not contain sperm, and are found in a significant number of queens. Queens with black spermatheca are not accepted by the bee colony, they do not lay eggs, despite having a high number of viable spermatozoa in the spermathecal (Porporato et al., 2015).

Table 3. *Developmental anomalies of the queen bee*

Anomaly	Cause	Clinical signs	Measures for prevention and control	Literature source
Stunted - dwarf queen	Insufficient nutrition of the larva of the future queen bee, environmental factors- mechanical, chemical, metabolic, actinic and infectious factors, queen rearing technique	Stunted queens—they barely reach the size of a worker bee and are sterile	Proper selection and breeding of queen bees, measures of good beekeeping and good hygiene practices	Lolin, 1991; Plavša and Pavlović, 2018; Bojanić Rašović, 2018b; Porporato et al., 2015; Santrač et al., 2021
Deformations of the queen's wings	Environmental factors, genetic factors	Appearance of front and rear rudiments, inability to fly and natural fertilization	Proper selection and breeding of queen bees, measures of good beekeeping and good hygiene practices	Lolin, 1991
Ovarian hypoplasia	Hereditary factors, environmental factors, most likely viruses	Reduced ovarian size, sterility	Proper selection and breeding of queen bees, measures of good beekeeping and good hygiene practices	Fyg, 1964; Lolin, 1991; Plavša and Pavlović, 2017; Porporato et al., 2015
Fallopian tube underdevelopment	Insufficient nutrition of the larva of the future queen bee, environmental factors- mechanical, chemical, metabolic, actinic and infectious factors, queen rearing technique, genetic factors	Stunting of one or both fallopian tubes, there is no connection between the ovary and the vagina	Proper selection and breeding of queen bees, measures of good beekeeping and good hygiene practices	Lolin, 1991; Porporato et al., 2015
Anomalies of the spermatheca (lack of spermatheca, small spermatheca, change in spermatheca color, abnormal shape of spermatheca	Genetic factors, the effect of biotic and abiotic factors on the development and health of the queen bee	Spermathecae do not contain sperm, queen bee not accepted by colony, no eggs laid	Proper selection and breeding of queen bees, measures of good beekeeping and good hygiene practices	Lolin, 1991; Porporato et al., 2015

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Factors influencing the occurrence of diseases and anomalies in the queen honey bee

Queen quality and health is influenced by a number of genetic and environmental factors (Hatjina et al., 2014; Porporato et al., 2015; Amiri et al., 2017; Arslan et al., 2021; Hubhachen et al., 2025). Genetic traits are transmitted to colonies through selected queens and drones (Czekońska et al., 2015; Metz and Tarpy, 2019), which is controlled through a selection program. Queens are exposed to various environmental stressors during different stages of their life. Abiotic stressors directly and indirectly affect the development, health, and reproductive characteristics of honey bee queens, posing a threat to their vitality and survival. These factors determine queen characteristics such as weight, diameter and volume of spermatheca, sperm count, number of oviducts (Payne and Rangel, 2018; Arslan et al., 2021). In addition to genetic factors, the successful egg-laying of queens is influenced by numerous environmental factors, such as: climatic conditions, air humidity and temperature, availability of food, toxicity of substances from agricultural activities (pesticides), micro and nanoplastics, stress during transport of queens, presence of predators, presence of disease agents and development of diseases and anomalies (Porporato et al., 2015; Amiri et al., 2017, 2025; De Souza et al., 2019; Abou-Shaara et al., 2021; Pineaux et al., 2023; Valizadeh et al., 2025). These factors also include season, flora, colony strength, queen cell properties, rearing techniques, number and age of transplanted larvae, drone quality, etc. (Büchler et al., 2024; Prešern and Škerl, 2019). Given their importance, drone breeding and the study of their biology are not given the necessary attention (Arslan et al., 2021). Lack of food in the early stage of the queen's life causes a decrease in her body mass and negatively affects the development of the ovarioles of hatched queens grown in vitro (Linksvayer et al., 2011). Nutritional deficit in young queens can significantly affect the reproductive potential of the queen. It leads to low sperm viability in the queen spermatheca (DeGrandi-Hoffman and Chen, 2015). Factors that affect worker bee health, such as nutrition, can significantly affect queen egg laying. Due to the lack of pollen, queens lay fewer eggs with a lower protein content (Fine et al., 2018; Fèvre et al., 2024; Fine et al., 2025). Transport stress and extreme temperatures also negatively affect the queen. Temperature differences during queen transport are a stressors that lead to higher queen mortality. Both low and high temperatures can occur during transport, with both negatively affecting queen fertility (Pettis et al., 2016; McAfee et al., 2020). It has been found that queen fertilization success is 59% in cold, bad weather and 82-100% in good, warm weather (Akongte et al., 2024). Exposure to extreme heat (≥40°C) for just 2 h can kill stored sperm (Pettis et al., 2016). Abiotic and biotic stressors, in addition to affecting the quality of queen egg laying, also affect her development in the larval and pupal stages. These influences can lead to the appearance of various diseases, as well as congenital defects in the queen. The consequences of the effects of stress factors depend on the physiological state of the queen, the degree of exposure and the duration of the stress. They can be lethal or sublethal, with biological, physiological or behavioral changes that can be transmitted to the next generation (Preston et al., 2019; Al-Ghzawi et al., 2022; Amiri et al., 2025). Neonicotinoids negatively affect reproductive performance, mating success, and queen survival (Williams et al., 2015; Gajger et al., 2017; Amiri et al., 2025). Direct exposure of developing queens to high doses of thiamethoxam reduces the survival of queen larvae and adult mated queens exposed to this pesticide during the larval stage had reduced sperm count and viability (Gajger et al., 2017; Kozii et al., 2021b). Exposure to coumaphos, tau-fluvalinate, and amitraz during queen development has negative effects on queen health and quality in adulthood (Rangel and Tarpy, 2015; Walsh et al., 2020).

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High doses of coumaphos in queen wax lead to a decrease in body weight and lower ovary weight in newly hatched queens (Pettis et al., 2004; Amiri et al., 2025). Coumaphos has been found to have negative effects on sperm vitality. The widely used fungicide boscalid has also been found to affect the reproductive capabilities of the queen. Boscalid is an inhibitor of the enzyme succinate dehydrogenase and is most often used to treat rapeseed, because it prevents the respiration process in the mitochondria of fungi. However, it also affects the metabolism of bees. Exposure of young queens to sublethal doses of boscalid leads to reproductive disorders, a dramatic increase in queen mortality, and a reduced number of spermatozoa stored in the spermatheca of surviving queens. Queens exposed to boscalid had a weak expression of the gene responsible for coding the synthesis of vitellogenin, a protein involved in egg yolk formation (Pineaux et al., 2023). Insecticides such as fipronil and thiacloprid significantly increase mortality in bees infected with nosemosis (Tlak Gajger et al., 2017; Kozii et al., 2021b). A sudden drop in temperature in spring and late autumn leads to sperm degeneration. Degeneration of drone sperm can also occur in the summer months due to sudden changes in temperature. The influence of unfavorable weather conditions also affects the success of mating and reproduction. The success of queen bee fertilization in cold weather is 59%, and in warm weather 82-100% (Tlak Gajger et al., 2017). Dziechciarz et al. (2025) also found that queen fertilization success was poorer in poor weather conditions. The bee-eater (*Merops apiaster*) is a predator that can significantly affect the mating success of queens. It has been found that the mating success of queens in the absence of the bee-eater is 80%, while in the presence of this bird the success rate is 46.67% (Ali and Taha, 2012). Invasive tests used to test the queen's traits also lead to stress that affects her health and productive capacity. Therefore, queen bee selection and production centers should apply non-invasive sampling methods to monitor queen bee quality (Tarpy et al., 2012; Rakić, 2020; Kanelis et al., 2023). Weather conditions significantly affect nectar availability to bees. Heavy rainfall during flowering dilutes nectar, which reduces the attractiveness of plants to bees (Le Conte and Navajas, 2008). Drought also negatively affects bees, as they cannot find sufficient food in nature. Some plants do not produce nectar at all during drought (Le Conte and Navajas, 2008; Rucker et al., 2018). Bee flight is negatively affected by low temperatures (<10°C), high temperatures (>40°C), wind speeds above 2–7 m/s, rainy weather (>3–5 mm of rain/day), and fog (Vincze et al., 2025). Fluctuations in temperature and humidity affect not only feeding and reproduction, but also susceptibility to diseases (vanEngelsdorp et al., 2008). Water scarcity also has a stressful effect on bee colonies (Vincze et al., 2025). However, the impact of stress factors on queens, both heat and other stressors, has been incompletely investigated. Genetic selection of queens can greatly reduce the incidence of infectious diseases in bee colonies. In bee colonies that do not have good hygienic behavior, which is a trait that the queen transmits to her offspring, clinical symptoms of the disease appear. Therefore, queen rearing is one of the most important activities on which the state of beekeeping in the country depends (Facchini et al., 2021; Arslan et al., 2021; Büchler et al., 2024; Rehman et al., 2024).

Selection and breeding of the queen honey bee

The health and quality of the queen bee are crucial for the development of beekeeping and the formation of strong bee colonies. The queen is responsible for almost all the properties of the bee community, such as strength, calmness, disease resistance and honey production. Throughout Europe and most countries in the world, the invasion of bee colonies by the mite *V. destructor* represents the main problem of beekeeping. Different

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breeds and lines of honey bees possess different defense mechanisms, which are being further enhanced through selection (Bubalo and Berg, 2004). *Nosema* spp. is also a major threat to modern beekeeping, especially in countries where winters are long and mild, and humidity is high due to the proximity of the sea. The influence of viral infections of the queen on her reproductive abilities should be investigated further. It should be borne in mind that the quality of the queen is not only the result of her own reproductive potential, but also her success in mating, which is evaluated by the number of stored sperm in the queen's spermathecal (Hatjina et al., 2014).

In order to achieve all the positive characteristics of the queen, selection and proper breeding are necessary (Facchini et al., 2021; Arslan et al., 2021; Büchler et al., 2024; Rehman et al., 2024). Queen bee breeding is the basis of successful beekeeping, and genetic improvement of the population can be achieved by implementing selection rules. The goals of queen bee breeding and selection are to increase yield, tolerance to diseases—especially varroosis, calmness of the bees, reduction of reproductive drive, and speed of spring development (Büchler et al., 2024). Queen bee selection involves the systematic mating of selected individuals from which offspring with the desired characteristics are expected, while queen bee breeding is the production of mated queens for beekeeping purposes. The goal of breeding cannot be achieved without selection, and selection cannot be carried out without the goal of breeding (Katušić, 2022). Due to the very complex reproduction of bees, which implies multiple mating of the queen, long flight distances, male haploids, selection of queens is a very challenging area of science (Dorić, 2016). The health condition of the queen, as well as the ability of the bee colony to prevent the occurrence of certain diseases, is of increasing importance in breeding programs. Bee colonies with multi-drone queens were found to have significantly lower disease intensity and higher colony vitality than colonies led by single-drone queens. Hygienic behavior of the bee colony is a natural defense against the diseases American foulbrood and chalky brood disease, varroa, therefore it is an important hereditary trait that is determined through the offspring of the queen (Hatjina et al., 2014). The breeding program must not lead to the destruction of the biological diversity of the honey bee, but should improve the production characteristics and adaptability to the climatic and grazing conditions of the region (Kezić and Bubalo, 1998; Dražić, 2004). The two basic methods used in queen rearing are non-larva transplanting or larval transplanting. The essence of breeding queens by transplanting is placing young larvae aged 12-24 h in queen cells that stand vertically in the hive. The larvae feed the nurse bees with royal jelly. After 11 to 12 days from transplanting, the queens can be moved to the nuclei, because then they are ready for queen hatching. The success and quality of the produced queens depends on the strength, nutrition and health of the bee colony, the quality of the equipment and the production plan (Dorić, 2016). Assessment of breeding value is carried out in most European countries using standard methods such as selection index calculation (Anon., 2019, 2024; Büchler et al., 2024). Using the BLUP animal model represents a genetic evaluation, and its result is the expected value of the offspring of the selected individual in relation to the population for the investigated trait. However, it should be kept in mind that the influence of the queen bee and the bee workers can be negatively correlated, which negatively affects the success of the selection (Kovačić, 2018; Büchler et al., 2024; Hatjina et al., 2014). The BLUP method takes into account the influences of the bee colony, the queen bee mother and external factors (Anon., 2024). The experience of many countries has shown that the selection of queens should focus on the selection of autochthonous breeds. Natural selection is the natural response of the bee population to

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changes in the environment and the consequences of pests and diseases. The race of bees is the result of the influence of climate, flora, enemies, i.e. natural selection (Dorić, 2016). Within a certain race there are ecotypes adapted to specific climatic conditions. The diversity of races and ecotypes of *A. mellifera* is a consequence of a long-term, continuous process of adaptation. This diversity represents a very valuable biological potential that should be preserved as a basis for future selection and development (Dorić, 2016). Genetic diversity can be a problem for species survival in disturbed and changing habitat conditions. Habitat loss, intensive agriculture and climate change are also leading to reduced genetic diversity and species extinction (Dorić, 2016). To date, 27 subspecies of the honey bee *A. mellifera* have been described (Ruttner, 1988). The subspecies *A. mellifera mellifera* and *A. mellifera iberiensis* are found in Western and Northern Europe, and the subspecies *A. mellifera ligustica*, *A. mellifera carnica*, *A. mellifera macedonica* and *A. mellifera cecropia* are found in Central and Southeastern Europe. The dark European bee *A. mellifera mellifera* occupies the largest area of Europe, from France, Scandinavia, Great Britain to Poland and Ukraine. *A. mellifera iberiensis* inhabits the area of Portugal, Spain and the Balearic Islands. The Italian bee *A. mellifera ligustica* naturally inhabits the Apennine peninsula where it is isolated on the Alps in the north and the Mediterranean Sea in the south. *A. mellifera macedonica* occupies the territory of Bulgaria, Greece, Macedonia, parts of Romania, Ukraine and Turkey, and *A. mellifera cecropia* the territory of southern Greece. The gray bee *A. mellifera carnica* is widespread in Austria, Slovenia, Hungary, Croatia, Bosnia and Herzegovina, Albania, Serbia and Romania (Kovačić, 2018). The honey bee *A. mellifera carnica* is also widespread in Montenegro, where it is the only autochthonous subspecies (Anon., 2024). Gray bee *A. mellifera carnica* is autochthonous and the only subspecies of honey bee in Croatia, and it is characterized by successful wintering, explosive spring development, industriousness and good collecting activity, low aggressiveness and pronounced swarming instinct. Three basic ecotypes of the gray bee *A. mellifera carnica* are known in Croatia: Pannonian, subalpine and Mediterranean. The subalpine or mountain ecotype is widespread in the area of Lika and Gorski Kotar, the Pannonian in the Pannonian lowland, and the Mediterranean in the coastal and island areas (Dražić et al., 1999; Kezić et al., 2003). According to Stevanovic et al. (2010), two autochthonous races inhabit the territory of the Republic of Serbia: *A. mellifera carnica* (“kranjska medonosna pčela”, domestic honeybee) which occupies the northwestern part of Serbia and *A. mellifera macedonica* (“makedonska pčela”) which spreads to the southeast. Between them, in the central part of Serbia, lies the zone of hybridization of these two subspecies of honey bee. The diversity of autochthonous subspecies and ecotypes of honey bees in Serbia is endangered because the law allows the breeding of only one subspecies, *A. mellifera carnica*, as well as due to the application of modern beekeeping techniques such as migratory beekeeping, artificial selection and uncontrolled trade in queens. Therefore, locally adapted populations, i.e. ecotypes, cannot be distinguished in Serbia. Only the population from the Pešter Plateau (Pešterska visoravan) is distinguished. *A. mellifera carnica* is an autochthonous subspecies preserved in all five geographical areas Slovenia: Prekmurje, Štajerska, Gorenjska, Dolenjska and the Central Region (Gregorc and Smodiš Škerl, 2015). Loss of biological diversity can be a problem for the survival of the species in disturbed and changing habitat conditions. Habitat loss, intensive agriculture and climate change also lead to a decrease in genetic diversity and species extinction. Protection of the genetic diversity of *A. mellifera* could be based on natural selection. It has been found that greater genetic diversity within a honey bee colony reduces the

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negative impacts of pathogens and parasites. Independent free-living bee colonies have greater mutual relatedness and greater genetic diversity compared to cultivated bee colonies. The loss of the indigenous subspecies *A. mellifera macedonica*, which inhabited the southern regions of Serbia, and the loss of genetic diversity within the subspecies *A. mellifera carnica*, clearly indicate that the future of the honey bee in Serbia is not promising. One of the measures to protect the genetic diversity of honey bees, which would prevent unwanted gene flow and hybridization between independent and cultivated bee colonies, as well as between cultivated bee colonies from remote areas of Serbia, could be to ban the movement of hives, i.e. migratory beekeeping, during the mating and swarming season (Patenković et al., 2022). In Vojvodina, an autonomous province of Serbia, *A. mellifera carnica* and its ecotype *A. mellifera carnica banatica* are bred (Pihler, 2008; Mamić, 2013).

Queen honey bee selection program in Montenegro

The queen bee selection program in Montenegro has been implemented since 2007. In Montenegro is grown *A. mellifera carnica* (“Kranjska pčela”), which due to its good qualities is grown in almost the entire area southeast of the Alps, i.e. in the countries of the Balkan Peninsula (*Figs. 2, 3* and *3*) This race is the most suitable for grazing conditions in Montenegro. The most characteristic feature of this race is the rapid spring development. The queen bee starts laying eggs early, already with the first intake of pollen. “Kranjska pčela” has a good orientation ability and does not have a strong propensity for predation. The mass of the worker bee body of the domestic *A. mellifera carnica* is 0.1 g, while the mass of the drone, i.e. the queen, is 0.2 g. The length of the worker bee body of domestic *A. mellifera carnica* is 12 to 14 mm, the drone is 15-17 mm, and the queen is 15-20 mm. The length of the tongue of domestic *A. mellifera carnica* is 6.4 to 6.8 mm (Anon., 2024). Preservation of the diversity of the *A. mellifera carnica* bee breed in the territory of Montenegro is achieved by preserving local ecotypes of this breed (Anon., 2024; Bojanić Rašović, 2025b). Two ecotypes of domestic *A. mellifera carnica* have been identified in Montenegro: littoral and mountain. The main goals of the breeding program in beekeeping in Montenegro are: higher productivity of bee colonies, greater resistance to diseases, reduced aggressiveness of bees, reduction of the reproductive drive, peace on the comb, vitality of bee colonies, rapid spring development, better wintering of bee colonies. In addition to the stated goals, which are aimed at improving the performance of the bee population, a very important goal is the preservation of the genetic diversity—the diversity of the *A. mellifera carnica* in Montenegro. This implies the preservation of the local population of the *A. mellifera carnica* in a pure race, as well as the identification of its ecotypes in the territory of Montenegro. An authorized and registered breeding organization—the Center for Breeding and Selection of Queen Bees is responsible for the implementation of the breeding and selection program of queen honey bees. For each bee colony that is included in the selection, as well as for the queen bee, registry records are kept in accordance with the regulations. Examination of queen traits is carried out by performance control and progenic tests, as well as morphometric tests. The results showed the following average values of morphometric traits: tongue length (6.46 mm), fore wing length (9.06 mm), fore wing width (3.13 mm), fore basitarsus width 1.15 mm, fore basitarsus length 1.94 mm, femur length (2.90 mm), tibia length (3.11 mm), hind wing length 6.32 mm, hind wing width 1.65 mm. The cubital index ranged from 2.56 to 2.66, which corresponds to the typical racial characteristics of the autochthonous race of the honey bee *A. mellifera carnica*. It was established that the

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quality and productivity of bee colonies significantly depend on the influence of climatic conditions, honey flora and the ecotype or strain of this race of honey bee (Anon., 2024; Büchler et al., 2024). Queen selection is carried out by monitoring the queen's production capabilities, the number of eggs laid, the number of successfully hatched eggs, the number of ovarioles in the ovary, the queen's body mass, size of spermathecae and mating success, number of spermatozoa in spermathecae etc. (Gregorc and Smodiš Škerl, 2015). A high-quality queen has a higher body mass, a large spermatheca containing a large number of spermatozoa, and a large number of ovarioles in the ovaries. All of these traits are genetically inherited and queen selection is carried out according to them. However, these traits are also significantly influenced by environmental conditions (Anon., 2024; Büchler et al., 2024). In addition to morphometric studies, studies of the production characteristics of ecotypes of the domestic *A. mellifera carnica* in the territory of Montenegro were also conducted. It was determined that the quality and productivity of bee colonies are strongly correlated with climatic conditions, the capacity of the honey flora and the ecotype. The average nectar intake of queen bee colonies from the Sutomore area was 5.8 kg in the first and 8.75 kg in the second year of measurement. In queen bees from the territory of Bijelo Polje, the average nectar intake in the first year was 7.50 kg and in the second year of measurement was 11.63 kg (Anon., 2024).

Measures implemented to prevent and combat infectious diseases of the queen

Knowledge and prevention of diseases, their timely detection and treatment are one of the most important factors for preserving the health of the entire bee colony (Bojanić Rašović, 2018c; Bojanić Rašović et al., 2019; Nekoei et al., 2023). It is of great importance to detect diseases or anomalies of the queen in time and that the apiary always has a spare mated queen available. In all situations when the queen is sick or has a defect, the best measure to protect the bee colony is to urgently replace the queen (*Fig. 2*). It should be borne in mind that, when it comes to diseases that affect the entire bee colony, replacing the queen will not give results unless the entire colony is treated. Therefore, first of all, measures to prevent diseases that affect the entire bee colony should be adequately implemented. One of the basic measures is to provide the bee colony with enough quality food. Well-fed and well-groomed bee colonies have greater resistance to disease (Bojanić Rašović, 2020). Maintaining proper hygiene of hives and other beekeeping equipment, and choosing the right location for the apiary during the colder and warmer seasons can significantly help prevent the occurrence of diseases. In the colder months, hives should be placed in sunny places (*Fig. 3*). Regularly replacing the comb in the hive can also significantly affect disease prevention (Bojanić Rašović, 2022a). It is necessary to regularly disinfect beekeeping equipment and accessories. Drinking water for bees should be disinfected, because infectious disease agents, bacteria, viruses, and fungi can also be transmitted through water. Treating bees against nosema mold and varroa mites reduces the occurrence of viral diseases. Education and knowledge of beekeepers about bee diseases is of great importance in the fight against bee diseases. It is necessary to continuously educate beekeepers about preventive measures (Amiri et al., 2017; Bojanic Rasovic, 2022a). If a disease is suspected, beekeepers should contact the veterinary service for the purpose of diagnosing the disease and immediately stop the breeding, sale and distribution of potentially infected queen bees. In the event of an outbreak of infection in an apiary, it is necessary to act in accordance with legal regulations (Bojanić Rašović, 2018c, 2022a). Failure to comply with the law and quarantine when exporting or importing queen bees or

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bee colonies can lead to catastrophic consequences (Amiri et al., 2020b). The queen is in constant contact with worker bees, and during the breeding period with drones. Care should be taken to ensure that the queen is provided with food during transport and that no injuries occur. For transport, cages made of plastic with two compartments are used, with the larger one used to house the fertilized queen and young companion bees, and the smaller one for storing food. When removing the queen from the cage, care must be taken to avoid damage to the wings, legs and antennae. Queen bees should also be protected from sunlight (Bojanić Rašović, 2018c, 2022a). Genetic selection of the queen can greatly reduce the incidence of infectious diseases in the bee colony (Moritz et al., 2010; Santrač, 2021; Kaskinova et al., 2020; Büchler et al., 2024; Hubhachen et al., 2025). One of the main goals of the breeding program in beekeeping is to increase the resistance of bee colonies to diseases (Withrow, 2017; Rakić, 2020; Büchler et al., 2024). The bee colony is inspected for the presence of diseases at every selection inspection (*Fig. 4*). Resistance to diseases, especially to varroa, is a very important characteristic of a bee colony (Hubhachen et al., 2025). Special attention is also paid to detecting the symptoms of chalky bee brood. If the presence of chalky bee brood mummies is detected on the floor of the hive, in the honeycomb, or on the frame with the brood, the queens of those colonies are excluded from selection and reproduction. In bee colonies that do not have good hygiene behavior, clinical symptoms of the disease appear. A test to determine the hygienic behavior of the bees, which is very important for the defense and resistance of the colony to diseases, is carried out every year (Anon., 2024; Jašić et al., 2016; Büchler et al., 2024; Bojanić Rašović, 2025c).

Figure 2. *Queen bee replacement—with the help of a queen cage (bee apiary in Brežine, Lješanska Nahija, Montenegro) (Bojanić Rašović)*

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Figure 3. *Queen bee breeding apiary (Brežine, Lješanska nahija, Podgorica, Montenegro) (Bojanić Rašović)]*

Figure 4. *Overview of the honeycomb frame with bee brood (bee apiary in Brežine, Lješanska Nahija, Montenegro) (Bojanić Rašović)*

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Conclusion

Diseases and congenital anomalies of the queen are key factors that affect the health of the bee colony. Varroa, viruses and nosema are the most important factors that lead to the loss of bee colonies, as well as the queens themselves. However, there are also diseases that only queens suffer from. These are: melanosis, carrying immature eggs, carrying unfertilized eggs, blockage of the oviducts with sperm cells, blockage of the genital tract with excrement, catalepsy. Of the viruses, the deformed wing virus and virus that causes black queen cell disease are particularly important. In addition to the aforementioned diseases, various anomalies can also occur in the queen during her development. The most common and significant developmental anomalies of the queen bee are: queen bee stunting, deformed wings, ovarian hypoplasia, underdevelopment of the oviduct, spermatheca anomalies etc. Queen quality and health is influenced by a number of genetic and environmental factors. In order to prevent the occurrence of diseases of the bee colony, and therefore of the queen, it is necessary to respect the principles of good beekeeping practice and good veterinary practice. Genetic selection of queens can greatly reduce the incidence of infectious diseases in bee colonies. That is why the health of the queen bee, as well as the ability of the bee colony to prevent the occurrence of certain diseases, is of increasing importance in selection and breeding programs. Given the importance of the queen for the bee colony, queen diseases must be studied more thoroughly, with the aim of their prevention and control. In Montenegro is cultivated *A. mellifera carnica*, which has shown the best characteristics for this area. One of the main conditions for the protection of the species, successful selection and breeding of queens and development of beekeeping is a preserved and healthy environment.

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