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Seven honeybee virus detection on honeybee mites in Thailand

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Abstract
Thailand beekeeping has faced two main mites infections, there are *Tropilaelaps mercedesae* and *Varroa destructor* mites. They are very important for honeybee health and one of the economic losses factors of beekeeping in Thailand. In this study, we studied virus detection on the *Tropilaelaps mercedesae* and *Varroa destructor* by using the RT- PCR in Chiang Mai. Thirty *Tropilaelaps mercedesae* and *Varroa destructor* samples were detected with seven honeybee virus such as Deform Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV), Chronic Bee Paralysis Virus (CBPV), *Varroa* Destructor Virus-1 (VDV-1), Sacbrood Virus (SBV), Kashmir Bee Virus (KBV) and Black Queen Cell Bee Virus (BQCV). We found that *Tropilaelaps mercedesae* have infected four honeybee viruses. Such as DWV, ABPV, CBPV and VDV-1. At the *Varroa destructor*, there have three honeybee viruses infected. Such as DWV, ABPV, and VDV-1. The percentage detection of DWV and ABPV are 100% detected and VDV-1 is 77 in *Varroa destructor*. And the *Tropilaelaps mercedesae* have DWV is 100% infected, ABPV is 57% infected, CBPV is 30% infected and VDV-1 is 23% detected. When compared the virus infection between the *Tropilaelaps mercedesae* and *Varroa destructor* mites, *Tropilaelaps mercedesae* have more virus infection than *Varroa destructor*.

Keywords: *Varroa destructor*, *Tropilaelaps mercedesae*, honey bee virus and RT-PCR

Introduction
In Thailand, *Apis mellifera* L. European honeybee was introduced to Chulalongkorn University, Bangkok Thailand in the early 1940s by Professor Supachai Wattana. In 1953, Professor Saman Worakitta, Introduced European honeybee from Australia to Kasetsart University, Bangkok Thailand (Wongsiri, 1988) [48]. In the 1970s, there were imported large numbers of A. mellifera from Taiwan to Lamphun and Chiang Mai in northern Thailand for commercial purposes (Wongsiri *et al*., 1995, Akratanakul, 2000) [49, 1]. Today, *A. mellifera* beekeeping record in Thailand has more than 500,000 colonies and 1,000 beekeepers all over the country (Suwannapong *et al*., 2011) [43]. *A. mellifera* beekeeping was spread out in northern Thailand, especially in Chiang Mai province (Thapa and Wongsiri, 1997) [45]. And beekeeping has two main mites infections, there are *Tropilaelaps* mercedes and *Varroa* destructor mites.
Tropilaelaps mites are primary brood ectoparasites of the giant Asian honeybees (*Apis dorsata* and *Apis laboriosa*) (Delfinado-Baker *et al*. 1989) [20]. The mites can only survive in colonies with the presence of brood in which they readily feed and reproduce while inside the cells, and they can live 1-3 days on adult bees (Kiprasert 1984; Woyke 1984; Koeniger and Musaffar 1988; Rinderer *et al*. 1994) [29, 50, 30, 39]. *Tropilaelaps* mites were infected with *Apis mellifera* when imported to Asia regions. The first reports identified *Tropilaelaps clareae* was the most serious parasite of *Apis mellifera* colonies in Asia (Burgett *et al*. 1983; Anderson and Morgan, 2007) [11, 6]. It is the most serious threat to *Apis mellifera* colonies in the Philippines (Cervancia, 1993) [12]. In Thailand, *Tropilaelaps mercedesae* was found on *Apis cerana* brood (Anderson and Morgan, 2007) [6]. *Varroa* mites are originally parasitized mites from the Asian honeybee (*Apis cerana*) (Anderson and Fuchsm, 1998; Anderson 2000, Anderson and Trueman, 2000) [5, 4, 3]. The *Varroa* mite is considered a major pest of honeybees *Apis mellifera* (Crane, 1978) [14]. And *Varroa destructor* is the most important pest of *A. mellifera* and it plays a main role in losses

of honeybee colonies (Martin, 2001) [32]. *Varroa* females entering to the brood cells and reproduction on the worker or drone larvae about 20-40hr before the cells are sealed (Boot, *et al*., 1992) [10]. The adult female mite and progeny feed on the hemolymph of pupae from a single feeding site (Kanbar and Engels, 2003) [28].

Deform Wing Virus (DWV) infection detected to be widespread throughout the world. The symptoms of DWV in bees have crumpled, vestigial wings and bloated abdomen. Then infected Bees were dead after emergence infection. Sometimes asymptomatic bees can see in bee colonies. It can be seen healthy bee but heavily infected and the other strains of DWV elevated to the bees behaviour aggressive and affected to the sensory response, learning and memory in adults (Tentcheva *et al*., 2004, Muz and Muz, 2018) [44, 36]. Chronic Bee Paralysis Virus (CBPV) detected in adult bees from every continent, except South America. CBPV manifests itself in adult bees through two different type of symptoms, which are the trembling of the wings and bodies, and then they cannot fly and drop down in front of colonies. The other symptoms can be seen hairless, greasy black bees caused by nibbling attacks from healthy bees in the colony. They became flightless, tremble and die. At the larval and pupal stages also infected with the virus. In this condition can be detected in faecal material and is efficiently transmitted by feeding (Dietemann *et al*., 2012) [21]. Acute Bee Paralysis Virus (ABPV) is a common virus that detected in healthy colonies (Allen and Ball, 1996) [2] and reported in *Apis mellifera* from North America, Europe, Oceania, Asia, Africa, and the Middle East (De Miranda *et al*., 2010, Dietemann, *et al*., 2013, Ellis and Munn, 2005) [17, 21, 22]. When the bees infected by ABPV, they cannot control their body movement and their flight. The infected bees became hair loss, dark and shiny. The infested bees retreat on top bars and die there having their wings extended (De Miranda *et al*., 2010) [17]. *Varroa* Destructor Virus-1 (VDV-1) closely related to DWV but reported more specific to *Varroa* destructor (Ongus, *et al*., 2006) [38]. However, DWV and VDV-1 viruses replicate in *Varroa* mites as well as in honeybees (Ongus *et al*., 2004; Yue and Genersch, 2005; Zioni *et al*., 2011) [37, 52, 53] and both viruses have been detected at high titres in different honeybee tissues (Gauthier *et al*., 2007; Zioni *et al*., 2011) [25, 53]. VDV-1 and DWV appear to co-exist in bees and mites as part of the same species-complex (De Miranda *et al*., 2010; Gauthier *et al*, 2007; Moore *et al*., 2010; Martin *et al*., 2012) [17, 25, 34, 33].

Many kinds of bee virus are related to honeybee mites such as *Varroa* and *Tropilaelaps* mites. When any kind of mites was infected with *Apis mellifera* colonies, honeybee virus infection was followed. *Tropilaelaps mercedes* infected colonies were found Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV), Sacbrood Virus (SBV), Kashmir Bee Virus (KBV), Acute Bee Paralysis Virus (ABPV), and Chronic Bee Paralysis Virus (CBPV) (Forsgren, *et al*., 2009; Dainat, *et al*., 2009; Chanpanitkitchote, *et al*., 2017) [23, 13]. *Varroa* mites infected colonies were found DWV, ABPV, CBPV, BQCV, KBV, SBV, *Varroa* destructor virus-1 (VDV-1) and Slow Bee Paralysis Virus (SBPV) (De Miranda, *et al*., 2011; Francis, *et al*., 2013; Moore, *et al*., 2015; Roberts, *et al*., 2017) [18, 24, 35, 41]. *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 (Ongus, 2006) [38]. SBV is the most widely distributed of all the viruses detected in almost colonies throughout the world. SBV infects mainly larvae but also adult bees, although they present no disease symptoms. SBV have the clearest symptoms and appear a few days after capping. The virus was infected in adult bees without symptoms (Lee and Furgala, 1967) [31]. The Asian honey bee, *Apis cerana*, frequently faced with lethal by various kind of Sacbrood virus. Such as Thai Sacbrood Virus (TSBV), Chinese Sacbrood Virus (CSBV) or Korean Sac brood (Tentcheva *et al*., 2004) [44]. KBV was first isolated from adult A. mellifera and from *Apis cerana* in Kashmir, India, and later found in A. mellifera from Canada, the USA, New Zealand and Australia Spain and several other countries in Europe and Oceania. Kashmir Bee Virus (KBV) they did not show symptoms but they have a different lethal dose at individual and colony level. BQCV infection is more prevalent in adult bees than pupae. BQCV infection in honeybee has been reported in North America, Central America, Europe, Oceania, Asia, Africa, and the Middle East. The Black Queen Cell Virus (BQCV) has blackened queen cells walls with dead pupa. When Infected to the larval stage the larvae have a pale yellow colour sac and similar with sacbrood. The virus is present in adult bees but not significant symptoms (Dietemann *et al*., 2012) [21].

In this study, we take seven virus detections on thirty *Tropilaelaps mercedes* 15 honeybee colonies in Chiang Mai province and *thirty Varroa destructor* mites from Nan province in Thailand in 2017.

Materials and Methods

Thirty adults female *Tropilaelaps mercedease* were collected from a sealed brood of *A. mellifera* from 15 colonies in Chiang Mai Province and thirty adults female *Varroa destructor* were from a sealed brood of *A. mellifera* 15 colonies from Nan province. All mites samples were kept in Eppendorf tube and placed in the ice box for 24-hour safe carrying (de Miranda, *et al*., 2013) [16]. All mites samples were frozen at -40 ℃ deep freezer until RNA extraction procedure. Frozen honey bees were crashed in sterilized 1.5 ml tubes and add 1ml of TRIzol (Invitrogen) reagent per 50-100 mg of honeybee tissue, 0.2 ml of Chloroform per 1 ml of TRIzol, 0.5ml of 100% isopropanol and 1 ml of 75% ethanol were add by manufacturer procedure and add 30µl of the RNA free water and kept in - 40 ℃ until cDNA synthesis.

For the first strand cDNA synthesis system used Invitrogen Super Script RT-PCR kit. Prepared the new 0.2 ml of sterilized PCR tubes and the Invitrogen cDNA extraction kits procedure. For the first standard cDNA synthesis system used Invitrogen Super Script RT-PCR kit. In the order to synthesize cDNA, 3µl of total isolated RNA was mixed with 1 µl of Oligo (dT) 20 50 µM, 1µl of 10mM dNTP mix and 5 µl of DEPC treated water (OmniPur, USA). Then incubated at 65 ºC for 5 min. And add 2µl of 10x RT buffer (200mM Tris-HCl (pH8.4), 500mM KCL), 4 µl of 25mM MgCl2, 2 µl of 0.1M DTT, 1 µl of RNaseOUT TM and 1 µl of Super Script® III RT and incubated at 50 ºC for 50 minutes and 85 ºC for 5 minutes. Finally, add 1 µl of RNase H and incubated at 37 ºC for 20 minutes. And cDNA samples were Kept at -20 ºC for PCR amplification.

PCR was conducted using 1µl of cDNA sample, 1µl of 25 nmole DNA oligo primers 1.0µM of each forward and reverse primer showed in table (1) and add 0.25µl of Taq (Invitrogen) DNA Polymerase Recombinant, 0.25µl of 10mM dNTP, 0.75µl of 50 mM MgCl2, 2.5µl of 10x PCR buffer and sterile distilled water were added to total 25µl. PCR reaction was performed using an initial denaturation of 10 min at 95 ℃ followed by 40 cycles of 10s at 95 ℃, annealing step of 16s at 60 ℃, and extension for the 20s at 72 ℃ and Final extension of 10 min at 72 ℃.

To analysis, the significant differences between *Tropilaelaps mercedes* and *Varroa destructor* mites used SPSS (20).

Table 1: Virus Primers sequences

Virus Names	Sequence	References
Deform Wing Virus	For 5'- GGA TGG TCC GCG GCT AAG A-3' Rev 5'-CGG CAG ATA TAA CAG TAC TTG-3'	Sanpa and Chantawannakul, 2009
Varroa Destructor Virus-1	For 5'- GCA GAG GAG GCC AGT GCT -3' Rev 5'- TTT AAA TAG AAA GTC ACG AAT C -3'	Zinoi, *et al*., 2011
Acute Bee Paralysis Virus	For 5′-TGA GAACAC CTG TAA TGT GG-3′ Rev 5′- ACC AGA GGG TTG ACT GTG TG-3′	Sanpa and Chantawannakul, 2009
Chronic Bee Paralysis Virus	For 5′-AGT TGT CAT GGT TAA CAG GAT ACG AG-3′ Rev 5′-TCT AAT CTT AGC ACG AAA GCC GAG-3′	Sanpa and Chantawannakul, 2009
Black Queen Cell Virus	For 5′-GGA CGA AAG GAA GCC TAA AC-3′ Rev 5′-ACT AGG AAG AGA CTT GCA CC-3′	Sanpa and Chantawannakul, 2009
Kashmir Bee Virus	For 5′-GAT GAA CGT CGA CCT ATT GA-3′ Rev 5′-CAG TTA AGG GGT GTT GTT GC-3′	Sanpa and Chantawannakul, 2009
Sacbrood Virus	For 5′-GGA TGA AAG GAA ATT ACC AG-3′ Rev 5′-CCA CTA GGT GAT CCA CAC T-3′	Sanpa and Chantawannakul, 2009

Result

After detection of thirty *Tropilaelaps* mites and thirty *Varroa* mites RNA with seven honeybee virus. *Tropilaelaps* mites have infected four honeybee virus and *Varroa* mites have three. In *Tropilaelaps* mites have infected with Deform Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV), Chronic Bee Paralysis Virus (CBPV) and *Varroa* Destructor Virus-1(VDV-1). DWV is 100% infected, ABPV is 57% infected, CBPV is 30% infected and VDV-1 is 23% detected (Figure 1). And *Varroa* mites have infected with DWV, ABPV, and VDV-1. DWV and ABPV are 100% detected and VDV-1 is 77% detected (Table 2). There have four different virus infections were found such as single virus infection, two virus infections, three virus infection and four virus infections. When the comparing the number of virus combination infection in *Tropilaelaps mercedease* are more than *Varroa destructor* mites (Table 3). When we calculated the correlation between virus and mites by using SPSS software, we found that *Tropilaelaps* mites were significant at the 95% (P>0.05) correlated with the virus. *Tropilaelaps* mites are mostly related with DWV, ABPV, VDV-1 and CBPV viruses than *Varroa* mites.

Table 2: Comparing the percentage of virus detection on *Varroa destructor* and *Tropilaelaps mercedease* mites in Chiang Mai and Nan Province

No.	Virus detection on *Varroa destructor*	%	Virus detection on *Tropilaelaps mercedease*	%
1	DWV	100	DWV	100%
2	ACBP	100	ACBP	57
3	CBPV	0	CBPV	30
4	VDV-1	77	VDV-1	23

Table 3: Comparing the percentage virus combination infections in *Varroa* destructor and *Tropilaelaps mercedease* mites in Chiang Mai and Nan Province

Varroa destructor			*Tropilaelaps mercedease*		
No. of Virus	Type of infection	%	No. of Virus	Type of infection	%
1.			1	DWV	10
2.	DWV, ABPV	23	2	DWV, ABPV	37
				DWV, CBPV	13
				DWV, VDV-1	20
3.	DWV, ABPV, VDV-	77	3	DWV, ABPV, CBPV	17
	1			DWV, ABPV, VDV-1	3

Discussion

The *Tropilaelaps* mites breeding on their honeybee hosts are different behaviour with *Varroa* mites, in that a mature mated female enters a bee brood cell that contains a developing bee larva and the mother mites eat the hemolymph and produces several offspring that all feed on the hemolymph of the developing bee. *Tropilaelaps* mites have different action unlike *Varroa* mites, the survival of *Tropilaelaps* mites was depended to access to bee brood (larvae or pupae) for their feed, their mouthparts and body shape cannot feed on adult bees. Therefore, the *Tropilaelaps* mites cannot feed or survive for more than about 74 hours (Wilde, 2000) [46]. *Varroa* mites can feed on adult bees. *Tropilaelaps* mites can only survive for a few days in the absence of bee brood (Woyke, 1984; Koeniger and Musaffar, 1988; Rinderer *et al*., 1994) [50, 30, 39]. This limited food source restricts their ability to disperse, as they can only disperse on adult bees on which they cannot feed. *Tropilaelaps mercedesae* can be infected 90% of the brood in *A. mellifera* colonies (Kiprasert, 1984) [29].

Nowadays, there have 18 viruses have been identified from honey bees (Allen and Ball, 1996) [2]; many of them cause persistent infections in seemingly healthy bee colonies (Tentcheva *et al*., 2004) [44]. Honeybee viruses in mites support a putative role of mites in vectoring the viruses (Hung *et al*., 2000; Ongus *et al*., 2004; Tentcheva *et al*., 2004; Shen *et al*., 2005) [37, 44, 42]. The *Varroa* mite may transmit the viruses among bees and its feeding might induce or activate viral replication in some ways (Ball, 1983; Bailey and Ball, 1991) [9, 8]. *Varroa* mites in honeybee viral diseases, we quantitatively compared with and *Tropilaelaps* mites, *Tropilaelaps* mites found the more viruses infection. DWV, ABPV and VDV-1 are commonly infected in two mites species. In *Tropilaelaps* mites DWV is 100% infected, ABPV is 57% infected but in the *Varroa* mites, DWV and ABPV are 100% infected. When compare VDV-1 virus there have VDV-1 is 77% in *Varroa* mites and in the *Tropilaelaps* mites have VDV-1 is 23% infected. Therefore, Virus infection on *Tropilaelaps* mites is more infected than *Varroa* mites. When they infected high infestations on *A. mellifera* brood, the adult bees with deformed wings (Dejong *et al*., 1982; Burgett *et al*., 1983) [19, 11] and they becoming reduced body weights (Kiprasert, 1984) [29]. There was no treatment on the infected colonies the *Tropilaelaps* mites population rapidly increased to high levels and invariably lead to the death of entire colonies (Atwal & Goyal, 1971; Ritter, 1988; Woyke, 1985) [7, 40, 51]. *Varroa* infestation is an important factor in bee mortality and colony collapses. Sometimes, *Varroa* kills an apparently healthy colony in a few months. However, little is known about how *Varroa* mites kill bees. Its frequent co-occurrence with severe viral infections in honeybee colonies,

it is considered that mites could activate latent viral infections (Allen and Ball, 1996) [2].

Conclusion

Both *Tropilaelaps* and *Varroa* mites are brood parasitic mites, but *Varroa* mites can be infected on the adult stage of bees than *Tropilaelaps* mites. In the brood infected condition, the population of *Tropilaelaps* mites are faster than *Varroa* mites, in this condition *Tropilaelaps* mites have more chance to destroy the bee colonies than *Varroa* mites. And *Tropilaelaps* mites can carry more virus than *Varroa* mites. Therefore we can conclude *Tropilaelaps* mites are more dangerous than *Varroa* mites for the commercial beekeeping in Chiang Mai and Nan province.

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References

1. Akratanakul P. Apiculture development in Thailand, in: Proc. VIIth Int. Conf. on Tropical Bees: Management and diversity, and Vth Asian Apicultural Association Conf., Chiang Mai, Thailand, IBRA, 2000, pp.395-398.
2. Allen MR, Ball BV. The incidence and world distribution of honey bee viruses. BeeWorld. 1996; 77:141-162.
3. Anderson DL, Trueman JWH. *Varroa* jacobsoni (Acari: Varroidae) is more than one species, Exp. Appl. Acarol. 2000; 24:165-189.
4. Anderson DL. Variation in the parasitic bee mite *Varroa* jacobsoni Oud. Apidologie. 2000; 31:281-292.
5. Anderson DL, Gibbs AJ, Gibson NL. Identification and phylogeny of sporecyst fungi (Ascosphaero spp.) using ribosomal DNA sequences. Mycological Research. 1998; 102(5):451-547.
6. Anderson DL, Morgan MJ. Genetic and morphological variation of bee-parasitic Tropilaelaps mites (Acari: Laelapidae): new and re-defined species. Exp. Appl. Acarol. 2007; 43:1-24.
7. Atwal AS, Goyal NP. J Apic. Res. 1971; 10:137-142.
8. Bailey L, Ball BV. Honey bee pathology (2nd Ed.). Academic Press; London, UK, 1991, pp. 31-34.
9. Ball BV. The association of Varroa jacobsoni with virus diseases of honey bees. Exp. Appl. Acarol. 1983; 19:607-613.
10. Boot WJ, Calis NM, Beetsma J. Differential periods of varroa mite invasion into worker and drone brood cells of honey bees. Exp. Appl. Acarol. 1992; 16:295-301.
11. Burgett MD, Akratanakul P, Morse RA. *Tropilaelaps* clareae: a parasite of honey bees in South-East Asia. Bee World. 1983; 64:25-28.
12. Cervancia CR. Philippines beekeeping status of research and development. In: Proc. Beenet Asia: Workshop on Priorities in R&D on Beekeeping in Tropical Asia. Kuala Lumpur, Malaysia, 1993, pp. 49-63.
13. Chanpanitkitchote Pichaya, Chen Yanping, Evans Jay, Li Wenfeng, Li Jianghong, Hamilton Michele *et al*. Acute bee paralysis virus occurs in the Asian honey bee Apis cerana and parasitic mite Tropilaelaps mercedesae. Journal of Invertebrate Pathology, 2017, 151. 10.1016/j.jip.2017.11.009.
14. Crane E. The varroa mite. Bee World. 1978; 59:164-167.
15. Dainat B, Ken T, Berthoud H, Neumann P. The ectoparasitic mite *Tropilaelaps* mercedesae (Acari, Laelapidae) as a vector of honeybee viruses. Insectes Sociaux. 2009; 56(1):40-43.
16. de Miranda JR, Bailey L, Ball BV, Blanchard P, Budge G, Chejanovsky N *et al*. Standard methods for virus research in Apis mellifera. In V Dietemann; JD Ellis; P Neumann (Eds) The Coloss Beebook, Volume II: standard methods for Apis mellifera pest and pathogen research. Journal of Apicultural Research, 2013, 52(4).
17. de Miranda JR, Cordoni G, Budge G. The acute bee paralysis virus-Kashmir bee virus–Israeli acute paralysis virus complex. J Invertebr. Pathol 103 (Supplement), 2010, S30-S47.
18. de Miranda Joachim, Gauthier Laurent, Ribière Magali, Chen YP. Honey Bee Viruses and Their Effect on Bee and Colony Health. Honey Bee Colony Health: Challenges and Sustainable Solutions, 2011, 71-102. 10.1201/b11318-9.
19. Dejong D, Morse EW, Eickwort GC. Mite pests of honey bees. Annual Review of Entomology. 1982; 27:229-252.
20. Delfinado-Baker M, Baker EW, Phoon ACG. Mites (Acari) associated with bees (Apidae) in Asia, with description of a new species. American Bee Journal. 1989; 129(9):609-613.
21. Dietemann V, Pflugfelder J, Anderson D, Charrière Jd, Chejanovsky N, Dainat B *et al*. Varroa destructor: research avenues towards sustainable control. Journal of Apicultural Research. 2012; 51:125-132.
22. Ellis JD, Munn PA. The worldwide health status of honey bees. Bee World. 2005; 86:88-101.
23. Forsgren E, De Miranda JR, Isaksson M, Wei S, Fries I. Deformed wing virus associated with *Tropilaelaps mercedesae* infesting European honey bees (*Apis mellifera*). Experimental and Applied Acarology. 2009; 47(2):87-97.
24. Francis RM, Nielsen SL, Kryger P. *Varroa*-virus interaction in collapsing honeybee colonies. PloS one. 2013; 8(3):e57-540.
25. Gauthier L, Tentcheva D, Tournaire M, Dainat B, Cousserans F, Colin ME *et al*., Viral load estimation in asymptomatic honey bee colonies using the quantitative RT-PCR technique. Apidologie. 2007; 38(5):426-435.
26. Highfield AC, El Nagar A, Mackinder LC, Laure MLN, Hall MJ, Martin SJ. Deformed wing virus implicated in overwintering honeybee colony losses. Applied and environmental microbiology. 2009; 75(22):7212-7220.
27. Hung ACF, Peng YSC, Shimanuki H. Nucleotide sequence variations in Kashmir bee virus isolated from *Apis mellifera* L. and *Varroa* jacobsoni Oud. Apidologie. 2000; 31:17-23.
28. Kanbar G, Engels W. Ultrastructure and bacterial infection of wounds in honey bee (*Apis mellifera*) pupae punctured by Varroa mites. Parasitol. Res. 2003; 90:349-354.
29. Kiprasert C. Biology and systematics of the parasitic bee mite *Tropilaelaps* clareae Delfinado and Baker (Acarina: Laelapidae). MSc thesis, Kasetsart University; Bangkok, Thailand, 1984.
30. Koeniger N, Musaffar N. Lifespan of the parasitic honeybee mite *Tropilaelaps* clareae on Apis cerana, dorsata and mellifera. Journal of Apicultural Research. 1988; 27:207-212.

31. Lee PE, Furgala B. Viruslike particles in adult honey bees (*Apis mellifera* Linnaeus) following injection with sacbrood virus. Virology. 1967; 32(1):11-17.
32. Martin SJ. The role of *Varroa* and viral pathogens in the collapse of honeybee colonies: A modelling approach. J Appl. Ecol. 2001; 38:1082-1093.
33. Martin SJ, Highfield AC, Brettell L, Villalobos EM, Budge GE, Powell M *et al*., Global honey bee viral landscape altered by a parasitic mite. Science. 2012; 336(6086):1304-1306.
34. Moore Jonathan, Jironkin Aleksey, Chandler Dave, Burroughs Nigel, Evans David, Ryabov Eugene. Recombinants between Deformed wing virus and Varroa destructor virus-1 may prevail in Varroa destructor-infested honeybee colonies. The Journal of general virology. 2010; 92:156-61.
35. Moore PA, Wilson ME, Skinner JA. Honey bee viruses, The Deadly *Varroa* Mite Associates. BeeHealth, 19.
36. Muz D, Muz MN. A molecular epidemiological study of black queen cell virus in honeybees (*Apis mellifera*) of Turkey: the first genetic characterization and phylogenetic analysis of field viruses. Apidologie. 2018; 49:89-100.
37. Ongus JR, Peters D, Bonmatin J, Bengsch E, Valk JM, Van Oers MM. Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite *Varroa* destructor. J Gen. Virol. 2004; 85:3747-3755.
38. Ongus JR, Roode EC, Pleij CW, Vlak JM, Van Oers MM. The 5′ non-translated region of Varroa destructor virus 1 (genus Iflavirus): structure prediction and IRES activity in Lymantria dispar cells. Journal of general virology. 2006; 87(11):3397-3407.
39. Rinderer TE, Oldroyd BP, Lekprayoon C, Wongsiri S, Thapa R. Extended survival of the parasitic mite *Tropilaelaps* clareae on adult workers of Apis mellifera and Apis dorsata. Journal of Apicultural Research. 1994; 33:171-174.
40. Ritter W. Varroa jacobsoni in Europe the tropics and subtropics. In GR Needham; RE Page, Jr; M Delfinado-Baker CE Bow (Eds). Africanized honey bees and bee mites. Halsted Press; Chichester, UK, 1988, 349-359.
41. Roberts JM, Anderson DL, Durr PA. Absence of deformed wing virus and *Varroa* destructor in Australia provides unique perspectives on honeybee viral landscapes and colony losses. Scientific Reports. 2017; 7(1):69-25.
42. Shen M, Yang X, Cox-Foster D, Cui L. The role of varroa mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. Virology. 2005; 342(1):141-149.
43. Suwannapong G, Yemor T, Boonpakdee C, Benbow ME. Nosema ceranae, a new parasite in Thai honeybees. Journal of Invertebrate Pathology. 2011; 106:236-241.
44. Tentcheva D, Gauthier L, Zappulla N, Dainat B, Cousserans F, Colin MF *et al*., Prevalence and seasonal variations of six bee 148 M. Shen *et al*. / Virology 342 (2005) 141-149 viruses in *Apis mellifera* L. and Varroa destructor mite populations in France. Appl. Environ. Microbiol. 2004; 70:7185-7191.
45. Thapa R, Wongsiri S. *Eupatorium odoratum*: A honey plant for beekeepers in Thailand. Bee world. 1997; 78(4):175-178.
46. Wilde J, Woyke J, Neupane KR, Wilde M. Comparative evaluation tests of different methods to control *Tropilaelaps clareae*, a mite parasite in Nepal. In Proceedings of the 7th International Conference on Tropical Bees and 5th AAA Conference, 2000.
47. Winston ML. The honeybee colony: Life history. The hive and the honeybee Hamilton Illinois: Dadant & Sons Graham JM. Apicult. Res. 1992; 37:69-78.
48. Wongsiri S. The Effect of import of *Apis mellifera* L. To Thailand. Proceedings of Fourth International Conference on Apiculture in Tropical Climate. Cairo, Egypt, 1988, 162-167.
49. Wongsiri S, Chen P. Effects of agricultural development to honey bees in Thailand. Bee World. 1995; 76(1):3-5.
50. Woyke J. Survival and prophylactic control of *Tropilaelaps* clareae infesting *Apis mellifera* colonies in Afghanistan. Apidologie. 1984; 15:421-434.
51. Woyke J. *Tropilaelaps clareae*, a serious pest of *Apis mellifera* in the tropics, but not dangerous for apiculture in temperate zones. American Bee Journal. 1985; 125:497-499.
52. Yue C, Genersch E. RT-PCR analysis of deformed wing virus in honeybees (*Apis mellifera*) and mites (*Varroa destructor*). Journal of General Virology. 2005; 86(12):3419-3424.
53. Zioni N, Soroker V, Chejanovsky N. Replication of *Varroa destructor* virus 1 (VDV-1) and a *Varroa destructor* virus 1–deformed wing virus recombinant (VDV-1–DWV) in the head of the honey bee. Virology. 2011; 417(1):106-112.