Diagnosis of varroosis taking into account modern requirements

A N Sotnikov, M I Gulyukin, Y G Isaev and E A Gulyukin
Federal State Budget Scientific Institution “Federal Scientific Centre VIEV”
Ryazanskiy prospekt, 24, 1, Moscow, 109428, Russia

E-mail: 5er@inbox.ru

Abstract. Pollination is an indispensable service for the ecosystem in which bees are distributed, therefore the health of bee colonies is one of the most important conditions, primarily for plant biodiversity, and is extremely important for sustainable and cost-effective agricultural production. Honey bees are susceptible to various diseases. Currently, the invasion caused by the tick Varroa destructor covers every apiary, taking the scale of panzootia. The fight against tick invasion is carried out, achieving only temporary success. Varroa destructor, parasitizing on a bee, opens the gates of a viral infection into its body, which significantly aggravates the situation and leads to the phenomenon of “Colony collapse disorder (CCD)”, reducing the number of families. Ordinary beekeepers and scientific researchers apply various methods of diagnosing invasion. Most of these methods have a significant error - from 2 to 12%, which, in some cases, exceeds the safe threshold for varroa tick content in the bee family. Therefore, science must develop new, humane and accurate methods for determining the degree of affection of the bee family with the varroa tick. Currently, work is underway at the Federal State Budget Institution «Federal Scientific Centre VIEV» (FSC VIEV) on accurate methods for the intravital diagnosis of varroosis.

1. Introduction
Honey bees are one of the most important pollinators of wild and cultivated flowering plants. The value of pollination conducted by them is many times higher than the value of the beekeeping products produced. Pollination is an indispensable service for the ecosystem in which bees are widespread, therefore the health of bee colonies is one of the most important conditions, primarily for plant biodiversity, and is extremely important for sustainable and cost-effective agricultural production.

Honey bees are susceptible to diseases caused by parasites, fungi, bacteria and viruses, and can also be exposed to various pests, predators and adverse environmental factors (including human activities).

Currently, the invasion caused by the tick Varroa destructor covers every apiary, taking the scale of panzootia. The fight against tick invasion is carried out, achieving only temporary success. Varroa destructor, parasitizing on a bee, opens the gates of a viral infection into its body, which significantly aggravates the situation and leads to the phenomenon of “Colony collapse disorder (CCD)”, reducing the number of families in individual apiaries by 30-60%, which leads to a significant decline in crop yields around the world [1].

Varroosis (Varroatosis) is a disease that affects honey bees (of the Apis genus), which are caused by ticks of the Varroa genus (mainly Varroa destructor). Being an ectoparasite of an adult honey bee and its brood, the tick spreads by direct contact of adult bees as a result of movements of infected bees and...
Female ticks parasitize mainly on young intra-beehives, attaching to the intersegmental partition between the third and fourth abdominal segments on the left side. At the age of the owner more than 7 days, the tick moves to the chest and head. It is rarely found on bees that bring water to the nest, since they have the greatest secretion of the Nasonov gland. In the brood during the active period of life of the nest of bees, up to 70-90% of ticks can be contained. In the summer, the winter generation of the female parasite is replaced by a new one.

The breeding rate of the tick depends on many factors: climatic and natural conditions, bee breeds, family strength, the ratio of bee and drone brood in it during the season, etc. The brood located in old combs (more than 2-3 years of operation) is affected the tick is much larger than in freshly built combs. Female ticks hibernate on bees, penetrating deeply between the abdominal segments, and feed on hemolymph. To maintain viability, a female tick needs 5.5 μg of hemolymph, if it is in the body of a wintering bee on average 4.3. The resistance of the tick in the external environment depends on temperature and humidity. In a hungry state, under optimal conditions for life, the parasite lasts up to 5-7 days, in a sealed brood at 20°C - up to 30-40 days. Loss of 5-10% of water by the body of the parasite complicates nutrition and the ability to reproduce (in 50% of females). They die after the loss of 10-20% of water [4].

2. Diagnosis of varroosis

The mite of varroa should be distinguished from other gamasid mites and braules found on bees.

Studies performed using molecular diagnostic methods have revealed, in some cases, infection of bees with viruses of more than 90%. Up to five to six types of viruses of different taxonomic affiliations can be simultaneously present in a bee colony strongly affected by a tick of varro [5].

To date, regulatory authorities in the field of veterinary medicine have come to the conclusion: since the invasion of the Varroa tick cannot be eliminated by the available methods, it was decided to limit the presence of the Varroa tick in the bee colony to a level of 4% hardening. According to clause 5.3.1. the current “Instructions on measures for the prevention and elimination of diseases, poisonings and main pests of bees”, the diagnosis of varroosis is made on the basis of visual detection of ticks on bees, brood and wax-pepper crumbs from the bottom of the hive in an apiary or veterinary laboratory, taking into account the epizootic situation . The viability of bee colonies is predicted by three degrees of damage: weak - up to two, medium - up to four and strong - over four ticks per 100 bees and in 100 cells of a drone or bee brood from the middle of the nest. Farms that have bee families with the first two degrees of damage are considered conditionally prosperous and show as prosperous in veterinary reporting [6,14].

This level was included at one time to determine the quality of the treatment by the beekeeper, when they allowed to use the drug bipin (the active substance is amitraz). To determine the degree of hardening in the veterinary laboratory in the spring, dead bees are sent from the bottom of the hive after wintering from 10% of apiary bee families. This is due to the fact that during the winter period, ticks showered at the bottom of the hive from the action of the drug and due to natural death. Bees in this period are not active and do not clean out dead bees, ticks or wax crumbs. Such studies were carried out at the very initial stage in detecting affection of apiaries with a tick of varroa. The results of such studies at the moment cannot be considered complete, due to the fact that it is impossible to determine the degree of damage to the bee family. It can only be argued that the bee family is affected by a tick of varroa [8, 9, 11, 13].

One of the most informative and quite affordable ways to determine tackiness is the method of sampling individuals. This method can be used by both veterinarians in the laboratory and ordinary beekeepers in the field. It should be carried out in late autumn, when there is no brood in the bee families, the flight of bees and the transfer of parasites from family to family are stopped. Due to the fact that
female ticks are attached under the tergites of young bees, the selection of bees in the amount of 100 individuals or more is carried out by sweeping the bees from each street in equal amounts, starting from the edge of the nest to the center in a transparent cellophane (polyethylene) bag, excluding getting the uterus in the sample. A label with the family number is placed in the package, examined for the presence of the uterus, and if it is found, the uterus is released into the family. In the laboratory or directly in the apiary, the bees are boiled with boiling water, adding a small amount of washing powder. Boiling water with washing powder is poured into a transparent glass jar in a 700 ml jar, a sample of bees is placed there, mixed thoroughly with a glass rod for two to three minutes. After that, the bees are removed from the can and placed on white filter paper, their number is calculated, inspecting for the presence of ticks stuck in the limbs of the bees, they are collected. Water from the can is filtered through a four-layer gauze filter. Collect and count ticks remaining on the filter. Calculation of the affection of the bees of the family, expressed as a percentage. The presented diagnostic method allows you to get a general idea about the situation with the tick in the apiary, but compared with the acute experience (when they kill the whole bee colony and analyze it) it has a significant error - from 2 to 12%, which exceeds, in some cases, a safe threshold by the content of the tick of varroa in the bee family.

One of the visual methods for diagnosing ticks of varroa is when live bees are placed between transparent polymer plates the size of a CD. Bees sandwiched between two plates are examined for the presence of female ticks on one side and the other, the number of bees and ticks detected is counted, and then the bees are released into the hive along with the tick. Bees remain alive - it is humane, but the tick continues to parasitize [8,10,11,12].

Known diagnostic methods for dusting bees using finely divided powder of talc, chalk or other substances that act on the pre-mite of the tick, dusting the bees, and then counting the number of ticks remaining in the powder. This method is probably effective when the female varroa tick moves around the bee, but does not fit, in the case when the female attached to the bee under tergite, the attached tick will not fall off from dusting and will not be displayed in the results. On this principle of action (on the pre-mite tick), drugs were proposed in which acaricides were present, but because of their low efficiency they were not widely used in practice.

3. Conclusion
Many ordinary beekeepers, and even agricultural specialists, still use the empirical approach, determining the hardening of the apiary by how many or few mites showered after the treatment, not having an idea of the exact degree of damage by varroa infestation [17,18,19].

So, scientific researchers and beekeepers are faced with the problem: there are no effective methods for the intravital diagnosis of varroa ticks in the bee family - the bee family has been treated, the drug has shown its effective effect on the parasite, but absolutely accurate counting is impossible, leaving the bee family subjected to treatment alive.

Following the principles of humane treatment of animals, as well as for a more accurate determination of the percentage of mites, it is necessary to introduce humane methods for diagnosing the infestation of bee colonies with tick-borne invasion.

For this, all individuals of the investigated bee colony must be immobilized for a long time and manually inspected each bee colony, counting and mechanical removal of the parasite, freeing the bee colony with full certainty (100%). The work is very laborious, the best time for such studies is at the beginning of wintering. After the diagnosis, it is necessary to leave the bee family for further life. This will allow the beekeeper to save the number of their own bee families and develop them in the next season, avoiding the acquisition of new families.

At this time, the team of the laboratory for bee diseases at the Federal State Budget Institution «Federal Scientific Centre VIEV» (FSC VIEV) and Sciences is working on this issue. Science will provide veterinarians with not only a method of treatment, but together with the diagnosis and mechanical cleaning of bees from ticks of all families, which will improve apiaries [7,11,12,14,15].
Acknowledgments
Work is done within the approved state task and the plan of researches to Federal State Budget Institution «Federal Scientific Centre VIEV» (FSC VIEV) for 2019-2021 year without attraction of additional sources of financing.

The financing was obtained in the frames of State Assignment from the federal budget without the attraction of additional financing sources. The authors confirm the absence of any conflict of interest.

References
[1] Sotnikov A N, Gulyukin M I, Gulyukin A M, Isaev Y G and Shabeykin A A, 2020 Epizootic process in quarantine bee diseases IOP Conference Series: Earth and Environmental Science 421(8) DOI:10.1088/1755-1315/421/8/082029
[2] 2018 OIE Terrestrial Animal Health Code (France: OIE)
[3] Grobov O F, Guzeva L N, Mikityuk V V, Batuev Yu M, Obukhov M L and Chernov K S 1985 Varroa mite - carrier of bees Disease Causative Agents Beekeeping 6 15
[4] Sadovnikova E F, Gisko V N and Pankiv E M 2019 Varroaosis of bees: recommendations - Vitebsk: VGAVM 32
[5] Udina I G, Kunizheva S S, Zlobin V I, Grishechkin A E, Kalashnikov A E, Krivtsov N I and Uchaeva V S 2010 Detection of the wing strain virus in the honey bee Apis mellifera L. in the Moscow Region by RT-PCR. Questions of Virology 55 37-40
[6] “Instruction on measures for the prevention and elimination of diseases, poisoning and the main pests of bees” approved by the Ministry of Agriculture of the Russian Federation on 08/17/1998 13-4-2/1362
[7] Luchko M A, Sotnikov A N and Stafford V V 2015 On the widespread use of chemotherapeutic drugs and antibiotics in beekeeping Proceedings of the VIEV 78 257-62
[8] Sotnikov A N 2016 Bee diseases and modern beekeeping One World - One Health 12-15 362-5
[9] Sotnikov A N, Gulyukin M I, Stafford V V, Luchko M A and Volodko D V 2017 Winter work with bee families in the apiary Veterinary medicine and feeding 2 28-31
[10] Grobov O F 2010 The role of varroa in the mass death of bees Proceedings of the VIEV 76 160-5
[11] Sotnikov A N, Gulyukin M I, Volodko D V, Konovalova T V, Stafford V V and Tolmacheva V A 2019 Toolkit for the maintenance, feeding, diagnosis, treatment and prevention of bee varroosis outside the nest in order to obtain organic beekeeping products (Moscow: RSKHN)
[12] Batuev Ju M, Drinjaev V A, Berezina L K, Novik T S and Tihomirova O I 2010 Varroa mit resistance to drugs Beekeeping 1 24-5
[13] Dorozhkin V I, Smirnov A M, Suvorov A V, Gunenkova N K and Isaev Y G 2016 Results of the coordination of scientific research on veterinary sanitation, hygiene and ecology for 2011-2015 Russian journal Problems of Veterinary Sanitation, Hygiene and Ecology 2(18) 6-10
[14] Smirnov A M, Dorozhkin V I, Suvorov A V, Gunenkova N K and Isaev Y G 2016 Coordination of scientific research: a new stage and new tasks Veterinary and feeding 1 8-10
[15] Shabeykin A A, Gulyukin A M, Stepanova T V, Kozyreva N G and Ivanova L A 2019 Risk assessment for interspecies transmission of enzootic bovine leukemia IOP Conf. Ser.: Earth Environ. Sci. 315 042036 https://doi.org/10.1088/1755-1315/315/4/042036
[16] Gulyukin A M, Belimenko V V, Shabeykin A A, Drosheva E A and Laishevtsev A I 2020 Epizoototical geo-information systems IOP Conf. Ser.: Earth Environ. Sci. 421 042013 https://doi.org/10.1088/1755-1315/421/4/042013
[17] Fernandez P J and White W R 2010 Atlas of transboundary animal diseases (France: OIE)
[18] Belimenko V V 2016 Protozoan diseases in pets (Moscow: Infra-M) DOI: 10.12737/17436
[19] Belimenko V V, Rafienko V A, Drosheva E A, Laishevtsev A I and Kapustin A V 2019 Application of geoinformational systems for veterinary geology IOP Conference Series: Earth and Environmental Science 315 032015