CHAPTER 3.2.7.

VARROOSIS OF HONEY BEES (INFESTATION OF HONEY BEES WITH *VARROA* SPP.)

SUMMARY

The mite, Varroa destructor (*formerly identified as* Varroa jacobsoni), *is a parasite of honey bees. It feeds on the preimaginal host stages within the sealed brood cells and penetrates the intersegmental skin between the abdominal sclera of adult bees to ingest haemolymph and fat body tissues. While feeding, V.* destructor *transmits viruses – deformed wing virus, acute bee paralysis virus, Israeli acute paralysis virus and Kashmir bee virus, among others. Without treatment of the honey bee colony, the number of parasites steadily increases with the growth of the bee population and its increasing brood activity leading to the collapse of the colony within 1–4 years. The clinical signs of infestation that mainly occur late in the season, are an effect of virus infections rather than the effect of direct parasitism by the mite itself. The life span of the mite depends on temperature and humidity but, in practice, it can survive from some days to a few months.*

Detection of the agent: *There are three methods to collect* V. destructor *mites and quantify colony infestation levels: the examination of colony debris, of adult honey bees or of brood cells. Adult mites can be collected in the debris, which is all material originating from honey bee bodies or bee honeycombs that falls to the bottom of the colony where a removable board may be installed. Examination of the debris-can be undertaken after the application of a medication that kills the mites directly or that forces them to drop off the bees so that colony infestation can be measured, or it can be done without medication to quantify the natural mite mortality. Adult bees can be examined to detect and quantify the presence of mites, which provides information about the dispersal phase mite population. The examination of brood provides information about the reproductive mite population.* Varroa destructor *prefers drone to worker brood, therefore the infestation in drone brood might be higher compared with worker brood.*

Serological tests: *No serological tests are applicable.*

Requirements for vaccines: *No biological products are available.*

A. INTRODUCTION

Varroa mites are parasites of brood and adult honey bees (species of the genus *Apis*), originally infesting native Asian honey bees closely related to *Apis cerana* (Dietemann *et al*., 2013). Four obligate ectoparasitic species have been described: *Varroa jacobsoni, V. underwoodi, V. rindereri* and *V. destructor* (Figure 1). Two haplotypes of the species *V. destructor*, the Korean and the Japanese/Thailand haplotype, parasitise *Apis mellifera* (Figure 2; Dietemann *et al*., 2013). The Korean haplotype has spread worldwide, while the distribution of the Japanese/Thailand haplotype is more restricted, being only reported in Japan, Thailand and the Americas (Anderson & Trueman, 2000). In 2008, the species *V. jacobsoni* was also found for the first time parasitising *A. mellifera* in the Pacific island country of Papua New Guinea, where it has been extensively damaging honey bee colonies (Roberts *et al*., 2015). The recent host shift presents a serious threat to world apiculture. It should be mentioned that until 2000 *Varroa* mites that affect *A. mellifera* were erroneously assumed to be *V. jacobsoni* (Anderson & Trueman, 2000).

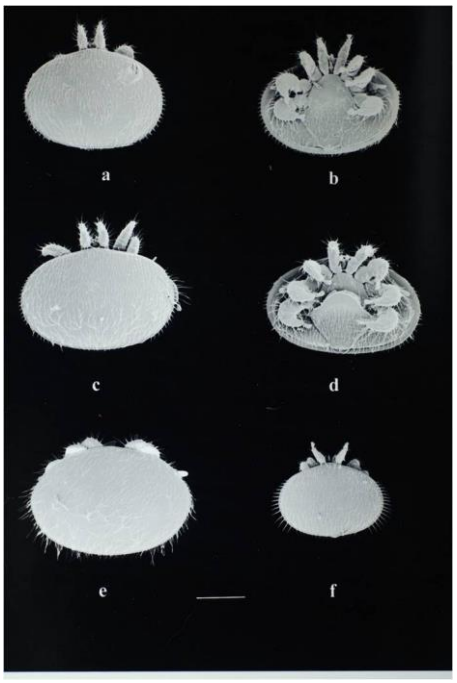

***Fig. 1**. The four species of* Varroa*:* V. jacobsoni (*dorsal view: a, ventral view: b);* V. destructor (*dorsal view: c, ventral view: d);* V. rindereri (*e*)*;* V. underwoodi (*f*).
Photograph by Denis Anderson (*Anderson & Trueman, 2000; Dietemann* et al.*, 2013).*

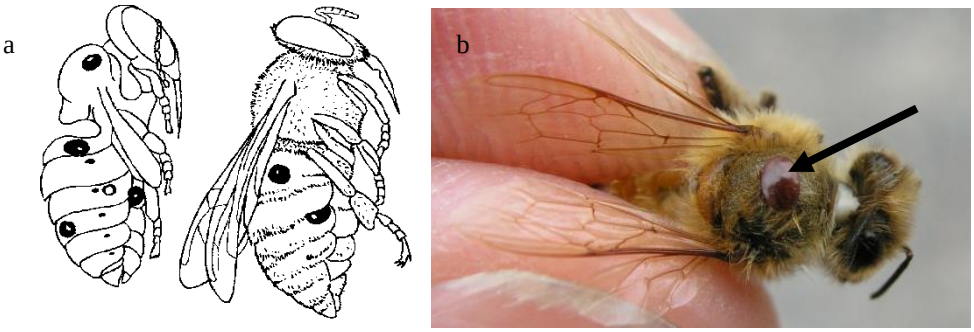

***Fig. 2** a)* Varroa *on pupa and adult bee. Left: pupa with four* Varroa *female mites. Right: worker bee with two female mites.*
b) Varroa (arrow) *on* A. mellifera*. Photograph by Dr Rob Manning.*

Varroa destructor has spread outside its native range since the 1960s, colonising other areas for the first time after it successfully shifted from the original host, *A. cerana,* to the Western honey bee, *A. mellifera* (Rosenkranz *et al*., 2010), to which it is now highly adapted. Decades later, *V. destructor* is present in most countries exploiting *A. mellifera* and only very few mite-resistant honey bee populations exist worldwide (Locke, 2016). Varroosis, also called varroatosis, is currently considered the largest threat to apiculture worldwide. Traditionally this disease has been defined as the infestation of honey bees with *Varroa* spp. However, due to the growing knowledge of viruses vectored by *V. destructor* and their confirmed important role in *Varroa*-induced colony collapse, this definition is no longer representative of the full process of the disease in *A. mellifera* (Genersch *et al*., 2010; Rosenkranz *et al*., 2010). An accurate and globally agreed definition of varroosis in *A. mellifera* is in progress among the scientific community, with efforts put into clarifying the precise role of *V. destructor* itself and the role of the different viruses carried by the mite, and thereby contextualising their relative importance in the complex of clinical signs observed. Until then, for the purposes of this *Terrestrial Manual* chapter, varroosis is linked to the detection of *Varroa* spp., regardless of the occurrence of clinical signs (see Section B. *Diagnostic techniques*).

There are more than 20 known viruses identified in honey bees, and it has been proven that *V. destructor* can act as a vector for deformed wing virus (DWV), acute bee paralysis virus (ABPV), Kashmir bee virus (KBV) and Israeli acute paralysis virus (IAPV), among others (Yañez *et al.,* 2020). Most of these honey bee viruses are positive-strand RNA viruses belonging to the Order Picornavirales (picorna-like viruses). Within this Order, DWV belongs to the Family *Iflaviridae,* and ABPV, KBV and IAPV to the Family *Dicistroviridae* (McMahon *et al*., 2018). Among all the viruses mentioned, currently the DWV is most closely associated with *Varroa* infestation (McMenamin & Genersch, 2015): it is well adapted to the life cycle of the mite (Di Prisco *et al*., 2016) and the only case where the role of *V. destructor* as a biological vector has been proven, with effective replication of the virus in mite tissues

(Yue & Genersch, 2005). Before the occurrence of *V. destructor*, honey bee viruses had been considered a minor problem to honey bee health, mainly present as subclinical infections, but since the dispersion of the mite they have been involved in large colony losses worldwide, exhibiting a markedly increased virulence (McMenamin & Genersch, 2015; Meixner *et al*., 2014). This is not surprising considering that the direct injection of the virus through mite bites is far more efficient than any of the other transmission routes, requiring fewer viral particles to establish infection, and also generating higher virus titres in affected honey bees (Brettell *et al*., 2017). At the same time, the level of infestation of *V. destructor* that causes colony damage appears to have decreased over time (fewer mites cause the same level of damage at the colony level than in the past). Another factor that points to the synergistic action of both pathogens is that the mite can trigger the replication of latent viruses already present in the honey bees, acting as an activator of endogenous viral infections (Di Prisco *et al*., 2016).

1. Life cycle, population dynamics and clinical signs

Varroa destructor lacks a free-living, bee-independent stage (Genersch, 2010). Instead, the life cycle of female mites consists of two distinct phases: the dispersal phase, where *V. destructor* parasitises adult bees, taking the opportunity to use them as a transport vehicle within the colony or between colonies, and the reproductive phase, where the mites parasitise drone or worker larvae just before cell-capping and reproduce within the sealed drone and worker brood cells (Figure 3). Male mites are short lived and can only be found inside the sealed brood cells during the reproductive phase (Rosenkranz *et al.,* 2010).

In the dispersal phase (Traynor *et al*., 2020) *V. destructor* usually inserts itself between the abdominal sternites in adult bees (Nazzi & Le Conte, 2016; Ritter, 1980) where it penetrates the intersegmental membranes in order to ingest haemolymph and fat body tissues (Ramsey *et al*., 2019). During this process the mite may acquire viral particles from covertly infected bees, and then, when it parasitises the next bee or larvae, it may directly inject these viruses into the haemocoel of the host (McMenamin & Genersch, 2015). Sometimes, *V. destructor* can also be found between the head and thorax or between the thorax and abdomen.

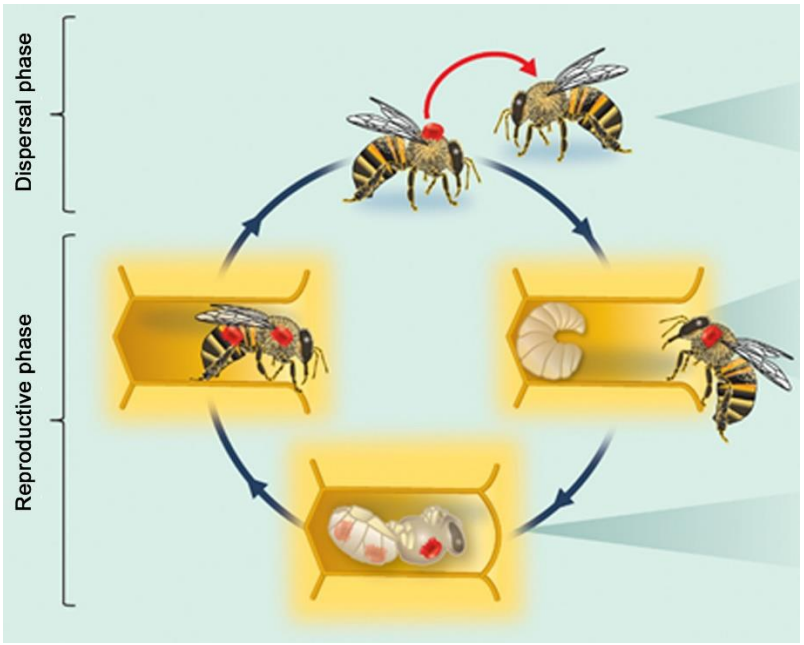

Fig. 3. *Simplified life cycle of* V. destructor*, with the dispersal and reproductive phases clearly shown (Nazzi & Le Conte, 2016).*

The reproductive phase starts when the female mite, guided by specific cues, leaves the adult bee host to invade a suitable brood cell just before capping. *Varroa destructor* prefers drone brood over worker brood (Fuchs, 1992). Factors that support this selection are, among others: (1) the longer duration of the invasion period (Boot *et al*., 1992), (2) the more frequent and intensive tending of the drone larvae by nurse bees (Calderone & Kuenen, 2003), thereby improving incrementally the chances of *V. destructor* to reach the suitable host cell, and (3) more attractive chemical cues (Le Conte *et al*., 1989). Mites are rarely found in queen cells, which might be repellent to them (Calderone *et al*., 2002).

After the invasion of the brood cell, the female *Varroa* stay immobile at the bottom of the cell within the larval food. Once the cell has been sealed and the larvae have consumed the rest of the food, the female mite starts feeding on the bee larvae and initiates oogenesis within 26 hours for the females, 30 hours for males. Specific host signals trigger egg laying in the *Varroa* female, starting around 3 days after the capping, generally with a male egg

(unfertilised) and followed by up to five or six female eggs (fertilised) in 30-hour intervals. The offspring mites hatch a few hours after oviposition and pass through proto- and deutonymph stages until they become sexually mature: after approximately 5.8 days for females and 6.6 days for males (Rosenkranz *et al.,* 2010). During this time they repeatedly consume haemolymph and fat body tissues from the pupae developing in the cell at the same feeding site. As soon as the first female reaches sexual maturity the male mates with it, triggered by female sex pheromones, until the next female is mature. As the entire process occurs in the capped brood cell, the duration of the post-capping period is the limiting factor that determines the number of emerging and mated female mites. Two to three mated daughter mites may develop in a single infested drone brood cell, and one to two in a worker bee cell. Once the bee has completed its development and hatches, the mated daughter mites along with the mother mite, leave the cell with the emerged bee, whereas immature daughter mites and the male mite will die. The female adult mites can be transmitted between individual honey bees within the same colony or might even be spread to a new host colony through foraging and drifting workers, searching for suitable brood cells to lay eggs to start a new generation. Mites prefer nurse bees to forager bees, both for the likelihood of later being taken to a brood cell and for avoiding the risks related to the outside activities of forager bees (Figure 4; Nazzi & Le Conte, 2016; Rosenkranz *et al.,* 2010).

Under field conditions the life span of *V. destructor* mites may vary from some days to a few months, depending on temperature and humidity, and two to three reproductive circles can be accomplished. The population growth of *Varroa* mites is highly variable and depends on traits of the host, the parasite and the environment (Meixner *et al.,* 2014). Features of the mites include reproductive capacity and life span; features of the host include genotype, brood availability, presence of drone brood, colony size, behaviour (effective grooming, hygienic behaviour, swarming and absconding); and finally environmental factors such as climate, nectar flow and the density of surrounding honey bee colonies as well as their health status play an important role in population dynamics. Despite this variety in population growth, the course of the disease in *A. mellifera* is usually lethal, except in some areas, such as tropical Latin America (Rosenkranz *et al.,* 2010) and some parts of Africa (Strauss *et al.,* 2013).

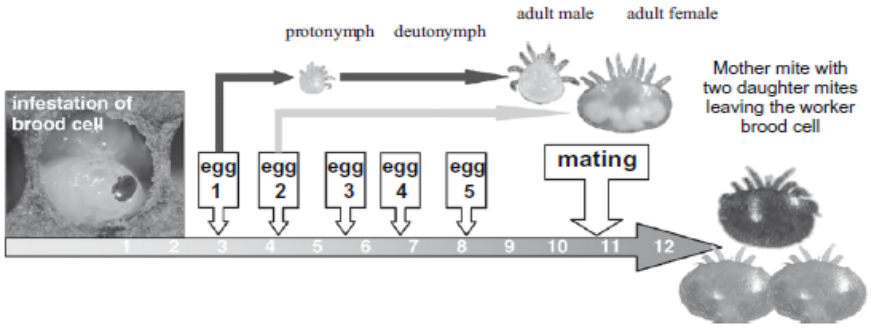

Fig. 4. *Oviposition and development of* Varroa *in brood cells of worker bee (up to about day 9 in unsealed brood, up to about day 21 in sealed brood) Reprinted from* J. Invertebr. Pathol., ***103****, Suppl. 1: S96–119, Rosenkranz P., Aumeier P. & Ziegelmann B., Biology and control of* Varroa destructor*, Copyright (2010), with permission from* Elsevier.

Varroa destructor infested colonies of *A. mellifera* have been reported to die after 1–4 years if not systematically treated, although sometimes the process is faster and they can succumb in a few months, especially if nearby colonies are collapsing. Under temperate conditions, damage at the colony level mainly appears during autumn and winter when the host population declines, the relative parasitisation increases and consequently the long-living winter bees are damaged (Figure 5; Rosenkranz *et al*., 2010).

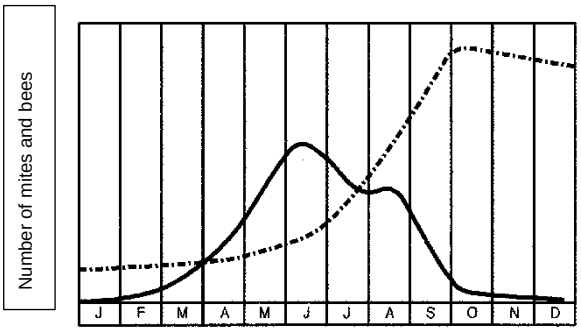

Fig. 5. *Graph of populations of bees and mites over 1 year in a temperate Northern Hemisphere climate: brood numbers (solid line); mite numbers (broken line).*

At the individual level, the direct pathogenic effects of the mite's feeding activities are: injuries in the cuticle, substantial depletion of haemolymph and fat body tissues and impairment of the immune system, the brood being the most sensitive host stage. Bees parasitised in the adult stage are only damaged if the infestation is severe. Parasitism during the host development results in a significant reduction in the size and weight of the hatching honey bee and increased disease susceptibility (De Jong *et al.,* 1982). Parasitised worker bees have a shorter life span, start foraging earlier and have a reduced capacity for non-associative learning, orientation and homing ability (Kralj & Fuchs, 2006). Parasitised drones have decreased flight performance and a shorter life span. Sperm production may be considerably reduced (Duay *et al.,* 2002).

At the colony level, low and moderate infestation rates often remain undetected, with clinical signs that may not be evident. Colonies have a reduced reproductive capacity however, as there is a reduced number of males available for mating, those that survive have a lower chance of succeeding, and infested colonies produce fewer swarms. There can also be a decreased growth in the honey bee population and, therefore, the honey yield can be diminished. Once a moderate infestation is reached, irreversible colony damage becomes more likely, especially in climates with a seasonal fall in temperate when the mite population still increases while the host population is decreasing (Figure 5). Brood care, social behaviour and worker bee tasks are negatively affected, leading to a weakening of the entire colony and eventually to its collapse (Le Conte *et al.,* 2010).

The clinical signs of a honey bee colony collapsing from varroosis include: high mortality at the hive entrance, rapid loss of the adult bee population, malnourished, crawling and crippled bees (with deformed wings and shortened abdomen due to the biological vectoring of DWV [figure 6]), direct visualisation of dispersal phase *Varroa* mites, scattered brood, brood cells with wax cappings fissured, sunken or partially removed, or with white patches on the cell wall (faecal accumulation site of mites), dead uncapped larvae and supersedure (replacement) of queens (Rosenkranz *et al.,* 2010).

a b

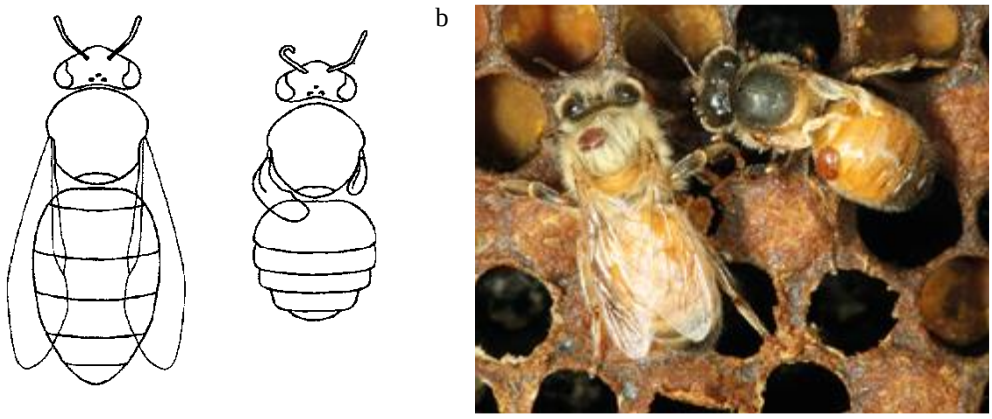

Fig. 6 *a) Effect of Varroa on bee morphology. Left: normal bee appearance. Right: bee heavily attacked by mites. This newly emerged bee has a deformed wing and reduced abdominal volume. b) Photograph by Fanny Mondet, INRAE.*

B. DIAGNOSTIC TECHNIQUES

As varroosis is established in many countries, diagnosis in these countries is not only focused on the presence or absence of *V. destructor* in honey bee colonies, but also on the mite density levels where the levels start to be damaging. Damage thresholds can be expressed in a variety of ways including (1) the infestation rate of adult bees (dispersal phase mites/100 bees), (2) daily natural mite fall and (3) total mites per colony (Dietemann *et al*., 2013). At the global level, the damage threshold of varroosis in *A. mellifera* cannot be established, and neither can a fixed infestation rate nor the number of mites per colony throughout the year. As is the case for mite population dynamics, damage thresholds are highly variable and depend on the interaction between genotype and environment together with beekeeping management practices and the time of year, resulting in substantial differences between regions (Rosenkranz *et al*., 2010). Nevertheless, in different regions of North America and Europe damage thresholds have been estimated: in the early beekeeping season it is considered to be 1–3% infestation rate of adult bees and 1–10 daily mites naturally fallen; and in the late beekeeping season the thresholds are considered to be 3–10% infestation rate of adult bees and 3000–4000 total mites per colony (Rosenkranz *et al*., 2010).

Table 1. *Test methods available for the diagnosis of infestation of honey bees with Varroa spp. and their purpose*

Method	Purpose					
	Population freedom from infestation	Individual animal freedom from infestation prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infestation – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent						
Morphological identification	+++	+++	++	++	++	–
Detection methods	+++	+++	+++	+++	+++	–

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.

1. Morphological identification of the agent

The predominant mite stage to be found are adult females, as they are the only ones that can survive outside the capped brood cells. The *V. destructor* female mite is a dark reddish/brown colour and has a flat, oval-shaped body approximately 1.1 mm in length × 1.5 mm in width × less than 0.5 mm in height, covered with short hairs (setae). The males are smaller than females, pear-shaped to triangular and white/light yellow in colour. Immature stages are also white/cream. All stages can be seen with the naked eye.

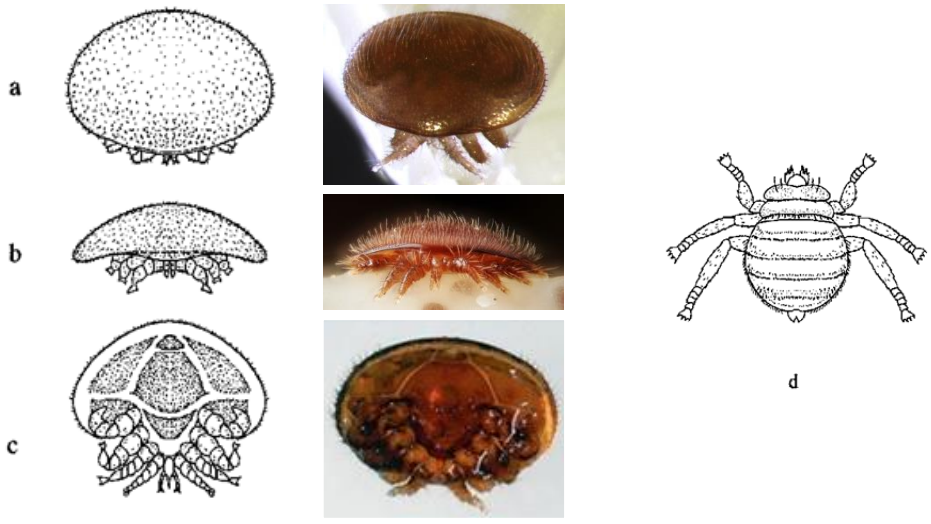

Fig. 7. *Diagram of* Varroa destructor (*formerly* Varroa jacobsoni *Oudemans*) (*female*).
a) Dorsal aspect. b) Anterior aspect. c) Ventral aspect. Note the flat shell-like back and four pairs of legs.
d) The bee louse (Braula coeca, *female*). *Note the lack of shell-like back and only three pairs of legs.*
First photo from M.O. Schäfer, Friedrich-Loeffler-Institut; Second photo from Gilles San Martin (https://aristabeeresearch.org/fr/varroa/); Third phot from National Bee Unit (http://www.bee-craft.com/talking-about-varroa-2/).

Varroa jacobsoni is morphologically close to *V. destructor*. It differs slightly in size, adult females of *V. destructor* being significantly larger and less spherical in shape than females of *V. jacobsoni*. However, as size criteria are difficult to appreciate, molecular techniques are advised for a confident differential diagnosis between *Varroa* species (Anderson & Fuchs, 1998).

Under some circumstances, the *Varroa* mite may be confused with the bee louse, *Braula coeca* (Figure 7). The latter is round, not oval, and being an insect, has only three pairs of legs. A number of different species of mite may be associated with *Varroa* mites on bees, but these are easily distinguished. In addition, other parasitic mites, such as those of the *Tropilaelaps* spp., are known to cause similar damage to bee colonies as the *Varroa* mites.

2. Detection of the agent

Three groups of different diagnostic techniques have been described: debris examination, bee examination and capped brood examination. Natural mite fall and adult and brood examination are only reliable for colonies with medium to high infestation rates with adequate amounts of brood (>3000 brood cells) that are not collapsing, whereas the debris examination after acaricide treatment with a product efficiency >95 % is reliable in all cases.

2.1. Debris examination

An easy method of diagnosis of varroosis is by examination of the debris generated by bees themselves, where fallen/dead mites can be found. This diagnostic technique can provide two different types of information: (1) natural mite fall and (2) mite mortality after acaricide treatment (Dietemann *et al*., 2013).

2.1.1 Natural mite fall

Apart from giving information about the natural death rate of the mite population, this non-invasive test can provide information about the grooming behaviour of the bees and, when brood is present, also about the mites detected and removed from infested brood cells by worker bees. Therefore, natural mite fall can provide information about dispersal and reproductive mite population. This technique is also recommended for monitoring activities.

i) Procedure:

Place a white or light-coloured covered insert with a screen mesh on the floor of the hive.

Considerations:

a) Arthropods such as ants must be prevented from accessing the insert, if not they will pillage the mites and thus bias the results (Dainat *et al.,* 2011). Protective measures include covering the insert with sticky material (e.g. grease, petroleum jelly, glue, gauze or impregnated absorbent paper). The sticky covering may also prevent live mites from climbing back into the hive.

b) The diameter of the screen mesh has to be small enough to avoid bees from reaching the insert and removing the mites, and big enough to allow the mites to fall through (3–4 mm).

ii) After a given time, count the mites present on the insert.

Considerations:

a) As there may be a large variety in the daily mite mortality, and given that many mites and a lot of debris makes counting difficult, the insert can be placed during 1–2 weeks to obtain a more reliable relationship between mite death rate and mite population. The counting and insert replacement frequency can be performed every 7 days or less, depending on local conditions. Consequently, the results can be expressed on a 24-hour basis.

b) A guide placed above the insert can be used for easier counting.

c) When mite fall is high, a subsample of the mites can be counted. One example to use in the field is a checker-board or sheet with a checked pattern to count mites from selected squares. In the laboratory another option is to examine large amounts of debris using a flotation procedure (see Section B.2.1.3).

2.1.2. Mortality after acaricide treatment

Procedure

i) Place a white or light-coloured insert covered with a screen mesh on the floor of the hive.

Considerations: The same as for step i) in Section B.2.1.1.

ii) Apply an acaricide with >95% efficiency.

iii) After a given time, count the mites present on the insert.

Considerations:

a) Given the rapid action of acaricides and to ease counting, mite counting frequency should be done every 3–7 days or less.

b) Depending on the acaricide applied, the length of the counting period differs:

1) If persistent acaricides unable to penetrate capped brood (i.e. most synthetic acaricides) and non-persistent acaricides capable of penetrating wax cappings (e.g. formic acid) are used, mite fall should be counted for 3 weeks. This period covers the dispersal phase mite mortality and the mortality during or after the capped stage (approximately 12–16 days) of developing bees. The results can be expressed on a 24-hour basis.

2) If non-persistent acaricides that do not penetrate capped brood are used, honey bee colonies should not have capped brood (either because of seasonal factors or because of management practices, such as caging the queen for 22 days before the treatment). As all mites are in the dispersal phase, the period of counting can be shorter. The results can be expressed on a 24-hour basis.

c) A guide and subsampling methods can also be used to facilitate counting, as well as the flotation procedure (see Section B.2.1.3).

Some countries demand the diagnostic application of certain medications to prove the absence of mites.

2.1.3. Flotation procedure

i) Dry the debris for 24 hours.

ii) Flood the debris with 70% industrial alcohol.

iii) Stir continuously for around 1 minute or, if debris contains wax or propolis particles, stir for 10–20 minutes.

iv) Identify and observe the mites that float to the surface.

2.2. Bee examination

In this second method three different effective tests can be used: alcohol wash, soapy wash and powdered sugar test. As adult bees are examined, this method provides information on the dispersal phase mite population. Samples are taken from uncapped brood frames, where bees have significantly more mites than brood-less frames (Rosenkranz *et al*., 2010).

It has to be noted that the infestation in one apiary can vary widely from other apiaries, even when all of them belong to the same beekeeper. Likewise, even in an apiary, the infestation can vary widely between colonies.

2.2.1. Alcohol wash test

This technique is invasive because bees quickly succumb when submerged in alcohol: 70–75% ethanol can be used.

Procedure:

i) Select any uncapped brood frame and check that the queen is not present. Hold the frame at approximately 10 degrees from the vertical.

ii) Take a sample of 300 bees using a jar (with a mark at the level of 100 ml of water, which is the volume occupied by 300 bees) by sliding it up and down so that the bees tumble in.

Considerations:

a) It is advised to verify that this volume (100 ml) corresponds to 300 bees for the particular subspecies of interest, as there are variations in size among *A. mellifera* subspecies.

iii) Rap the jar on a hard surface to be sure the bees are at the marked line; add or subtract bees as needed.

iv) Alcohol is added to the jar to cover the 300 bees, the lid is closed and the jar is shaken and moved in a circular motion for 1 minute to dislodge the mites.

Considerations:

a) In the laboratory a more precise method is by mechanical shaking for 30 minutes.

b) Instead of adding alcohol to the jar, the bees can be poured into a shallow dish or similar container filled with enough alcohol to cover them.

v) The content of the jar or container is then poured over a 3–4 mm sieve, which will trap the adult worker bees, into a clear plate or bowl. Check the jar or container for mites sticking to the sides.

Considerations:

a) Another option is to use a double sieve system, with the upper coarse sieve of 3–4 mm and the lower fine sieve of <0.5 mm, to collect all the mites, and then flushing the bees and mites with large amounts of warm water.

vi) The mites are collected and counted. The results can be expressed as a percentage of infestation, dividing the number of mites dislodged by the number of bees in the sample and then multiplying by 100.

2.2.2. Soapy wash test

As in the case of the alcohol wash test, this technique is invasive, killing the bees during the process. Detergents such as dish-washing soap can be used. To avoid too much foaming, which would make counting the mites difficult, low concentrated solutions are recommended, ranging from 0.2–1%.(1–5 ml of dish-washing soap in 500 ml of water). The procedure is the same as the alcohol wash test, with just two more considerations to take into account.

Procedure:

i) Select any uncapped brood frame and check that the queen is not present. Hold the frame at approximately 10 degrees from the vertical.

ii) Take a sample of 300 bees using a jar (with a mark at the level of 100 ml of water, which is the volume occupied by 300 bees) by sliding it up and down so that the bees tumble in.

Considerations:

a) It is advised to verify that this volume (100 ml) corresponds to 300 bees for the particular subspecies of interest, as there are variations in size among *A. mellifera* subspecies.

b) At the laboratory, the bees can be anaesthetised with ether, or by cooling at 4°C for 15 minutes or –18°C for 5 minutes, before undertaking the wash.

iii) Rap the jar on a hard surface to be sure the bees are at the marked line; add or subtract bees as needed.

iv) Detergent solution is added to the jar to cover the 300 bees, the lid is closed and the jar is shaken and moved in a circular motion for 1 minute to dislodge the mites.

Considerations:

a) In the laboratory a more precise method is by mechanical shaking for 30 minutes.

b) Instead of adding detergent solution to the jar, the bees can be poured into a shallow dish or similar container filled with enough detergent solution to cover them.

v) The content of the jar or container is then poured over a 3–4 mm sieve, which will trap the adult worker bees, into a clear plate or bowl. Check the jar or container for mites sticking to the sides

Considerations:

a) If there is too much soapy foam, extra water or alcohol can be added to eliminate it.

b) Another option is to use a double sieve system, with the upper coarse sieve of 3–4 mm and the lower fine sieve of <0.5 mm, to collect all the mites, and then flushing the bees and mites with large amounts of warm water.

vi) The mites are collected and counted. The results can be expressed as a percentage of infestation, dividing the number of mites dislodged by the number of bees in the sample and then multiplying by 100.

2.2.3. Powdered sugar test

This technique is not as invasive as the alcohol and soapy wash tests, and following it, bees can be placed back in their colony where they will be cleaned by the other bees. As well as its diagnostic use, this test is also recommended for monitoring activities, as is also the case for natural mite fall.

The principle of this technique is that dusting with powdered sugar will dislodge mites from their host, possibly because the sugar particles adhere to the ambulacra (distal segment of the mite legs), and thus affected mites lose their grip and fall off the bee. High humidity conditions decrease the efficacy of this test, as the powdered sugar collects moisture and clumps together. It is recommended to use fresh powdered sugar.

Procedure:

i) Select any uncapped brood frame and check that the queen is not present. Hold the frame at approximately 10 degrees from the vertical.

Considerations:

a) If greater precision (C ≤ 0.1 or h ≤ 0.5) is needed, sampling has to be done in three different brood frames, repeating the steps described below three times.

ii) Take a sample of 300 bees using a jar (with a mark at the level of 100 ml of water, which is the volume occupied by 300 bees) by sliding it up and down so that the bees tumble in.

Considerations:

a) It is advised to verify that this volume (100 ml) corresponds to 300 bees for the particular subspecies of interest, as there are variations in size among *A. mellifera* subspecies.

b) The lid of the jar must be modified: the centre part is replaced by a 2–3 mm hardware cloth or mesh.

c) To achieve a precision higher than C ≤ 0.1 or h ≤ 0.5, 900 bees in total have to be sampled.

iii) Rap the jar on a hard surface to be sure the bees are at the marked line, and add or subtract bees as needed. Once the volume is correct close the lid.

iv) Pour 1–2 table spoons (at least 7 g) of powdered sugar through the mesh or cloth, and roll the jar to cover all the bees with sugar

v) Let stand for 1 minute (in the shade in case of high temperatures so the bees do not overheat).

vi) Turn jar upside down over a clear plate or pan, and shake it for 1 minute at least, or until no more mites came out.

a) In the field, when wind may cause mites to be lost, the plate or pan can be filled with water.

vii) The mites are collected and counted. The results can be expressed as a percentage of infestation, dividing the number of mites by the number of bees in the sample and then multiplying by 100.

Considerations:

a) A drop of water can be added to the plate or pan to dissolve the powdered sugar and facilitate the counting, if it has not been previously filled with water.

viii) Reintroduce the sampled bees in the top of their colony or at the colony entrance.

2.3. Capped brood examination

For the third method, capped drone brood is examined, if available, otherwise capped worker brood is examined. As this technique is performed on the brood, it provides information about the reproductive mite population. It has to be taken into account that *V. destructor* prefers drone brood to worker brood, therefore the infestation rate in drones will be higher. This method is invasive – it will kill the sampled pupae – and is not always feasible in certain regions at certain times of year where brood may not be present (Rosenkranz *et al*., 2010).

Procedure:

i) Select 200 brood cells from the brood box.

Considerations:

a) Taking the samples from more than one brood frame will account for the spatially irregular infestation by *Varroa* mites. One option is to select four samples of 50 cells from different

frames (dimensions of the cut: 25 × 25 mm: it contains approximately 50 worker cells or 40 drone cells).

ii) Examine them for the presence of mites.

Considerations: There are different methods:

a) Individual opening of each capped cell:

Examine each pupa and its cell, especially the bottom, for the presence of mites or their faeces (white spots). The results after can be expressed as:

1) Percentage of infested cells (dividing the number of infested cells by the total number of opened cells, and then multiplying by 100)

2) Total number of mites on capped brood (multiplying the average number of mites per pupa by the number of capped brood in the colony). The number of capped brood can be calculated using a grid.

b) Washing the brood over a double sieve system:

1) Cut out the selected sample/samples from the frame/frames

2) Remove the cappings of the brood cells with a knife

3) Wash the brood cells directly into a sieve system with warm water from a hand-held shower

4) Collect the mites in the lower fine sieve (mesh width < 0.5 mm) while the brood is gathered in the upper coarse sieve (mesh width 2–3 mm)

5) Place the contents of the sieve on a bright plate, where the mites can be easily identified and counted

6) The results after can be expressed as the average number of mites per cell.

2. Serological tests

No serological tests are available for routine laboratory diagnosis.

C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

There are no vaccines available.

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Illustrations by Karl Weiss, extracted from Bienen-Pathologie, 1984. Reproduced with the kind permission of the author and Ehrenwirth-Verlag, Munich (Germany). Photographs by Dr Denis Anderson, Dr Rob Manning, Dr Nazzi, Dr Le Conte, Dr Rosenkranz, Dr Mondet, Dr Schäfer and Dr San Martin.

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NB: There are OIE Reference Laboratories for infestation of honey bees with *Varroa* spp. (varroosis) (please consult the OIE Web site: https://www.oie.int/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3). Please contact the OIE Reference Laboratories for any further information on diagnostic tests and reagents for bee diseases

NB: FIRST ADOPTED IN 1989 AS VARROASIS. MOST RECENT UPDATES ADOPTED IN 2021.