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Original Scientific Article

DETECTION OF SIX HONEYBEE VIRUSES IN CLINICALLY AFFECTED COLONIES OF CARNIOLAN GRAY BEE *(APIS MELLIFERA CARNICA)*

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Summary: This research describes the detection of six honeybee viruses in samples of clinically affected Carniolan gray bee collected between 2007 and 2009 on the territory of Slovenia. Using one-step reverse transcription-PCR (RT-PCR), 60 bee samples originated from 45 apiaries were screened for the presence of six honeybee viruses. Samples were found positive for acute bee paralysis virus (ABPV = 40%), black queen cell virus (BQCV = 83,3%), chronic bee paralysis virus (CBPV = 18,3%), deformed wing virus (DWV = 70%), Kashmir bee virus (KBV = 1,7%) and sacbrood bee virus (SBV = 8,3 %). Mortality and paralysis were often evident in the apiaries and could be connected with ABPV and/or CBPV infections. Both viruses were detected in clinically affected apiaries with high bee mortality and with paralysis symptoms showing flightless bees, trembling and crawling at the hive entrance. The severity of clinical manifestation with high bee losses were associated with higher number of viruses detected in the samples. Among virus positive samples, 27% of them were infected with one virus, 30% with two viruses, 25% with three viruses and 15% of samples contained four viruses simultaneously. The results of this study provide data about the detection of several bee viruses in affected bee colonies and viral infections in Carniolan bee have to be investigated by further research.

Key words: honeybee; *Apis mellifera carnica*; bee viruses, RT-PCR; diagnosis; epidemiology

Introduction

Honeybees (*Apis mellifera* L.) are a critical player in the production of many fruit, vegetable and seed crops grown throughout the country and worth of millions euro in value to agriculture each year. The Carniolan bee *(A. mellifera carnica)* is the subspecies of the Western honey bee that has naturalized and adapted to the Slovenia geographic area, the Southern part of the Austrian Alps and North Balkan countries. It is indigenous bread and one of the most popular bee races. In Slovenia, it is protected under the law and introducing other bee species is not allowed. Carniolan bee is gentle, not aggressive, has good sense for orientation and is though less drifting to a neighbouring hives. In the winter it is able to survive with not much honey stores, which is good feature for the areas with long winters. The increased number of honeybee colonies losses in our country during the last decade has resulted great interest in honeybee pathology and viruses have emerged as one of several candidates for these losses. Viruses are probably the least understood part of honeybee pathology mainly because of the lack of information of the objective data about viral disease outbreaks. With rapid dissemination of the ectoparasitic mite varroa (*Varroa destructor)* and bee losses, viral honeybee diseases have been

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detected in Europe and worldwide in last years (1-6). When varroa is spreading among bees in the colony and between apiaries to the long distances *varroa* is considered as an important vector for many viruses. In addition because of sucking bee haemolymph, varroa suppresses its immunity (7).

The most commonly observed bee viruses are single stranded RNA viruses and include *Acute bee paralysis virus* (ABPV), *Black queen cell virus* (BQCV) and *Kashmir bee virus* (KBV) which are classified as members of the genus *Cripavirus* (family *Dicistroviridae*), *Deformed wing virus* (DWV) and *Sacbrood bee virus* (SBV) are assigned to the genus *Iflavirus* and *Chronic bee paralysis virus* (CBPV) which also possesses an RNA genome, but is not picorna-like and remains unclassified. Five of these viruses, ABPV, DWV, KBV, SBV and CBPV can cover and over infection with clinical signs that can be identified by beekeepers, while the majority of the bee virus infections are believed to cause persistent, usually inapparent infections (8).

The great diversity of viruses isolated from honey bees, the lack of specific clinical signs and partial or complete sequencing of several RNA viruses of the honeybee has resulted in the development of several RT-PCR methods and applied for the diagnosis of ABPV, BQCV, CBPV, DWV, KBV and SBV (9-17). Clinical signs and laboratory diagnostics supported the presence of ABPV, DWV and SBV in Slovenia already in 2004 (18).

Between 2007 and 2009, the increased losses of bee colonies have been reported in Slovenia. In this paper we present the results of first survey of bee viruses, with the prevalence of six RNA viruses in samples collected in the apiaries in five different geographic regions, covering whole country. The aim of this survey was also to establish the routine laboratory molecular methods for specific detection of honey bee viruses and to obtain comprehensive insight into the correlation between the presence of honeybee viruses and their clinical manifestation in bee colonies.

Materials and methods

Sixty samples of dead worker Carniolan bee (Apis mellifera carnica) were collected from 45 different apiaries located in five geographical regions in Slovenia (Primorska, Gorenjska, Dolenjska, Štajerska, Prekmurje). Bee samples were collected from January 2007 to December 2009 and were mostly associated with abnormal behaviour of bees, mortality or sudden colony losses. Each sample consisted of a pool of 50 to 100 bees of the same beekeeper; in few apiaries more than one sample was collected from the same apiary where clinical manifestation of the infection was present. Samples were collected by the veterinarians – specialists for bee diseases and samples were as soon as possible sent to the Virology department (National Veterinary Institute, Slovenia) where they were stored at low temperature (less than minus 60 °C) until used. For the virus analysis, 10 to 15 bees were randomly selected from each bee sample and placed into the sterile plastic bags. The samples were homogenized in RPMI (Gibco) with homogenizer (IUL masticator). After homogenization, the sample suspensions were centrifuged for 15 min at 2.500 rpm. The supernatant was recovered and 140 µl of the supernatant was used for the extraction of total RNA using QIAamp viral RNA mini kit (Qiagen, Germany) according to the manufacturer's instructions. Each RNA sample was tested for the presence of nucleic acids of six viruses (Table 2). A part of viral genome of ABPV, BQCV, CBPC, DWV, KBV and SBV was amplified by RT-PCR using specific primer pair (Table 1) and One-Step RT-PCR kit (Qiagen, Germany) reagents according to the manufacturer's instructions. Reaction mixtures without RNA served as negative controls, and verified positive samples of each virus as a positive control. The reaction was performed in a total volume of 25 µl as follow: 15 µl of nuclease free water, 5 µl of 5 x PCR buffer, 1 µl of dNTP mix (containing 10 mM of each dNTP), 0,5 µl of stock solution of 20 µM of each primer, 1 µl of one step RT-PCR enzyme mix and 2 µl of RNA template. The primers used in the assay are shown in Table 1. The amplification program included reverse transcription stage at 50 °C for 30 min, followed by an initial PCR activation step at 95 °C for 15 min. This was followed by 40 cycles of 94 °C for 30 sec, 54 °C for 30 sec (for DWV and CBPV, annealing temperature was 60 and 55 °C, respectively) and 72 °C for 1 min and final extension step at 72 °C for 10 min. The reaction was performed using T1 Biometra Thermocycler and PCR products were visualized in 1,8% agarose gel with 0,5 µg/ml ethidium bromide and subsequent visualization under UV light. The size of each PCR product was compared to the 100-bp DNA ladder (Fermentas, Germany) and results for each virus were interpreted as positive or negative according

Table 1: Sequence of primers used in this study

Primers (5'-3')	Position in genome	Product (bp)	Reference
Acute bee paralysis virus			
ABPV-1 (cat att ggc gag cca cta tg)	8114 - 8512	398	11
ABPV-2 (cca ctt cca cac aac tat cg)	(capsid protein)		
Black queen cell virus			
BQCV-F (tgg tca gct ccc act acc tta aac)	7850 - 8550	700	12
BQCV-R (gca aca aga aga aac gta aac cac)	(structure polyprotein)		
Chronic bee paraliysis virus			
CBPV1-1 (tca gac acc gaa tct gat tat tg)	147-716	570	16
CBPV1-2 (act act aga aac tcg tcg ctt cg)	(RNA polymerase)		
Deformed wing virus			
DWV F (agg cga cat ggg aac agg)	1312-1815	504	18
DWV R (caa ctt cac cct cgc cat ca)	(capsid protein)		
Kashmir bee virus			
KBV 1 F (gat gaa cgt cga cct att ga)	5406 - 5820	414	8
KBV 1 R (tgt ggg ttg gct atg agt ca)	(RNA polymerase)		
Sacbrood bee virus			
SBV-F (gct gag gta gga tct ttg cgt)	(4957-5781)	824	14
SBV-R (tca tca tct tca cca tcc ga)	(structure polyprotein)		

to the expected size of DNA fragment (Table 1). A limited number of PCR amplicons, specific for each of six bee viruses, were purified using Wizard PCR Prep DNA Purification System (Promega) and sequenced to confirm the specificity of RT-PCR assays. The obtained nucleotide sequences of each virus were analyzed with DNASTAR program (Lasergene, USA) and compared with the published sequences in GenBank database using National Centre for Biotechnology Information (NCBI) to specify the amplicons for each PCR.

Results

60 samples, originated mainly from the affected Carniolan bee colonies, from 45 apiaries in five Slovenian geographic regions (Primorska, Gorenjska, Dolenjska, Štajerska, Prekmurje), were collected and tested for the presence of six bee viruses by specific RT-PCR methods. In the majority of apiaries, where samples were collected, clinical signs of disease were reported with one or more symptoms confirmed by veterinarian specialists: sudden bee losses, high mortality of colonies (more than 50% of bee colonies losses in the apiary), mortality (less than 50% of bee colonies losses), bee paralysis, wing deformities, varroa infestation and affected bee brood (Table 2). All these bee colonies have had a history data with varroa infestation and were intensively treated by acaricides in 2008 and 2009. Three samples of apparently healthy bees (samples with numbers 20, 21 and 22, Table 2) were collected in the year 2009. Twenty-four samples (40%), 50 (83,3%), 11 (18,3%), 42 (70%), 1 (1,6%) and 5 (8,3%) of 60 samples were positive for ABPV, BQCV, CBPV, DWV, KBV and SBV, respectively (Table 2). The majority of bee samples (70%), collected from apiaries where bee losses, paralysis or varrosis were reported were positive for two to four different viruses (30% of bee samples contained two viruses, 25% three viruses and 15% four viruses), while in 16 bee samples only BQCV, CBPV or DWV was detected (Table 3). The severity of clinical manifes-

tations with high bee losses was associated with higher number of viruses detected in the samples (Table 2). SBV was detected in five samples from five apiaries where typical signs of the diseased sac brood were observed. When more than one sample was collected from different colonies of the same apiary, very similar number and patterns of detected viruses was observed. Two of three bee samples collected from apiaries with no symptoms of disease were positive only for BQCV and no nucleic acids of other five viruses were detected. Regional differences in the distribution of six viruses were not ascertained (Table 2). Nucleotide sequences of the amplified product were determined and confirmed the expected bee virus using BLAST at the NCBI. BLAST sequence alignment of each obtained sequence with published sequences has resulted in 96% sequence identity for ABPV (381 nucleotide-nt), 99% for BQCV (561 nt), 99% for CBPV (522 nt), 97% for DWV (471 nt), 99% for KBV (402 nt) and 95% identity for SBV (711 nt) (data not shown). According to the obtained sequence data for six bee viruses from this study the primers used for PCR amplification are specific for detection of determined bee viruses.

Table 2: Honeybee viruses (ABPV, BQCV, CBPV, DWV, KBV and SBV) detected in sixty bee samples from five geographic regions in Slovenia

Sample number	Geografic area	Year of sampling	ABPV	BQCV	CBPV	DWV	KBV	SBV	Clinical symptoms	No. of viruses detected
1	Primorska	2008	-	+	-	-	-	-	Paralysis	1
2	Gorenjska	2009	-	+	-	+	-	-	Mortality and varrosis	2
3	Prekmurje	2009	+	+	-	+	-	-	Paralysis	3
4	Gorenjska	2008	-	+	-	+	-	-	High mortality	2
5	Štajerska	2008	+	+	-	+	-	-	High mortality and varrosis	3
6	Štajerska	2008	+	+	+	+	-	-	High mortality	4
7	Štajerska	2008	+	+	+	+	-	-	High mortality	4
8	Gorenjska	2008	-	+	-	+	-	-	Mortality	2
9	Štajerska	2008	+	+	-	+	-	-	Paralysis	3
10	Štajerska	2007	+	+	-	+	-	-	High mortality	3
11	Štajerska	2007	+	+	-	+	-	-	High mortality	3
12	Gorenjska	2008	-	+	-	-	-	-	Mortality	1
13	Štajerska	2007	+	+	-	+	-	-	Mortality	3
14	Štajerska	2007	+	+	-	+	-	-	High mortality	3
15	Štajerska	2007	+	+	+	+	-	-	High mortality	4
16	Štajerska	2007	+	+	-	-	-	-	High mortality	2
17	Štajerska	2007	-	+	+	+	-	+	Mortality	4
18	Gorenjska	2009	+	+	-	+	-	-	Mortality	3
19	Prekmurje	2009	-	+	-	+	-	-	Mortality	2
20	Dolenjska	2009	-	+	-	-	-	-	No symptoms	1
21	Prekmurje	2009	-	+	-	-	-	-	No symptoms	1
22	Prekmurje	2009	-	-	-	-	-	-	No symptoms	0
23	Prekmurje	2009	+	+	-	+	-	-	Paralysis	3
24	Dolenjska	2009	-	-	+	-	-	-	Paralysis	1
25	Dolenjska	2009	-	+	-	-	-	+	Affected brood	2
26	Štajerska	2009	+	+	-	-	-	+	Mortality	3
27	Primorska	2009	+	+	-	+	-	+	Affected brood	4
28	Prekmurje	2009	-	+	-	-	-	-	Paralysis	1
29	Štajerska	2009	-	+	-	+	-	-	Mortality and paralysis	2
30	Prekmurje	2009	-	+	-	-	-	-	Paralysis	1
31	Prekmurje	2009	-	+	-	-	-	-	Varrosis and paralysis	1
32	Prekmurje	2009	-	+	-	-	-	-	Paralysis	1
33	Prekmurje	2009	-	+	-	-	-	-	Varrosis and paralysis	1
34	Prekmurje	2009	+	+	-	-	+	-	Mortality	3
35	Prekmurje	2009	-	+	-	+	-	-	Mortality and paralysis	2
36	Prekmurje	2009	-	-	-	+	-	-	Paralysis, deformed wings	1

Sample number	Geografic area	Year of sampling	ABPV	BQCV	CBPV	DWV	KBV	SBV	Clinical symptoms	No. of viruses detected
37	Štajerska	2009	-	+	-	+	-	-	Varrosis	2
38	Štajerska	2009	-	+	-	+	-	-	Varrosis	2
39	Štajerska	2009	+	+	-	+	-	-	Mortality and paralysis	3
40	Gorenjska	2009	-	-	-	+	-	-	Mortality	1
41	Gorenjska	2009	+	+	-	+	-	+	High mortality	4
42	Gorenjska	2009	+	-	-	+	-	-	Mortality and varrosis	2
43	Gorenjska	2009	+	-	-	+	-	-	High mortality	2
44	Štajerska	2009	-	+	-	-	-	-	Mortality	1
45	Štajerska	2009	-	-	-	-	-	-	Mortality	0
46	Štajerska	2009	+	+	+	+	-	-	Mortality and varrosis	4
47	Štajerska	2009	-	+	-	+	-	-	Mortality and varrosis	2
48	Gorenjska	2009	-	+	-	+	-	-	Mortality	2
49	Gorenjska	2009	-	+	-	+	-	-	Mortality	2
50	Gorenjska	2009	-	+	-	+	-	-	Mortality	2
51	Štajerska	2009	+	+	+	-	-	-	Mortality and paralysis	3
52	Gorenjska	2009	-	+	+	+	-	-	Mortality and varrosis	3
53	Gorenjska	2009	+	+	+	+	-	-	Mortality and varrosis	4
54	Gorenjska	2009	+	+	+	+	-	-	Mortality and varrosis	4
55	Gorenjska	2009	-	-	-	+	-	-	Mortality and varrosis	1
56	Gorenjska	2009	-	+	+	+	-	-	Mortality and varrosis	3
57	Gorenjska	2009	-	+	-	+	-	-	Mortality and varrosis	2
58	Primorska	2009	-	-	-	+	-	-	High mortality	1
59	Primorska	2009	-	-	-	+	-	-	Mortality	1
60	Prekmurje	2009	-	+	-	+	-	-	High mortality	2
Number (%) of positive samples			24 (40)	50 (83,3)	11 (18,3)	42 (70)	1 (1,7)	5 (8,3)		

Legend: - RT-PCR negative result, + RT-PCR positive result, high mortality (more than 50 % of bee colonies losses reported in the apiary), mortality (less than 50 % of bee colonies losses reported in the apiary).

Table 3: The occurrence of virus infections in Carniolan honeybee samples

No. of viruses	Detected viruses	No. of samples	Percent of samples
0 virus	-	2	3,3%
1 virus	BQCV or CBPV or DWV	16	26,7%
2 viruses	BQCV, CBPV, DWV	18	30%
3 viruses	ABPV, BQCV, CBPV, DWV	15	25%
4 viruses	ABPV, BQCV, CBPV, DWV, SBV	9	15%
5 viruses	-	0	0%
6 viruses	-	0	0%
	Total	60	100%

Discussion

The honeybee colonies losses worldwide during the last decade increased the interest of bee toxicology and bee pathology. In the last few years, the diagnostic methods for the honeybee viruses changed from serological to PCR-based methods.

In this research article we describe the molecular-genetic evidence of six viruses in the samples of the Carniolan gray bee collected in Slovenia in the years 2007-2009. Of the six viruses identified by RT-PCR, BQCV had the highest prevalence (83,3%), which supports published data that BQCV can persist in the colony as an unap-

parent infection in adult bees for a longer period. BQCV affects mainly developing queen larvae and capped brood. Bailey and co-workers (19) reported that infections of bees with BQCV were strongly associated with the infestation of the protozoan *Nosema apis*, a parasite of honey bees. Although adult bees are often infected with BQCV, they normally do not exhibit the disease symptoms.

With the 70% prevalence, DWV was the second most prevalent virus. The veterinarians did not often report about typical symptoms for the DWV infection, such as shrunken, crumpled wings, reduced size and discoloration of bees but they described the symptoms of paralysis and high mortality which could be associated with the detection of other two viruses, ABPV and CBPV. High prevalence of DWV infection in the Carniolan bee is similar to the prevalence reported previously in other bees (3, 20).

Mortality and paralysis were often evident in the apiaries with ABPV and/or CBPV infections. Both viruses were detected in clinically affected apiaries where flightless bees, trembling and crawling of bees at the hive entrance were observed. Also the observations of black and hairless bees rejected by the healthy ones were reported by our veterinarians. In several affected Carniolan colonies, the bees left the queen with few workers or the bees were unable to fly and died within few days in thousands. ABPV was detected in the apiaries in some European countries and with its high prevalence of 40% is widespread also in Slovenia. In France 58% (20) of apiaries were infected with ABPV, in Hungary 67% (11) and in Austria 68% (3).

In our study SBV, was detected rarely (8,3%) only in adult bees or larvae. Much higher (86%) SBV prevalence was reported in France (20), 48% in Austria (3) and 100% in Uruguay (21). In Slovenia all five SBV positive cases were detected in the apiaries where the clinical manifestation of sac brood disease was recorded. This is in agreement with previous observations that this virus infection can be readily and easily diagnosed by the typical clinic symptoms of the honeybee brood disease.

KBV was detected only in one sample out of sixty tested samples in our study. It has already been reported that this virus is less prevalent in the cases where many viruses were present in the same honeybee population (1-3, 20, 22).

In two previous studies in Slovenia 24 and 4 samples had already been examined for the presence of DWV, ABPV, SBV and KBV in 2004 (18) and 2006 (3) respectively. In both studies ABPV, DWV and SBV were identified from randomly collected samples. In this study almost all collected samples were obtained from the colonies with clinical symptoms of bee disease and from the previously published data, the detection of bee viruses was expected. However, three viruses previously not detected in Slovenia were confirmed in our study. Surprisingly, 70% of examined bee samples had more than one virus present at the same time suggesting that many honeybee viruses are widely represented on our territory. Among all virus positive samples, 27% of them were infected with one virus, 30% with two viruses, 25% with three viruses and 15% of samples contained four viruses simultaneously. The results of this study provide the evidence of the detection of several bee viruses in affected Carniolan bee colonies and these viruses have to be investigated by further research. However, it is evident that the majority of Slovenian apiaries with affected bee colonies have multiple virus infections. Another study already confirmed that different viruses could be detected also in a single bee, in the colony (14). Ninety-two percent of the apiaries in France were found positive for at least three different viruses (31% of the apiaries contained three viruses, 36% four viruses and 25% five viruses) (20). The percentage of multiple virus infections in France apiaries was even higher than it was determined in our study.

Although the detection of varroa mite was not a specific topic of our study, its significance in horizontal transmission of viruses among bees could not be ignored. Honeybees have non-specific defence system as mechanical barriers with cuticle and hair and the non specific immune system. With varroa introduction, this system fails because the varroa sucks haemolymph and cause anaemia with reducing immune system, perforate the cuticle and introduce viruses. High varroa infestations could affect the virus distribution in bee colony (5, 20). Varroa suck the bee haemolimph with their strong mouth apparatus and directly injects viruses (ABPV, BQCV, DWV, KBV, SBV) in the bee body. The finding of bee viruses in varroa strongly supports the important role of varroa in the viral infections of bees (20, 23). In Slovenia, the national varroa treatment campaign started in 2009 lowered the varroa infestation and probably prevented even harder colony losses. However, the treatment with acaricides had limited success be-

cause of possible resistance and reintroduction of varroa. In our research we found that many apiaries still suffered from varrosis in the year 2009 (Table 2). In conclusion, we confirmed that the varroa is present in our area as elsewhere, it plays an important role in the honeybee pathology and it is together with different bee viruses one of the main causative agent for the bee losses.

The apiaries, which are infested with varroa and co-infected with ABPV, BQCV, CBPV and DWV as a single or multiple infections could potentially be at high risk for the colony losses. The experiments done on the artificially inseminated queens with the semen originated from the brothers of the drones, from which virus-positive semen was collected, showed that the transmission of viruses through sperm in most cases do not overt signs of the disease (24). Another data demonstrated that queens can harbour multiple viruses, although they show no clinical signs of the infection (1). This supports the theory of co-evolution of the honey bees and the viruses which has resulted in a balance allowing both partners to survive. However the clinical manifestation of the virus infections in the honey bee colony has dramatically changed when varroa emerged and started to spread the viruses among the bee colonies with varroa infested drifting bees. This new route of virus transmission probably resulted in the majority of detected bee losses between 2007 and 2009. The need for the development of new methods such as multiplex RT-PCR and real – time PCR reported by other authors also applies to our laboratory. These methods will allow the simultaneous detection of different viruses in a single reaction, allowing us to screen honeybee viral infections in a large number of samples within short time and less money.

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DOKAZ ŠESTIH ČEBELJIH VIRUSOV V ČEBELJIH DRUŽINAH KRANJSKE ČEBELE *(APIS MELLIFERA CARNICA)* S KLINIČNO SLIKO OBOLENJA

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Povzetek: Raziskava opisuje dokaz šestih čebeljih virusov v vzorcih klinično obolelih čebeljih družin kranjske čebele zbranih na področju Slovenije med letoma 2007 in 2009. Z uporabo metode reverzne transkripcije in verižne reakcije s polimerazo (RT-PCR) smo na šest čebeljih virusov pregledali 60 vzorcev čebel, ki so izvirali iz 45 čebelnjakov. Virus akutne paralize čebel (ABPV) smo dokazali v 40 % vzorcev, virus črnih matičnikov (BQCV) v 83,3 % vzorcev, virus kronične paralize čebel (CBPV) v 18,3 % vzorcev, virus deformiranih kril (DWV) v 70 % vzorcev, kašmirski virus čebel (KBV) v 1,7 % vzorcev, virus mešičkaste zalege (SBV) pa v 8,3 % od pregledanih vzorcev. V čebelnjakih smo ob odvzemu vzorcev pogosto zabeležili smrtnost in paralizo čebel, ki bi ju lahko povezali z laboratorijsko ugotovitvijo okužb z ABPV in CBPV. Oba virusa smo dokazovali pri klinično prizadetih čebeljih družinah skupaj z zabeleženo visoko smrtnostjo in z znaki paralize pri čebelah, zaradi česar čebele niso bile sposobne leteti in so umirale na tleh pred čebelnjakom. Intenzivnost opisane klinične slike z opisanimi izgubami čebel v čebelnjaku se stopnjujejo z ugotovitvijo večjega števila dokazanih različnih virusov v vzorcu. Med pozitivnimi vzorci smo pri 27 % ugotavljali okužbo z enim virusom, pri 30 % vzorcev okužbo z dvema različnima virusoma, pri 25 % vzorcih s tremi virusi, 15 % vzorcev pa je sočasno vsebovalo štiri različne viruse. Z rezultati te študije smo dokazali številne viruse v vzorcih prizadetih čebeljih družin, zato je potrebno virusnim okužbam kranjske čebele v prihodnje nameniti posebno pozornost.

Ključne besede: medonosna čebela; *Apis mellifera carnica*; virusi pri čebelah, RT-PCR; diagnostika; epidemiologija