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INFECTIOUS DISEASES OF BEES AND THEIR IMPACT ON THE VITAL ACTIVITY AND HONEY PRODUCTIVITY OF HONEY BEE COLONIES IN UKRAINE

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Summary. The article presents the results of the study of the mass mortality of honey bee colonies in different apiaries from different regions of Ukraine. The epizootic status of 102 honey bee colonies was studied and 607 samples of pathological material were analyzed in 2021–2023. According to the results of the monitoring of the epizootic situation in Ukrainian beekeeping it was found that the share of parasitic diseases (54.4%, 74.0%, 69.3%) constantly prevails over infectious diseases. It was noted that the incidence of varroosis in honey bees (34.4%, 71.4%, 41.47%) remains the highest among other diseases. Against the background of severe damage to honey bee colonies by the *Varroa* mite, infectious diseases began to appear in an atypical form, which significantly complicates their differential diagnosis. A probable increase in the incidence of nosemosis in adult bees and the detection of *Nosema* cysts in the intestines of bees, both in spring and summer, and the detection of cysts in honey indicate the spread of another pathogen, *Nosema cerana*, which causes nosemosis in the summer season. The reason for the periodic mass death of bees in Ukraine is the combination of a high number of parasitic *Varroa* mites in the honey bee colony with the presence of bee infection with microsporidia *Nosema* spp. These pathogens negatively affect the immunity of the honey bee colony, and cause exacerbation of latent infections in bees, which leads to a decrease in the number of honey bee colonies, weakening their viability and reducing the quality of honey products

Keywords: parasitic diseases, mites, microsporidia, fungi, viruses, bacteria

Introduction. The health and viability of the honey bee colony should be the basis of beekeeping because bee pollination is the main guarantee of maintaining the biodiversity of flora and fauna (Galatyuk, 2010).

However, studies by US scientists show that the benefits of bee pollination of entomophilous crops are 150 times higher than the cost of beekeeping products (Klatt et al., 2013; Traynor et al., 2016).

Without bee pollination, there would be no livestock production, as it would be impossible to obtain animal feed. Improving the food security level in Ukraine requires increased production, processing, and storage of agricultural products and the safety of their consumption.

This fully applies to the beekeeping industry, which provides: pollination of entomophilous crops, contributing to their yield; produces raw materials for many industries and exclusive bee products for human consumption (Polishchuk and Haidar, 2008; Ratnieks and Carreck, 2010; Zakhariya, Davydova and Gotska, 2020).

However, beekeeping is suffering significant losses due to several negative factors. The biggest problem is the periodic mass death of bees — a phenomenon known worldwide as 'colony collapse disorder' (Arnauta and Kalachniuk, 2017).

In Ukraine, in 2014–2015, about 40% of honey bee colonies died (Efimenko, Halat and Odnosum, 2014), and in 2020, 2022 and 2023, massive bee mortality was observed. The causes of death were various pesticide poisonings, consumption of honeydew honey during hibernation, and infectious and invasive diseases (Yefimenko and Odnosum, 2017). Laboratory tests of dead colonies revealed pathogens of invasive diseases in 60% of them.

The study aimed to find out what caused the massive loss of honey bee colonies in different apiaries from different regions of Ukraine.

Materials and methods. The study was conducted from 2021 to 2023 in the Laboratory of Veterinary Sanitation, Parasitology and Honey Bee Diseases of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine). In 2021 the pathological material of 60 apiaries from 17 regions of Ukraine was examined, a total of 372 samples of pathological material were examined, 278 samples of dead adult bees, and 94 samples of brood. In 2022, 23 apiaries in 5 regions were inspected and 140 samples of pathological material were examined. In 2023, 19 private apiaries in Kharkiv, Poltava, and Sumy regions of Ukraine were inspected. A total of 95 samples of pathological material were examined.

Pathological material was taken from 10% of honey bee colonies. The material was examined by group and individual methods. Microbiological, mycological, and parasitological studies were carried out according to existing methods (Fasulati, 1971).

Bee corpses were taken from the middle layer of dead bees formed at the bottom of the hive. Live bees were taken from the upper bar of the frames. Adults and brood

were examined for the presence of ectoparasites (detection and counting of *Varroa destructor* mites). To detect acarapidosis, 200 g of garbage from the bottom of the hive, a bee brood on the honeycomb from the bottom edge of 3–15 cm, and 100 live bees were selected. Bees were dissected and tracheal microscopy was performed for the presence of *Acarapis woodi* mites; microscopic examination of the intestinal contents for the presence of *Nosema* spp. spores and *Malpighamoeba mellificae* cysts.

Microscopy of the intestinal contents of the flight bee for the presence of pathogenic microflora and inoculation into nutrient media.

Laboratory diagnosis of the affected brood included:

— smear microscopy;

— cultivation of the pathogen on nutrient media and determination of its culture properties.

To exclude foulbrood diseases, honeycombs of 10×15 cm with brood were selected. In case of suspicion of membranous brood, 10×15 cm honeycombs were preserved in 50% glycerin solution. In case of suspicion of septicemia, paratyphoid, gafniosis, and polybacteriosis, 50 live bees were taken from the hive. For microscopy, smears were made from the larval suspension and stained with Gram stain to detect the vegetative form of the pathogen. The vegetative form is Gram-positive (appears blue or purple), and to detect spores, the smears were stained with a 2% alcohol solution of carbol fuchsin. Brownian motion is characteristic of *Paenibacillus larvae* spores. To detect Brownian motion, a carbol fuchsin-stained smear from larval suspensions was washed off with water and covered with a layer of immersion oil while still wet. After the smear was dried, it was examined under a microscope. The Brownian motion of spores was observed in the field of view in the water droplets in the immersion oil.

The diagnosis of bee virus diseases was made using PCR, 50 bees preserved in a 50% glycerol solution were examined.

Results and discussion. In 2021, we examined the pathological material from 60 apiaries from 17 regions of Ukraine, a total of 372 samples of pathological material were examined, of which 278 samples were dead adult bees and 94 samples were brood, the data are presented in Table 1.

Table 1 — Share of bee diseases in Ukrainian apiaries in 2021

No.	Region	The number of apiaries	The number of samples	Number of positive samples for pathogens			
				viral	bacterial	mycoses	parasitic
1	Kharkiv	20	92	6	20	5	25
2	Poltava	5	20	6	2	12	15
3	Sumy	5	30	6	5	–	17
4	Dnipropetrovsk	3	18	6	4	–	15
5	Donetsk	2	12	5	2	–	6
6	Luhansk	2	12	5	2	–	8
7	Lviv	2	12	1	2	6	7
8	Kyiv	2	18	1	2	–	15
9	Odesa	4	24	1	3	12	14
10	Kirovohrad	1	10	1	6	–	5
11	Mykolaiv	1	10	3	6	–	6
12	Ternopil	1	10	3	6	–	4
13	Chernihiv	3	24	4	–	–	18
14	Zhytomyr	2	18	1	2	8	14
15	Ivano-Frankivsk	3	24	1	–	–	15
16	Rivne	2	20	1	9	–	14
17	Zaporizhzhia	2	18	1	8	–	14
Total		60	372	52	79	38	202
				Share of bee diseases, %			
				13.9	21.3	10.4	54.4

According to the results of epizootological survey of apiaries and laboratory examination of pathological material in 2021, it was found that the proportion of parasitic diseases was significantly higher than bacterial diseases — 33.1%, viral diseases — 40.5%, and mycoses — 44.0%. The following bacterial pathogens were isolated from the dead bees and affected brood: American foulbrood pathogen *Paenibacillus larvae* — 4.3%; European foulbrood pathogen *Melissococcus pluton* — 15.3%, and *Paenibacillus alvei* — 1.7%. Our research is close to the results obtained by Stupak and Masliy (2009).

Among viruses, PCR was used to diagnose chronic bee paralysis virus (CBPV) isolated in 13.2% of samples,

and deformed wing virus (DWV) in 0.7% of samples. Among the mycoses, the pathogen of chalkbrood (*Ascosphera apis*) was recorded in Kharkiv, Lviv, Poltava, Zhytomyr, and Odesa regions, and the pathogen of aspergillosis (*Penicillum fusarium*) in Poltava Region.

In 2021, varroosis was detected in 34.4% of samples. Mite infestation in colonies averaged 0.91 ± 0.36%, which did not exceed the conditional level of colony well-being.

Thus, in Kharkiv Region, the average infection of a colony was 2.17 ± 1.42%, in Poltava Region — 1.00 ± 0.71%, in Ivano-Frankivsk Region — 0.15 ± 0.05%, in Odesa Region — 0.73 ± 0.16%, in Kyiv Region — 0.28 ± 0.06%, in Rivne Region — 0.11 ± 0.01%. Differences in bee infection by *Varroa* mites depended on many factors, including abiotic factors: a long bribe-free period in bees, significant changes in the thermo-hydro regime in spring and summer, and biotic factors: bee breeds, diversity of the nectar base. However, the main influence was exerted by the anthropogenic factor, in particular, non-compliance with the recommendations for veterinary and sanitary measures at apiaries during the year (Arnauta and Kalachniuk, 2017).

The share of nosemosis cases amounted to 47.9%. The disease was recorded in all regions. Nosemosis (*Nosema* spp.) was detected in high degree '++++' (more than 1,000 spores in the field of view of the microscope) in 14.6% of samples, average '+++' — 30.1%, weak '++' — 24.0%, and single spores '+' — 31.3% of samples. Infection with *Nosema* spp. spores were recorded in not only autumn and spring but also in summer, which gave us reason to suspect the presence of another pathogen, *Nosema ceranae*, which causes nosemosis in the summer. Characteristic clinical signs confirmed the presence of the disease: a significant decrease in the number of adult bees in the hive, a reduction in flight activity and honey productivity, and, as a result, a substantial weakening of the strength of the honey bee colony as a whole. Our assumptions coincide with the results obtained by other scientists (Efimenko, Halat and Odnosum, 2014; Odnosum, Yefimenko and Soroka, 2018; Yefimenko et al., 2014).

Most bee infections by pathogens of contagious diseases were detected in apiaries whose technological direction is honey collection (Kisil and Fotina, 2018; Sklyar, Gerasymova and Shkromada, 2017), especially during the nomadism period in areas with insufficient sown areas of entomophilic crops. Violation of veterinary and sanitary measures, such as the density of honey bee colonies per unit area, as well as the transportation of apiaries during the season to different regions without certification and laboratory diagnostic tests for the presence of pathogens in the colonies are the main factors in the spread of pathogens.

In 2022, 23 apiaries in 5 regions were inspected, and 140 samples of the pathological material were analyzed. According to the results of the epizootic survey of apiaries and laboratory examination of pathological material in 2022, it was found that the proportion of infectious diseases (26%) is significantly lower than parasitic diseases (74%).

Varroosis was diagnosed in 100 samples of pathological material, which amounted to 71.4% compared to other diseases. The prevalence of mite infection averaged 0.95 ± 0.34 %, which does not exceed the conditional level of colony well-being. Thus, in Kharkiv Region, the average prevalence was 1.17 ± 1.12%, in Poltava Region — 1.00 ± 0.25%, in Zaporizhzhia Region — 0.25 ± 0.05%, in Ternopil Region — 0.83 ± 0.11%, in Sumy Region — 1.25 ± 0.05%. In 2022, bee infection with *Varroa* mites depended mostly on anthropogenic factors, which were associated with military operations.

Nosema was diagnosed in 68 samples. The disease rate was 48.6%. The disease was found everywhere. Nosemosis was detected in high degree '++++' in 14.8% of samples, average '+++' — 17.6%, weak '++' — 17.6%, and single spores '+' — 50.6%. The average and slight degree of damage decreased slightly, and the number of single cysts increased by 19.9%.

The mixed course of bee diseases was detected in 8 apiaries, which represented 35% of the examined apiaries, the proportion of diseases was 57.14%. Thus, a mixed course of varroosis with non-communicable bee diseases (chemical toxicosis, pollen toxicosis) (10%), varroosis with nosemosis (46%), and varroosis with chalkbrood (1.14%) were detected. Musiienko, Kirik and Tsyt (2018) obtained similar results and developed methods of combating mixed forms of infectious diseases in honey bees.

In 2023, surveys were conducted in 19 private apiaries in Kharkiv, Poltava, and Sumy regions of Ukraine. A total of 95 samples of pathological material were examined. According to the results of the study of the epizootic situation in 2023 in the apiaries of Kharkiv, Poltava, and Sumy regions of Ukraine, it was found that the proportion of invasive diseases averaged 79.33% and was significantly higher than infectious diseases (20.67%). The share of varroosis was 41.47%, nosemosis — 27.86%, foulbrood — 16.85%, mycosis — 14.34%, and viruses — 3.48%. According to the results of the research, out of 19 private apiaries, five were healthy in Kharkiv Region, seven in Poltava Region, and one in Sumy Region, which is 73.7% of the total number of the examined apiaries.

Additionally, honey samples were tested for the presence of pathogens according to DSTU 8684:2016 (SE 'UkrNDNC', 2016). The results of the study of honey samples from beekeeping farms of different forms of ownership for contamination with pathogens of invasive bee diseases: nosemosis (*Nosema* spp.), amoebiasis (*Malpighamoeba mellificae*), varroosis (*Varroa destructor*), tracheal mites (*Acarapis woodi*), mermitidosis (nematodes) (*Mermis*), and braulosis (*Braula coeca*, *B. smitzi*, *B. orientalis*) are presented in Table 2.

Table 2 — Contamination of honey harvested in 2022 with pathogens of invasive bee diseases

No.	Region	Botanical origin	*Nosema apis*	*Nosema cerenae*	*Malpighamoeba mellificae*	*Varroa destructor*	*Acarapis woodi*	*Mermis*	*Braula coeca*, *B. smitzi*, *B. orientalis*
1	Kharkiv	sunflower	+	–	–	+	–	–	–
2		various herbs	+	–	–	–	–	–	–
3	Zaporizhzhia	various herbs	+	–	+	+	–	–	–
4	Poltava	various herbs	+	+	–	–	+	–	–
5	Ternopil	various herbs	+	–	–	+	–	+	–
6	Sumy	sunflower	+	–	–	+	–	–	–

The causative agent of nosemosis (*Nosema apis*) was identified in honey; microscopy revealed mature oval, ovoid cysts of the parasite measuring 4.5–7.5×2–3.5 μm with a smooth, three-layer shell 0.2–0.3 μm thick. The pathogen *Nozema cerenae* was identified in only one sample. It was also possible to identify the causative agent of amoebiasis (*Malpighamoeba mellificae*) in honey in the form of oval cysts 5–8 μm in size, with a smooth, dense shell (up to 1 μm thick) in Gram-stained smears.

Larvae of *Mermis albicans*, the causative agent of nematodosis, were identified in honey by their size length of 0.74 mm, and diameter of 0.034 mm. The pathogens of varroosis (*Varroa destructor*) and tracheal mites (*Acarapis woodi*) were detected in individual cases.

During the study the pathogens of invasive diseases were identified in honey samples from unfavorable apiaries (Table 3).

Table 3 — Contamination of honey harvested in 2023 with pathogens of invasive bee diseases

No.	Region	Botanical origin	*Nosema apis*	*Nosema cerenae*	*Malpighamoeba mellificae*	*Varroa destructor*	*Acarapis woodi*	*Mermis*	*Braula coeca*, *B. smitzi*, *B. orientalis*
1	Kharkiv	sunflower	+	+	–	+	–	–	–
2		various herbs	+	–	–	+	–	–	–
3	Poltava	sunflower	+	+	–	+	+	–	–
4		various herbs	+	–	–	–	–	–	–
5	Sumy	sunflower	+	–	–	+	–	–	–
6		various herbs	+	–	–	–	–	–	–

According to the results of the research, *Nosema apis* was identified in honey samples from apiaries unfavorable for diseases — 5 samples, *Varroa destructor* — in 4 samples, *Acarapis woodi* — in one sample. *Nosema cerenae* was identified in two samples from Kharkiv and Poltava regions. In honey samples where *Nosema apis* were detected, the mass fraction of water was higher (18.61%), while the average mass fraction of water was 17.59%. In the samples where *Varroa destructor* was detected, the diastase number was 11.20 Gothe units, while the average was 18.24 Gothe units. In samples of honey from unfavorable farms where *Ascosphaera apis* were detected, the mass fraction of water was 1.5% higher than the average.

Conclusions: According to the results of monitoring the epizootic situation in Ukrainian apiaries, it was found that the share of parasitic diseases (54.4%, 74,0%, 69.3%) constantly exceeds the infectious diseases.

It was noted that the incidence of varroosis in bees (34.4%, 71.4%, 41.47%) remains the highest among other diseases. Against the background of severe damage to honey bee colonies by the *Varroa* mite, infectious diseases began to appear in an atypical form, which significantly complicates their differential diagnosis. Thus, from an epizootiological point of view, varroosis should be considered as one of the main factors reducing the overall resistance of the entire honey bee colony as an integral biological organism.

A significant increase in the incidence of nosemosis in adult bees and the detection of *Nosema* cysts in the intestines of bees, both in spring and summer, as well as the detection of cysts in honey, indicates the spread of another pathogen — *Nosema cerana*, which causes nosemosis in the summer season. This leads to a significant decrease in the number of adult bees in the hive, a decrease in honey productivity, a significant weakening of the strength of the honey bee colony in the summer, and the departure or death in the fall.

The reason for the periodic mass death of bees in Ukraine is the combination of a high number of parasitic

mites *Varroa* in a honey bee colony with the presence of bee infection with microsporidia *Nosema* spp. These two pathogens negatively affect the immunity of the honey bee colony and cause exacerbation of latent infections in bees, which leads to a reduction in the number of honey bee colonies, weakening their viability and lowering the quality of honey products.

Prospects for further research. More research is needed to determine the impact of *Nosema cerana* and other pathogens on the quality of honey produced by bees.

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