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Prevalence of multiple viral diseases associated with honey bees colony collapse and control of disorders

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Abstract
The paper briefly examines major virus diseases of honey bee occurring in wide geographical bee keeping areas. Honey bee viruses cause colony to abscond; kill larvae, pupae and adult populations; and loss of honey bee products such as honey and wax. Black queen cell virus affects queen pupae; bee virus X reduces life span of bees; bee virus Y is associated with Nosema; chronic bee paralysis results infected bees tremble, listless crawlers, often hairless, black with greasy wings become opaque; deformed wing virus deforms wing and shortened life span; Kashmir bee virus is harmful if associated with other pathogens; and Kakugo virus affects brain and increases aggression. An active, integrated management of infection sources and other stressors is essential to minimizing virus titers with best management practices. Routinely inspect colonies for possible disease, have a thorough knowledge of symptoms and identify when colonies are slow to build up or have sporadic brood patterns indicating that brood has been pulled out and removed. For more viral protection, follow judicious treatment practice and hygiene, clean comb, replace contaminated equipment, fallow of natural forage, proper nutrition, eases stress, strong immune system, and maintain healthy bee stock. Comb replacement and requeening are the best practical responses to a virus infection. Other future avenues of virus control include breeding hygienic bee strains that detect brood diseases and remove infected individuals from colonies or breeding of resistance to infestation.

Keywords: Honey bee, Viruses, Paralysis, Pest control Anomalies, Varroa mite

1. Introduction
Honeybees (*Apis mellifera*) have been managed for honey and wax production for at least 4500 years. Hive products (honey, wax, royal jelly, propolis) remain globally valuable. However, of far greater value is the contribution made by honeybees to agricultural crop pollination. Industrial-scale migratory beekeeping is critical for many high-value crops such as top fruit, almonds and blueberries. The only large-scale managed pollinator honeybees account for a significant proportion of the estimated values provided by insect pollination to global agriculture. Infections of the disease ranging from chronic to highly virulent can result loss of honey bee's population and loss of honey bee products such as honey, wax and also cause honey bees to abscond and death [1, 2].

Viruses of the honey bee typically infect the larval or pupa stages, but the symptoms are often most obvious in adult bees. Many of these viruses are consumed in pollen or the jelly produced by nurse bees that are fed to developing bees. Many viruses are also transmitted by Varroa. Mite, when feeding on the hemolymph and it transfers the viruses directly into the open circulatory system, which reaches every cell in the insect body. Native bees are generally better adapted to the climate, changes in food supply and the pathogens present, etc. Importation of foreign bee species and races is therefore unnecessary [3, 4]. Therefore, an attempt has made in this article to present the information on the various honey bee's virus diseases and assessment made on the economic loss associated with their presence on life of honey bees.

2. Honeybee Viruses
Honey bee's virus diseases are problems for bee keeping practice in various localities of world. The success of apiculture is entirely influenced by these diseases causing pathogenic organisms and various pest creatures. The economic loss associated with the presence of honey bee diseases and pest has been estimated in some works and significant losses are reported [5]. Honey bee virus diseases include the followings:-

2.1. Acute bee paralysis virus/ Kashmir bee virus/ Israeli acute paralysis virus
Acute bee paralysis virus (ABPV), Kashmir bee virus (KBV) and Israeli acute paralysis virus (IAPV) are three closely related viruses that are largely symptomless, but they can be lethal at individual and colony level, particularly when transmitted by *Varroa destructor*, which is an active vector of these viruses [6]. These viruses are characterized by the ability to kill both pupae (after injection) and adult bees (after injection or feeding very rapidly; 3-5 days after inoculation with sufficient virion loads. This exerts a strong negative selection pressure on the transmission by varroa, since infected pupae fail to complete development, preventing the release of infectious mites from the pupal cells. The association of these viruses with varroa infestation is therefore unstable and much influenced by the presence of other viruses that are better adapted to transmission by varroa [7, 8, 9].

2.2. Black queen cell virus
The main symptoms for black queen cell virus (BQCV) consist of blackened cell walls of sealed queen cells containing dead pro-pupae. Diseased larvae have a pale yellow appearance and tough sac-like skin, much like sacbrood. The virus is present in adult bees but without obvious symptoms. Symptoms of BQCV are limited to queen larvae, and the immature dies and turns black after its cell is sealed. There may be an association between Black queen cell virus and Nosema disease. Treating

colonies with Fumidil-B® to control Nosema may help to keep this disease at bay [10].

2.3. Aphid lethal paralysis virus & Big Sioux River virus

Aphid lethal paralysis virus (ALPV) is a common intestinal dicistrovirus of several major agricultural aphid pests, associated with aphid population declines. Big Sioux River virus (BSRV) is closely related to *Rhopalosiphum padi* virus (RhPV), another common intestinal dicistrovirus that uses the plant vascular system to transmit horizontally between aphids. Both can be detected infrequently at very low background levels in adult honey bees throughout the year, with a sharp quantitative increase during late summer when bees often feed on honeydew (aphid excreta) during low nectar flows. It is unclear therefore whether these viruses are incidental or truly infectious in bees. Either of these may be related to Berkeley bee picorna-like virus (BBPV), which has not yet been sequenced [11].

2.4. Deformed wing virus/ Kakugo virus/ Varroa destructor virus-1/ Egypt bee virus

The symptoms for deformed wing virus (DWV) consist of bees with crumpled and vestigial wings and bloated abdomen and infected bees die soon after emergence. Asymptomatic bees can also be heavily infected, though with lower titres than symptomatic bees. The virus is detected in all other life stages as well, but without obvious symptoms. Kakugo virus (KV) and other strains of DWV have been associated with elevated aggression in bees, although naturally aggressive bee races are not more infected with DWV than gentle bee races. The DWV also affects sensory response, learning and memory in adults [12]. The Varroa destructor virus-1 (VDV-1) is genetically closely related to DWV, but is reported to be more specific to *Varroa destructor* than to bees. However, both viruses replicate in varroa mites as well as in honey bees; both have been detected at high titres in different honey bee tissues; both have been found in regions where *V. destructor* is absent and natural recombinants between them have been found. The VDV-1 and DWV, therefore, appear to co-exist in bees and mites as part of the same species-complex. Egypt bee virus (EBV) is serologically related to DWV, but has no known symptoms in adults, pupae or larvae [13].

2.5. Sacbrood virus/ Thai sacbrood virus

Beekeepers rarely consider sacbrood a serious threat; however recent estimates suggest that one larva killed by the sacbrood virus contains enough virus to kill over one million larvae. More research needs to be conducted on the sacbrood virus since it is unknown how the virus is actually transmitted to the larvae in nature, why severe outbreaks occur only during the build-up season, or how the virus persists from year to year. The clearest symptoms of sacbrood virus (SBV) appear a few days after capping, and consist of non-pupated pale yellow larvae, stretched on their backs with heads lifted up towards the cell opening, trapped in the unshed, and saclike larval skin containing a clear, yellow-brown liquid. The virus is also present in adult bees, but without symptoms. Symptoms of sacbrood are partially uncapped cells scattered about the frame or capped cells that remain sealed after others have emerged. Diseased individuals inside cells will have characteristically darkened heads which curl upward. The dead prepupa resembles a slipper inside the cell. Diseased prepupae fail to pupate and turn from pearl white to pale yellow to light brown and finally, dark brown. The skin is flaccid and the body watery. The dark brown individual becomes a wrinkled, brittle scale that is easily removed from the cells (unlike American foulbrood). The presence of sacbrood-infected larvae produces a spotted appearance of the brood combs, which is a condition shared with all other brood diseases. The larvae die extended on the lower side of the sealed cells, and after they die, a part or all of the cappings may be removed by the adult bees. The skin of the dead larva does not rot as it does if the larva has died of foulbrood. Instead, it remains tough and encloses the watery contents like a sack, giving the disease its name. The head of the dead larva darkens more rapidly than the rest of the body and stays upright in the cell. The elevated head of the completely dried larva remains readily visible in the cell and such a scale is easily removed from the cell [14, 15, 16].

Sacbrood is most common in the spring, usually affecting only a few cells in a comb. Diseased larvae are most commonly seen in spring, but the disease normally clears quickly with rapid expansion. However, the Asian honey bee, *Apis cerana*, frequently suffers from lethal sacbrood epidemics caused by a closely related strain of SBV, variously called Thai sacbrood virus (TSBV), Chinese sacbrood virus (CSBV) or Korean sacbrood. The genetic differences of these strains with the SBV infecting *A. mellifera* are minimal. The SBV-infected adults cease to attend brood or eat pollen, start foraging much sooner than normal, and only forage nectar, rarely pollen. These may be behavioral adaptations by *A. mellifera* to prevent sacbrood epidemics, since SBV is shed in the hypopharyngeal secretions fed to larvae and combined with pollen to make bee-bread. Occasionally very susceptible queen may have large numbers of affected larvae. The disease usually requires no treatment and in severe cases, the colony should be requeened with a young queen from a different strain of bees [17, 18].

2.6. Slow bee paralysis virus

Slow bee paralysis virus (SBPV) is characterized by the paralysis of the front two pairs of legs of adult bees, a few days before dying, after inoculation by injection. The virus is associated with, and transmitted by, *V. destructor*. Despite this association, SBPV is rarely detected in bee colonies. The SBPV can also be detected in larvae and pupae, but produces no symptoms in these [19].

2.7. Chronic bee paralysis virus/ Satellite virus

Chronic bee paralysis virus (CBPV) manifests itself in adult bees through two distinct set of symptoms. One set consists of trembling of the wings and bodies and a failure to fly, causing them to crawl in front of the hive in large masses. They often have partly spread dislocated wings and bloated bodies as well. The other set of symptoms consists of hairless, greasy black bees caused by nibbling attacks from healthy bees in the colony. They soon also become flightless, tremble and die. The virus also infects the larval and pupal stages, can be detected in fecal material and is efficiently transmitted through contact and feeding. The CBPV is sometimes associated with a small 'satellite' virus; chronic paralysis satellite virus (CBPSV; originally called chronic bee paralysis right is a typical example of a CBPV infected bee. The CBPV is identified as a cause of adult bee paralysis after long shiny and black. These bees in infected hives are often found isolated, motionless or shaking on the top bars. When the colony is smoked they do not tend to move down between the frames as the other 'normal' bees do.

Abdomens may also be distended and the wings dislocated. They cannot fly and so can also be seen crawling in front of the hives [20, 21].

Suspicion is that the tracheal mite *Acarapis woodi*, is the culprit of the paralysis. The CBPV is extracted from naturally paralyzed bees as one of the first viruses isolated from honey bees and has since been detected in adult bees of *A. mellifera* from almost every continent. The CBPV mainly attacks adult bees and causes two forms of 'paralysis' symptoms in bees. The most common one is virus associate CBPVA, which has a unique genome and capsid protein to CBPV and is of unknown significance to symptomatology. The photograph is characterized by an abnormal trembling of the body and wings, crawling on the ground due to the flight inability, bloated abdomens, and dislocated wings. The other form is identified by the presence of hairless, shiny, and black-appearing bees that are attacked and rejected from returning to the colonies at the entrance of the hives by guard bees. Both forms of symptoms can be seen in bees from the same colony. The variation in the disease symptoms may reflect differences among individual bees in inherited susceptibility to the multiplication of the virus. The close contact of overcrowded bees breaks hairs from the cuticle, allowing CBPV to spread from diseased bees to healthy bees via their exposed epidermal cytoplasm. It is likely that any factors that result in decreased foraging activities and crowded conditions in the bee colonies may lead to disease outbreaks of CBPV. It has been reported that CBPV is very widespread and infects most bees and can cause mortality in bee colonies particularly during long periods of confinement [22].

2.8. Cloudy wing virus

The symptoms for cloudy wing virus (CWV) consist of opaque wings of severely infected adult bees, with lower titres resulting in asymptomatic infected bees. It cannot be propagated in larvae or pupae. It has an unpredictable incidence and no regular associations with other pathogens or pests. Like chronic bee paralysis satellite virus, it has a small particle and very small genome, but they are serologically unrelated and their single capsid proteins are of different size [23].

2.9. Bee virus X/ Bee virus Y

Bee virus X (BVX) is largely symptomless in adult bees and does not multiply in larvae or pupae. It is associated with the protozoan *Malpighamoeba mellificae* that causes dysentery in winter bees. Bee virus Y (BVY) is serologically related to BVX and is similarly symptomless in adult bees, larvae or pupae. It is associated in adult bees with the dysentery inducing microsporidium *Nosema apis.* Both viruses are common, BVY is more so than BVX, with strong peaks in late winter for BVX and early summer for BVY [24].

2.10. Lake Sinai virus-1, virus-2

Lake Sinai virus-1 (LSV-1) and Lake Sinai virus-2 (LSV-2) are two closely related viruses that are identified in through a mass metagenomic sequencing survey of honey bee colonies. Their genome organization and sequences place them together with CBPV, in a unique family somewhere between the Nodaviridae and Tombusviridae. Both viruses are common and very abundant at peak incidence. The LSV-1 is more common than LSV-2, and present throughout the year with a peak in early summer. The LSV-2 has a very sharp incidence and abundance peak in late winter with low incidence and abundance the rest of the year. These viruses have also been detected, with similar incidences and titres, in historical European honey bee samples. The LSV-1 and LSV-2 have strong similarities in capsid and genome size, seasonal incidence, predominantly adult-based infection and absence of overt symptoms with Bee virus Y and Bee virus X, respectively, and may therefore be related [25].

2.11. Arkansas bee virus & Berkeley bee virus

Arkansas bee virus (ABV) and Berkeley bee picorna-like virus (BBPV) are two viruses first identified, of which very little is known other than that they often occur together. They have no known symptoms in adult bees or brood and BBPV has typical capsid and genome size characteristics of the Dicistro and Iflaviruses [26].

2.12. *Apis mellifera* filamentous virus

The *A. mellifera* filamentous virus (AmFV) is a baculovirus-like DNA virus that has no physical symptoms. It renders the haemolymph of adult bees milky white with rod-shaped viral particles, when examined by electron microscopy [27].

2.13. Apis iridescent virus

The symptoms for Apis iridescent virus (AIV) are similar to the adult flightless clustering symptoms of CBPV. It is only known to occur in adult bees and a partial sequence of AIV has been published [28].

2.14. Chronic Bee Paralysis (Hairless Black Syndrome)

Viruses are pieces of genetic material that parasitize a host cell, making the cell to produce more viruses. Symptoms of chronic bee paralysis are limited to adults, and individuals exhibit an abnormal trembling motion of the wings and body. Bees appear incapable of flight and may be seen crawling up the stems of grass in front of the hive. The abdomens may be bloated and the wings partially spread or dislocated. Bees afflicted with the virus may appear shiny and greasy because of the lack of hair, which should not be confused with robbing bees. Also, adult bees are chewed by other bees and harassed by guard bees at the entrance to the hive (again may be confused with signs of robbing). Adult bees die within a few days of the onset of symptoms. The virus is spread from bee to bee by direct body contact. Food exchange does not appear to be an important mode of spread. Bees vary genetically in susceptibility; therefore requeening is a good practice if symptoms appear [29, 30].

2.15. Deformed Wing Virus (DWV)

This virus disease most regularly reported in Varroa-infested colonies is due to a member of the Flaviridae, which as a picorna-like virus and is distantly related to poliovirus (an enterovirus). Although DWV is found in the majority (95%) of colonies, irrespective of the Varroa status, it replicates to high levels and causes developmental deformities (malformed, atrophied wings and abdominal stunting) in mite-infested colonies. Significantly, high levels of DWV also reduce worker's lifespan; this is thought to be the major contribution to overwintering losses where worker numbers are critical. A study on the biology, pathogenesis and transmission of DWV in honeybees, using a combination of molecular, reverse genetic and systems-based methods has been carried out. In recent studies, it has been demonstrated that a recombinant form (RF) of DWV predominates after Varroa transmission. Researchers have investigated the virus population in the bee before and after

transmission, the response of the bee to virus infection (e.g., by microarray analysis of changes in host gene expression) and the sequence of the virus population replicating in the Varroa mite. Bee pupae are susceptible to infection at the white-eyed stage. The virus multiplies slowly which permits the infected individual to survive to adulthood. The newly-emerged adult has misshapen wings and soon dies. The results of these studies will have implications for the diagnosis and potential therapeutic treatment of DWV-mediated infections in managed honeybee colonies [31, 32].

3. Detecting and Quantifying of Viruses

There are numerous techniques available for detecting and quantifying viruses; particularly studies on molecular methods. Most of these studies detect only a small portion of the viral genome or the capsid proteins, and almost all require some sort of amplification, either of the target (most of the nucleic acid-based detection technologies) or the detection signal (most of the protein-based detection technologies). Both are important considerations to bear in mind when interpreting virus diagnostic data. Secondly, despite the popular classification of molecular assays as either 'qualitative' (presence/ absence) or 'quantitative' (concentration), ultimately all assays are quantitative, while qualitative assays are simply quantitative assays with a detection threshold (a visible color; a band on a gel; a fluorescence level; a C_q value; a statistical index). This is an important consideration, since there are many factors besides the initial virus amount that can influence whether or not an assay reaches a detection threshold, such as degradation of the sample, changes to storage-extraction procedures, assay deterioration Furthermore, the molecular and mathematical rules underpinning any assay are the same whether this assay is 'qualitative' or 'quantitative'. The only difference is that in 'quantitative' assays these rules are specifically acknowledged and accounted for, whereas in 'qualitative' assays they are often ignored. It is therefore advisable to approach any experiment or assay from a quantitative perspective first, and include the appropriate controls for threshold-conversion to 'qualitative' data, if this is desired [33, 34].

3.1. Enzyme-Linked Immuno Sorbent Assay (ELISA)

There are many versions of the ELISA, using different blocking agents, primary/ secondary antibodies, reporter enzymes and their specific colorimetric substrate solutions for detection and quantification [35, 36]. They generally fall into one of two major categories:-

3.1.1. Normal ELISA

In conventional ELISA, the sample is adsorbed directly into the wells, to be detected by the specific antibody. This antibody is either conjugated directly to an enzyme; usually either horse radish peroxidase or alkaline phosphatase, or more commonly is detected in a subsequent incubation by a commercial enzyme-conjugated protein that recognizes antibodies in genera.

3.1.2. Sandwich ELISA

In sandwich ELISA, a modified version of the primary antibody is adsorbed to the well first, in order to capture the virus particles after the sample is added. The captured virus particles are then detected as before, either with the reporting enzyme directly conjugated to the detecting antibody or with an extra incubation using an antibody-detecting protein conjugated to the reporter enzyme. The sandwich ELISA is cleaner and much more sensitive than conventional ELISA, but has a less predictable relationship between virus concentration and signal (depending on which component in the assay is limiting).

3.2. RT-(q) PCR

The most common current methods for honey bee virus detection are based on Reverse Transcription Polymerase Chain reaction (RT-PCR), essentially the PCR amplification of cDNA. A detailed coverage of the principles and practices of PCR is found in studies and the beekeeping papers on molecular methods. A reduced version, including those elements specifically relevant to virus detection, has been presented [37].

3.3. Microarrays

Multiplexing is far more effective through microarrays, which is an ordered array of hundreds of molecular probes specific for different target RNAs bound to a solid support, usually a slide. Most microarray technology has been developed for nucleic acid probes, although protein-based arrays are also being developed. The hybridization of RNA target sequences to these probes to these probes can be detected by a variety of methods, including PCR and sequencing. Numerous honey bee microarrays have been designed, including honey bee immune gene-pathogen arrays and a honey bee virus array. Microarrays are being superseded for research purposes by high-throughput sequencing technologies, but retain a future in routine screening applications, due to their adaptability and high multiplexing capacity [38].

Researchers proposed a honeybee-mite-virus model that incorporates (1) parasitic interactions between honeybees and the Varroa mites; (2) five virus transmission terms between honeybees and mites at different stages of Varroa mites: from honeybees to honeybees, from adult honeybees to phoretic mites, from honeybee brood to reproductive mites, from reproductive mites to honeybee brood, and from honeybees to phoretic mites; and (3) Allee effects in the honeybee population generated by its internal organization such as division of labor. Researchers provide completed local and global analysis for the full system and its subsystems. Analytical and numerical results allow having a better understanding of the synergistic effects of parasitism and virus infections on honeybee population dynamics and its persistence. Interesting findings from this work include (a) Due to Allee effects experienced by the honeybee population, initial conditions are essential for the survival of the colony. (b) Low adult honeybee to brood ratios have destabilizing effects on the system, generate fluctuated dynamics, and potentially lead to a catastrophic event where both honeybees and mites suddenly become extinct. This catastrophic event could be potentially linked to Colony Collapse Disorder (CCD) of honeybee colonies. (c) Virus infections may have stabilizing effects on the system, and could make disease more persistent in the presence of parasitic mites. The model illustrates how the synergy between the parasitic mites and virus infections consequently generates rich dynamics including multiple attractors where all species can coexist or go extinct depending on initial conditions. The findings may provide important insights on honeybee diseases and parasites and how to best control them [39].

4. Management Practices for Viral Disease Control

Viruses are pieces of genetic material that parasitize a host cell, making the cell to produce more viruses. No vaccines or medications are available for any of the honey bee viruses; however, new RNA Silencing technology may soon provide a means to reverse virus symptoms in bee viruses. Until then, good sanitation practices are the key to prevention of diseases. Comb replacement and requeening are the best practical responses to a virus infection. The other best management against many honey bee viruses is aimed at elimination of infection sources. When beekeepers are suspect to have a disease in colony, take a sample and send it to be identified at research Centre. Viruses persist in normal, healthy colonies, only to explode during times of stress. Many viruses are only damaging when in combination with another stressor like Varroa mite or Nosema. Active, integrated management of Varroa and other stressors is essential to minimizing virus titers. For learning of more about reducing stressors with best management practices include routinely inspect colonies for possible disease. Have a thorough knowledge of symptoms and identify when colonies are slow to build up or have sporadic brood patterns, indicating brood has been pulled out and removed. For more information on best management performs, follow judicious treatment practice and hygiene, clean comb, replace contaminated equipment, fallow of natural or forage, proper nutrition, eases stress, strong immune system, and healthy bees. Other best management avenues of virus control include use of hygienic bee strains that can detect brood diseases and remove infected individuals from colonies or breeding of resistance to infestation. Specific resistance to viruses is not yet considered in most breeding programs, however, there is evidence of specific viral resistance in honey bees, and there has been at least some attempt to breed resistance to infestation [40, 41].

5. Breeding Resistance Variety of Bees

Some hives appear to be more resistant to different honey bee diseases than others due to their hygienic behavior. Theses colonies have an ability of their adult bees to uncap and remove affected broods which can reduce the spread of infection to the whole colony. This hygienic behavior has been explained as being controlled by two recessive genes, one for uncapping and one for removal of larvae. So, it is recommended to selective breeding of varieties of honey bees colony by evaluating the hygienic behavior in different honey bee species found in the states. Breeding resistant variety of colonies should be practiced through selective rearing of queen from the resistant colony of bees [42].

6. Conclusion

The Honey bee (*Apis mellenifera* L.), is prone to infect with virus pathogenic organisms with infections of disease ranging from chronic to highly virulent resulting loss of honey and also causing honey bees to abscond and death. The key for protecting honey bee colonies from harmful diseases, parasites and other problems is the ability to identify problems at the earliest. This publication designed might assist to beekeepers in learning to recognize the symptoms of common maladies of the honey bee and to administer for approved treatments or control measures. Currently, the apicultural industry depends heavily on chemical control treatments to keep managed colonies alive. Without such control the mite populations in the colony will grow exponentially and the honey bee colony will succumb to the development of overt virus infections that are vectored typically by the mite within few (three) years. Some hives appear to be more resistant to different honey bee diseases than others due to their hygienic behavior. An integrated management of infection sources and other stressors is essential to minimize virus titers with best management practices. Further future avenues of virus control include breeding of hygienic bee strains that can detect brood diseases and remove infected individuals from colonies or breeding of resistance to infestation. Specific resistances to viruses are not yet considered in most breeding programs. There is an indication of specific viral resistance in honey bees and there has been at least some attempt to breed resistance to viruses. Additionally, encouraging of research areas for controlling honey bee viruses in the use of gene silencing are entitled as Review on RNA interference (RNAi). The researchers are developing this method and the consumer products may be available in the near future as RNAi technology continues to become more efficient and inexpensive.

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