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Study of Acute Bee Paralysis Virus in Some Regions of the Republic of Armenia

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ABSTRACT

Bees are of great importance in agriculture. Recently, beekeeping has been threatened by various viral diseases, which forces beekeepers to look for new solutions in the fight against bee diseases.

Epizootological and laboratory research was carried out in two regions (marzes) of the Republic of Armenia: Aragatsotn (Ashtarak community) and Tavush (Dilijan community). The clinical signs of the studied bees coincided with the symptoms of acute bee paralysis virus (ABPV). As a result of laboratory research, acute bee paralysis virus was confirmed in Tavush marz.

Introduction

Armenians have been engaged in beekeeping since ancient times. Honey, beeswax, royal jelly, bee venom, etc. are obtained from beekeeping (Markosyan, et al., 1984).

There are over 4000 bee species in the order of Hymenoptera, including those that are social or solitary, native or introduced, managed or wild. Honey bee colonies consist of approximately 35 000 individual bees, including sterile female workers, a few hundred male bees (called drones), and a single reproductive female queen bee. Honey bee colonies typically survive multiple years, while the longevity of individual worker bees depends on their caste (i.e., from six weeks to four months for worker bees, approximately eight weeks for drones, and several years for queen bees) (McMenamin, et al., 2018). The honey bee *Apis mellifera* is an insect that plays an important role in agriculture by pollinating a wide variety of crops and flowers (Morse and Calderone, 2000), increasing their yield (Taranov, 1948). The dependence of worldwide crops on pollinators is extremely deep and during 2005 the global economic value of insect pollination was estimated to be $212 billion a year (Gallai, et al., 2009). The economic value of honey bee pollination is estimated as several billion dollars; hence, the health of honey bees is an ongoing concern. Although the number of managed honey bee colonies worldwide are steadily increasing, it is not enough to meet the increasing demand for pollination in agriculture. Recent large-scale losses of managed honey bee colonies in some parts of the world and the decline of

wild pollinators have raised awareness and concern of the lack of pollinators (de Oliveira, et al., 2021).

In recent decades, beekeepers around the world have encountered serious honey bee colony losses. During the winter of 2006-2007, a phenomenon called Colony Collapse Disorder (CCD) mysteriously wiped out honey bee colonies from most parts of the United States but the cause of CCD remains unknown. Several factors such as the emergence of new parasites and pathogens, poor nutrition due to loss of forage land and monocropping, excessive and inappropriate use of pesticides and other environmental threats have been supposed to be the reason of widespread bee population declines. Among the potential factors reported so far, viruses have been suggested to be one of the major risk factors that negatively affect bee health (Pichaya Chanpanitkitchote, et al., 2018). The disappearance of bees not only leads to a drastic decrease in the number of many plant and animal species, but also to a reduction in natural resources. Based on the above, it is necessary not to ignore the fall of bees and to apply appropriate medical and preventive measures in time.

The diseases of bees are infectious and non-infectious. The main distinguishing feature of infectious diseases from non-infectious ones is the emergence and development of the epidemiological process (Grigoryan, 2002). Depending on the nature of pathogens, infectious diseases are classified into: prion, viral, bacterial, protozoal and fungal infections. Infection can be direct, that is, via contact with bees, and indirect, through infected feed, water, and vectors. Most often, bees are infected through food and water, rarely through the respiratory tract. Many studies have indicated that honey bee viruses can be shared by wild bee populations of bumble bees and solitary bees. While horizontal transmission of pathogenic viruses between honey bees and wild bees is thought to be ubiquitous and occurs through shared foraging spaces, the prevalence and intensity of these honey bee viruses in wild bee populations are not well documented (Laura J. Jones, et al., 2021).

Recently, beekeeping is increasingly threatened by viral diseases, and new strains of viruses appear that were previously unknown. To date, twenty-two viruses have been described in the honeybee, several of which have been linked to Varroa parasitism (Fanny Mondet, 2014). The mite Varroa jacobsoni plays an important role in the spread of viral diseases, which serves as a reservoir and transmitter of the virus (Glinsky and Jarosz, 1992). Virus infection of the family is directly related to the number of Varroa jacobsoni ticks present in it. Therefore, all the factors that directly or indirectly influence the levels of mites will be also influencing the presence of the viruses (Ana Molineri, et al., 2017). In high infestations, pathogens multiply rapidly to lethal limits. Mites are easily infected with the virus from sick bees and transfer it to healthy bees. The impact of viruses on their hosts is exacerbated by the stressors faced by bee populations, including parasites, poor nutrition, and exposure to chemicals (Grozinger and Flenniken, 2019). For example, survival in honeybees infected with viral pathogens is reduced under a monofloral diet (Dolezal, et al., 2019).

The Acute Bee Paralysis Virus (ABPV), is among the diseases that pose a serious risk to beekeeping enterprises globally. It was discovered for the first time during laboratory works on the identification of the causative agent of bee paralysis, i.e. the Chronic Bee Paralysis Virus (CBPV). Pathogens refer to RNA-containing viruses (www.pcheloved.ru). The virus is characterized by its resistance to various esters. It can persist in the bodies of dead bees for about 6 months (www.rsn.tomsk.ru). The acute bee paralysis virus (ABPV) has similar symptoms as the chronic bee paralysis virus(CBPV). However, acute is a term used to refer to increased mortality in bees, unlike with CBPV. Just like most other honey bee diseases, the Acute Bee Paralysis Virus can easily be transmitted from one bee colony to the other and across different hives. Bees are social insects that interact while foraging, making it almost impossible to completely eradicate most of the diseases that affect them. Humans also participate in the spread of diseases through their various activities such as: hive inspection, movement of bees during pollination services, importation of honey bees, and other unhygienic practices. The Acute Bee Paralysis Virus is present in most parts of the world. Countries such as the United States of America, Africa, Asia, Europe, and the Middle East have reported cases of ABPV. Scientists have pointed out that ABPV does not belong to any virus genus but is rather described or grouped under the picornaviruses order Dicistroviridae family. These refer to viruses that affect insects and are described as cricket paralysis viruses. The virus is so common and is spread widely in bee colonies, that it has become almost impossible to completely eliminate it (www.beekeepclub.com).

Acute bee paralysis virus (ABPV) was originally discovered during laboratory experiments as a cause of asymptomatic infections of adult bees (Benjeddou, et al., 2001). ABPV could attack all stages of honeybees, but the most favorable hosts for virus multiplication were the pupae (Chen, et al., 2005a).

The accumulation of viral particles in the brain and especially in the hypopharyngeal glands prove the

foodborne transmission of the virus through the salivary and gland secretions of infected adult bees used to feed the young larvae or mixed in the pollen (Benjeddou, et al., 2001). Infected larvae either die before they are sealed in brood cells if large amounts of virus particles were ingested, or survive to emerge as healthy infected adult bees (Bailey and Ball, 1991).

In some cases ABPV is characterized by the rapid death of adult bees; previously the lethally infected adults show a rapidly progressing paralysis, including trembling, inability to fly, and the gradual darkening and loss of hair from the thorax and abdomen (Maori, et al., 2007a). ABPV can cause infection without obvious symptoms.

Materials and methods

Virus diseases of bees are common all over the world. They are usually underestimated by beekeepers: they can cause serious economic losses if they are associated with other bee diseases. Virtually all viruses are present in apiaries in a latent or asymptomatic form (i.e, no symptoms are seen in the hive). Precipitating events, such as other hive diseases or stressors, can lead to the development of infection and the death of bees or the destruction of colonies and/or affected combs. Seasonal factors and the area where the apiary is located greatly affect the occurrence of bee viruses. To this day many bee viruses have been identified and classified, but there is insufficient information on their worldwide distribution (www.fao.org). This was another reason to start research.

Our goal was to identify the epizootological situation of viral diseases in the Tavush and Aragatsotn marzes of the Republic of Armenia and to confirm the disease in bees with clinical signs of acute paralysis virus by laboratory examination. Laboratory testing is required for the diagnosis of the Acute Bee Paralysis Virus. The tests were carried out in the past in countries such as the United States, Austria, Hungary, and Germany. The UK has also been at the forefront when it comes to the diagnosis of ABPV.

In order to find out the reasons for the decline of bees, the studies were carried out in field and laboratory conditions. The corpses of the bees that died from diseases were collected with tongs and subjected to visual observation. In the samples taken, clinical signs were weakly expressed. Ticks were present on some samples brought from Tavush and Aragatsotn marzes. The abdomen of the bees was swollen and color changed. Some of the samples from the Dilijan consolidated community of Tavush marz showed slight hair loss in the abdomen, which raised suspicion of ABPV. Such hair loss was not present in the samples brought from the Ashtarak community of Aragatsotn marz. The beekeepers said that before the fall, a trembling of the wings was noticed, and a large number of falls occurred in a certain period. According to the beekeepers, the bees in question were gathered in front of the hives and did not fly even when the beekeeper approached.

Obtained samples were placed in cooler bag and immediately sent to the laboratory. The presence of virus in all investigated samples was detected by polymerase chain reaction (PCR) with a Q1600 Real time PCR device. Nucleic acid extraction was performed using a HiGene™ Viral RNA/DNA Prep Kit (BIOFACT), according to the manufacturer's instructions. After, using the REVERTA-L kit (AmpliSens Biotechnologies), the samples were subjected to reverse transcription.

The PCR data were implemented using BioMaster HS-Taq PCR Kit following the manufacturer's protocol. All cDNAs were amplified by PCR for the related viral target and amplicons were visualized on a 2 % agarose gel and stained by GelRed (Biotium, USA).

Oligonucleotide primer pairs were employed in PCR assays:

F: TTATGTGTCCAGAGACTGTATCCA

R: GCTCCTATTGCTCGGTTTTTCGGT

Our results show the presence of ABVP in honey bees in the Tavush marz.

Results and discussions

The research was carried out in the communities of 2 regions of the Republic of Armenia: Dilijan consolidated community of Tavush marz and the Ashtarak united community of Aragatsotn marz. In the course of the research, we collected dead bees in the mentioned communities. According to the clinical signs, the cause of the recession was ABPV.

According to the research, ABPV was detected in the bees brought from the Dilijan enlarged community by the results of laboratory research, and ABPV was not confirmed in the samples brought from Ashtarak unified community.

A secondary cause of bees becoming infected with ABPV was the removal of bees infected with the disease, during which the bees became infected. The identification of the causes of the primary infection of bees needs more extensive research. In Dilijan community, research was conducted with 3 beekeepers, and in Ashtarak's united community of Aragatsotn marz – with 4 beekeepers. Only one of the 3 beekeepers in the Dilijan community had

infected hives; 5 out of 7 hives were infected, that is – about 70 %.

Vaccines in non-human creatures are not new. Inoculating an insect, however, is very different. In typical vaccines, either a dead or weakened version of a virus is introduced into an animal, whose immune system is then able to create antibodies to fight the disease. Insects, however, don't have antibodies, meaning they don't have the same type of immune response (www.smithsonianmag.com). Like most viral diseases, the Acute Bee Paralysis Virus cannot be controlled by medication (www.beekeepclub.com). This is the problem and the easiest way to solve it is the correct organization of bee treatment and hive disinfection.

Beekeepers were advised to disinfect beehives, position them away from each other to reduce contact between colonies, start fighting against ticks, treat bees with antibiotics, give bees immunostimulants and additional food, drain honey from infected beehives, remove beeswax, melt it and replace it.

Conclusion

According to epidemiological and laboratory research, acute paralysis of bees was detected in the Dilijan consolidated community of Tavush marz. As a result of the laboratory tests of bees based on clinical signs, the acute paralysis of bees in the Ashtarak united community of Aragatsotn marz was not confirmed.

According to the assessment of the epidemic situation in the two marzes, the conducted studies should be large-scale and of continuous nature. At the same time, it is necessary to identify the ways to spread the acute paralysis viral disease of bees and to develop effective measures to fight (treat) it.

An adequate response to the causes of death of bees from acute bee paralysis requires the creation of standardized diagnostics based on clinical signs (if there are clinical signs), and the creation of a regional baseline, which could help beekeepers accurately diagnose at the local level.

Knowing that the immune response of insects differs from the immune response of animals, it is necessary to make the correct organization of bee treatment and hive disinfection.

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