6-2019

Honeybees in the UAE: Diseases, Associated Microbial Communities, And Parasitic Varroa Mites

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HONEYBEES IN THE UAE: DISEASES, ASSOCIATED MICROBIAL COMMUNITIES, AND PARASITIC *VARROA* MITES

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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Environmental Sciences

Under the Supervision of Dr. Mohammad Ali Al-Deeb

June 2019

Declaration of Original Work

I, Maitha Saif Abdullah Masoud Alshamsi, the undersigned, a graduate student at the United Arab Emirates University (UAEU), and the author of this thesis entitled "*Honeybees in the UAE: Diseases, associated Microbial Communities, and Parasitic Varroa Mites*", hereby, solemnly declare that this thesis is my own original research work that has been done and prepared by me under the supervision of Dr. Mohammad Ali Al-Deeb, in the College of Science at UAEU. This work has not previously been presented or published, or formed the basis for the award of any academic degree, diploma or a similar title at this or any other university. Any materials borrowed from other sources (whether published or unpublished) and relied upon or included in my thesis have been properly cited and acknowledged in accordance with appropriate academic conventions. I further declare that there is no potential conflict of interest with respect to the research, data collection, authorship, presentation and/or publication of this thesis.

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Abstract

Apis mellifera honeybees are the most important pollinator worldwide. Numerous diseases and parasites are causing the decreasing numbers of the *A. mellifera* honeybee. The main objective of this thesis was to study the *A. mellifera* honeybee pathogens and parasites along with the *Varroa destructor* mite. The study included (1) Studying bacterial communities associated with *A. mellifera* and *A. florea* in the UAE. (2) Conducting a molecular detection of *A. mellifera* honeybee pathogens in the UAE. (3) Studying *Varroa* mites and *Varroa*-associated pathogen in the UAE. DNA and RNA were extracted from *A. mellifera* honeybees and *V. destructor* mites. Subsequently, PCR assays were conducted. Sequencing results revealed 23 bacterial operational taxonomic units (OTUs) in both *A. mellifera* and *A. florea* honeybees. Another result, *Nosema ceranae* parasite was detected, while *Nosema apis* parasite was not found in any of the *A. mellifera* honeybee samples. In addition, two viral pathogens were detected in both *A. mellifera* honeybee and *V. destructor* mites. The species of the *V. destructor* mite was confirmed using DNA sequencing. This study is the first of its kind in the UAE. Further studies are needed to investigate the effect of these pathogens on honey production in the UAE.

Keywords: Metagenomics, *Apis mellifera*, *Varroa destructor*, *Nosema ceranae*, *Nosema apis*, DWV, *Varroa destructor* virus-1, bacterial community diversity, next-generation sequencing.

Title and Abstract (in Arabic)

النحل في دولة الإمارات العربية المتحدة: الأمراض، المجتمعات الميكروبية المصاحبة، وعثة الفاروا الطفيلي

الملخص

نحل العسل هو من أهم الملقحات في جميع أنحاء العالم. تتسبب العديد من الأمراض والطفيليات في تناقص أعداد نحل العسل. الهدف الرئيسي من هذه الأطروحة هو دراسة مسببات الأمراض إلى جانب عثة الفاروا المدمرة. أولاً، دراسة المجتمعات البكتيرية المرتبطة بـنحل العسل ونحل العسل القزم في دولة الإمارات العربية المتحدة. ثانيًا، إجراء الكشف الجزيئي لمسببات أمراض نحل العسل في الإمارات العربية المتحدة. ثالثًا، دراسة عث الفاروا ومسببات الأمراض المرتبطة بالفاروا في الإمارات. تم استخراج الحمض النووي والحمض النووي الريبي من نحل العسل وعثة الفاراوا. في وقت لاحق، أجريت فحوصات للنحل وعثة الفاراوا. وتم كشف النتائج المستخرجة من تفاعل البوليميراز المتسلسل عن 23 وحدة تصنيف تشغيلية بكتيرية في كل من نحل العسل ونحل العسل القزم. وفي نتيجة أخرى، تم اكتشاف النوسيما سيرانا الطفيلي، بينما لم يتم العثور على النوسيما آبيس الطفيلي في أي من عينات نحل العسل. بالإضافة إلى ذلك، تم الكشف عن فيروسيين في كل من نحل العسل وفي عثة الفاراوا وقد تم تأكيد أنواع سلالة الفاروا المدمرة باستخدام تسلسل الحمض النووي. هذه الدراسة هي الأولى من نوعها في دولة الإمارات العربية المتحدة. هناك حاجة إلى مزيد من الدراسات لاستقصاء تأثير هذه الأمراض على إنتاج العسل في دولة الإمارات العربية المتحدة.

مفاهيم البحث الرئيسية:الميتاجينومية، نحل العسل، الفاراوا المدمرة، نوسيما سيرانا، نوسيما آبيس، فيروس الجناح المشوه، فيروس الفاراوا المدمر، تنوع مجتمع البكتيريا ، تسلسل الجيل التالي.

Acknowledgements

Many thanks to my supervisor Dr. Mohammad Ali Al-Deeb who inspired me in the insects' field, to his helpful guidance and endless help and patience in teaching me all the new techniques in the lab.

All my love and thanks to my beloved mother who supported me to complete the master studying and took care of my son while I was at the university. A special thanks to my husband who encourage me to complete master studying and for supported me in this journey.

I'd like to thank Ms. Nighat Perveen for her help and support in the lab, for not just being a lab research assistant but a friend.

Dedication

To my beloved mother

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List of Abbreviations

A. florea	*Apis florea*
A. mellifera	*Apis mellifera*
BLAST	Basic Local Alignment Search Tool
DWV	Dwarf Wing Virus
N. apis	*Nosema apis*
N. ceranae	*Nosema ceranae*
NCBI	National Center for Biotechnology Information
OTUs	Operational Taxonomic Units
PCR	Polymerase Chain Reaction
V. destructor	*Varroa destructor*
VDV-1	*Varroa destructor* Virus type 1

Chapter 1: Introduction

1.1 Overview

Honeybees are insects that come under order Hymenoptera and family *Apidae* and showed complete metamorphosis (Blüthgen and Klein, 2011). Honeybees populations are widely influenced by climate change and pesticides. In addition, honeybees hugely reduced by insecticides, the indiscriminate use of insecticides affect the honeybee hives and reduce it sharply in Pakistan (Perveen and Ahmad, 2017).

1.2 Relevant Literature

1.2.1 Honeybee Impact in the Environment

1.2.1.1 Honey Production

Honey is the distinctive compound that honeybees produced from flowers nectar. The color and composition of the honey is dependent on the different types of flowers that the honeybees feed on. Generally, honey is composed of about 17-20% water, 76-80% glucose, fructose, pollen, wax and mineral salts. Honeybee history goes back millions of years. However, the earliest known evidence of human-honeybees interaction could be 8000 years ago (Purbafrani et al., 2014). Honey is the most primary product that honeybees produce. The demand for honey increased while the number of honeybees colony starts to decrease since 70's, the reduction is continuing until now and it's a worldwide dilemma (Lozano et al., 2019). Honey is used in the prevention and treatment of some diseases. In ancient Egypt, honey was applied on the wound for treatment purpose, after applying the wine. More recently, scientist discovered that honey plays a role as an antimicrobial agent in wound infection, indeed, it kills

Staphylococci. Additionally, it works as anti-inflammatory agent (Ulloa-Gutierrez, 2008).

1.2.1.2 Pollination of Plants

Honeybees are the most important pollinators in the world. The value of honeybees mostly comes from producing hive products such as honey, wax and royal jelly. In addition, it helps in pollinating valuable crops such as alflaalfa that is used to feed cattle which produce meat and milk. Honeybees pollinate about one-third of the crops that feed the world, it's estimated that the total economic value of pollination is 153 billion $, which represent 9.5% of the value of the world agricultural output for human food in 2005 (Li et al., 2012). Many flowering plants including important consumable crops rely on honeybees to pollinate them, almost 90% (Burkle et al., 2013). Honeybee species are characterized by particular functional traits that facilitate pollination services (Blüthgen and Klein, 2011).

Using pesticides is an important agricultural activity that eliminate pest from the crops, but in the same time, it poisons both pollen and nectar, causing a frightening threat to honeybee colony that depend on these elementary food sources. Additionally, using insecticides to eliminate diseases from the crops, such as parasites, could be followed by a lot of negative effects on honeybee colony, by poising the nectar that honeybees feed on (Kasiotis et al., 2014).

1.2.1.3 Pollen

In addition to water, honeybees consume pollen and nectar as their main sources of food. Pollen is considered as the main source of protein, fats and minerals.

Especially the new brood, pollen help them to fully develop tissue (Keller et al., 2005). Pollen consumption differs from caste to other, in adult workers, it depends on the age and behavioral tasks that are performed by workers, while the queen consumes a large amount of pollen due to its task in the colony. The amount of pollen differs among colonies due to the different behaviors of honeybees, the collection and store of pollen increased when the brood level increased in the colony (Hellmich and Rothenbuhler, 1986).

1.2.2 Honeybee Life Cycle

Honeybee life cycle has four main distinct stages or phases, egg, larva, pupa and finally an adult. In honeybee's colony, there are three different castes drones, workers, and the queen (Figure 1). In contrast to the queen, both drones and workers show physiological, morphological, and behavioral differences (Li et al., 2016). The main task of the queen is mating and laying eggs, it lays one egg per wax cell. The egg stays in vertical position in the first day, binds in the second day and lays down in the third day. After three days, the egg hatches into a larva. All the larva feed on royal jelly for the first three days. The queen keeps feeding on jelly throughout her life, while workers and drones feed on bee bread which is a mix of pollen and honey prepared by the workers. The larval period varies among different castes, 5-5.5 days for queen, 6 days for workers and 6.5 days for drones. After the fully development of the larva, workers cap the cell with wax. Then the honeybee enters a new stage, pupa, this stage also varies from caste to another, 7.5 days for queen, 12 days for workers and 14.5 days for drones. This stage is inactive, the pupa depends on the stored fat, small hair starts showing in the honey body, the head, thorax, abdomen and wings start to be

visibly distinguishable. Finally, the adult stage, after the full development of the honeybee, it becomes an adult that takes its way out of the cell (Humagain, 2017).

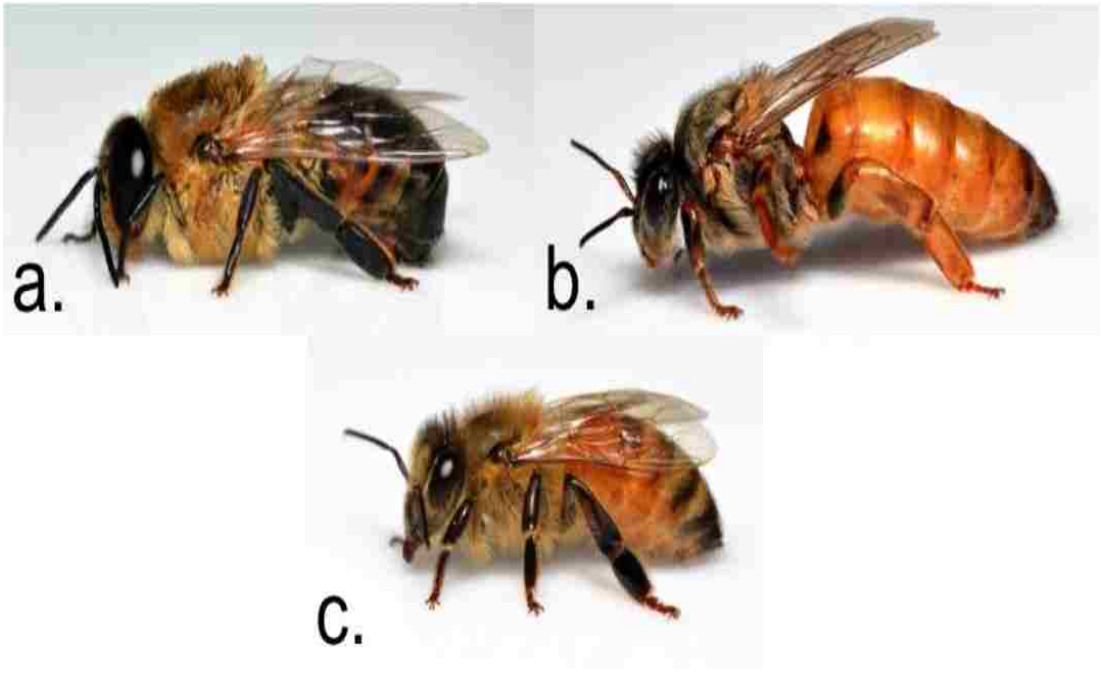

Figure 1: Honeybee castes: a) drone, b) queen, and c) worker (Mortensen et al., 2018).

1.2.3 Honeybees Species in the UAE

In this research, we will study two species of honeybees, *A. mellifera* and *A. florea*.

1.2.3.1 *A. mellifera*

The western honeybee, *A. mellifera*, is one of the most economically important honeybees, it is one of the nine recently described members of the genus *Apis*. It has been transported from its native countries, Europe and Africa throughout the world for beekeeping purposes (Klee et al., 2007). Figure 2 shows the anatomy of *A. mellifera*

honeybee worker. Among all the *Apis* species, only *Apis cerana* and *A. mellifera* are domesticated by man because of their hidden nesting habit. Behavioral limitations of the dwarf and giant honeybees' species, particularly their practice of open-air nesting, prevent them from being kept in man-made hives for reasonably long periods, while hiving colonies in specially constructed containers is essential in that it enables the colonies to be manipulated. *A. mellifera* honeybee species is one of the domesticated species by human. It's also known as European honeybee. Moreover, this species can be manipulated and kept in hives. New technologies and methods have been used to maximize the utilization of their potential. *A. mellifera* is similar in habits to the *A. cerana* honeybee species, both species built multiple combs parallel to each other. Actually, the combs are built in the hollows of trees, in walls or in shady places (Yadav et al., 2017). *A. mellifera* honeybee species occurs in different geographical areas, extending from Scandinavia in the north to the Cape of Good Hope in the south and from Dakar in the west to Oman in the east. More clearly, this species found between 1000 m above the sea level and 3700 m, its survival shows a significant result in both extreme hot and arid zone conditions, like United Arab Emirates. That's mean that this species could adapt to different climates region (Ruttner et al., 1978; Dutton et al., 1981).

A. mellifera population decreased due to several factors. In Asia, both *A. cerana*, the native honeybee species and *A. mellifera*, the introduced honeybee species, are used for beekeeping purposes. These two species have faced a lot of diseases that affected the production of honey. Specifically, infections of wide range of microorganisms such as fungi, bacteria, viruses, and microsporidian parasites. The

disease that is affecting honeybee's colony the most is *Nosemosis*. It is caused by two identified species of microsporidia, *N. apis* and *N. ceranae* (Sinpoo et al., 2018). Many studies are conducted to investigate *A. mellifera* hives collapse worldwide. *Varroa* mite is one of the main reason to the colony collapse, which infect *A. mellifera* and cause a huge devastation in the colony (Li et al., 2012).

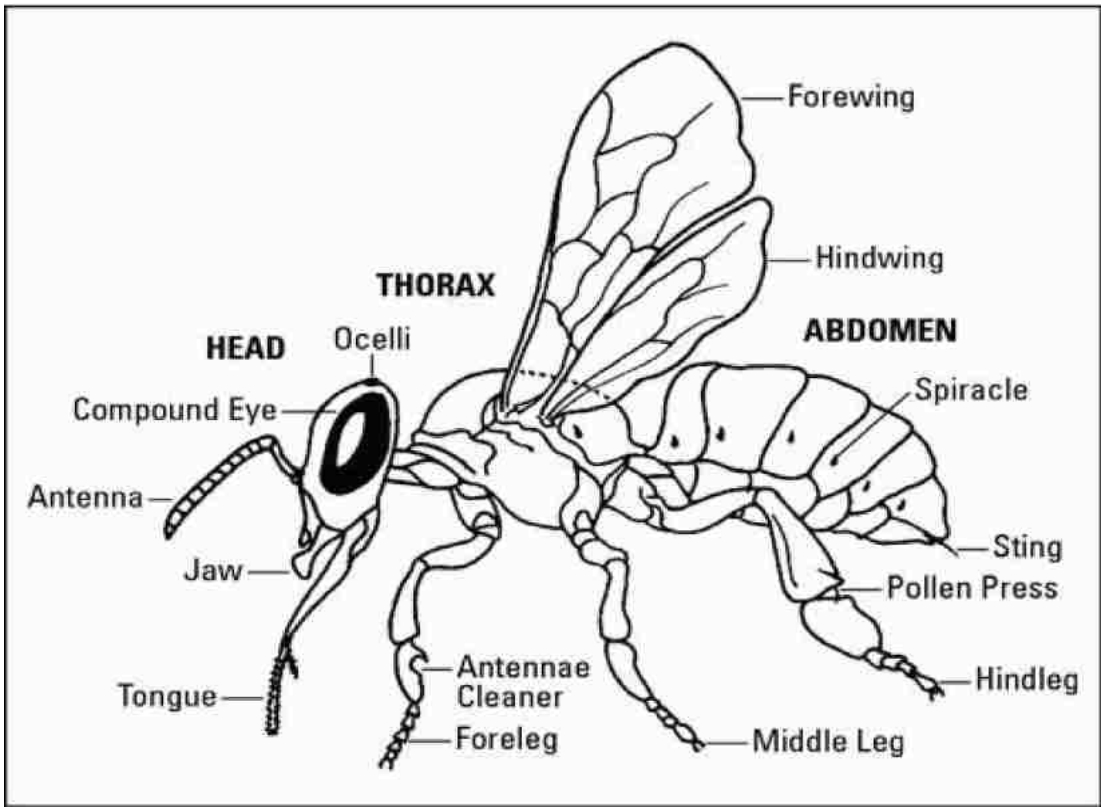

Figure 2: Anatomy of honeybee worker (Blackiston, 2009).

1.2.3.2 *A. florea*

A. florea is phylogenetically the most basal honeybee species (Karpe et al., 2016). Figure 3 shows *A. florea* honeybee species morphology. *A. florea* is known as the dwarf or little honeybee and is found in Asia. These bees build a single comb, exposed nest. Nests are built around a twig of a shrub or a branch of a tree. This species

considered the smallest honeybee species in both the size of the body and the size of the nest. *A. florea* honeybee species distribution is generally confined to warm areas, i.e. Pakistan, Iran, Oman, India and Sri Lanka. Its distribution almost ceases at altitude above 1500 m and is absent in north of the Himalayas. Actually, it can be seen in tropical forest and cultivated areas (Yadav et al., 2017).

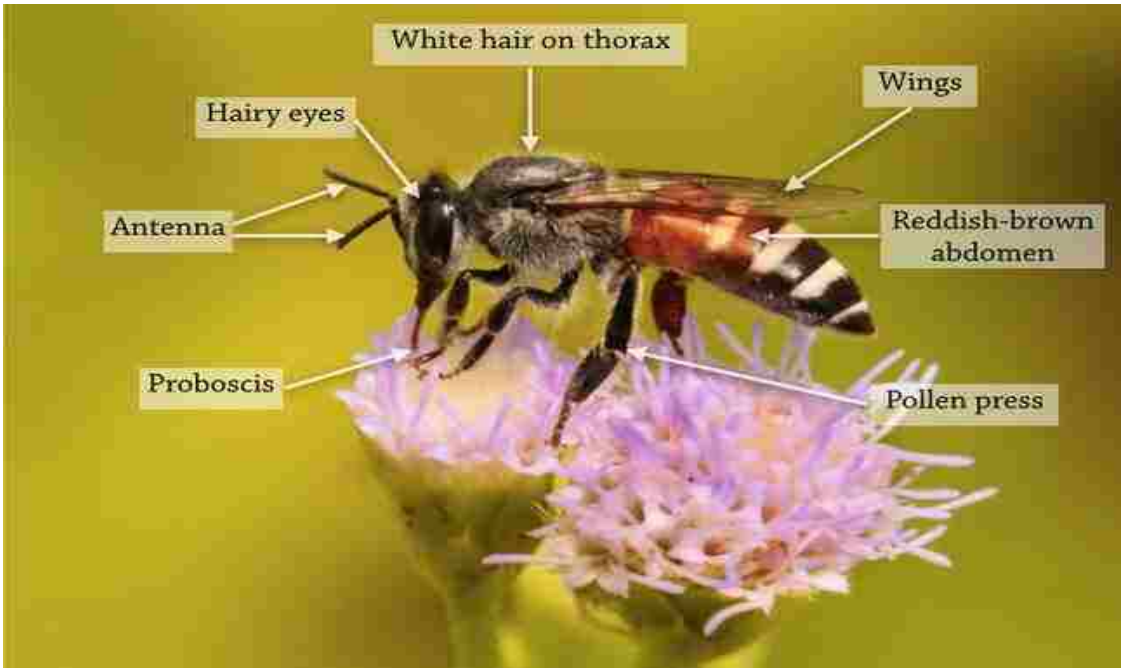

Figure 3: *A. florea* honeybee species morphology (Varma, 2017).

1.2.4 Honeybee Pathogens and *Varroa* Mite Associated Pathogens

Honeybee diseases are wide and occur because of different pathogens and parasites, in this project we will try to figure some of them.

1.2.4.1 *Nosema* Pathogen (*Nosemosis*)

The class of microsporidia is a huge group of parasites that spread in nature widely and infect a huge number of insects (Chen et al., 2018). Microsporidia are

intercellular single cell, spore forming fungal parasites with a wide range of hosts, infecting both invertebrates and vertebrates. *Nosema* has a widespread genus of parasitic microsporidia, usually infecting invertebrates such as *amphipoda*, *orthoptera*, *lepidoptera* and *hymenoptera* (Sinpoo et al., 2018). *Nosemosis* attacks adult honeybees and causes a serious damage to the colony (Chen et al., 2012). *Nosema* is a microsporidia pathogen, which competes with the honeybee for nutrition and exerts an energetic stress on them where it infects the honeybee gut and its well known to cause a suite of metabolic changes in the host (Mayack and Naug, 2009). There are different types of *Nosema*, first discovered species was *N. apis* which firstly discovered in European honeybees, *A. mellifera*. The second one is *N. ceranae*, which was firstly discovered in Asian honeybees, *A. cerana* in 1996 (Li et al., 2016). Different *Nosema* species has different number of polar filament coils and that is one tool that helps to differentiate between the different species of *Nosema* (Fries et al., 1996). Figure 4 shows the life cycle of *N. ceranae* and *N. apis*.

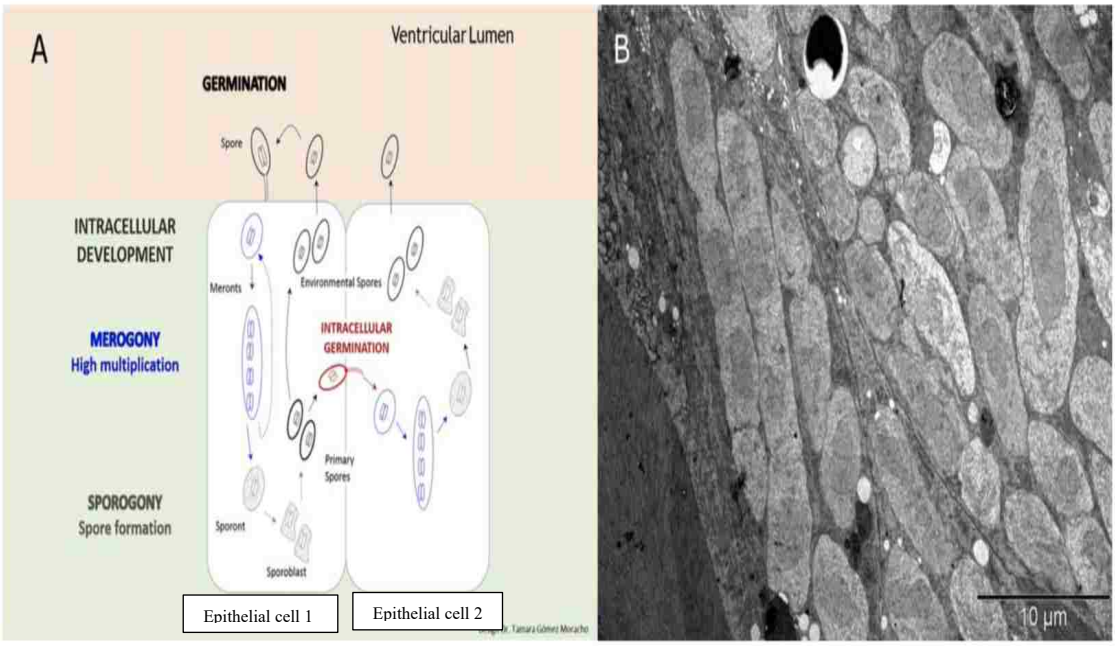

Figure 4: *N. ceranae* and *N. apis* life cycle in the cell: A) The life cycle of *N. ceranae* and *N. apis*. B) Honeybee cell infected with *N. ceranae*, shows different stages of parasitic life cycle (Martín-Hernández et al., 2018).

New species of *Nosema* is currently reported, *N. ceranae* which is recently replacing *N. apis* throughout the world, its exceed its prevalence over it (Klee et al., 2007). *N. ceranae* was first discovered in *A. cerana* and then it has emerged as a potentially virulent pathogen of *A. mellifera*, later on, *N. ceranae* has been associated with colony collapse of *A. mellifera* (Li et al., 2012). *N. ceranae* has infected *A. mellifera* and spread worldwide, which is possibly the main reason to decline *N. apis* species, at least in warmer climates (Sinpoo et al., 2018). The spread of the pathogen through *A. mellifera* is considered recent (Maside et al., 2015).

The newly emerged pathogen causes a lot of effects in *A. mellifera* honeybees. Mostly, workers are more vulnerable to the pathogen, while the queen and the drones are less vulnerable to it (Collison, 2017). The transmission of the pathogen takes place

through the oral-fecal route, severe diarrhea and inability of the digestion and absorption of nutrients and the production of food secretions (Chapman et al., 2017). *N. ceranae* induces the disruption of the bio-element in the honeybee. However, more research is needed to investigate the consequences of these changes on honeybee health during the infection of *N. ceranae* pathogen (Ptaszyńska et al., 2018). In addition to the disruption of bio-element, *N. ceranae* causes a disruption in oxidation balance on the gut of *A. mellifera* honeybee (Paris et al., 2017). There is a risk that infected bees will not be able to fly back to the colony and dies outside while collecting pollen, mostly adult bees and that's cause honeybee colonies to collapse. More clearly, *N. ceranae* is one of the main reason to depopulate the colony (Higes et al., 2008), and one of the most typical symptoms of colony collapse syndrome (Oldroyd, 2007).

The transmission of the pathogen is highly influenced by foraging, since the hunger rate increases, the foraging rate is increased also, thus makes the chance of the transmission of the pathogen through the flowers higher (Mayack and Naug, 2009).

Using the same pathogen load, *N. ceranae* results in more mortality in caged bees than *N. apis*, which means that *N. ceranae* would results in a particularly severe metabolic stress in its new host, *N. apis* (Sinpoo et al., 2018). The median life span of infected and non-infected honeybees with *N. apis* is between 18 to 54 days, while the honeybees that are infected with *N. ceranae* will died within 8 days after the exposure to the pathogen. Thus, this shows that *N. ceranae* is more pathogenic than *N. apis*. In addition, colonies that are infected with *N. ceranae* would have a high rate of death without treatment (Higes et al., 2008). *N. ceranae* causes a higher rate of metabolic stress in its new host than *N. apis*, by imposing an energetic stress on infected bees,

leading to decrease the honeybee appetite and causing hunger which lead to increase the mortality of honeybees (Mayack et al., 2009). Increasing the hunger results in some numerous behavioral effects at both individual and colony level which could leads to increase the exchange rates of the food within the honeybees (trophallactic) and that lead to increase the transmission of the pathogen within the colony (Colla et al., 2006).

There are different methods to eliminate *Nosemosis*, such as using a plant treatment to reduce *N. ceranae* pathogen, two plants are discovered, *Artemisia dubia* and *Aster scraber*, which both eliminate the spore development (Kim et al., 2016).

1.2.4.2 Viruses

One of the most important pathogens that affect *A. mellifera* honeybee colonies is viruses. There are eighteen viruses that have been identified with the ability of infecting honeybees. In addition, most of the virus infections doesn't lead to a clearly defined symptom. Many of the viruses are transmitted by the ectoparasitic mite *V. destructor*. The transmission happens when the *Varroa* feed on the hemolymph of the honeybee. *Varroa* mite was transmitted from *A. cerana* honeybee species to *A. mellifera* honeybee species in the middle of 20th century, and spread worldwide. Australia believed to be the only country that is *Varroa*-free (Chantawannakul et al., 2006).

1.2.4.2.1 Deformed Wing Virus (DWV)

Deformed wing virus (DWV) is an RNA virus mostly associated with *V. destructor*, causes a huge loses in honeybee colonies of *A. mellifera* (Brettell et al., 2017). Figure 5 shows the different between the normal wings and deformed wing

honeybees that infected with DWV. DWV has several viral strains, the most known are DWV-A, DWV-B and DWV-C as they differ in their virulence (Bradford et al., 2017; Schroeder et al., 2017). Deformed wing virus is associated with *Varroa* mite appearance in the colony, although the virus still shows in the colonies with the absence of *Varroa* mite. It is a picorna RNA virus. It's related to *V. destructor* virus type 1 (Ryabov et al., 2014). The pathogeny of the virus is low, lack of hive management and stress from *V. destructor* mite infestation lead to a latent infection of the virus. All the life stages of honeybee are susceptible to DWV attack, with high susceptibly of pupa stage. The virus is believed to be transmitted through mite saliva, which has immunosuppressive proteins. These proteins helps to activate and facilitate the virus transmission (Tantillo et al., 2015). Some recent studies have shown that DWV could have been co-evolved with the European honeybee, *A. mellifera*, while the original strain of the virus has low prevalence and low virulence (Santamaria et al., 2017). There are a large number of shared strains of DWV among *A. cerana* and *A. mellifera*, however the virus is considered to be more widespread in the *A. mellifera* colonies, causing more losses. Overall, the virus has more efficient transmission route in the *A. mellifera* and/ or *A. mellifera* has a greater susceptibility to the virus or to the mite (Yañez et al., 2015). The virus is more virulent when *Varroa* mite appears in the colony (Highfield et al., 2009). The virus is transmitted through *Varroa* mite by anthropogenic activities, where the mites are carried across the borders worldwide causing the pathogen to spread. The queen is considered the main source of transmission of the virus to the colony, and the level of the virus in the queen determines the infection level in the colony (Amiri et al., 2018). In order to eliminate

the transmission of the virus among honeybees, removal of the *Varroa* mite through borders is mandatory (Wilfert et al., 2016).

Figure 5: The difference between normal wings and deformed wings that infected by DWV (Tehel et al., 2019).

1.2.4.2.2 VDV-1

V. destructor virus type 1 is a picornavirus same as DWV, but less common than DWV, and its appearance is less in both honeybees and mites. The appearance of VDV-1 is associated with DWV infected colony. There are few studies about this virus (Cornman, 2017). *V. destructor* virus type 1 (VDV-1) is sharing about 84% similarities at the nucleotide level to DWV and 95% at the amino acid level (Ongus et al., 2004). The virus appears in both the honeybee and the *Varroa* mites (Moore et al., 2011).

1.2.4.3 *Varroa* Mites and Associated Pathogens

Varroa mites are considered as one of the most destructing enemy of honeybee colony worldwide, it has been discovered from 100 years in Java, Indonesia and its

first host was the Asian honeybee, *A. cerana*. The *Varroa* mites is belong to the genus of *Varroa*, and it is classified under *Varroidae* family (Dietemann et al., 2013).

Varroa mite is a native parasite of *A. cerana* throughout Asia. *Varroa* mites has been reported as causing damage worldwide. The main effect of *Varroa* infestation is to weaken the honeybee colonies and lead to decrease the honey production (FAO, 2006). There are four species of *Varroa* mites: *Varroa jacobsoni*, *Varroa underwoodi*, *Varroa rindereri* and *V. destructor*. *V. destructor* is the most dangerous species to honeybees colonies (Oldroyd, 1999).

V. destructor was transmitted from its original host *A. cerana* to *A. mellifera* in the middle of the 20th century, and has caused distressing damage to *A. mellifera* since that time (Li et al., 2012). The mite attacks *A. mellifera* honeybees in different stages of its life cycle, through that, the mite transmits several viral diseases and weakens the bee by feeding on its blood, which causes hemolymph loss. Over all, this makes the parasite one of the major pest of *A. mellifera* (Tantillo et al., 2015). *Varroa jacobsoni* has effects on Asian honeybees (*A. cerana*). Pesticides has been used to control the disease. In Europe, maximum residue amount (MRL) has been applied to control the amount of pesticides, this means the honey products are considered as inappropriate to use, if the level exceeds it. Pesticide residue has become a concern lately, because it enters the human food cycle and causes diseases (Lozano et al., 2019). Figure 6 shows the life cycle of *Varroa* mite in a worker brood cell, which is the mite favorite cell, due to the larger space of brood cell. Mites enter the cell just before workers cap it and hide under larval food in the very bottom of the cell, the female mite (Figure 7) lay the egg inside the cell near the top, which develop into a male. Then it lay a second

egg that develops into a female, after the maturation of the male and the female they mate. The female mite lays more eggs that develop into females. When the brood is fully mature and ready to go out of the cell, the original female mite and mated females attached to the body of the brood, while the male and unmated females die inside the cell (Oldroyd, 1999; Tantillo et al., 2015). For mite's collection, it could be easily collected from the caped brood cells. A white fecal deposit inside the cell is a certain sign of the infection. In *A. cerana*, it's found in drone cells only, while in *A. mellifera* it could be found in both drone and worker cells (Koeniger et al., 2002).

Regarding bacterial community in *Varroa* mite, it has less diverse bacterial community than honeybees, although they share some bacteria. Some of the bacteria that transfer from mite to honeybee might be harmful. *Diplorickettsia* is the most abundant bacteria in *Varroa* mites.

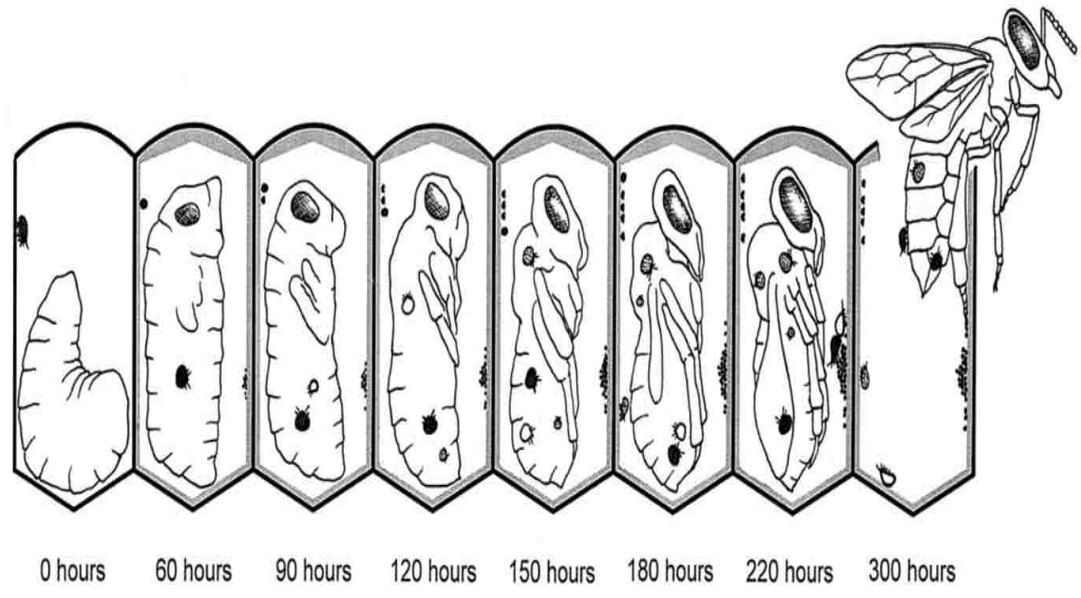

Figure 6: *Varroa* mite life cycle in *A. mellifera* worker brood (Oldroyd, 1999).

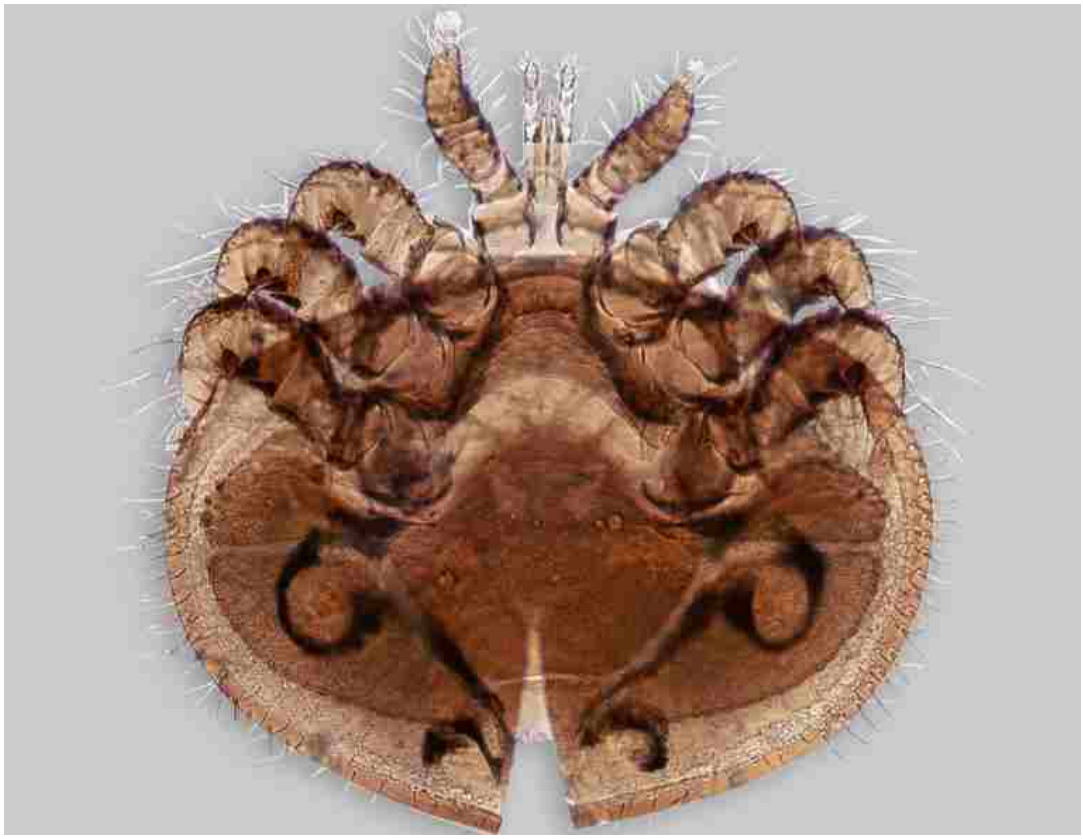

Figure 7: Female *V. destructor* mite, ventral view (Klimov et al., 2016).

1.2.5 Bacterial Communities Associated with Honeybees in the UAE

1.2.5.1 Sanger DNA Sequencing

Sanger has invented his method in 1977 (Totomoch-serra et al., 2017), which has gone under a big transformation, from expensive complicated industry into large production business. Actually, the price of DNA sequencing using Sanger method has fallen dramatically after the appearance of Moore's law(Moore, 1965). Sanger has invented dideoxynucleotide, which inhibit DNA polymerase from its function. In the past, bacterial communities used to be investigated by culture techniques, while

nowadays it's much easier to investigate bacterial communities using the new sequencing platforms, although the culture technique is still used (Mardis, 2008).

1.2.5.2 Microbial Communities

Some bacterial communities are linked with many diseases that affect human health, e.g. inflammatory bowel diseases and obesity (Turnbaugh and Gordon, 2009). American foulbrood disease (AFB) & European foulbrood disease (EFB) are one of the most economically significant diseases of honeybees and it's distress contributes to colony collapse disorder (Ghorbani-nezami et al., 2015). *A. mellifera* honeybees act as a vector for *Paenibacillus larvae* spores, which cause American foulbrood disease (AFB) (Erban et al., 2017b). Some other parasites and pathogens could lead to European foulbrood disease, like *Melissococcus plutonius*, a bacterium that infects *A. mellifera* larvae, which cause a serious damage to colonies of *A. mellifera* (Li et al., 2017). European foul brood usually associated with weak colony that has a low food reserves, attacking the colonies in spring season resulting in huge losses (Erban et al., 2017a).

In invertebrates, studying bacterial communities is very important due to its role in regulating different physiological and behavioral issues in animals. Bacterial communities regulate digestion, animal behavior and immunity development (Budachetri et al., 2016). In human being, the microbial community in gastrointestinal is between 300 to 500 bacterial species, which is more than any tissue in human body, these bacteria help in food digestion, detoxifying of harmful molecules, providing nutrients, a defending wall against the invading pathogens and

parasites and regulating the development and immunity of the body (Kwong and Moran, 2016; Quigley, 2013). The gut of a worker honeybee contains only 9 species of bacteria. The microbial community in honeybees are similar to those in mammals, although the microbiota of honeybees is simpler. Honeybee microbial community in the gut could be cultured easily in the laboratory, while in mammalian it's difficult to be cultured (Kwong and Moran, 2016).

There is some evidence that microbial community in honeybee's gut affect its health, as similar in the mammal's gut bacteria. Actually, there are different species of bacteria in different honeybee species and in different part of the honeybee (Engel and Moran, 2013). Figure 8 shows the different bacterial community in different parts of the honeybee.

Disruption some of these bacteria could make the honeybee more susceptible to *N. ceranae* pathogen, and that's show the importance of microbial community in maintaining the immune system of honeybee (Chen et al., 2011).

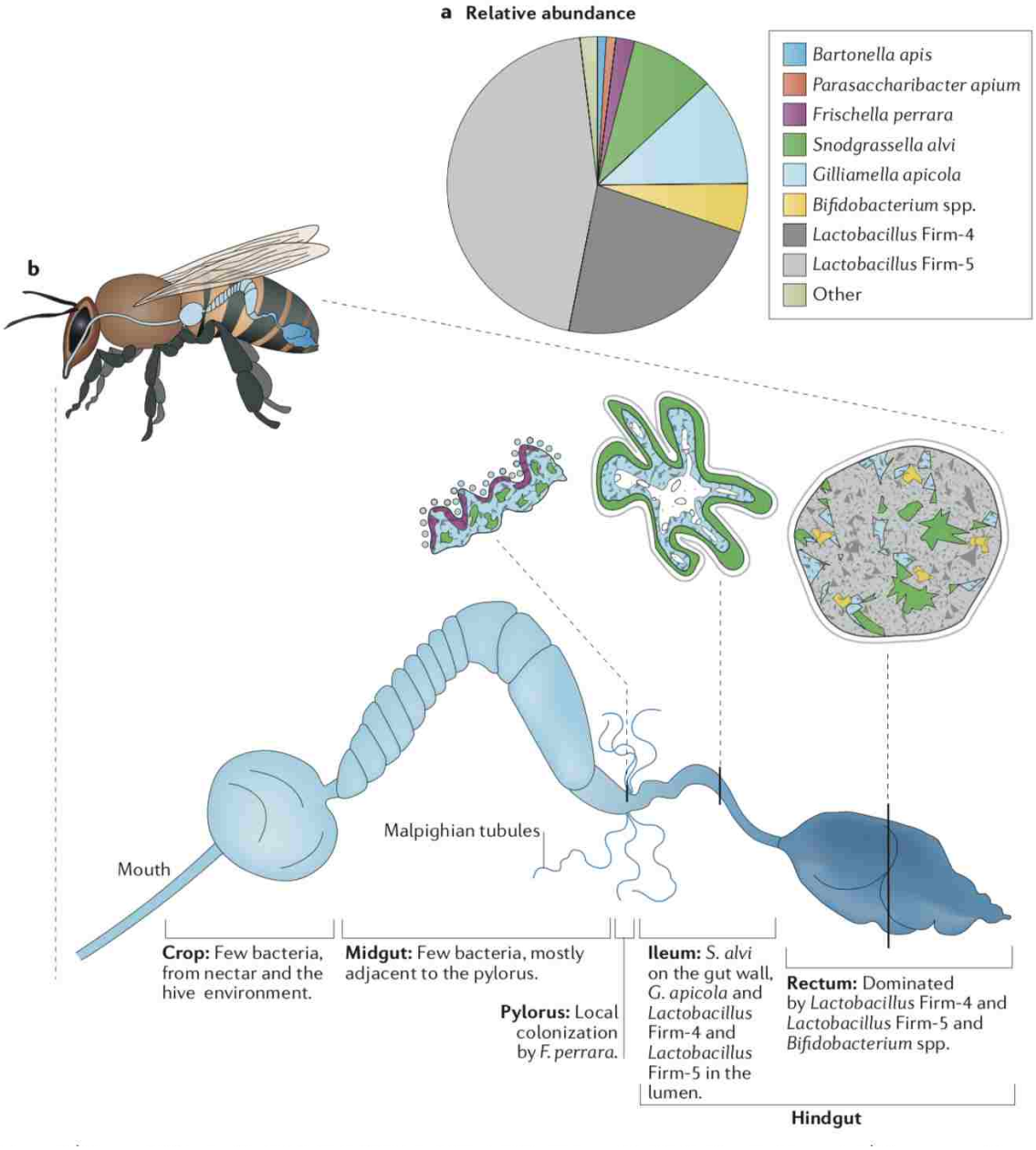

Figure 8: The composition and locative organization of bacterial communities in the honeybee gut (Kwong and Moran, 2016).

Chapter 2: Methods

2.1 Sample Collection

2.1.1 Honeybee Sample Collection

108 samples of *A. mellifera* honeybees have been collected in 2014 and 2015, from all around United Arab Emirates, Al Ain, Abu-Dhabi, Sharjah, Ras AlKhaima and Fujairah. The samples have been kept in freezer under -80°C. *A. florea* honeybees have been collected from Al Ain in 2017.

2.1.2 *Varroa* Mites Sample Collection

V. destructor mite samples were collected from Al Ain city in 2014 and 2015.

2.2 DNA Extraction

DNA was extracted from *A. mellifera* and *A. florea* honeybee samples, using Maxwell 16 DNA Purification Kit following the manufacturer's protocol. DNA was recovered in 300 µl of elution buffer for each sample. The concentration and the quality of the DNA were measured by Nano drop device.

2.3 Next Generation Sequencing (NGS) Mi-Seq Workflow

For microbial community analysis, the honeybee DNA samples were shipped to South Korea, to be sequenced by Macrogen Company. Next generation sequencing has been used for metagenomics records regarding bacterial communities in *A. mellifera* and *A. florea* honeybees. The workflow of Next Generation Sequencing (NGS) Mi-Seq steps are simplified in Figure 9. First: sample preparation, second:

library construction, third: sequencing, and finally raw data.

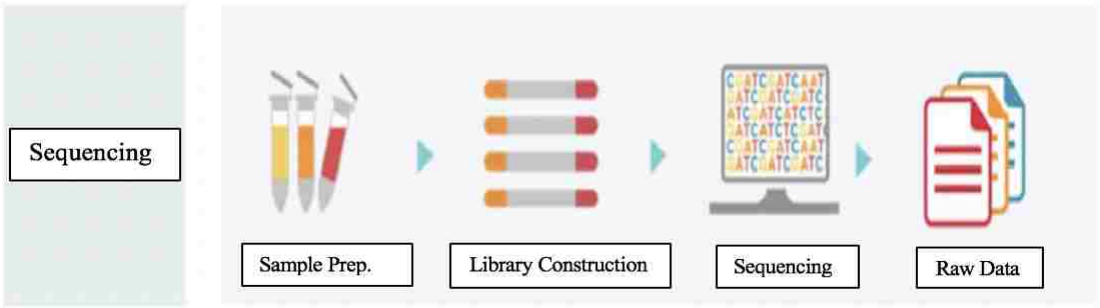

Figure 9: Next Generation Sequencing (NGS) Mi-Seq workflow

Next Generation Sequencing (NGS) Mi-Seq data analysis steps are shown in Figure 10. First: preprocessing and clustering, second: taxonomic assignment and third: diversity statistics.

Figure 10: Next Generation Sequencing (NGS) Mi-Seq workflow data analysis

2.4 RNA Extraction

RNA was extracted from adult frozen bees using Trizol reagent; 1 ml of Trizol was added to the sample and homogenized it. After incubation at room temperature for five min, 200 µl of chloroform was added and the sample mixed by inverting it several times. Incubation was done at room temperature for 2 min, then centrifugation at 12000 xg at 4°C degree for 15 min, after that 400 µl of the aqueous phase was transferred to a fresh tube and precipitated by using 700 µl of isopropanol by mixing the sample by inverting it and incubating it at room temperature for 10 min. Then, centrifugation was done at 7500 xg at 4°C degree for 5 min, and the supernatant was removed, and the pellet was washed by adding 1000 µl of 75% ethanol. Centrifugation was done at 7500 xg speed at 4°C degree for 5 min. Then, the supernatant was removed and the sample was left to air dry for 20 min. Finally, the RNA was dissolved by adding 100 µl of the RNA storage solution. The concentration and the quality of the RNA were measured by Nano drop device.

2.5 PCR

PCR was performed for DNA specifically for *N. ceranae* pathogen, using the primer pair: *N. ceranae* F and *N. ceranae* R (Martin-Hernandez et al., 2007) (Table 1). The primers were used together for the PCR reaction and produce a 218 bp amplicon. Negative control was used in PCR reactions. For each PCR reaction, 5 µL of DNA template was used for the 25 µL PCR. The PCR thermocycler profile was 40 cycles of a 95°C for 5 min, 95°C for 30 sec, and 61.5°C for 30 sec, 72°C for 40 sec with a final extension of 10 min at 72°C. Amplicons were run on a 1.5% agarose gel

electrophoresis, and products were visualized under UV light.

PCR reactions were performed to detect *N. apis* pathogen using the primer pair: *N. apis* F and *N. apis* R, which produce a 321 bp amplicon (Martin-Hernandez et al., 2007) (Table 1). Negative control was used in PCR reactions. For each PCR reaction, 5 µL of DNA template was used for the 25 µL PCR. The PCR thermocycler profile consisted of 40 cycles of a 95°C for 5 min, 95°C for 30 sec, and 61.5°C for 30 sec, 72°C for 40 sec with a final extension of 10 min at 72°C. Amplicons were run on a 1.5% agarose gel electrophoresis, and products were visualized under UV light.

Table 1: *N. ceranae* and *N. apis* primers

Primer Sequences	**Target**
218MITOC (For) 5`-CGGCGACGATGTGATATGAAAATATTAA-3` 218MITOC (Rev) 5`-CCCGGTCATTCTCAAACAAAAAACCG-3`	*N. ceranae*
321APIS (For) 5`-GGGGGCATGTCTTTGACGTACTATGTA-3` 321APIS (Rev) 5`-GGGGGGCGTTTAAAATGTGAAACAACTATG-3`	*N. apis*

2.6 RT-PCR

Multiplex RT-PCR was used to detect both DWV and VDV-1 using DWV F, DWF R, VDV-1 F and VDV-1 R primers (Chen et al., 2004; Soroker et al., 2011)(Table 2). After RNA extraction, the multiplex RT-PCR was performed using Qiagen one step RT-PCR kit. A 25 µl total reaction mix was prepared for the multiplex PCR by adding 5 µl of 5x QIAGEN OneStep RT-PCR buffer, 1 µl dNTP mix (containing 10 mM of each dNTP), 1.5 µl of each primer, 1µl of Qiagen OneStep RT-

PCR enzyme mix, 2.5 µl of RNA and both DWV R, DWV F and VDV-1 R and VDV-1 F primers were added to the same samples. Each PCR was 40 cycles of 50°C for 30 min, 95°C for 15 min, 95°C for 30 sec, 72°C for 1 min and 72°C for 10 min. Amplicons were run on a 1.5% agarose gel electrophoresis, and products were visualized under UV light.

Table 2: DWV and VDV-1 primers

Primer Sequences	Virus
(For) 5`-CTTACTCTGCCGTCGCCCA -3` (Rev) 5`-CCGTTAGGAACTCATTATCGCG -3`	DWV
(For) 5`-GAAGTCGAATACTTGTGTATAGT-3` (Rev) 5`-ATTACTGATTGAAATGGGGAC-3`	VDV-1

2.7 DNA Extraction from *Varroa* Mite

DNA was extracted from *Varroa* mites using Chelex method. The 10% Chelex solution was prepared in ddH_2O. An amount of 500 µl of Chelex 10% was added to *Varroa* mites and 7 µl Proteinase K at 20 mg/ml was added too. Incubation was done at first for 1 hr at 56°C then for 15 min at 100°C. Then, samples were stored at -20°C.

2.8 RNA Extraction from *Varroa* Mite

RNA was extracted from *Varroa mite*s using RNA easy mini kit. 350 µl of buffer RLT was added to mites. Centrifugation was done for 3 min, at the maximum speed, then the supernatant was transferred to a new tube. One volume of 70% ethanol was added and mixed it well by pipetting without centrifugation. An amount of 700 µl of the sample was transferred into the RNAeasy column and was centrifuged for 15

sec at >8000 xg. After centrifugation, the flow-through was discarded. A 700 µl of buffer RW1 was added and centrifuged the sample for 15 sec at >8000 xg and discarded the flow-through. A 500 µl buffer RPE was added and centrifuged the sample for 15s at >8000 xg and discarded the flow-through. Again, a 500 µl buffer RPE was added and centrifuged the sample for 2 min at >8000 xg. The RNA easy column was placed in a new 2 ml collection tube and centrifuged the sample at full speed for 1 min to dry the membrane. Then the RNAeasy column was placed in a new 1.5 ml collection tube. Finally, a 30 µl of RNAase-free water was added to the sample and stored it at -20°C.

2.9 PCR for *Varroa* Mite Identification

V. destructor species was identified by using LCO and HCO primers (Folmer et al., 1994) (Table 3). After DNA extraction from *Varroa* mites, PCR was done to identify *V. destructor* species. A total 25 µl reaction mix was prepared by adding 5 µl of DNA, 1 µl of both F and R primers, 12.5 µl of PCR mix and 5.5 µl H_2O. The samples were placed in the PCR following this protocol: 95°C for 10 min, 95°C for 30 sec, 45.5°C for 1 min, 72°C for 1 min and a final extension at 72°C for 10 min. Amplicons were run on a 1.5% agarose gel electrophoresis, and products were visualized under UV light.

Table 3: *V. destructor* mite primers

Primer Sequence	Target
(LCO) 5`- GGTCAACAAATCAAAAGATATTGG -3` (HCO) 5`- TAAACTTTCAGGGTGACCAAAAAATCA -3`	*V. destructor* mite

2.10 RT-PCR

RT-PCR has been done for *Varroa* mite samples, using QIAGEN OneStep RT-PCR Kit. In purpose of detection two viruses, DWV and VDV-1. A 25 µl total reaction mix for a singleplex PCR was prepared by adding 5 µl of 5x RT-PCR buffer, 1 µl dNTP mix (containing 10 mM of each dNTP), 1.5 µl of each primers, 1µl of RT-PCR enzyme mix, 2.5 µl of RNA and four primers were added DWV R, DWV F, VDV-1 R and VDV-1 F separately for the samples (Chen et al., 2004; Soroker et al., 2011). The PCR conditions were: 40 cycles of 50°C for 30 min, 95°C for 15 min, 95°C for 30 sec, 72°C for 1 min and 72°C for 10 min. Amplicons were run on a 1.5% agarose gel electrophoresis, and products were visualized under UV light.

Chapter 3: Results

3.1 Ecological Parameters: Community Richness & Diversity Indices

A quantitative measure was used to evaluate the richness and the diversity of microbial community in *A. mellifera* and *A. florea* honeybees. The OTUs (Operational Taxonomic Units) values show that *A. mellifera* has a slightly more richness than *A. florea* regarding bacterial community with 17 and 14 count, respectively (Figure 11).

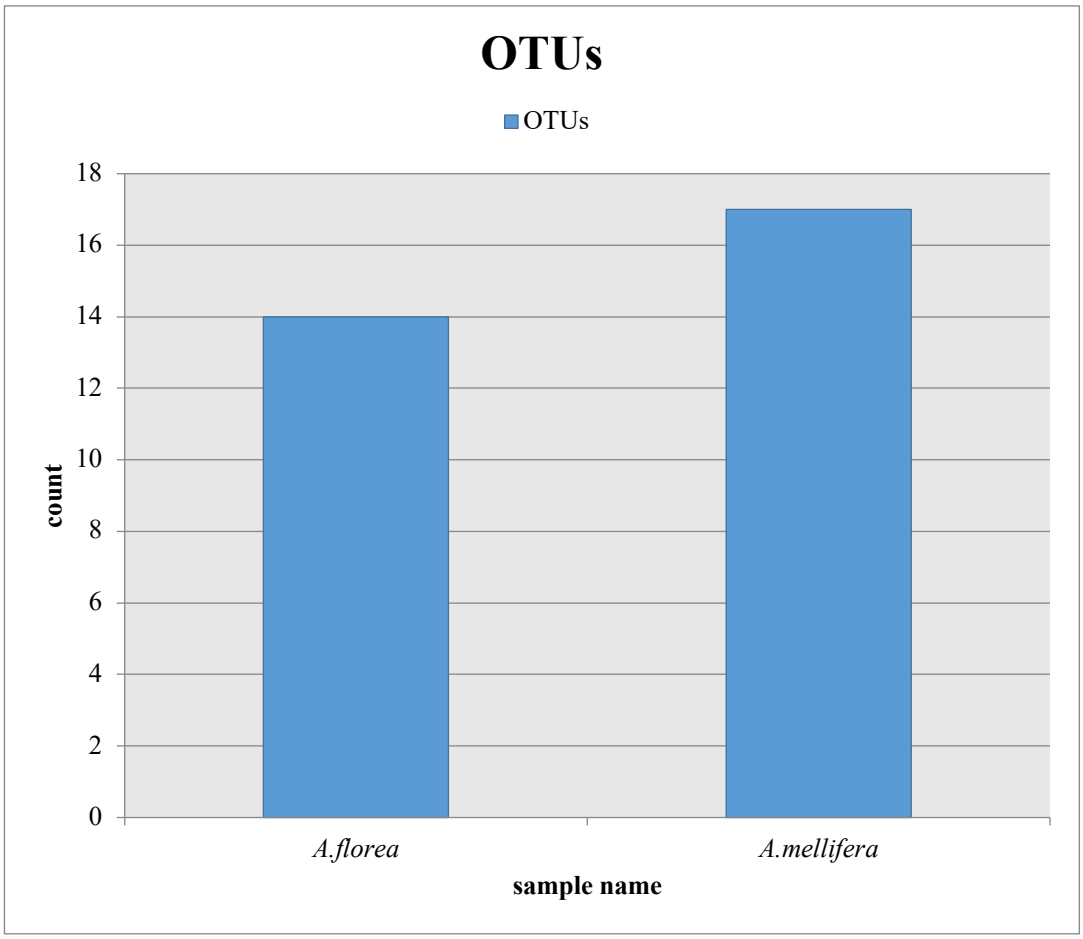

Figure 11 OTUs of microbial communities in *A. florea* and *A. mellifera*

The Simpson's index readings are higher n *A. mellifera* (3.1) than *A. florea* (2.1). For Shannon's index *A. meillfera* (0.8) was higher as compared to *A. florea* (0.7) (Figure 12).

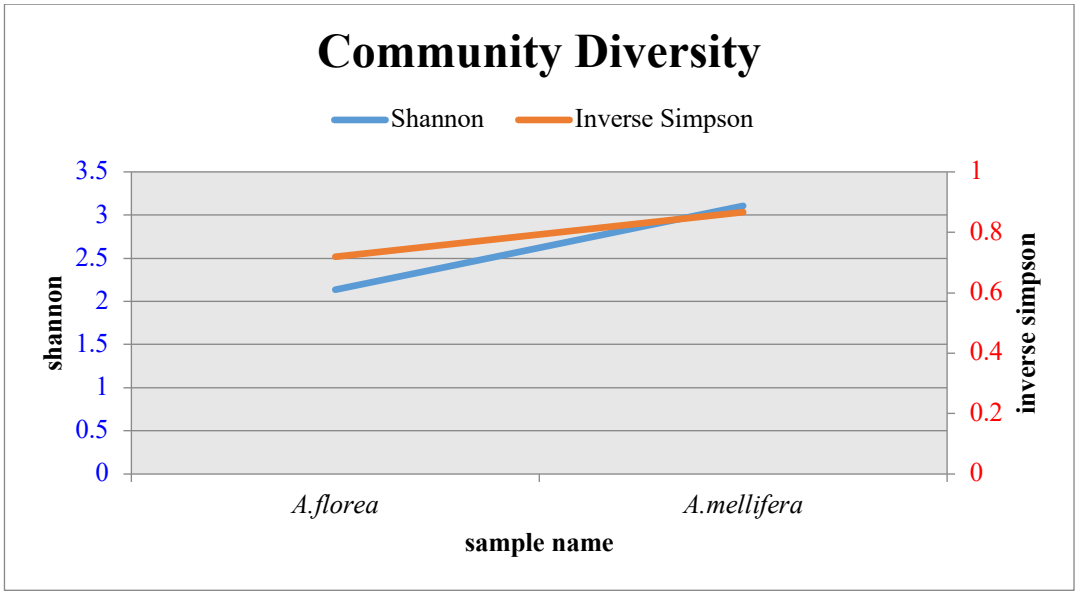

Figure 12: Difference of bacterial community diversity between *A. florea* and *A. mellifera* by using Shannon index and inverse Simpson index.

3.2 Taxonomic Assignment

The bacterial community diversity associated with *A. mellifera* and *A. florea* are presented here at taxonomical levels as dominant bacterial family and genus, because the other levels mainly contain uncultured bacteria and other, with no specification.

After clustering and processing, a total of 86,273 sequences were obtained in this study (46,222 and 40,051 sequences for *A. florea* and *A. mellifera* samples, respectively).

Taxonomic profiling revealed five main bacteria phyla including *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Cyanobacteria*, and *Bacteroidetes*. The bacterial phylum *Firmicutes* was dominant in *A. florea* (86.2%), while *Proteobacteria* was prevalent in *A. mellifera* (66.5%). However, the phylum *Bacteroidetes* was only found in *A. mellifera* (1.4%). *Actinobacteria* was more prevalent in *A. florea* (10.7%) as compared to *A. mellifera* (1.3%) and *Cyanobacteria* was confirmed only in samples of *A. florea* (2.7%) (Figure13).

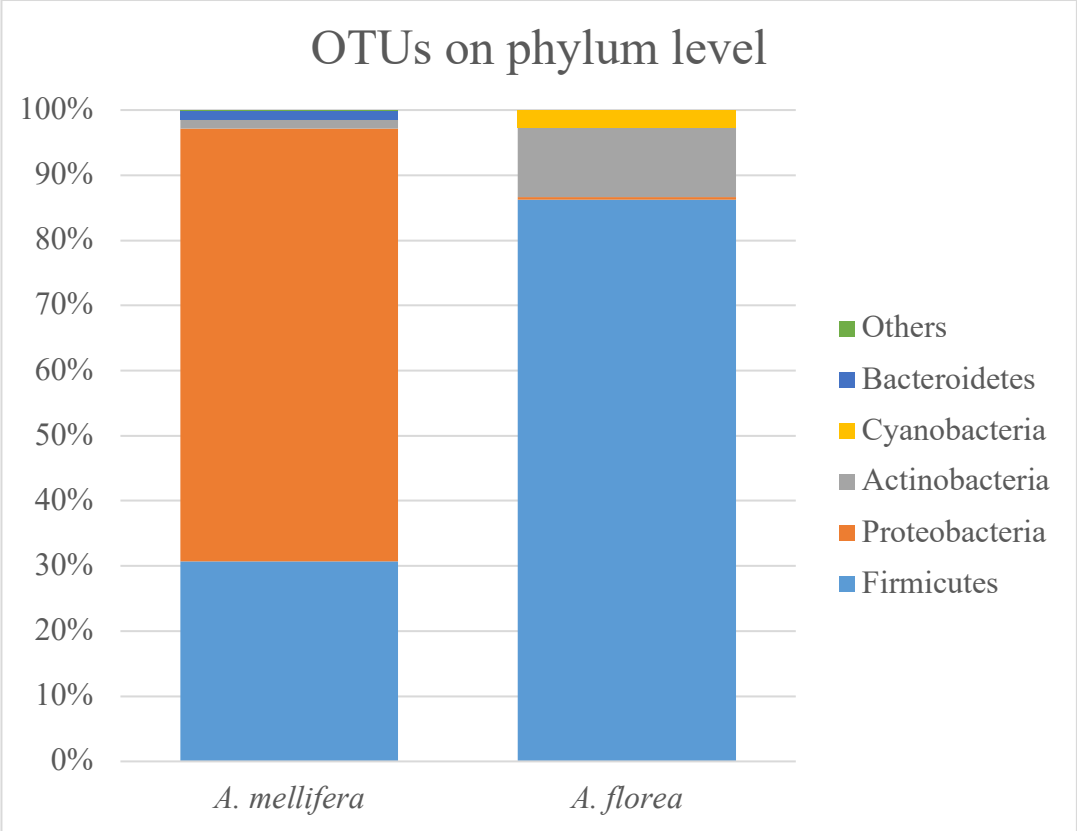

Figure 13: Taxonomy of the OTUs at the phylum level for *A. mellifera* and *A. florea* honeybees.

Seven bacteria classes were detected in both honeybee species. They include *Bacilli*, *Gammaproteobacteria*, *Betaproteobacteria*, *Actinobacteria*, *Chloroplast*, *Alphaproteobacteria*, and *Flavobacteriia*. The bacterial class *Bacilli* has the highest percentage in both *A. florea* and *A. mellifera* with 86.2% and 30.7% respectively. The least one in *A. mellifera* was *Actinobacteria*, with 1.3%, while the lower abundant bacteria class in *A. florea* was *Chloroplast*, with 2.7% (Figure 14).

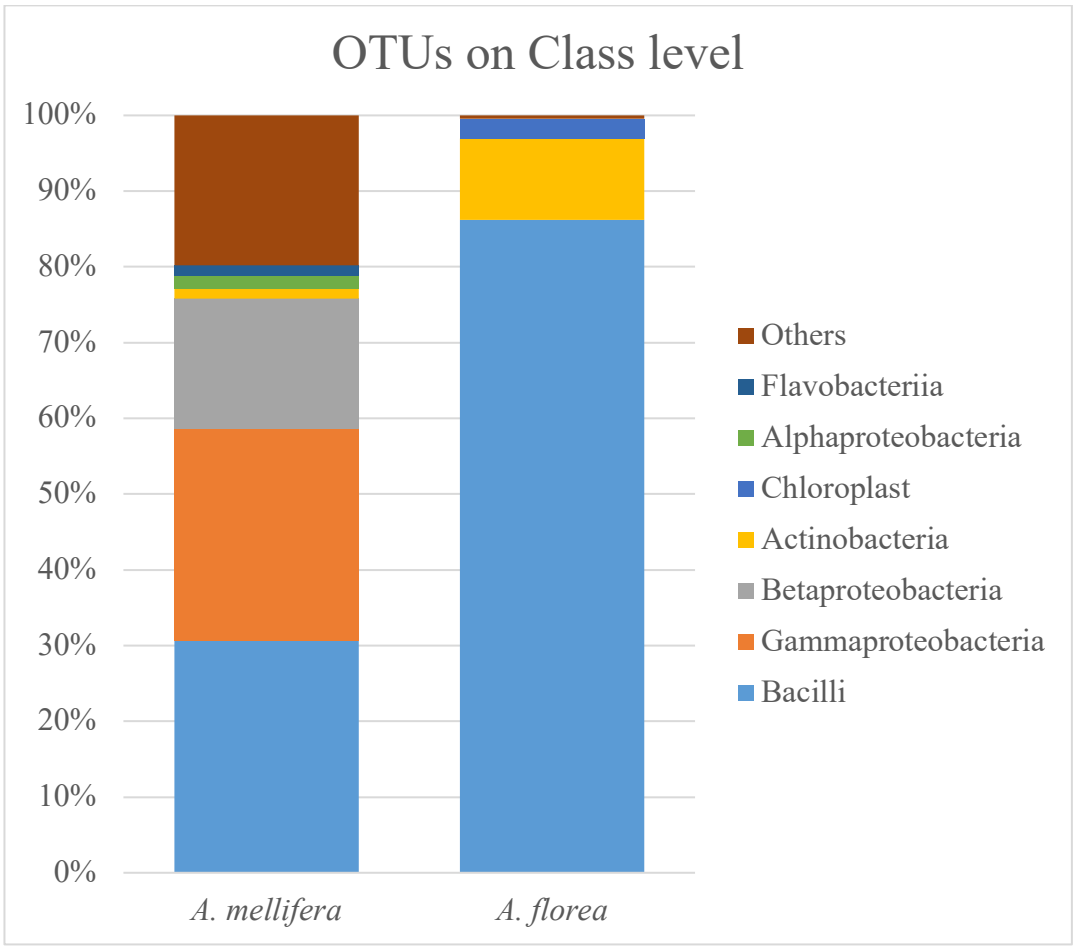

Figure 14: Taxonomy of the OTUs at the class level for *A. mellifera* and *A. florea* honeybees.

In terms of order, there are 12 bacterial orders that includes *Lactobacillales*, *Orbales*, *Neisseriales*, *Bifidobacteriales*, *Enterobacteriales*, *Rhodospirillales*, and *Flavobacteriales*. The top most abundant were *Lactobacillales* in both *A. mellifera* and *A. florea* honeybees with 86.2% and 30.7%, respectively. The least abundant at order level in *A. florea* is *Actinomycetales* with 0.1%, while *Bifidobacteriales* was the less abundant in *A. mellifera* with 1.3% (Figure 15).

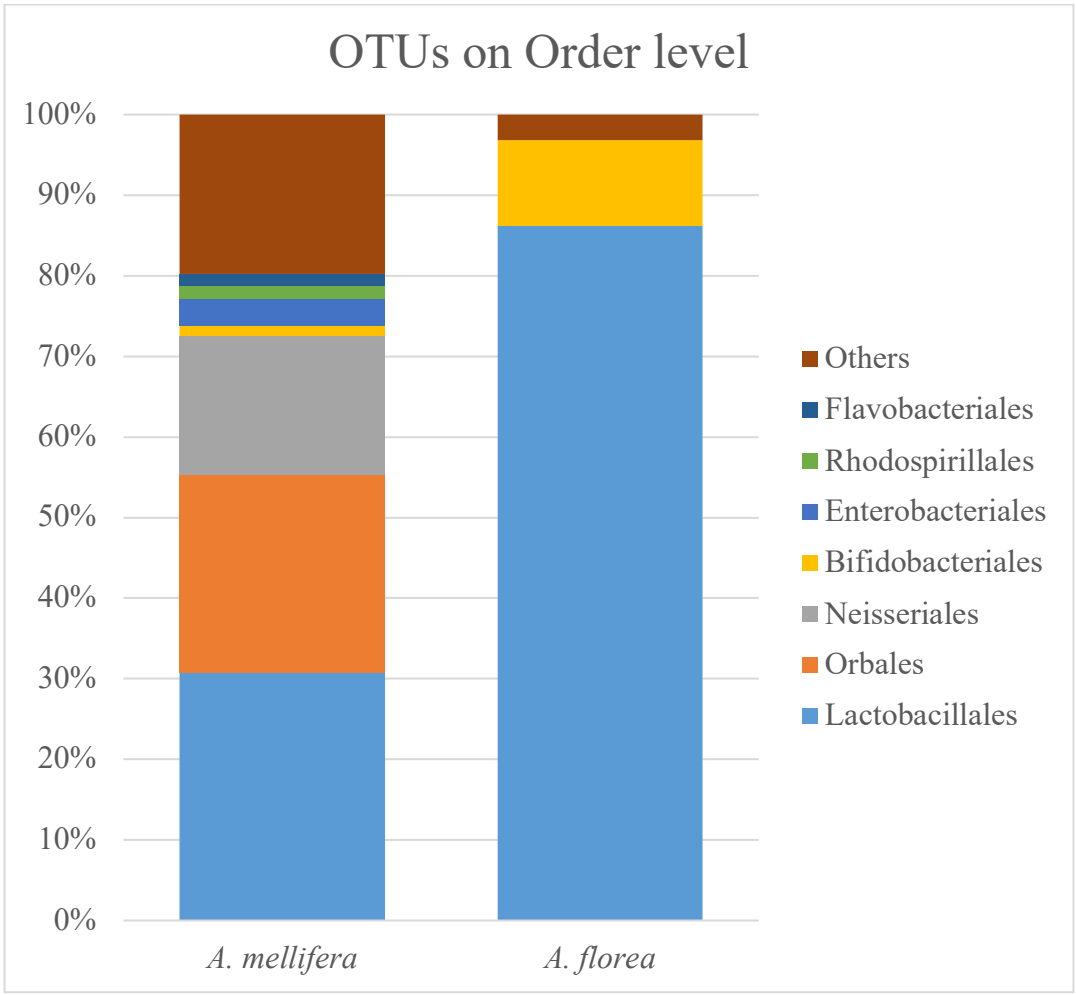

Figure 15: Taxonomy of the OTUs at the order level for *A. mellifera* and *A. florea* honeybees.

The taxonomy of the OTUs at the family level for *A. mellifera* and *A. florea* honeybees contained 14 families, it includes *Neisseriaceae, Bifidobacteriaceae, Acetobacteraceae, Flavobacteriaceae, Microbacteriaceae, Enterobacteriaceae, Orbaceae, Chloroplast, Lactobacillaceae*, and *Leuconostocaceae*. The highest percentage in both *A. mellifera* and *A. florea* honeybee species was *Lactobacillaceae* with 30.7% and 86%, respectively. Moreover, the smallest percentage in *A. florea* was *Microbacteriaceae* with 0.1%, while *Bifidobacteriaceae* was the smallest percentage in *A. mellifera* with 1.3% (Figure 16).

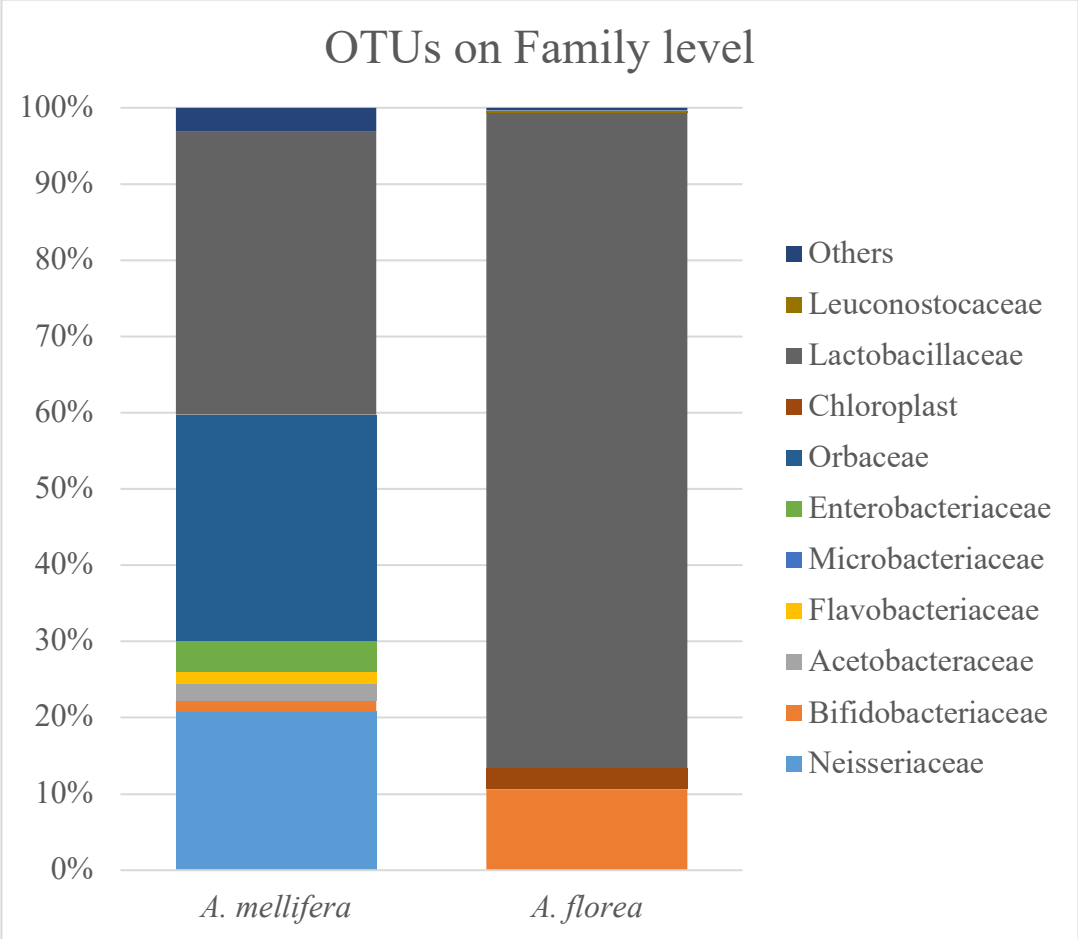

Figure 16: Taxonomy of the OTUs at the family level for *A. mellifera* and *A. florea* honeybees.

In the genus level, the taxonomic profiling shows 17 main bacterial genera. They include *Okibacterium*, *Bifidobacterium*, *Arsenophonus*, *Enterobacter*, *Frischella*, *Gilliamella*, *Streptophyta*, *Lactobacillus*, and *Fructobacillus*. The top most abundant one was *Lactobacillus* in both *A. florea* and *A. mellifera* honeybees, with 52.8% and 23.5%, respectively. The least abundant in *A. florea* honeybees was *Okibacterium* with 0.1%, whereas *Arsenophonus* with 0.1% in *A. mellifera* honeybees (Figure 17).

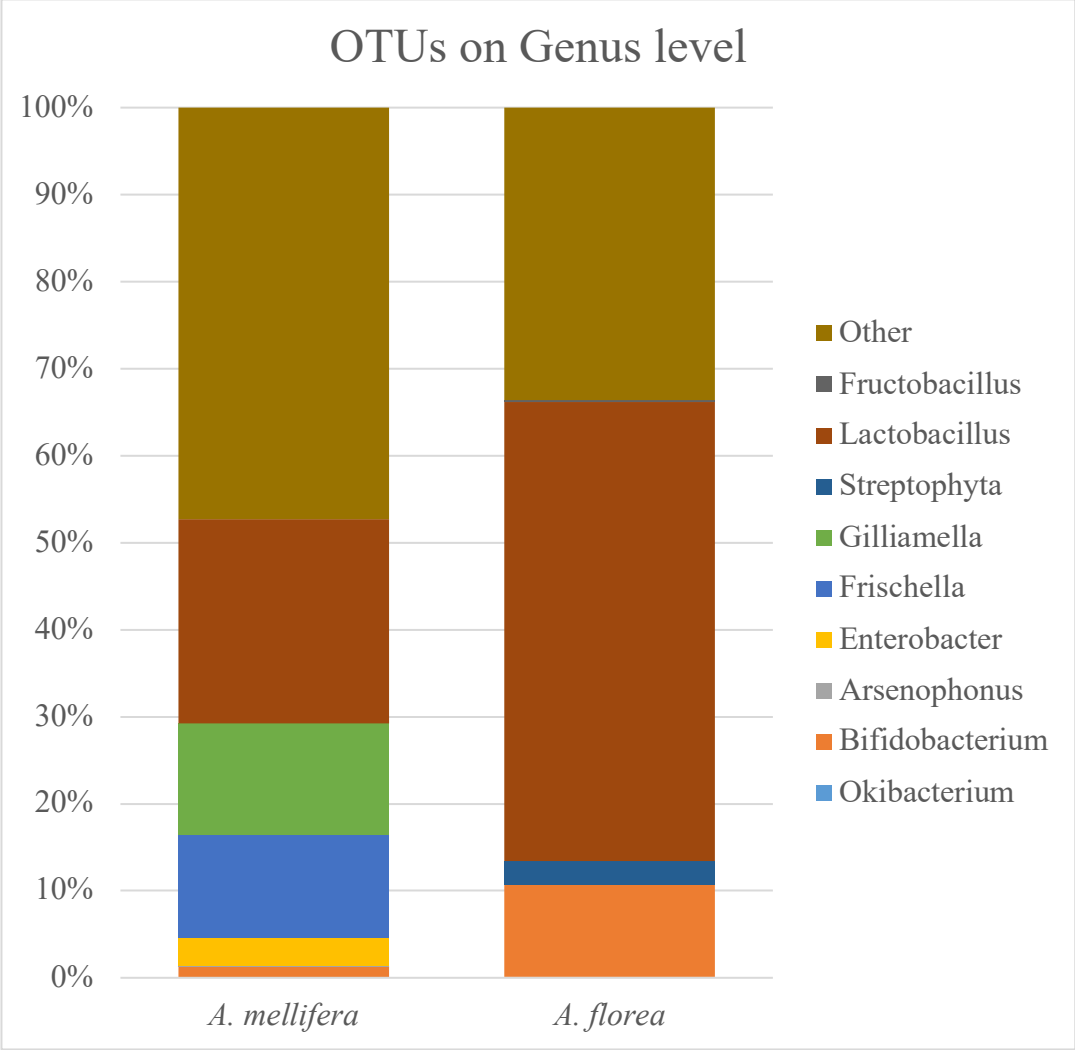

Figure 17: Taxonomy of the OTUs at the genus level for *A. mellifera* and *A. florea* honeybees.

The last level is the species level and 19 bacteria species were found. The only known one was *Fructobacillus fructosus* with 0.2% in *A. florea* and 0.0 % in *A. mellifera*. The remaining species were classified as uncultured and others (Figure 18).

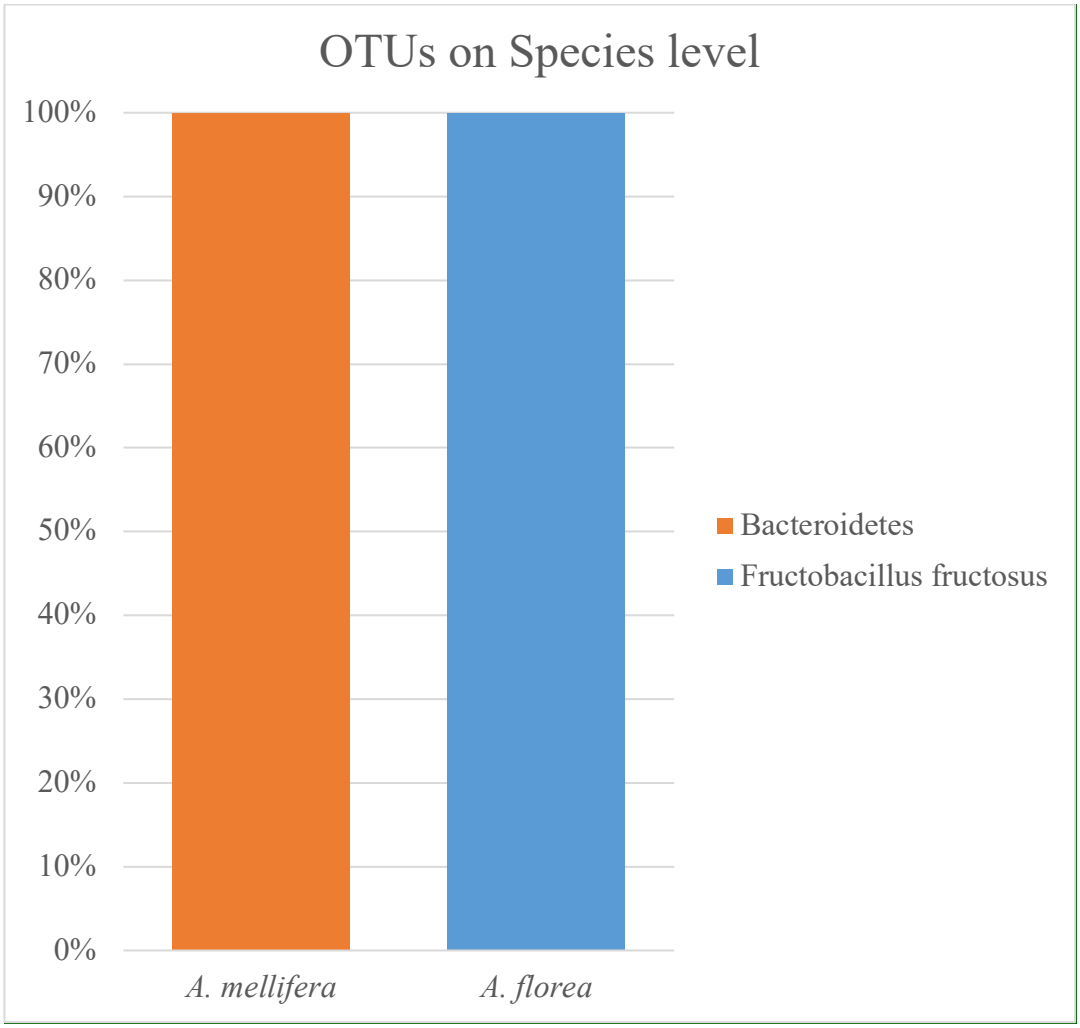

Figure 18: Taxonomy of the OTUs at the species level for *A. mellifera* and *A. florea* honeybees.

3.3 *Nosema ceranae* Pathogen

N. ceranae pathogen has been detected in *A. mellifera* honeybee samples from 2014 and 2015. 24 samples out of 108 were generated the diagnostic 218 bp PCR amplicon of *N. ceranae*, indicating the presence of the pathogen in the sampled colonies, with 22.22%. The samples were taken from different emirates, Al Ain, Abudhabi, Fujairah and Sharjah with 58%, 4%, 13%, 4%, 21% respectively. Figure 19, shows the band located in 218 bp amplicon.

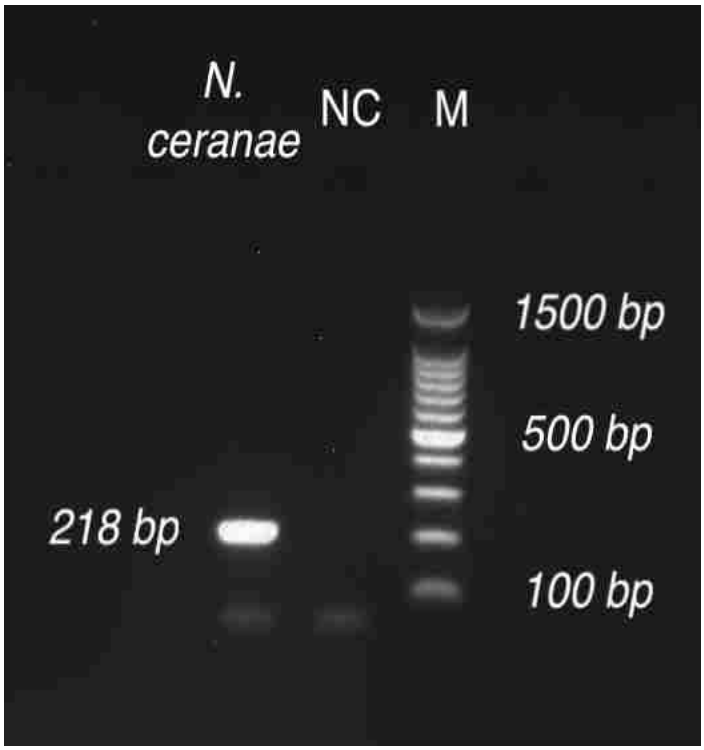

Figure 19: *N. ceranae* pathogen in adult honeybees.

Positive samples of the *N. ceranae* in *A. mellifera* honeybee were sequenced in Korea. By using NCBI BLAST database, the 10 best matches species were labeled in the table 10. The results confirmed our work, showing that the sequence belong to the

N. ceranae pathogen. The most similar strain was *Nosema ceranae* strain GD2 (Accession number: MF099642.1) with 100% similarities (Table 4).

Table 4: Molecular identification of *N. ceranae* isolated from *A. mellifera* honeybee in UAE based DNA, similarities between this pathogen and GenBank species using NCBI BLAST.

Best match species	Query Cover	E value	Identification	Accession number
Nosema ceranae strain GD2	100%	2.00E-80	100.00%	MF099642.1
Nosema ceranae strain BJ2	100%	2.00E-80	100.00%	MF099638.1
Nosema ceranae strain BJ1	100%	2.00E-80	100.00%	MF099637.1
Nosema ceranae strain N5E	100%	2.00E-80	100.00%	KC708005.1
Nosema ceranae strain N5D	100%	2.00E-80	100.00%	KC708004.1
Nosema ceranae strain CR1	100%	2.00E-80	100.00%	JQ639307.1
Nosema ceranae isolate MEX2 16S	100%	2.00E-80	100.00%	HM802210.1
Nosema ceranae strain GD1	99%	7.00E-80	100.00%	MF099641.1
Nosema ceranae strain BJ4	99%	7.00E-80	100.00%	MF099640.1
Nosema ceranae strain BJ3	99%	7.00E-80	100.00%	MF099639.1

3.4 *Nosema Apis* Pathogen

N. apis pathogen was not detected in any samples out of 108. The PCR was conducted using species-specific primers *N. apis-F and N. apis-R.* The expected band size was 321 bp amplicon (Figure 20).

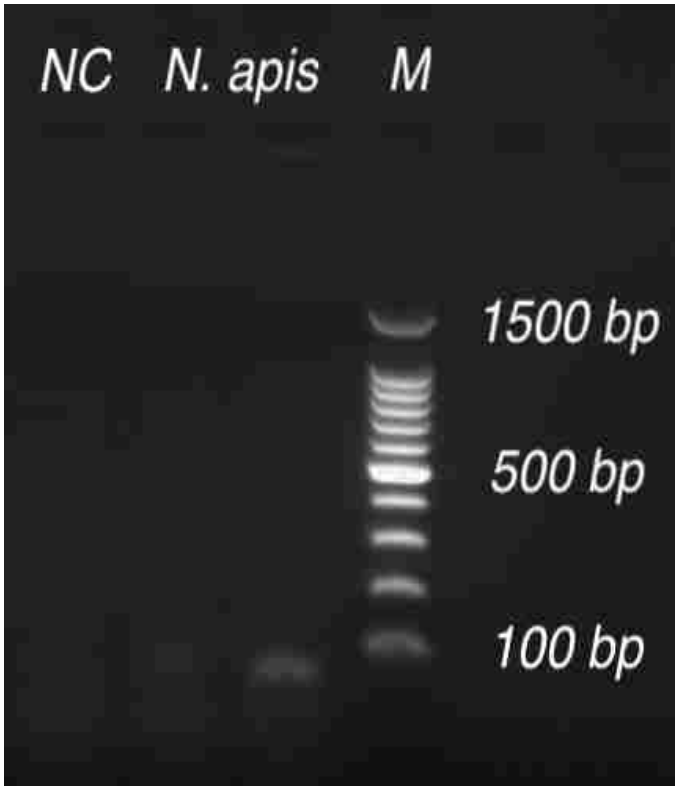

Figure 20: Agarose gel 1.5% showing no band for the *N. apis* pathogen.

3.5 Two Viruses in *A. mellifera* Honeybee

A multiplex RT-PCR was conducted to detect two viruses in *A. mellifera* honeybee samples 2014-2015. DWV and VDV-1 have been detected. As in Figure 21, the DWV band in 190 bp, while VDV-1 band in 1000 bp. DWV has been detected in 14 out of 103 samples with 14%, while *V. destructor* virus type 1 has been detected in 1 sample out of 103 samples. The honeybee samples of *A. mellifera* were taken from RasAlkhaima, Sharjah and Al Ain. For DWV, it was 14%, 7% and 79% respectively.

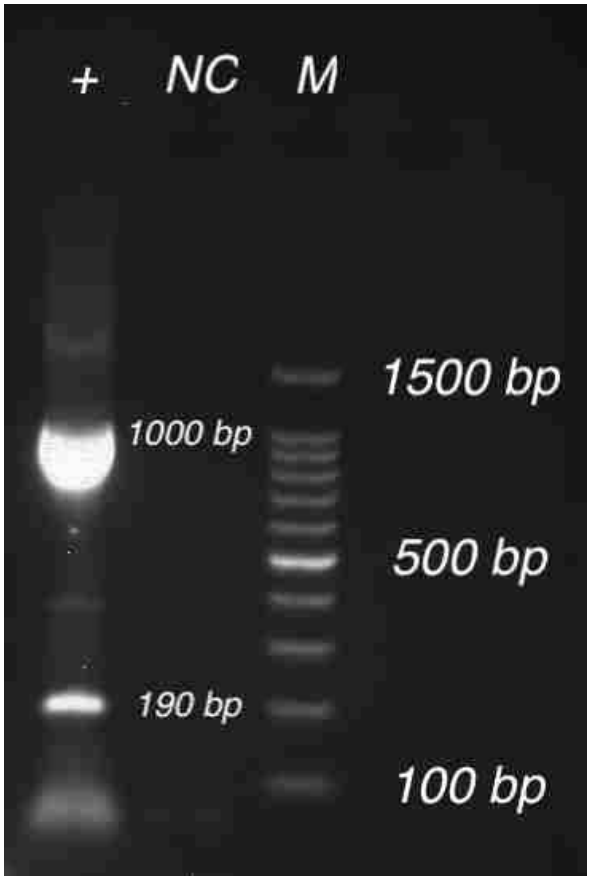

Figure 21: Deformed wings virus and *V. destructor* virus type 1 has been detected.

3.6 *V. destructor* Species Identification

PCR was conducted to identify *V. destructor* species, using LCO and HCO specific-primers. The band was detected on 929 bp amplicon (Figure 22).

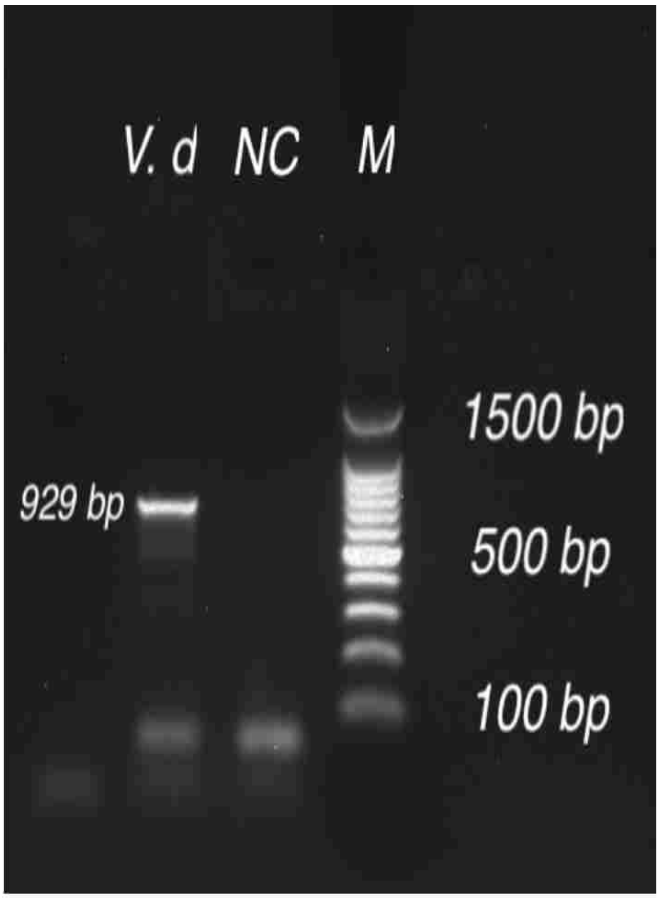

Figure 22: *Varroa* mite identification PCR for *V. destructor* species, the band located on 929 bp.

Positive samples of the *V. destructor* mite species were sequenced in Korea. The sequences of our samples have been searched against GenBank using BLAST database; the eight best matches species were labeled in the Table 5. The results of the sequence confirmed our work, showing that the sequence belongs to *V. destructor* mite species. The most similar isolate is *V. destructor* isolate A-1 (Accession number: KR528385.1) with 100% similarities.

Table 5: Molecular identification of *V. destructor* mite in UAE based DNA, similarities between our results and GenBank species using NCBI BLAST.

Best match species	Query Cover	E value	Identification	Accession number
V. destructor isolate A-1	100%	0	100.00%	KR528385.1
V. destructor isolate FII-1	100%	0	100.00%	KR528381.1
V. destructor isolate Peshter1-Suvi_Do	100%	0	100.00%	JX970939.1
V. destructor isolate Serbia1-Belgrade	100%	0	100.00%	JX970938.1
V. destructor isolate AmK1-4China-Xishuanbanna	100%	0	100.00%	GQ379060.1
V. destructor isolate AmK1-1Korea-Seoul	100%	0	100.00%	GQ379056.1
V. destructor mitochondrial partial cox1 gene	100%	0	99.84%	LN873226.1
V. destructor mitochondrial partial cox1 gene	100%	0	99.67%	LN873222.1
V. destructor isolate AcK1-3China-Hunan	100%	0	99.67%	GQ379059.1
V. destructor isolate AmJ1-1Taiwan-Taichung	100%	0	99.51%	GQ379069.1
V. destructor mitochondrial partial coi gene	100%	0	99.51%	AJ784872.1
V. destructor isolate EII-3	100%	0	99.35%	KR528383.1
V. destructor isolate CI-3	100%	0	99.19%	KR528379.1
V. destructor isolate AcV1-4Thailand-BangChangtay	100%	0	99.19%	GQ379064.1
V. destructor isolate Vd-CP10	97%	0	100.00%	KY865185.1
V. destructor isolate Vd-CP9	97%	0	100.00%	KY865184.1

Based on BLAST result, it shows high similarity with our sequence, with 100% namely *V. destructor* isolate A-1. Thus, the Neighbor-Joining homology tree revealed *V. destructor* mite in the 86 group (Figure 23).

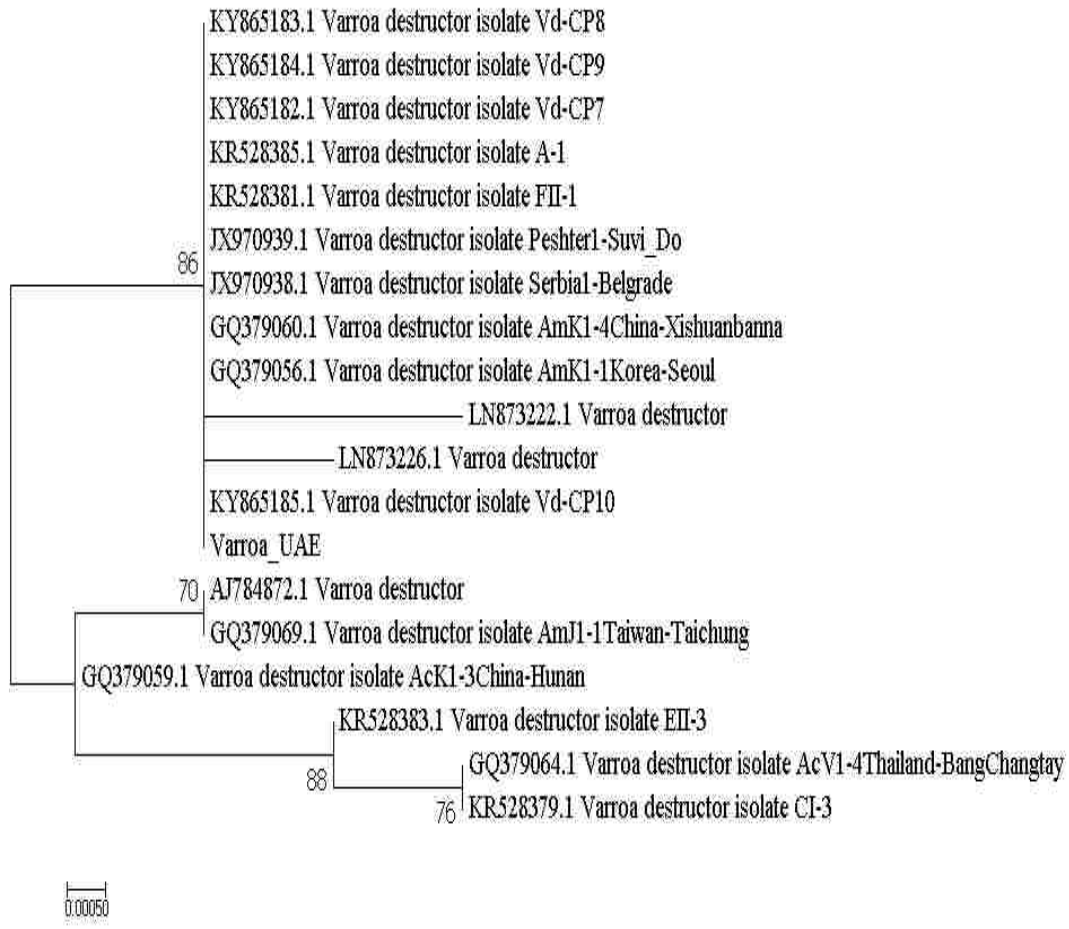

Figure 23: Neighbor-Joining homology tree of the *V. destructor* mite compared with other sequences of the GenBank. 16 sequences with the highest similarity values after a BLAST search were used for phylogenetic analysis. Bootstrap values (1000 replications) are indicated at each node.

3.7 Two Viruses in *V. destructor* Mite

The two viruses were detected in *Varroa* mite samples 2014-2015. As in Figures 24 and 25, the DWV band located in 190 bp, while VDV-1 band located in 1000 bp.

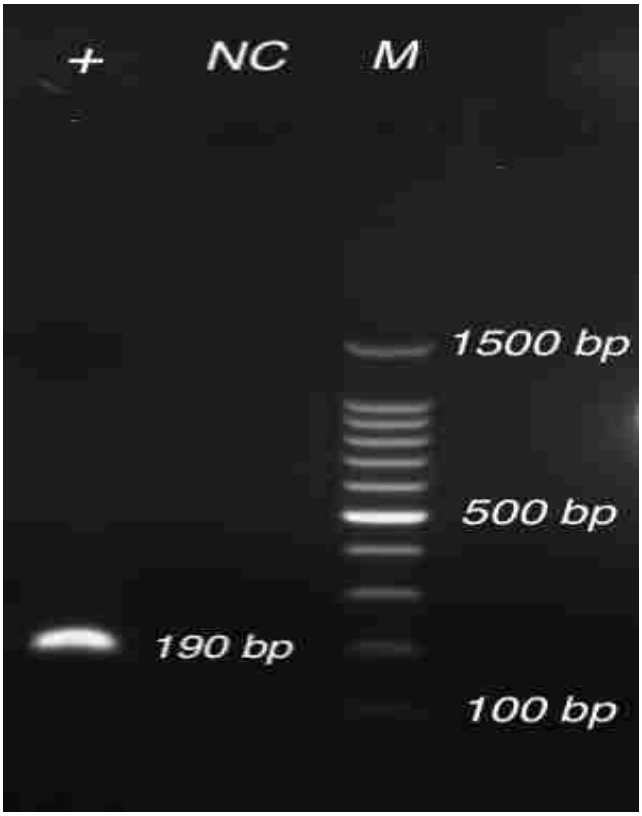

Figure 24: Deformed wings virus (DWV) in *Varroa* mite

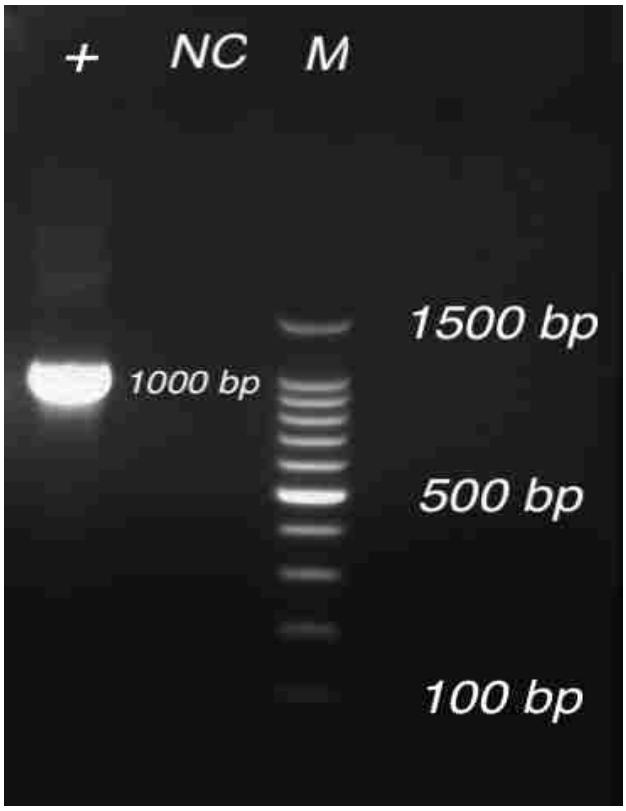

Figure 25: *V. destructor* virus-1 (VDV-1) in *Varroa* mite

Positive samples of the DWV in *V. destructor* mite were sequenced in Korea. Using NCBI BLAST database, the best 10 matches species were labeled in the Table 6. The results have supported our work, showing that the sequence belong to DWV virus. The most similar isolate is Deformed wing virus isolate JO15 (Accession number: KT591942.1) with 96.73% similarities.

VDV-1 in *V. destructor* mite positive samples were sent to Korea for DNA sequencing. By using NCBI BLAST database, the 8 best matches species were labeled in Table 7. The results have supported our work, showing that the sequence belong to VDV-1 virus. The most similar strain is Varroa destructor virus 1 (Accession number: AY251269.2) with 97.94% similarities.

Table 6: Molecular identification of DWV isolated from V. destructor mite in UAE based DNA, similarities between this virus and GenBank species using NCBI BLAST.

Best match species	Query Cover	E value	Identification	Accession number
Deformed wing virus isolate JO15	100%	8.00E-64	96.73%	KT591942.1
Deformed wing virus isolate Sy-Lat6	100%	2.00E-60	95.42%	KX530467.1
Deformed wing virus isolate DZ3	100%	2.00E-60	95.42%	KT591931.1
Deformed wing virus isolate DZnm24	100%	2.00E-60	95.42%	KT591927.1
Deformed wing virus isolate DZnm40	100%	2.00E-60	95.42%	KT591903.1
Deformed wing virus isolate DZnm39	100%	2.00E-60	95.42%	KT591902.1
Deformed wing virus isolate DZne34	100%	2.00E-60	95.42%	KT591897.1
Deformed wing virus isolate JOn30	100%	2.00E-60	95.42%	KT591895.1
Deformed wing virus isolate JOn29	100%	2.00E-60	95.42%	KT591894.1
Deformed wing virus isolate EGm25	100%	2.00E-60	95.42%	KT591890.1

Table 7: Molecular identification of VDV-1 isolated from *V. destructor* mite in UAE based DNA, similarities between this virus and GenBank species using NCBI BLAST.

Best match species	Query cover	E value	Identification	Accession number
Deformed wing virus clone	97%	0	98.49%	HM162359.1
Synthetic construct clone DWVinfVVD9	97%	0	98.27%	KT215905.1
Deformed wing virus isolate VDV-1-DWV-No-9	97%	0	98.27%	HM067438.1
Varroa destructor virus 1	97%	0	97.94%	AY251269.2
Deformed wing virus	97%	0	96.87%	KX783225.1
Deformed wing virus clone	97%	0	90.75%	HM162357.1
Deformed wing virus	97%	0	90.75%	HM067437.1
Synthetic construct clone	97%	0	90.65%	KT215904.1

Dorsal and Ventral view of *V. destructor* mite are shown in Figure 26, 27.

Figure 26: *V. destructor* mite, dorsal view (Photo credit: Mohammad Ali Al-Deeb)

Figure 27: *V. destructor* mite, ventral view (Photo credit: Mohammad Ali Al-Deeb)

Chapter 4: Discussion

4.1 Bacterial Communities Associated with *A. mellifera* and *A. florea* in the UAE

The study of microbial community is very important as it has an effect on the immune system of the honeybee and has a role in participating in the metabolic processes. The digestive system is affected by the number and the type of the microbial community in the gut (Quigley, 2013).

In this study, a comparison between *A. mellifera* and *A. florea* microbial communities was conducted. Honeybees microbial community has not been studied before in United Arab Emirates, so this is the first study in the UAE and in the region. The diversity of the bacteria in honeybee samples indicated that the identified OTUs were classified into 5 phyla, 7 classes, 12 orders, 14 families, 17 genera and 19 species.

Lactobacillaceae family was detected as the highest percentage in both *A. florea* and *A. mellifera* honeybee species with 86% and 30.7%, respectively. Acosta et al. (2015) found the similar results as the *Lactobacillaceae* family percentage was the highest in *A. mellifera* honeybees. In addition, they found that *Lactobacillaceae* was one of the main families that was responsible for the specificity of the beebread, and that's including other families of bacteria like *Neisseriaceae*, *Flavobacteriaceae* and *Acetobacteraceae*. They also found it has the highest percentage among beebread. Moreover, this family is involved in fermentative processes or in the controlling the changes in the pH of the beebread.

In this study, *Lactobacillus* was the dominant genus in both *A. florea* and *A.*

mellifera honeybees, with 52.8% and 23.5%, respectively. *Lactobacillus*, is considered one of the honeybee symbiotic bacteria (Hubert et al., 2016). In addition, *Lactobacillus* is one of the anaerobic bacteria (Kwong and Moran, 2016), and has a role in sugar utilization and the breakdown of carbohydrates (Moran, 2015). Engel and Moran (2013) found that *Lactobacillus* was one of the dominant bacteria in *A. mellifera* honeybee as in the current study. It is present in the worker bee gut in particular (Yun et. al, 2018). Similarly, in *A. cerana* honeybee species, *Lactobacillus* was one of the dominant bacteria at genus level, in addition to *Snodgrassella*, and *Gilliamella* (Huang et al., 2018).

Generally, there were a difference between *A. mellifera* and *A. florea* microbial communities. Thus, because of the different location that the samples have taken. All *A. florea* honeybee samples were from Al Ain, while *A. mellifera* honeybee samples were from Al Ain, Abu Dhabi, Fujairah and RasAlkhaima. A study conducted on *A. cerana* workers, shows that feeding has a significant effect on the differential of the microbial community in the bee (Huang et al., 2018)

4.2 Molecular Detection of Honeybee Pathogens in the UAE

The work is reported in this thesis, represents the first work to study molecular detection of *N. ceranae* in the United Arab Emirates. The parasite *N. ceranae* has been detected in nearby countries, like Saudi Arabia (Abdel-Baki et al., 2016).

4.2.1 *N. ceranae*

The prevalence of *N. ceranae* is highly presented in *A. mellifera* honeybees (Jack et al., 2016). *N. ceranae* is one of the microsporidian parasites, that release it spores in the host (Fries, 2010). It has a pathogenic effect on the honeybees, resulting in the reducing of the workers lifespan, and this decreases the colony lifespan as well, as the main job of the workers is to collect pollen and nectar, which means that the main food supply for the honeybee colony will be decreased, resulting in colony collapse (Bernklau et al., 2019). The transmission of the pathogen occurs by the ingestion of *N. ceranae* spores through the contaminated food or water. In addition, the spores are transmitted through the process of exchanging food between honeybees (Martín-Hernández et al., 2018).

In this study, the pathogen has been detected in *A. mellifera* honeybee samples from 2014 and 2015. The pathogen has been detected in 24 samples out of 108, with 22.22%. This is considered a relatively high prevalence compared to our small sample size.

In the past few years, the pathogen has spread and increased in number all over the world causing a dramatic loss in *A. mellifera* honeybee colonies. In Poland, the pathogen has risen from 2001 until 2008, resulting in a huge *A. mellifera* honeybee colony loses (Ptaszyńska et al., 2012). This indicated the ability of this parasite to multiply and reproduce in massive amounts. The same situation occurred in Chile, *N. ceranae* had widely infected *A. mellifera* honeybees (Arismendi et al., 2017). Similarly, the pathogen has reached Texas honeybee's in the United State Of America (Chapman et al., 2017). Latvia, a country in European Union, has indicate a wide

spread of the pest distribution which occurred in all the regions of the country (Reimane et al., 2017). In Bulgaria, they found that the prevalence of *N. ceranae* exceed *N. apis*. They used the same primers and similar PCR condition (Shumkova et al., 2018).

The similarity is 99.46% between our results and the sequenced analysis, this result is supporting our work and guarantee that *N. ceranae* has been detected in our samples.

4.2.2 *N. apis*

N. apis pathogen has not been detected in this study. Compared to *N. apis*, the prevalence of *N. ceranae* exceeds the expansion of *N. apis* worldwide. The transmission of both *N. ceranae* and *N. apis* are through the spores, so the reason could be the spore's multiplication of *N. ceranae* is higher than *N. apis* (Sinpoo et al., 2018). In addition, *N. ceranae* is showing better reproduction process in warmer areas than *N. apis* (Van der Zee et al., 2014), as the weather of United Arab Emirates is warm most of the year (Beeatna, 2019), making it a better environment for *N. ceranae* than *N. apis*.

N. apis seems to be less common infecting *A. mellifera* honeybees in the whole world similarly to our results. In Canada for example, *N. ceranae* was more widespread than *N. apis* (Emsen et al., 2016). In Chile, there was no detections of *N. apis* at all (Arismendi et al., 2017).

4.2.3 Viruses

Two viruses have been detected in this study, *V. destructor* virus type 1 and deformed wings virus. Samples of *A. mellifera* honeybees from 2014-2015 have been used. DWV has been detected in 14 out of 103 samples, while *V. destructor* virus type 1 has been detected in 1 sample out of 103 samples. Our study is the first study to detect *Varroa* mite viruses in the UAE. The DWV seems to be more common and widespread than VDV-1.

4.2.4 DWV

DWV is one of the destructive viruses of *A. mellifera* honeybee. It affects the health and the survival of the honeybee colony (Levin et al., 2019). The existence of DWV is associated with the *V. destructor* mite, although the virus can show with the absence of *V. destructor* mite (Roberts et al., 2017). Moreover, it was detected in Sweden in mite resistant colony (Thaduri et al., 2018). This raises the importance of studying the existence of DWV in both honeybee colonies and in the *Varroa* mite as it will be discussed below.

This virus is widespread worldwide. DWV has been detected in *A. mellifera* honeybee in the Middle East and North Africa (Haddad et al., 2015). In Serbia, DWV was the most prevalent virus among the other ones, and that was due to the association of this virus with *V. destructor* mite (Cirkovic et al., 2018). The same situation was in Iran, DWV considered the most prevalent among all other viruses in all different Iranian provinces (Nabian et al., 2017). Most of the studies showed that DWV is the

most prevalence virus attacking *A. mellifera* honeybee, including Argentina (Giacobino et al., 2016).

Compared to our work to similar studies, (Cirkovic et al., 2018) used the same primers and similar PCR conditions, resulting in the detection of DWV virus in *A. mellifera* honeybee. Similar study in Syria using RT-PCR, detected DWV in *A. mellifera* honeybee as the major virus among the others (Elbeaino et al., 2016).

A recommendation which could be suggested to eliminate the virus by studying the toxic saliva protein of *Varroa* mites that lead to risen the DWV load in the adults of *A. mellifera* honeybees (Zhang and Han, 2018). This could help to produce a biopesticides that does not affect both honeybee and the honey.

4.2.5 *V. destructor* Virus Type 1

Another virus that destroy honeybee colony is VDV-1, which is a very closely related to DWV (Thomasson et al., 2017). VDV-1 is considered the most prevalent virus in *A. mellifera* honeybee along with DWV (Gauthier et al., 2011). In the USA, VDV-1 is widespread causing a higher pathogenicity in *A. mellifera* honeybee than DWV.

In this study, we found a very low percentage of our samples infected by VDV-1, with only 1%. Our study was in agreement with other similar studies that investigated both DWV and VDV-1 in USA, in which they found similar results, VDV-1 was less common than DWV (Ryabov et al., 2017). Another study shows that both the incidence and the titer of VDV-1 is less than DWV (Cornman, 2017). In general, few studies investigated VDV-1 than DWV, and this shows the negative influence of DWV in *A. mellifera* honeybee than VDV-1.

The presence of both of the DWV and VDV-1 makes it hard to produce a treatment, as viral evolution considered a dilemma to develop a treatment to control viral infections (Shreeve et al., 2013). More studies are suggested to find a treatment for the recently widespread virus.

4.3 *Varroa* mites and *Varroa* Associated Pathogens in the UAE

4.3.1 *V. destructor* Species Identification

V. destructor is one of the most destructive ectoparasite of *A. mellifera* honeybee and could lead to colony collapse if no good management has been taken in concern (Gracia et al., 2017). The mite feeds on the hemolymph of the honeybee, causing a huge losses of the bees in the colony (Kraberger et al., 2018).

V. destructor species has been identified in this thesis research by conducting a PCR on *Varroa* mite's samples from Al Ain (2014-2015) using LCO and HCO primers. Identifying the sequence of *V. destructor* genome can help us to compare the analysis with a model organisms (Street et al., 2009).

The pest is widely expanded and infected most of *A. mellifera* honeybee worldwide with the exception of Australia (Roberts et al., 2017). In Texas, United State Of America, *V. destructor* mites infected 76% of the colonies that been investigated by (Chapman et al., 2017).

The results of DNA analysis support our work, as the sequence shows that it belongs to *V. destructor* isolate A-1 with 100% similarities. Similar study has detected *V. destructor* isolate Serbia1-Belgrade cytochrome oxidase subunit I (cox1)

(Accession number: JX970938.1), which has 99.53% similarities with *V. destructor* species. Additionally, this strain has been first discovered in Serbia (Gajić et al., 2016).

A good recommendation is using a mite resistance colony (Guzman et al., 2019). Another suggestion is to use a biological control agent, *Bacillus thuringiensis* bacteria for example. It's widely available in different habitat and has proof it successfulness in eliminating *V. destructor* mite without affecting *A. mellifera* adults and larva bees (Alquisira-Ramírez et al., 2017).

4.3.2 Two Viruses in *Varroa* Mite

Two viral diseases have been detected in this research, *V. destructor* virus type 1 and deformed wings virus. Samples of *Varroa* mite from 2014-2015 has been used.

The existence of DWV is associated with *V. destructor* mite, although the virus shows with the absence of *V. destructor* mite (Roberts et al., 2017). The interaction between these two leads to the lowering the life span of *A. mellifera* honeybee, and cause colony collapse worldwide, and that's occur by imposing stress on the immune system of the honeybee (Zhao et al., 2019). This, show the importance of studying this virus in *V. destructor* mite. Most of the studies investigate the viruses in *A. mellifera* honeybee, while few are focusing in the viruses in the *V. destructor* itself (Levin et al., 2016).

4.3.2.1 DWV

DWV is one of the most affecting viruses to the *A. mellifera* honeybee colonies and it has a major role in the survival of this species (Levin et al., 2019). The synergistic interaction between DWV and *V. destructor* mite lead to decrease the

lifespan of *A. mellifera* honeybee, thus raise the importance of studying this objective (Zhao et al., 2019). Studies have been shown that the existence of *V. destructor* mite in the colony increase DWV titers (Dolezal et al., 2016). Similar study has detect DWV in the *V. destructor* mite in Czechia (Erban et al., 2015).

In this study, DWV was detected in *V. destructor* mites. A pool of five samples has been used to conduct a PCR and detect the virus. Similar study has detect the virus in 100% of the samples in Mexico, thus showing the high prevalence of the virus in the mites (Anguiano-Baez et al., 2016). The level of DWV virus shows a significant increase after exposing the honeybees to *V. destructor* mite, and the virulence of the virus has a positive correlation with the *V. destructor* level in the colony (Emsen et al., 2016). Moreover, this shows the link between *V. destructor* mite and DWV, as the viral load increase with the existence of this mite.

A good suggestion is to use propolis (antimicrobial plant resins), as its proven to reduce DWV in infected colonies by just applying the propolis in the colony (Drescher at el., 2017).

The sequenced DNA analysis shows that the band we detect in our work belongs to Deformed wing virus isolate JO15 (Accession number: KT591942.1) with 96. 73% similarities. As a result, this confirm that our work is accurate, and the band belongs to DWV.

4.3.2.2 VDV-1

VDV-1 is a virus that infect *A. mellifera* honeybees, which is very close to DWV (Levin et al., 2016).

To exclude any PCR-biased amplification in our results and to help us studying virus diversity. After sequencing, the most similar species was *V. destructor* virus 1 with 97.94% similarities. The result supports our work and assured us that VDV-1 has been detected in our samples. Similar study has been detect *V. destructor* virus 1 (GenBank accession number: AY251269.2) and its been identified as DWVB, as there are two variants that belong to DWV, which are DWVA and DWVB (Bradford et al., 2017). Same species has been detected by (Ryabov et al., 2014) in *A. mellifera* honeybee.

Similar study has detected VDV-1_{VVD} (GenBank accession number: HM067438.1) and VDV-1_{DVD} (GenBank accession number: HM067437.1), which both are a recombinant genomes between VDV-1 and DWV (Moore et al., 2011). Similar to Moore, they detect both VDV-1_{VVD} and VDV-1_{DVD} (Dalmon et al., 2017). As all studies show the recombination between DWV and VDV-1.

Chapter 5: Conclusion

In conclusion, *A. mellifera* honeybee has an important role in the environment. Honeybees are valuable insects due to the significant value of honeybee's product, like honey and wax and their major role as pollinators. The honeybee colonies are decreasing rabidly worldwide due to several factors. In this thesis, we investigated the existence of certain pathogens and parasite in the UAE *A. mellifera* honeybees and the harmful mite *V. destructor*. *Nosema ceranae* and two viral diseases were detected. *N. ceranae* was detected in *A. mellifera* honeybees while *N. apis* was not found. Both DWV and VDV-1 viruses were detected in both *A. mellifera* honeybee and *V. destructor* mite. The identity of the mites was confirmed by DNA sequencing. Moreover, the microbial community of both *A. mellifera* and *A. florea* were analyzed and compared. Further studies are needed to investigate the possibilities of introducing pathogens and parasites through the imported honeybees from different countries, e.g. Egypt. Applying effective quarantine measures would be useful too.

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