

Detection of acute bee paralysis virus by RT-PCR in honey bee and *Varroa destructor* field samples: rapid screening of representative Hungarian apiaries

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Abstract – A two years survey was undertaken to determine the occurrence of Acute Bee Paralysis Virus (ABPV) in field samples of adult bees and the parasitic mite *Varroa destructor*. To detect the viral nucleic acid we used polymerase chain reaction following reverse transcription (RT-PCR). We demonstrated the presence of ABPV RNA in 14 from 114 seemingly healthy colony samples collected from Hungarian honey bees. The investigation revealed that two third of the apiaries were infected with ABPV at a 12.2% infection rate. In seven other apiaries out of eight investigated (87.5%) the presence of the virus was detected from colonies following a sudden collapse; these colonies were simultaneously infected with *Nosema apis* or infested with *Varroa destructor*. Virus specific nucleic acid was also identified in the mites collected from two apiaries falling into the latter category. The amplicon of RT-PCR was sequenced and the nucleic acid sequence was aligned to the complete ABPV sequence deposited in the GeneBank database revealing a 93% homology.

acute bee paralysis virus / polymerase chain reaction / *Varroa destructor* / *Nosema apis* / honey bee

1. INTRODUCTION

Acute bee paralysis was diagnosed first by Bailey et al. (1963) as an inapparent infection of adult honeybees. Since that time the presence of the virus has been detected in several countries throughout Europe, in-

cluding Hungary (Békési et al., 1999). ABPV is considered to be a common infective agent present in a high proportion of apiaries, causing hidden infections (Hung et al., 1996) but resulting in losses only in colonies heavily infested with the parasitic mite *Varroa destructor* Anderson &

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Trueman 2000 (Ball, 1985; Ritter et al., 1984). This mite had been previously identified as *Varroa jacobsoni* Oud. 1904 but the type infesting *Apis mellifera* L. was recently taxonomically changed to *V. destructor* (Anderson, 2000; Anderson and Trueman, 2000). The mite is considered to act as an activator of the inapparent infection and also as a virus vector transmitting ABPV (Ball and Allen, 1988; Bowen-Walker et al., 1999). This supposition was supported by the detection of the virus in the mites by the use of indirect ELISA (Allen et al., 1986). The role of *Varroa destructor* as a predisposing factor and vector was also reported in the case of other honeybee pathogens (Abrol, 1996; Brødsgaard et al., 2000). The term “bee parasitic mite syndrome” has been used for the disease complex, that is observed in colonies infested with mites and infected with viruses simultaneously (Shimanuki et al., 1994; Hung et al., 1995) and accompanied with high mortality.

Several hypotheses has been formed to explain which effects are responsible for causing the symptoms. The feeding activities of *V. destructor* can reduce the protein content of the hemolymph (Glinski and Jarosz, 1984), cause weight loss, and reduce longevity in the parasitized bee (De Jong and De Jong, 1983). Furthermore there are hypotheses directly involving the ABPV. Faucon et al. (1992) showed that *V. destructor* could transmit ABPV into a bee’s haemolymph when the mite feeds. Ball (1989) showed that *V. destructor* collected from naturally infested colonies transmitted ABPV and other viruses to healthy test pupae. Adult bees in which the virus has been activated or injected by *V. destructor* are probably able to infect young larvae by secreting the virus in gland secretions that are fed to the larvae before the adult bee succumbs (Ball and Allen, 1988).

Studies on inapparent infections of ABPV (and Kashmir Bee Virus) by Shimanuki et al. (1994) suggest that the im-

pact of the mite is mainly activation and not transmission of the virus. Their work also indicated that mere piercing by the mite did not activate an infection. Referring to the laboratory experiments by Ball (1989) and Shimanuki et al. (1994) opens for the possibility that the detection of ABPV in the control pupae could have been the result of an activation of an inapparent infection, elicited by the feeding of the mite and not a transmission.

Besides the role of the mite (whether *V. destructor* transmits the virus or just activates an inapparent infection), the pathogenicity of the virus and its relationship to the mite infestation seems to be far from being understood. The importance and consequences of the viral infections of the honeybee, among which ABPV is one of the most frequent one in many countries (Vecchi et al., 1990; Ruzicka, 1991), is also not fully appreciated. For example in Britain where the parasitic mite *Varroa destructor* had not occurred in the time of their investigations, Bailey and Ball (1991) reported that ABPV had never been associated with disease or mortality in nature. The virus appeared to be contained within the tissues that are not directly essential to the life of the bee. Infectivity tests made by Bailey and Gibbs (1964) estimated that live adult bees in the summer could contain as much as 10^6 virus particles without showing signs of paralysis and without any increase in mortality. In such an inapparent infection the virus must be contained in non-vital tissues i.e. fat-body cells, and the replication of the virus must be suppressed. This statement is supported by the fact that in other tests, where the virus is injected directly into the blood, as few as 10^3 virus particles can cause acute paralysis (Ball, 1985). Activation of ABPV may happen by piercing the body wall of the bee, which then soon after will become systemically infected and succumb. Alternatively, when the mite pierces the tissues it causes damages which might enhance the release of the virus and allow it

to replicate. Another hypothesis is that the mite activates the virus by the introduction of foreign proteins such as the mite's digestive enzymes released into the blood while sucking.

Studies from Eastern Europe and America, reveal that ABPV may be a major cause of death in bee colonies infested with *Varroa destructor* (Batuev, 1979; Carpana et al., 1991; Österlund, 1998). What is more, according to records from Belize and Nicaragua ABPV was detected in large amounts in dead adult bees and diseased brood and yet it is reported that *Varroa destructor* is absent from both countries (Allen and Ball, 1996). In Hungary large amounts of ABPV were detected in 1998 during an outbreak when characteristic symptoms of paralysis were observed and other causes leading to increased mortality were excluded (Békési et al., 1999).

The difficulties of the diagnosis of ABPV may also contribute to the contradictory opinions on the significance of the infection. The conventional diagnosis of ABPV infection, like other bee viruses, is based on the detection of the virus from homogenates of bees using electron microscopy directly or from homogenates of pupae following inoculation with the test material (Vechci et al., 1990). The latter diagnostic method is labour and time consuming and also season dependent, since pupae can be collected only in spring and summer. Electron microscopy generally is complemented by agar-gel immunodiffusion (AGID) test, since several bee pathogen viruses are morphologically similar to ABPV. This procedure has a low sensitivity, requires the costly development of immune-reactive sera, and is not suitable for large-scale screening. The method of indirect ELISA worked out by Allen et al. (1986) was very sensitive but also immune serum dependent.

Recently the use of PCR in the direct diagnosis of bee virus infections was shown to be a very appropriate tool, to overcome

the aforementioned difficulties of the diagnosis of bee virus infections: it is not dependant on immune-serum, there are no cross-reactions and the diagnosis can be supported by genetic identification using the amplicons. These advantages were utilized by Benjeddou et al. (2001) when they developed an RT-PCR method, which was used for testing laboratory specimens containing ABPV in high concentration. Field samples were not included in their investigations and the sensitivity of the system was not compared to any other classical method of virus identification and/or diagnosis.

To collect information on the connection between virus infection, mite infestation and clinical symptoms observed in a colony or among an apiary, it is necessary to trace the spread and circulation of ABPV. For this work a sensitive, reliable and high throughput approach is needed. The polymerase chain reaction following reverse transcription (RT-PCR) has been successfully applied for the diagnostics of sacbrood virus (Grabensteiner et al., 2001), Kashmir bee virus (Hung and Shimanuki, 1999), and recently on black queen cell virus (BQCV) and ABPV as well (Benjeddou et al., 2001). In this latter report stock virus from artificially infected pupae was tested. In the present study, we report on a RT-PCR method for the detection of the ABPV genome in field specimens.

2. MATERIALS AND METHODS

2.1. Samples and sampling

Three categories of samples were investigated (Tab. I):

A: Apparently healthy adult bees, *A. mellifera*, were sent by twelve volunteers from the five regions of Hungary. The volunteering beekeepers had 30 to 150 colonies, and sent samples during the test period from three randomly selected colonies. The

Table I. Results of the survey of the RT-PCR test on samples sent by volunteering bee keepers. Numerators indicate how many samples proved to be positive (infected/infested) from a total indicated by the denominators. N = not tested (For various reasons: i.e. samples were not sent, one volunteer had to give up bee-keeping due to health problems, etc.). Samples from which mites were collected and tested with RT-PCR are signed with asterisk.

Code of the apiary	Samples							
	1999 spring		1999 autumn		2000 spring		2000 autumn	
	Virus	Mite	Virus	Mite	Virus	Mite	Virus	Mite
PT/B	1/3	0/3	1/3	1/3	0/3	0/3	0/3	0/3
KF/K	0/3	2/3	0/3	2/3	1/3	1/3	1/3	2/3*
PL/D	0/3	2/3	0/3	2/3	2/3	3/3	N	N
PF/B	0/3	1/3	0/3	0/3	0/3	0/3	N	N
BI/L	0/3	1/3	0/3	2/3	N	N	0/3	0/3
HD/K	0/3	2/3	0/3	1/3	N	N	0/3	0/3
CsP/K	1/3	3/3	0/3	0/3	N	N	N	N
SzL/B	0/3	0/3	0/3	1/3	0/3	0/3	N	N
SzL/Zs	2/3	2/3	0/3	3/3	1/3	2/3	0/3	3/3*
SzB/M	N	0/3	0/3	3/3	0/3	3/3	1/3	3/3*
PJ/K	N	2/3	1/3	3/3	0/3	1/3	0/3	3/3*
HM/K	N	3/3	2/3	2/3	0/3	0/3	0/3	0/3

same three colonies of the apiaries were tested in the spring (March–May) and in the autumn (August–October) of 1999 and 2000, all together 114 colonies were sampled. These samples contained 100–500 adult bees.

B: In addition, samples sent by beekeepers following a sudden collapse of several colonies (six apiaries: five pooled samples from five apiaries each collected from four colonies, and ten individual colony-samples sent by one apiary), and following unusually high winter mortality (further two apiaries, one pooled sample from four colonies of each apiary) were analysed. The amount of dead and moribund bees sent for investigation varied between 0.5–2.5 kg per apiary.

C: Four further symptomless apiaries not participating in the survey were also sam-

pled (pooled samples, each collected from four randomly selected colonies). These samples also contained 100–500 adult bees.

All samples were personally transported or sent by express mail in carefully wrapped paper sacks or boxes.

Cesium chloride gradient purified virus suspension from 100 white or light brown eyed pupae artificially infected with 10 µl of the ABPV isolated by our group in 1998 (Békési et al., 1999) served as positive control.

2.2. Parasitological investigations

The samples were checked for the presence of *V. destructor* mites, and mites from the different samples were collected separately for PCR testing.

2.3. Preparation of specimens for the PCR

Following parasitological investigations, 50 adult bees were homogenized in 10 ml phosphate buffered saline (PBS), centrifuged at 1 500 g for 10 min. Supernatants were transferred into sterile tubes and centrifuged again at 12 500 g for 15 min to clean them from cell debris and bacteria. Mite homogenates (if mites could be collected from the samples) were tested by PCR, and prepared according to the same protocol using 0.5 ml PBS. The number of mites collected from one sample varied between 1 and 300.

The viral RNA was isolated from the clear supernatants using QIAamp viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions, and reverse-transcribed into cDNA using oligo(dT) primer method with RevertAid™ First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania).

2.4. Titration

The sensitivity of the RT-PCR was tested on tenfold dilutions of the gradient purified virus suspension. The same suspension was also tested in agar-gel immunodiffusion (AGID), but in the latter test twofold dilutions of the same virus suspension were reacted with the ABPV specific rabbit serum.

2.5. Agar-gel immunodiffusion

Bee homogenates were measured into the 32 µl wells of 0.8% agar-gel produced according to standard methods (Hoskins, 1967) and reacted with ABPV specific antisera raised in rabbits and kindly provided by Dr. G. Topolska (Warsaw Agricultural University, Warsaw, Poland). Results were read after 48 hours incubation at 37 °C.

2.6. Primers

A pair of oligonucleotide primers were designed from the partial sequence of ABPV genome published by Ghosh et al. (1999) in GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>, accession number AF126050) using Primer 2.0 software (Scientific & Educational Software, Serial No. 50178). The code and the nucleotide sequences of the selected primers were: ABPV1 (5'-CATATTGGCGAGCCACTATG-3') and ABPV2 (5'-CCACTTCCACACA ACTATCG-3').

2.7. Amplification conditions

Amplification was performed in 50 µl reaction mixture containing 10 pmol deoxynucleoside triphosphate (dNTP) mix, 1.5 mM MgCl₂, 2 pmol of the appropriate primers, 2 µl cDNA and 1.5 U Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania). This reaction mixture was subjected to 40 cycles with an initial incubation at 94 °C for 3 min, followed by heat denaturation at 94 °C for 1 min, primer annealing at 55 °C for 1 min, and DNA extension at 72 °C for 1 min. Thereafter the samples were maintained at 72 °C for 2 min for the final extension.

We used the cDNA of the purified Hungarian isolate as positive control (the strain was propagated by inoculation of PCR-negative pupae as described above) and a reaction mixture without cDNA as negative control.

2.8. Identification of the PCR product

Following the RT-PCR reaction, 10 µl of the amplicon was electrophoresed in a 1% Tris borate-EDTA buffered agarose gel containing 0.5 µg/ml ethidium bromide, at 80 V for 1 hour. The bands were visualized by UV transillumination at 312 nm and photographed by a Kodak DS Electrophoresis

Documentation and Analysis System using the Kodak Digital Science 1D software. Product sizes were determined with the reference to λ phage DNA cleaved with *PstI* restriction enzyme.

2.9. Nucleotide sequencing and computer analyses

The PCR product amplified by ABPV1 and ABPV2 primers from inoculated pupae was electrophoresed in a 0.8% Standard Low- m_r Agarose Gel (Bio-Rad, Richmond, CA, USA) at 80 V for 2 hours. The position of the amplicon was checked with short transillumination, and then it was excised from the gel and extracted using QIAquick Gel Extraction Kit (Qiagen, Germany). Fluorescence-based sequencing PCR was performed at the Biological Research Centre of the Hungarian Academy of Sciences in Szeged, employing an AbiPrism 2.1.0 automated sequencing system. The primers used for sequencing were identical to those in the RT-PCR reaction.

The nucleotide sequences were compared using FASTA (NCBI) and BioEdit 4.7.8 software programs and verified by visual inspection. The multiple alignments

were performed using BioEdit 4.7.8 and Clustal W 5.a software programs.

3. RESULTS

3.1. Electrophoresis of the PCR product

Following the RT-PCR reaction with the ABPV1 and ABPV2 primers on the isolated RNA of the purified ABPV suspension an approximately 400 bp product was detected. By the amplification the virus signal was always detected in the artificially infected pupae but not in the non-infected ones (Fig. 1).

3.2. Titration

The results of the titration by RT-PCR are shown by Figures 2/A and 2/B. Shortly: in the AGID tests the suspension of artificially infected pupae gave positive reaction only with the concentrated and with the 1:2 to 1:16 dilutions of the homogenate. With the 1:32 diluted homogenate no visible precipitation line could be detected. The same virus suspension gave a clear, well visible

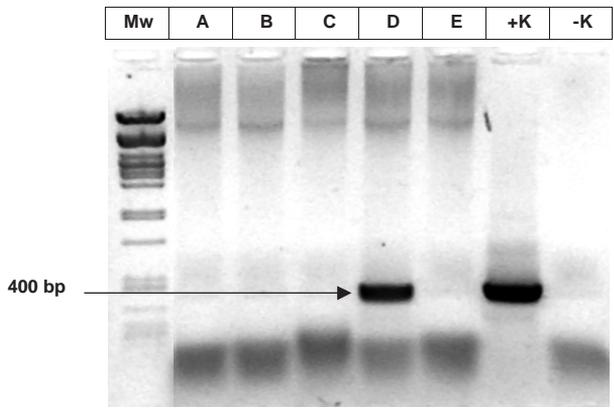


Figure 1. Diagnostic RT-PCR in agarose gel electrophoresis. Mw standard (*PstI* cleaved λ -phage DNA), A, B, C, E: negative field samples; D: positive field sample, +K: positive control (ABPV inoculated into pupae), -K: negative control.

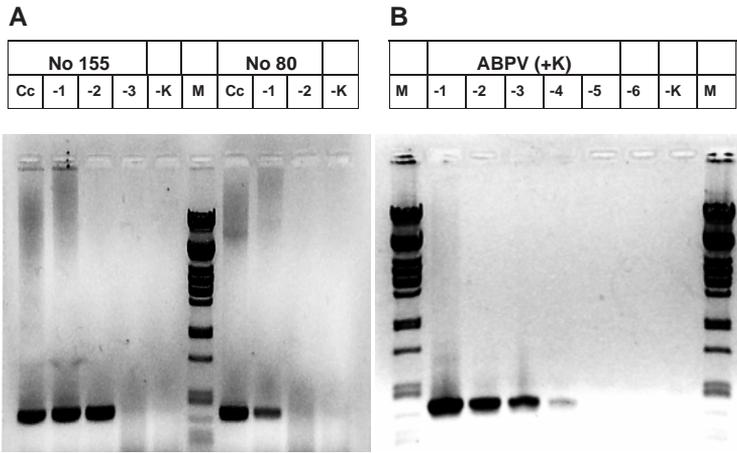


Figure 2. RT-PCR titration A: positive field samples: No. 155 (strong positivity) No. 80 (lower positivity), B: positive control: ABPV inoculated into pupae, Cc: undiluted homogenate, -1: tenfold dilution, -2: 100 fold dilution, -3: 1000 fold dilution, etc., M: standard (*Pst*I cleaved λ -phage DNA), -K: negative control.

band by PCR even if it was diluted 10^4 times (Fig. 2/B). Two strongly positive samples were also titrated (Fig. 2/A) for sake of testing the sensitivity on field samples too: neither of them was positive with AGID. Even the sample No. 155 giving the strongest signal in RT-PCR and showing a well visible band in 1:100 dilution was negative by AGID, as all field samples tested by AGID for comparison. The majority of these positive samples (like No. 80, KATKI/G, May 2000) when titrated with RT-PCR were positive in the not diluted and in the tenfold dilution.

3.3. Nucleotide sequencing and computer analysis

The fragment amplified with the ABPV1-ABPV2 primer-pair was sequenced and a 398 base long sequence was identified. The sequence was aligned to the GenBank database and the highest identity (93%) was found with the ABPV complete genome (Govan et al., 2000; AF150629) (Fig. 3). The sequence was deposited in the GenBank database under accession number

AY059372 (<http://www.ncbi.nlm.nih.gov/Genbank/index.html>).

3.4. Survey on the occurrence of ABPV in Hungarian apiaries

Besides investigations on artificially infected pupae using this diagnostic primer-pair, a survey was started on field samples collected from volunteering apiculturists living at different locations in Hungary. The samples were checked for the presence of *Varroa destructor*, and were tested by RT-PCR to detect ABPV specific nucleic acid, first only in the bees but later also in the mites.

Twelve apiaries had sent samples on a regular basis (Category A, no clinical symptoms or losses) and eight of them (66.6%) proved to be infected at least once within the test period of 2 years (Tab. I). Considering the individual colonies the infection rate was less, 14 from 114 colonies (12.2%). Since not all colonies sampled in an apiary in a certain season were positive, the infected and non-infected colonies may be present simultaneously. Furthermore,

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AF150629 ( 8115) CATATTGGCGAGCTACTATGTGCTATCGTATAGCTATAGTTAAAACAGCT
ABPV1-2 ( 1) .....C.....

AF150629 ( 8165) TTTCACACTGGTAGGTTAGGAATTTTCTTCGGACCTGGTAAGATTCCAAT
ABPV1-2 ( 51) .....C.....A.....T.A.....G.....G..

AF150629 ( 8215) AACGACGACGAAAGATAATATTTCCCGGACTTGACTCAGTTAGATGGAA
ABPV1-2 ( 101) ..T.....C.....A.....

AF150629 ( 8265) TTAAAGCGCCTTCTGATAACAATTACAAATACATCTTGGATCTAACTAAT
ABPV1-2 ( 151) CC.....C..C..C.....T.....A...T.G.....

AF150629 ( 8315) GATACGGAGATCACCATAAGGGTACCTTTTGTTTCAAACAAAATGTTCA
ABPV1-2 ( 201) .....

AF150629 ( 8365) GAAATCTACGGGAATTTATGGTGGAAATCTGAAAATAATTGGGATTTCT
ABPV1-2 ( 251) .....C.....

AF150629 ( 8415) CTGAATCATTACTGGATTTTGTGCATAAGACCTATTACAAAATTTATG
ABPV1-2 ( 301) .....C.....A...

AF150629 ( 8465) TGTCCAGAGACTGTATCCAATAATGTGTCGATAGTTGTATGGAAGTGG
ABPV1-2 ( 351) .....G..A.....A.....G.....

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Figure 3. Nucleotide sequence of the amplicon of the diagnostic PCR product (ABPV1-2) aligned with the complete sequence deposited in the GenBank (AF150629, Govan et al., 2000).

presence of the virus in a certain apiary was inconsistent, some colonies and apiaries were found negative in one season and became positive in the next. For example apiary PT/B sent bees from colony No. 62, 63 and 98 throughout the investigation period, of which No. 98 was infected in spring and autumn of 1999, but not in 2000. Contrary to that SzL/Zs was sending samples from colonies No. 24, 33 and 43 and in the spring of 1999 colonies No. 33 and 43 were infected, but in the spring of 2000, No. 33 was negative. In the autumn of 1999 and 2000 all three colonies were negative.

The eight apiaries sending samples for aetiological investigations (Category B) were infected in a much higher ratio. The virus-specific nucleic acid was detected in samples from seven apiaries (either in the bees, or in the mites, or in both), what means a positive rate of 87.5% (Tab. II). In one apiary colonies were tested individually: five out of ten (50%) were positive, a rate higher than the 12.2% found among the colonies of symptomless, regularly tested apiaries. The samples sent from these apiar-

ies struggling with high mortality and clinical symptoms had been pooled by the bee keepers from four colonies (see Methods) and arrived for aetiological investigations (including nosema infection, insecticide-intoxication, which latter was excluded by parallel investigations in each case). In the “problematic” cases seven of eight apiaries proved to be infected (87.5%) with ABPV, and the viral nucleic acid was also detected in mite homogenates. Although the sampling was different in Category A and B (apparently healthy bees and low number of mites on one side, dead and moribund bees, high number of mites on the other), the regular presence of the virus in the “problematic” apiaries is remarkable. The randomly selected colonies of the four symptomless apiaries not participating in the regular sampling, but also tested on request within the period (Category C) were negative.

From 6 samples, *Varroa destructor* were analysed for the presence of the virus. In the last phase of the survey (2000 autumn, 2001 spring) mites were also tested for the

Table II. Results of the aetiological investigations by ABPV RT-PCR test on samples sent by bee keepers struggling with problems due to unknown reason, but not participating in the survey (Category B). Samples from which mites were collected and tested with RT-PCR are signed with asterisk.

Code of the apiary	Sampling	Cause of investigation	Result of RT-PCR	Auxilliary diagnosis
CsSzM/K	Apr.1999	Depopulation	Positive	Nosemosis, no Varroa infestation
KATKI/G	May 2000	Depopulation	5 positive in 10	Heavy varroosis in 6 from 10*
HTGy/T	Febr. 2001	Poor wintering	Positive	Heavy varroosis, Varroa PCR positive*
GA/H	Febr. 2001	Poor wintering	Negative	Heavy varroosis, Varroa PCR positive*
SzI/Kh	May 2001	Paralysis, depopulation	Positive	Nosemosis, no Varroa infestation
KI/K	May 2001	Paralysis, depopulation	Positive	Nosemosis, no Varroa infestation
MI/Kh	May 2001	Depopulation	Negative	
SzGy/Kh	May 2001	Paralysis, depopulation	Positive	Nosemosis, no Varroa infestation

presence of ABPV. From the “regular” adult bee samples (Category A) containing 200–500 bees sent by the volunteers, a rather low number of mites could be collected (1–15), and the virus was not detected in their homogenates. In the “problematic” cases (Category B) a mass of dead bees sometimes swept from the bottom of the hives and weighing between 0.2–2.5 kg was sent, from which 200–300 mites could easily be collected. Although the presumed role of *Varroa destructor* mite as a virus carrier and a possible vector has been supported by the demonstration of the virus in the mites by our RT-PCR method and previously by ELISA (Allen et al., 1986), the presence of virus specific nucleic acid could not be demonstrated in some of the mite homogenates by our RT-PCR investigations, even if the colonies were heavily infested with mites, and simultaneously heavily infected with ABPV.

Only in 2 samples (pooled samples of 4 colonies of which all bees had died during the winter) was the virus detected. In the

negative samples (Tab. I) the number of the mites collected varied between 1 and 15, while from each of the 2 positive samples (Tab. II) 300 mites were retrieved. This indicates that not all the mites are carrying the virus, and the rearing number of mites tested will increase the probability of detection.

In addition to *Varroa destructor* infestation, nosema disease was also regularly observed in the “problematic” apiaries (Category B) together with ABPV infection. Heavy varroosis was observed in two while *Nosema apis* Zander was detected in four of the eight apiaries that sent samples for aetiological investigations and were found ABPV positive (Tab. II).

4. DISCUSSION

The diagnosis of viral infections in the honeybee has been rather complicated compared to other fields of veterinary virology. The lack of characteristic clinical symptoms and pathological alterations

makes the recognition of most diseases difficult. Since cell cultures of bee origin are not available, the only way of isolation and artificial propagation of viruses is the experimental infection of pupae. Furthermore, as bees do not produce antibodies against pathogens, the indirect determination of viral infections (widely used in other fields of veterinary praxis) is not possible. Electron microscopy and serological methods to detect sometimes very low amounts of viral antigen in field samples contribute to the difficulties described in the introduction. Therefore the RT-PCR method worked out to amplify unique regions of the viral nucleic acid present in the samples seems to be very promising in the diagnosis of bee virus infections.

The RT-PCR method worked out by our group is based on a primer pair (ABPV1 and ABPV2) designed within the structural protein region of the viral genome, producing an amplicon between base pairs 8107 and 8504. To test the reliability of our RT-PCR in the diagnostic work, the sensitivity of our system was compared to AGID, the only other widespread diagnostic method. It was not surprising, that the same virus suspension gained by artificial infection of pupae and giving a positive result up to 1:16 in the AGID test, proved to be positive up to 1:10⁴ dilution in RT-PCR.

Using this very sensitive, fast and specific method in a survey we have detected ABPV infection in apparently healthy bee colonies as well as in colonies with high mortality. Furthermore the virus was also detected in *Varroa destructor* samples collected from the mite infested colonies.

Besides varroosis, nosema infection was also frequently detected in the apiaries struggling with severe losses (Category B). This fact raises the possibility that virus infections may be activated or the losses caused by these infections may be enhanced by other predisposing factors in insects too. This phenomenon is frequently observed among the virus infections of ver-

tebrates, where predisposing factors (i.e. shipping, immunosuppression, crowding, etc.) or co-infections (chlamydia, mycoplasma) activate virus infections followed by bacterial secondary infections (Yates, 1982; Nordengrahn et al., 1996). None of the factors alone will lead to severe disease or economic losses, but the cumulative effect of the factors is frequently fatal. It seems, that the existence of these "polyfactorial" disease complexes may not be excluded in the case of invertebrates either. Our survey does not help to find an answer to the question whether the virus or the cofactor (varroosis, nosema disease) is more important, and which of them may be considered as primary agent.

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Résumé – Détection du virus de la paralysie aiguë des abeilles par PCR-RT dans des échantillons d'*Apis mellifera* et de *Varroa destructor*: criblage rapide de ruchers hongrois. La PCR suivie de transcription inverse (RT) est une technique moderne et très sensible pour mettre en évidence la présence d'acide nucléique virale dans des échantillons d'abeilles. La méthode a été utilisée pour montrer la présence du virus de la paralysie aiguë des abeilles (ABPV) dans des ruchers de différentes régions de Hongrie. Des abeilles fraîchement écloses ont été prélevées dans des colonies apparemment saines par des apiculteurs volontaires. On a vérifié l'infestation des abeilles par *Varroa destructor* Anderson & Trueman et la présence d'ABPV chez les acariens récoltés a été testée par PCR-RT. Les résultats sont visualisés par électrophorèse sur gel d'agarose (Fig. 1). Sur une période

de deux ans, 12 ruchers ont été échantillonnés au printemps et à l'automne et ont fourni 114 échantillons d'abeilles. Les tests ont montré un taux d'infection des colonies de 12,2 % et dans les deux tiers (66,6 %) des ruchers, on a trouvé au moins une fois au cours de la période d'étude des échantillons positifs (Tab. I), malgré le fait que tous ces ruchers ne présentaient pas de symptômes durant toute la période d'étude. Les colonies de huit autres ruchers, présentant un effondrement soudain ou un mauvais hivernage, ont été également étudiés. Sept des huit ruchers étaient infectés par l'ABVP (77,5 %) et infectés simultanément par *Nosema apis* Zander ou infestés par *V. destructor*. La présence de l'ABVP a pu également être mise en évidence chez certains acariens prélevés dans ces derniers ruchers.

virus de la paralysie aiguë des abeilles / varroose / nosérose / PCR

Zusammenfassung – Nachweis des Akuten Paralyse – Virus mit RT-PCR in Freilandproben von Honigbienen (*Apis mellifera*) und *Varroa destructor*: Schnelltest zur Überprüfung des Vorkommens in Ungarns Bienenständen. Die Polymerase – Kettenreaktion mit nachfolgender reverser Transkription (RT-PCR) ist eine moderne und sehr empfindliche Methode um Nukleinsäuren von Viren in Bienenproben nachzuweisen. Diese Methode wurde für einen Überblick über das Vorkommen von ABPV in Bienenständen in verschiedenen Orten von Ungarn genutzt. Geschlüpfte lebende Bienen von scheinbar gesunden Völkern wurden freiwillig durch Imker gesammelt. Die Bienen wurden auf eine Infektion mit *Varroa* geprüft und die dabei gesammelten Milben ebenfalls mit der RT-PCR Methode auf ABPV getestet. Die Ergebnisse wurden durch eine Agarose Gel Elektrophorese sichtbar gemacht (Abb. 1). Zwei Jahre lang wurden Proben von 12 Bienenständen im

Frühjahr und Herbst gesammelt, insgesamt 114 Proben. Mit den Tests wurde eine Infektionsrate von 12,2 % pro Volk nachgewiesen und bei 2/3 der Bienenstände wurden mindestens einmal innerhalb der 2 Jahre positive Proben gefunden (Tab. I), obwohl bei keinem der Bienenstände in dieser Zeit Krankheitssymptome auftraten. Die Völker von 8 weiteren Ständen, die plötzlich zusammengebrochen waren oder schlecht überwintert hatten, wurden ebenfalls überprüft. Sieben der 8 Stände waren von ABPV (87,5 %) und gleichzeitig mit *Nosema apis* oder *Varroa destructor* befallen. ABPV konnte auch bei einigen *Varroa* Milben nachgewiesen werden, die in diesen geschädigten Bienenständen gesammelt worden waren.

ABVP / *Varroa destructor* / *Nosema apis* / PCR

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