SOS HONEY BEE: DETECTION OF FOUR HIGHLY PATHOGENIC BEE VIRUSES IN APIARIES FROM REPUBLIC OF MOLDOVA

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ALBINA MELIFERĂ ÎN PERICOL: IDENTIFICAREA A 4 VIRUSURI PATOGENE PENTRU ALBINELE DIN REPUBLICA MOLDOVA

In colaborare cu savanții celui mai prestigios Centru de Excelență din Franța prin aplicarea metodelor molecular-genetice, în premieră pentru Republica Moldova, a fost demonstrată prezența a patru ARN-virusuri cu consecințe nefaste pentru familiile de albine, vectorul transmisibil fiind acarianul ***Varroa destructor.*** *De remarcat că până în prezent în lume sunt cunoscute 18 virusuri care afectează stupunele albinelor.*

Introduction

The scientific interest in viral diseases of the honeybee (*Apis mellifera* L.) has been increasing considerably during the past few years. At least 18 different viruses have been detected in honeybees so far. Viruses in certain cases may cause serious or even lethal disease in individual bees or the collapse of entire colonies without clinical symptoms [1]. Infestation with the ectoparasitic mite *Varroa destructor* is the major predisposing factor [3, 6]; however, a variety of other weakening circumstances may play a role in clinical manifestation of bee virus infections (e.g., *Nosema apis* infestation, intoxications, environmental pollution, and cold weather) [1].

In practical terms, six viruses are considered to be able to cause severe disease in honeybees, and hence they are most important in beekeeping. These are sacbrood virus (SBV), chronic bee paralysis virus (CBPV), black queen cell virus (BQCV), deformed wing virus (DWV), acute bee paralysis virus (ABPV), and Kashmir bee virus (KBV).

The study of the impact of viral diseases on honey bee colonies suffers from a lack of data concerning bee virus prevalence, particularly in asymptomatic colonies. The great diversity of viruses isolated from honey bees, the lack of specific clinical signs and the limited availability of tools for rapid and large-scale diagnoses are partially responsible for this situation. Complete or partial sequencing of several RNA viruses of the honey bee has allowed the recent development of highly sensitive methods for detection based on amplification by reverse transcription-PCR (RT-PCR) of specific viral sequences. Originally developed for diagnosis of human and animal RNA viruses, these methods have been successfully adapted to identify several RNA bee viruses, including SBV, KBV, CBPV, ABPV, BQCV, and DWV [5, 8, 9, 10].

We report here the first survey of the prevalence of bee RNA viruses in different apiaries from Republic of Moldova based on large-scale sampling of adult bees, brood, and varroa mites.

Material and Methods

Bee and varroa mite sampling. Beekeepers from different parts of the Republic of Moldova submitted bee samples from colonies suffering from symptoms of depopulation, sudden collapse, paralysis or dark color, and varroa infestation.

Sample preparation and PCR analysis. The frozen samples were crushed in a mortar in the presence of liquid nitrogen and were homogenized

in 10 mM Tris-400 mM NaCl buffer (pH 7.5). An aliquot of supernatant was used for extraction of total RNA with a Nucleospin RNA-II kit (Macherey-Nagel) used according to the supplier's recommendations. An average of 2 µg of total RNA was retrotranscribed at 25°C for 10 min and at 50°C for 1 h with a Thermoscript RT-PCR kit (Invitrogen) by using random hexamers.

PCR assays were done as follows. Five microliters of cDNA (1/10 dilution with water) was mixed with 2.5 µl of 10× buffer (100 mM Tris-HCl [pH 9], 500 mM KCl, 15 mM $MgCl_2$, 1% Triton X-100, 2 mg of bovine serum albumin per ml), 0.25 µl of a solution containing each deoxynucleoside triphosphate at a concentration of 20 mM, each primer at a concentration of 0.5 µM, and 1 U of *Taq* polymerase (Q-Biogen); the final volume of the mixture was 25 µl. The mixture was heated for 2 min at 95°C, and this was followed by 35 amplification cycles (30 s at 95°C, 30 s at 56°C, and 1 min at 72°C) and then by 7 min at 72°C to complete the polymerization. PCR products were analyzed by 1.5% agarose gel electrophoresis [10].

Results and Discussion

A total of 47 honeybee samples originating from different parts of Republic of Moldova, were investigated by RT-PCR for the presence of the six most important honeybee viruses.

A large majority of the apiaries were found to be infected by several viruses, as 76% of the apiaries were found to be positive for at least three different viruses. As a whole, higher virus frequencies were detected in adult populations than in brood populations. In adults during the DWV, SBV, BQCV, ABPV were found in 62%, 54%, 37% and 10%, respectively. CBPV and KBV viruses were never detected in bees.

SBV primarily affects the brood of the honeybee and results in larval death [7]. Infected larvae fail to pupate and ecdysis fluid aggregates around the integument, forming the "sac" for which the disease is named. Larvae change in color from a pearly white to pale yellow; after death, they dry out and change to a dark brown ship-shaped scab. Infection of adult bees is possible; the viruses are able to propagate in them, but the bees remain apparently healthy. Sacbrood virus appears mainly in spring, when the brood season begins and large numbers of infected young adults are present [7].

BQCV is common in adult bees; however, it clinically affects mainly prepupae or pupae of the queen, especially in spring and early summer [3]. The symptoms are similar to those of sacbrood. Infected queen pupae die and darken, and the cell walls get black.

DWV is mostly detected in *Varroa*-infected bees [4, 8]. The virus propagates slowly, and pupae infected at the white-eye stage of development may have malformed wings. The majority of infected bees do not show any symptoms.

ABPV commonly appears in apparently healthy bees; however, it has been presumed that this virus plays a role in cases of sudden collapse of honeybee colonies infested with *V. destructor*. Due to the spread of the varroa mite in Europe during the last decades, ABPV has gained more and more importance [2]. On one hand, the mite is a possible vector for the virus; on the other hand, it weakens the bees and activates viral infections [9].

Conclusion

To summarize, we have looked into the occurrence of the six most important honeybee viruses in diseased bee colonies and identified remarkable differences in the distribution pattern of the viruses in the different geographic regions of Moldova. These differences may be partly explained by differences in climate, landscape, and density of the bee populations; however, trade and exchange of infected animals, contaminated equipment, and bee products between apiaries, regions, or even countries may be of greater importance in the spread of viruses.

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References

1. Allen, M., and B. V. Ball. The incidence and world distribution of honey bee viruses. // Bee World, 1996, Nr. 77. – p. 141-162.

2. Ball, B. V. Varroa and viruses. *In* P. Munn and R. Jones (ed.), Varroa! Fight the mite. International Bee Research Association, Cardiff, Wales, 1997. - p. 11-15..

3. Ball, B. V., and M. Allen. The prevalence of pathogens in honey bee (*Apis mellifera*) colonies infested with the parasitic mite Varroa jacobsoni. // Ann. Appl. Biol., 1988, Nr.113. – p. 237-244.

4. Bowen-Walker, P. L., S. J. Martin, and A. Gunn. The transmission of deformed wing virus between honeybees (*Apis mellifera* L.) by the ectoparasitic mite

Varroa jacobsoni Oud. // J. Invert. Pathol., 1999. Nr. 73. – p. 101-106.

5. Grabensteiner, E., W. Ritter, M. J. Carter, S. Davison, H. Pechhacker, J. Kolodziejek, O. Boecking, I. Derakhshifar, R. Moosbeckhofer, E. Licek, and N. Nowotny. Sacbrood virus of the honeybee (*Apis mellifera*): rapid identification and phylogenetic analysis using reverse transcription-PCR. // Clin. Diagn. Lab. Immunol., 2001. Nr. 8. – p. 93-104.

6. Nordstom, S., I. Fries, A. Aarhus, H. Hansen, and S. Korpela. Virus infection in Nordic honey bee colonies with no, low or severe *Varroa jacobsoni* infestations. // Apidologie, 1999. Nr.30. – P. 475-484.

7. Ritter, W. Diagnostik und Bekämpfung von Bienenkrankheiten. Gustav Fischer Verlag Jena, Stuttgart, Germany. 1996. - p. 104-114.

8. Stoltz, D., X. R. Shen, C. Boggis, and G. Sisson. Molecular diagnostic of Kashmir bee virus infection. J. Apic. Res., 1995. Nr.34. – p. 153-165.

9. Tentcheva, D., L. Gauthier, S. Jouve, L. Canabady-Rochelle, B. Dainat, F. Cousserans, M. E. Colin, B. V. Ball, and M. Bergoin. Polymerase chain reaction detection of deformed wing virus (DWV) in *Apis mellifera* L. and *Varroa destructor*. Apidologie, 2004. Nr. 35:431-439.

10. Diana Tentcheva, Laurent Gauthier, Nathalie Zappulla, Benjamin Dainat, François Cousserans, Marc Edouard Colin, and Max Bergoin Prevalence and Seasonal Variations of Six Bee Viruses in *Apis mellifera* L. and *Varroa destructor* Mite Populations in France // Appl Environ Microbiol. 2004 Nr. 70(12). – p. 7185–7191.

Andrei Sârbu. Prăsadă. Tehnică mixtă, pânză. 1970-1972