PARALYTIC VIRUSES OF THE HONEY BEE

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ABSTRACT

The Acute bee paralysis virus, the Israeli acute paralysis virus, the Kashmir virus and the Chronic bee paralysis virus of the honey bee are actively involved in the worldwide continuous decrease in Honey bee *(Apis mellifera L)* colonies in the last years. The first three viruses belong to the same viral family, the *Dicistroviridae*, and induce quick paralysis and mortality, in contrast to the latter virus that is not classified yet. The former viruses bear a monopartite, and the latter a bipartite, positive strand RNA genome. Moreover, the route of infection of the three former viruses seems to require a vector while the latter does not. However the four viruses may become activated in covertly infected asymptomatic bees by still undefined stress factors, to cause overt lethal infections and substantial honey bee colony losses. Progress made in understanding their molecular structure, ways of infection and innate immune defenses of the honey bee, will contribute to improve management of honey bee colonies.

Key words: *Apis mellifera*, Acute bee paralysis virus, Israeli acute paralysis virus, Kashemir bee paralysis virus, Chronic bee paralysis virus

Anahtar Kelimeler: *Apis mellifera, Akut arı felci virüsü, İsrail akut felç virüsü, Kaşmir arı felci virüsü, Kronik arı felci virüsü*

INTRODUCTION

The worldwide continuous decrease in honey bee *(Apis mellifera L)* colonies observed in the last years brought attention of the public because of the important role that honey bees play in maintaining the diversity of plant species of our planet and in agriculture by assisting pollination of a wide variety of crops. A metagenomic microbiological survey performed in the United States showed high correlation between the presence of viruses in the colony, more specifically the recently discovered Israeli acute paralysis virus (IAPV), and colony collapse disorder CCD (Cox-Foster *et al.*, 2007).

The most common viral pathogens of honey bees are the Acute bee paralysis virus (ABPV), the Black queen cell virus (BQCV), the Deformed wing virus (DWV), the Israeli acute paralysis virus (IAPV), the Kashmir virus (KBV), the Sacbrood virus (SBV) and the Chronic bee paralysis virus (CBPV) (Bailey, 1967; Blanchard *et al.*, 2008; Chen & Siede, 2007; Cox-Foster *et al.*, 2007; de Miranda *et al.*, 2010; de Miranda & Genersch, 2010; Genersch *et al.*, 2006; Maori *et al.*, 2009; Ribiere *et al.*, 2010).

ABPV, BQCV, IAPV and KBV belong to the *Cripavirus* genus of the *Dicistroviridae* family of viruses. DWV and SBV belong to the *Iflavirus* genus of the *Iflaviridae* family and CBPV is still unclassified. This review will focus on viruses associated most frequently with development of paralytic diseases of the adult honeybee, namely IAPV, KBV, ABPV and CBPV and colony losses.

MORPHOLOGY AND GENOMIC ORGANIZATION

IAPV, ABPV and KBV

The genome of the dicistroviruses ABPV, IAPV and KBV is a monopartite positive-stranded RNA molecule of size varying from 9491 to 9613 bp coated with an icosahedral capsid shell forming a viral particle of diameter of about 30 nm [Genebank, (Chen & Siede, 2007; Christian 1998; de Miranda *et al.,* 2010; Maori *et al.*, 2007a)]. The viral genome is polyadenylated at its 3' end. It serves as template for replication and is also translated by the host

machinery to produce the viral proteins. Three viral proteins encoded in the viral genome VP1, VP2 and VP3 compose the viral capsomer and a forth VP4 seem to be internal to the viral particle and not exposed in its surface (de Miranda *et al., 2010*). The above proteins are encoded by a single open reading frame (ORF2) that follows the intergenic region, IGR (Fig.1A). ORF2 is translated as a single polypeptide that is assumed to be processed by the viral protease 3C-pro (Chen & Siede, 2007; de Miranda *et al.*, 2010; Maori *et al.*, 2007a). ORF1 encodes for a larger polypeptide that includes non-structural proteins that display high homology to the functional proteins of picorna-like viruses helicase, protease (3C-pro) and RNA dependent polymerase (RdRP). This polypeptide is also assumed to be processed by the putative viral protease. RdRP is involved in copying the positive viral strand into a complementary negative copy that serves as template for the amplification of new positive strands that are packaged into the viral particles by the *de novo* synthesized virion proteins. Also, a putative small viral protein VPg seems to be encoded by ORF1. By analogy to picorna viruses it is assumed to associate covalently with the viral genome, thus facilitating translation and replication. ABPV, IAPV and KBV genomes bear two IRES (internal repeat entry site): one at the 5-'UTR and a second one in the intergenic region (IGR). IRES are involved in efficient translation of the viral polypeptides eliminating the need of CAP-mediated host factors to assist this process, thus conferring advantage for translation of the viral RNA over the host mRNAs (Chen & Siede, 2007; de Miranda *et al.*, 2010).

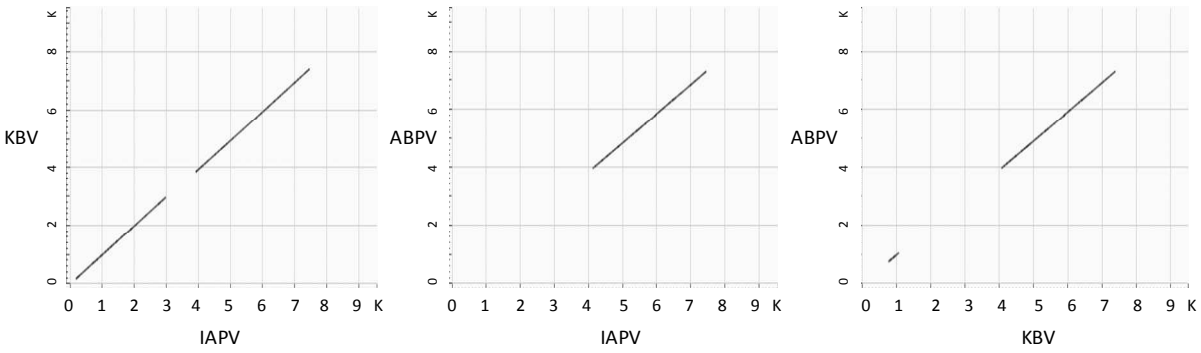

Fig.2. Dot-plot similarity matrix along the genomes of honey bee paralytic dicistroviruses based upon the BLAST results. The y and x-axis represent the length of each genome in Kilobases (K). The full lines show the regions along the viral genomes that share high similarity between the nucleotide sequences of the viruses plotted. The query sequence is represented on the X-axis and the numbers represent the bases/residues of the query. The subject is represented on the Y-axis and again the numbers represent the bases/residues of the subject. Alignments are shown in the plot as lines. (Zheng *et al*, 2000).

The similarity between the genomes of IAPV and KBV are higher than between both of them and ABPV. This becomes evident when their genomes are compared and plotted as a function of their nucleotide similarity (Fig. 2). The KBV-IAPV similarity extends through long regions of ORF1 and 2. This fact contributed to miss-identification of several strains of IAPV and KBV by RT-PCR, since some primers utilized were able to react indistinguishably with any of these viruses if present, or by antiserum raised against the KBV capsid in antibody-based methods such as ELISA, because of the similarity observed KBV and IAPV in the ORF2 region. This fact demands to take careful measures to identify correctly the virus present in the colony analyzed (for a detailed review of diagnostic methodology see the reference (de Miranda *et al.,* 2010).

CBPV

CBPV possess a bipartite positive-stranded RNA genome, with a 3674 bp sequence for the larger RNA1 molecule and 2305 bp sequence for the shorter RNA2. The 5’ ends of CBPV RNAs are capped and they lack poly-A tails at their 3’ ends. The viral particle is anisometric and mostly ellipsoidal and of about 20 nm width and 30-65 nm length (Bailey *et al.*, 1968; Olivier *et al.*, 2008a; Ribiere *et al.*, 2010).

RNA1 bears three ORFs including a putative RdRp, while RNA2 bears four ORFs (Fig.1B).

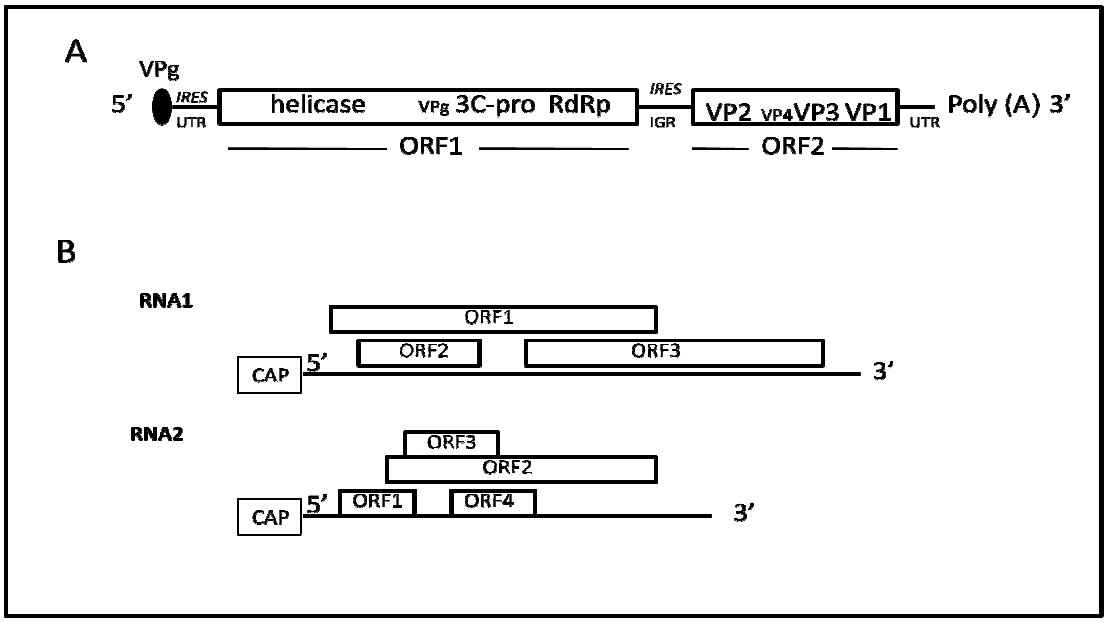

Fig.1. Genomic organization of honey bee paralytic viruses. A dicistroviruses: IAPV, ABPV and KBV. B. CBPV. ORF: open reading frame.

Western blot analysis revealed the presence of four polypeptides associated with the viral capsids with an approximate molecular weight of 75, 50, 30 and 20 kDa, respectively. Phylogenetic studies based on the amino acid composition of the conserved RdRp domains place this virus between the *Nodaviridae* and the *Tombusviridae* family clusters (Blanchard *et al.*, 2009; Ribiere *et al.*, 2010).

A satellite virus designed CBPVS was reported to be frequently associated with CBPV [for an extensive discussion please see the reference (Ribiere *et al.*, 2010)].

INFECTION AND PATHOLOGY

ABPV, IAPV and KBV have been shown to provoke acute paralysis upon their injection into the hemolymph of adult bees (Bailey *et al.*, 1963; Dall, 1987; Maori *et al.*, 2007a). Injected pupae and adult bees die between 3 to 6 days in contrast to slow paralysis produced by CBPV (Bailey *et al.*, 1963; Dall, 1987; Olivier *et al.*, 2008a; Ribiere *et al.*, 2010). The infected adults display increased paralysis, they tremble, are not able to fly, and die rapidly. Several studies indicated that the oral infectivity of these viruses is low and relatively large doses are required to provoke infection (about 9 log difference in the administered viral particles (Bailey *et al.*, 1963; Dall, 1987; de Miranda *et al.*, 2010; Maori *et al.*, 2009). In this respect, it is noteworthy that the mite *Varroa destructor*, a worldwide distributed ectoparasite of honey bees [reviewed in (Rosenkranz *et al.*, 2010)] can transmit ABPV with 50 to 80 % efficiency (Ball, 1985; Ball & Allen, 1988) and KBV with 70% (Chen *et al.*, 2004; Shen *et al.*, 2005b). In addition, the IAPV incidence in Israeli apiaries has been noted to increase in correlation with the seasonal increase in the *Varroa* population (Soroker *et al.*, 2010; NC, unpublished).

The above information may be relevant since ABPV and KBV have been associated with varroa-mediated colony losses (Todd *et al.*, 2007), IAPV was associated with CCD in the US, and KBV was suggested as a possible CCD marker as well (Cox-Foster *et al.*, 2007; vanEngelsdorp *et al.*, 2009).

ABPV, IAPV and KBV are usually present in many apiaries worldwide in asymptomatic covert infections that can be easily detected using RT-PCR and ELISA [for a geographical distribution and a comprehensive review of detection methods please see (de Miranda *et al.*, 2010)]. It has been proposed that various stress factors may induce changes in these silent infections provoking activation of virulent infections that could result in high mortality in the colony and eventually its collapse. *Varroa* has a dual role as a vector of bee viruses as well as activating asymptomatic virus

infections (Bailey *et al.*, 1979; Ball & Allen, 1988; Chen *et al.*, 2004; Hung *et al.*, 1996; Shen *et al.*, 2005a; Shen *et al.*, 2005b; vanEngelsdorp *et al.*, 2009). Moreover, it has been shown that expression of immune related genes of the honey bee decreased even after the mites were removed (Yang and Cox-Foster, 2005).

Anderson and Gibbs (1988) found that injection of different buffers that did not match the osmolarity of the bee hemolymph could activate KBV allowing them transition from non-detectable levels to levels detected by ELISA. Amplification of viruses dramatically occurs when *Varroa* mites parasitize honey bees (Shen *et al.*, 2005b). Also, it has been reported that viral amplification occurs by other means than just osmotic stress by *Varroa* saliva (Yang & Cox-Foster, 2005).

Interestingly, it was reported that IAPV sequences were carried in asymptomatic hosts and suggested that these sequences may have given protection to the host from lethal virus infection (Maori *et al.*, 2007b).

ABPV incidence increases in the summer and KBV and IAPV in the fall [(Bailey *et al.*, 1981; de Miranda *et al.*, 2010) and NC unpublished].

At the individual level, ABPV has been detected in the brain and hypopharingeal glands of adult bees (Bailey & Milne, 1969). KBV and ABPV have also been involved with oral routes of transmission such as through adult-larvae transmission, cannibalization of infected brood, etc. (Chen *et al.*, 2006a; Chen *et al.*, 2006b; Chen & Siede, 2007). KBV was reported to be present in queens and eggs (Shen *et al.*, 2005a). ABPV but not KBV were also detected in the semen (Yue *et al.*, 2006). Less data is available for transmission of IAPV.

Taken together, the above data implicate that these viruses could be transmitted either vertically from the queen through transovarial transmission and from drones to the queen via insemination, and horizontally from workers to larvae or other bees through brood food sources containing glandular secretions. At the colony level their ability to provoke high mortality in a very short period of time identifies them as an important factor involved in rapid losses of honey bee colonies observed worldwide.

Future research involving unified sensitive techniques will enable to extend our knowledge and understanding of ways of transmission and their specific characteristics associated with intrinsic properties of ABPV, IAPV and KBV.

CBPV

In contrast to the three viruses discussed above, CBPV infections are not correlated with the presence of other parasites in the beehive. CBPV-paralytic symptoms include clusters of trembling, flightless, crawling bees with some individual black, hairless bees standing at the hive entrance, carried out from the colony by their companion bees [(Bailey, 1976; Ribiere *et al.*, 2010) NC, unpublished observations]. Bees with bloated abdomens and partially spread dislocated wings were also observed. Masses of dead individuals have been observed piling up in front of the hives causing significant reduction in the bee population (Bailey, 1976; Ribiere *et al.*, 2010).

Collapse of heavily infected colonies that remain with the queen and a small group of workers and unattended combs was observed as well (Ribiere *et al.*, 2010).

CBPV infection was first naturally observed in infected colonies in contrast to ABPV and KBV that were first detected experimentally (Bailey & Woods, 1977; Bailey *et al.*, 1963; Ribiere *et al.*, 2010). CBPV infection can be propagated efficiently by spraying caged bees with bacteria-free extracts of paralyzed bees (Burnside, 1945, Bailey *et al.*, 1963).

Infection develops slowly and mortality appeared about 6 days post-treatment. Injection of worker honey bees with CBPV resulted in pronounced mortality at 5-7 days post-treatment (Bailey *et al.*, 1963; Ribiere *et al.*, 2010; Ribiere *et al.*, 2002). Injection, topical application and oral administration of CBPV required estimated infected doses of 100 viral particles, 10^7 genome copies and over 10^{10} particles, respectively (Bailey, 1976; Bailey *et al.*, 1963; Blanchard *et al.*, 2007). Successful infection by topical application of the virus was effectively achieved by removing the cuticular hair of the target bees (Bailey *et al.*, 1983). Addition of infected adults with paralytic CBPV symptoms to healthy honey bees under overcrowded conditions in cages results in efficient spread of the infection (Ribiere *et al.*, 2007). Thus, it appears that the virus is transmitted through the epidermis, once the bees are deprived from the protection conferred by the cuticular hair (Chen & Siede, 2007; Ribiere *et al.*, 2010). In this respect, outbreaks of CBPV-induced paralysis were detected in Israel when honey bee colonies were introduced into experimental

avocado net houses to assist pollination (amplified by RT-PCR and identified by subsequent sequencing). These bees suffered from cuticular breaks, denuded cuticular surfaces, and cuticular injuries that could easily facilitate initial infection and subsequent spread of CBPV (NC, unpublished).

Outbreaks of CBPV infections were frequently observed in the spring and in the summer, when the population in the colony is high and there are plenty of food resources, suggesting that increased body contact of highly active number of bees may facilitate infection and propagation of the virus (Ribiere *et al.*, 2002). The exact trigger of the infection is not clear and covert infections and external sources of contamination have been implicated. Interestingly, alternative hosts like *Camponotus vagus* and *Rufa formica* ants were also shown to be carriers of CBPV (Celle *et al.*, 2008).

In addition to the data discussed above, it should be added that infected individuals displayed high titer of up to 10^{13} CBPV genomic copies in symptomatic bees in contrast to 10^{4} copies in asymptomatic bees (Blanchard *et al.*, 2007). CBPV was detected in the head of symptomatic bees, more specifically in the brain, in the thoracic and abdominal nerve ganglia, in the hypopharingeal and mandibular glands (Blanchard *et al.*, 2007). These data and the detection of CBPV in specific regions of the brain involved in neurosecretive processes and other nervous tissue together with the symptoms accompanying CBPV infections support the hypothesis that CBPV is a neurotropic virus (Olivier *et al.*, 2008b).

CBPV is able to infect all the developmental classes of the colony, including the queen but it seems to prefer adult bees (Blanchard *et al.*, 2007; Chen *et al.*, 2006b; Chen *et al.*, 2005). Also, CBPV was found contaminating pollen (Chen *et al.*, 2006a).

Thus, the presented data indicate that CBPV may be transmitted in the food as well and even vertically by the queen. However the preferable mode of infection seems to be through the cuticular epidermis or even through injuries as described above (Ribiere *et al.*, 2010).

MANAGEMENT AND TREATMENT

Management of viral diseases requires detailed knowledge about the infectious agent, its ways of transmission and the conditions that facilitate the propagation of the epidemics that will eventually conduct to loss of the colony. At the individual level immune mechanisms of defense are activated to abort the viral infection.

RNA interference (RNAi) is a conserved mechanism of antiviral immunity in plants, vertebrates, and insects (Ding, 2007; Li, 2002). RNAi efficiently inhibits replication of RNA viruses by detecting dsRNA intermediates formed during their replication (Ding, 2007). A specific RNaseIII endonuclease Dicer binds and cleaves dsRNA to produce ds-small RNA fragments of 21–24 base pairs, called small interfering RNAs (siRNAs). The siRNAs are integrated into the RNA-induced silencing complex (RISC) that is activated and binds to homologous ssRNA resulting in its sequence-specific degradation. All essential components of the RNAi machinery are present in the honey bee genome, suggesting that RNAi is an important defense against viruses in honey bees (Weaver *et al*., 2007). Moreover, artificial introduction of RNAi was shown to inhibit IAPV replication (Maori *et al.*, 2009).

Also, adequate control and management of *Varroa destructor* infestation is vital to diminish substantive damage due to virus infections because of the active role the mite plays in transmitting and activating honey bee viruses and in weakening honey bee defenses [reviewed in (Rosenkranz *et al.*, 2010)].

Thus, we expect that research efforts to deepen our knowledge about RNAi and other mechanisms involving innate immunity defenses of the honey bee against viral pathogens, appropriate treatment against *Varroa*, and breeding for resistance to these pathogens, will highly contribute in better managing of viral-mediated colony losses.

CONCLUSION

In summary, ABPV, KBV, IAPV and CBPV have low oral infectivity and they establish in the colony covert infections of low virulence in most cases but it appears that diverse stress factors, which may involve facilitation of their access to the insect hemolymph (ABPV, KBV, IAVV and CBPV) or the cuticular epidermis (CBPV) and /or the appearance of virulent strains, may mediate the transition from covert to overt virulent infections of high mortality that result eventually in abrupt decrease of the adult bee population. Better understanding of the biology of ABPV, IAPV, KBV and CBPV infections, including honey bee mechanisms of resistance to infection, will enable to develop proper approaches to manage

viral infections of the honey bee, as well as breeding for resistance to viral pathogens.

Acknowledgements:

I acknowledge support for honey bee virus research by The Chief scientist Fund of The Israeli Ministry of Agriculture, The Israeli Honey Board and The Israeli Plant Growers Association under grant no.-1510-13108 and 09-1483-131. Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel #504/10

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GENİŞLETİLMİŞ ÖZET:

Giriş: En yaygın viral patojenler Akut Arı paralizi virüsü (ABPV), siyah kralice gözü virüsü (BQCV), deforme kanat virüsü (DWV),Israil akut paraliz virüsü (IAPV), Kaşmir arı virüsü (KBV), torba çürüklüğü virüsü (SBV) ve kronik arı felci (paralizi) virüsü (CBPV)'dür. Bu derlemede yetişkin arıda paralitik hastalıklara en çok neden olan IAPV, KBV, ABPV ve CBP virüsü ile koloni kayıpları değerlendirilecektir.

Morfoloji ve Genom Organizasyonu: Bu virüslerden IAPV, ABPV ve KBV morfolojileri ve genom organizasyonları açısından birbirlerine benzemektedir. Genom organizasyonu içerisinde bu üç virüsün taşıdıkları genetic material RNA'nın büyüklüğü, oluşturduğu protein ürünleri, taşıdıkları intergenik bölgeler karşılaştırılmıştır. IAPV ve KBV'nin birbirlerine daha çok benzer olduğu, ayrıca hangi genom bölgeleri açısından birbirlerine benzer olduğu yapılan çalışmalarla ortaya konulduğu belirtilmiştir. CBPV'nin ise daha küçük genoma sahip olduğu belirtilmiştir. Yapılan filogenetik çalışmalara göre bu virüsün *Nodaviridae* ve *Tombusviridae* virü ailesi kümeleri arasına yerleştirdiği yapılan bilimsel çalışmalarla gösterilmiştir.

Enfeksiyon ve Patolojisi:

Morfoloji ve genom organizasyonunda olduğu gibi ilk gruptaki benzer virüslerin enfeksiyonları ve nasıl hastalığa neden oldukları gerek doğal gerekse hastalığa neden olarak gösterilmiştir. Bu virüsler verilen arılarda ne gibi değişiklikler ve hastalık seyri yapılan çalışmalarla gösterilmeye çalışılmıştır. Bu virüslerin yetişkin balarılarından nerelerde bulunduğu ve ayrıca nasıl belirlenebileceği konuları yapılan çalışmalarla özetlenmiştir. ABPV görülmesi yaz aylarında sıklaşırken, KBV ve IAP virüslerinin görülme sıklığı ise sonbaharda artmaktadır. Bu virüsler diğer parazitleri ile korelasyon göstermektedir. Kovan bireyleri arasında ise bu virüslerin hem yatay hem de dikey olarak aktarıldıkları belirtilmiştir. Ana arıdan yumurtlama ile yavrulara aktarırken, erkek dölleme yolu ile ana arıya, işçiler ise besleme yolu ile larvalara virüsleri aktarmaktadırlar. Bu virüslerin koloni düzeyinde neden oldukları kayıplar daha sonra yoğun koloni kayıpları ile ilişkilendirilmektedir. Yukarıdaki virüslerin aksine CBPV'nin diğer parazitler ile korelasyon göstermediği ancak koloni içerisinde çok farklı şekillerde paralitik semptom gösterdiği belirtilmektedir. Bu semptomlardan bazıları uçamayan, sürünen siyah arılar, kovan önünde tüysüz siyah arılar, dışarı atılmaya çalışılan arılar, kovan önünde ölü arı birikimi ve koloni sayısında dramatic düşüş bu semptomlardan bazılarıdır. CBPV enfeksiyonunun yavaş seyretmesine rağmen ölümlerin kısa sürede meydana gelmektedir. Bu virus kovan içerisinde tüm gelişim sürecini enfekte etmekte, Ana arıyı da enfekte etmesine rağmen daha çok yetişkin arıları etkilemektedir. CBPV sal-

gını daha çok ilkbahar ve yaz aylarında görülmektedir.

Virüs Hastalıklarının Yönetimi ve Tedavisi: Virüs hastalıklarının yönetiminde hastalığa neden olan ajanın çok iyi bilinmesi nasıl hastalığa neden oluyor koloni içerisinde nasıl bulaşıyor koloninin zayıflamasına nasıl neden oluyor konularının kolonilerde çok iyi bilinmesi durumunda bu ajana karşı birey düzeyinde bağışıklık sisteminin harekete geçirilerek enfeksiyon önlenebilecektir. Yapılan çalışmalar RNAi teknolojisinin RNA virüslerinde replikasyonu engelleyerek hastalığı engellemektedir. RNAi, RNA replikasyonu sırasında ikili sarmal RNA ara ürünlerini belrilemekte ve replikasyona engel olmaktadır.

Sonuç: Özetle ABPV, KBV, IAPV ve CBPV düşük oral enfeksiyona sahiptir ve kovan içerisinde saklı enfeksiyona neden olur ve çoğu durumda düşük hastalığa neden olur, fakat değişik stres nedenlerinden dolayı arının hemolimfine (ABPV, KBV, IAPV ve CBPV) ya da kütiküler epidermise (CBPV) ulaşabilir ve dolayısıyla yüksek düzeyde ölüme neden olabilirler. ABPV, IAPV KBV ve CBPV enfeksiyonlarının biyolojisini daha iyi anlama ve enfeksiyona karşı balarısı direnç mekanizmasının anlaşılması, balarısı virus enfeksiyonlarına karşı daha iyi yaklaşımlar geliştirmede ve viral patojenlere dirençli soyların ıslahında yardımcı olacaktır.