

CHAPTER 2.2.1.

**ACARAPISOSIS OF HONEY BEES  
(INFESTATION OF HONEY BEES  
WITH *ACARAPIS WOODI*)**

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**SUMMARY**

*Acarapisosis or acariosis or acarine disease is a disease of the adult honey bee *Apis mellifera* L. and other *Apis* species. It is caused by the Tarsonemid mite, known as the tracheal mite, *Acarapis woodi* (Rennie). The mite is approximately 150 µm in size, and is an internal parasite of the respiratory system, living and reproducing mainly in the large prothoracic trachea of the bee. Sometimes they are also found in the head, thoracic and abdominal air sacs. Mites feed on the haemolymph of their host.*

*The pathogenic effects found in infected bees depend on the number of parasites within the trachea and are attributable both to mechanical injuries and to physiological disorders consequent to the obstruction of air ducts, lesions in the tracheal walls, and the depletion of haemolymph. As the parasite population increases, the tracheal walls, normally white and translucent, become opaque and discoloured with blotchy black areas, probably due to melanin crusts.*

*The mortality rate may range from moderate to high. Early manifestations of infection normally go unnoticed, and only when infection is heavy does it become apparent. This is usually in the early spring. The infection spreads by direct contact. Generally, only newly hatched bees under 10 days old are susceptible. Reproduction occurs within the tracheae of adult bees, where female mites may lay 8–20 eggs. There are 2–4 times as many females as males. Development takes 11–12 days for males and 14–15 days for females.*

**Identification of the agent:** *The parasites are demonstrated only by laboratory methods and under the microscope. The mites need to be observed inside the tracheae or removed from them to be observed microscopically. Several techniques are available for demonstrating the mites, such as dissection, grinding and staining.*

*The thoraces of suspect bees are dissected to expose the trachea. Each trachea is examined under a dissecting microscope (×18–20), where the mites will be seen through the transparent wall as small oval bodies.*

*Alternatively, larger samples of suspect bees can be ground or homogenised in water, followed by coarse filtration of the suspension, and centrifugation. The deposit is treated with undiluted lactic acid for 10 minutes. This is then mounted for microscopic examination.*

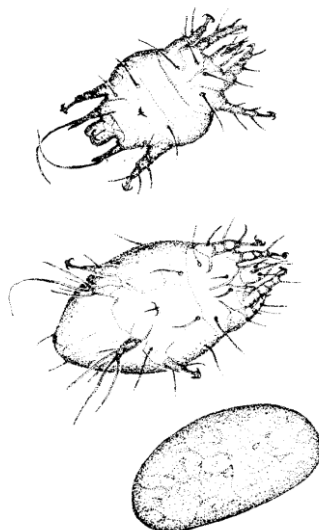
*The parasites may be stained by histological techniques so that they can be observed within the bee trachea. The tracheae are separated out, cleared with 8% potassium hydroxide, and stained with 1% methylene blue. This is the best method for large numbers of samples.*

**Serological tests:** *Serological tests are not available.*

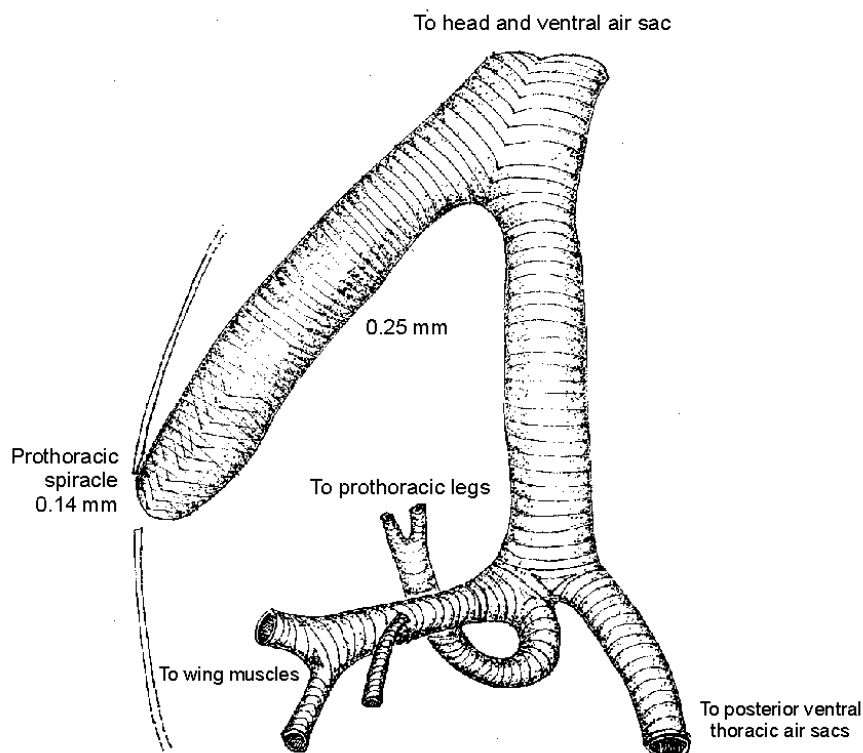
**Requirements for vaccines and diagnostic biologicals:** *There are no biological products available. Menthol crystals or oil patties made with vegetable oil (not animal fat) and white granulated sugar will keep mite levels under control.*

## A. INTRODUCTION

Acarapisosis is a disease of the adult honey bee *Apis mellifera* L. and other *Apis* species, caused by the microscopic Tarsonemid mite *Acarapis woodi* (Rennie). The mite is approximately 150 µm in size and is an internal parasite of the respiratory system (Figure 1). These tracheal mites enter, live and reproduce mainly in the large prothoracic tracheae of all bees, feeding on the haemolymph of their host (Figure 2). Sometimes they are also found in the head, thoracic and abdominal air sacs (Giordani, 1965; Wilson *et al.*, 1997).



**Fig. 1.** *Acarapis woodi* (Rennie). Top: Adult male, Centre: Adult Female, Bottom: Egg.



**Fig. 2.** Main thoracic tracheae of a honey bee where *Acarapis* is commonly found; light infestations are near the spiracle opening.

The pathogenic effects on individual bees depend on the numbers of parasites within the tracheae and are attributable both to mechanical injuries and to physiological disorders consequent to the obstruction of the air ducts, lesions in the tracheal walls, and to the depletion of haemolymph. As the parasite population increases, the tracheal walls, which are normally whitish and translucent, become opaque and discoloured with blotchy black areas, probably due to melanin crusts (Giordani, 1964).

The mortality rate may range from moderate to high. Early signs of infection normally go unnoticed, except for a slow dwindling in the colony size. Only when infection is heavy does it become apparent. This is generally in the early spring after the winter clustering period when the mites have bred and multiplied undisturbed into the longer-living winter bees. This applies mainly to the Northern Hemisphere where there are seasonal variations in the reproduction of bees.

Infection spreads from one bee to another by direct contact. Generally, only newly hatched bees under 10 days old, are susceptible. Attempts to rear *A. woodi* on artificial and synthetic diets have been unsuccessful, while culturing them on immature stages of the honey bee itself has been only partially successful (Giordani, 1970). The life span of the mites in dead bees is approximately 1 week. Reproduction occurs within the tracheae of adult bees, where female mites may lay 8–20 eggs. There are 2–4 times as many females as males; development takes 11–12 days for males and 14–15 days for females.

There are no reliable clinical signs for the diagnosis of acarapisosis as the signs of infection are not specific and the bees behave in much the same way as do bees affected by other diseases or disorders. They crawl around in the front of the hive and climb blades of grass, unable to fly. Dysentery may be present.

## B. DIAGNOSTIC TECHNIQUES

### 1. Identification of the agent

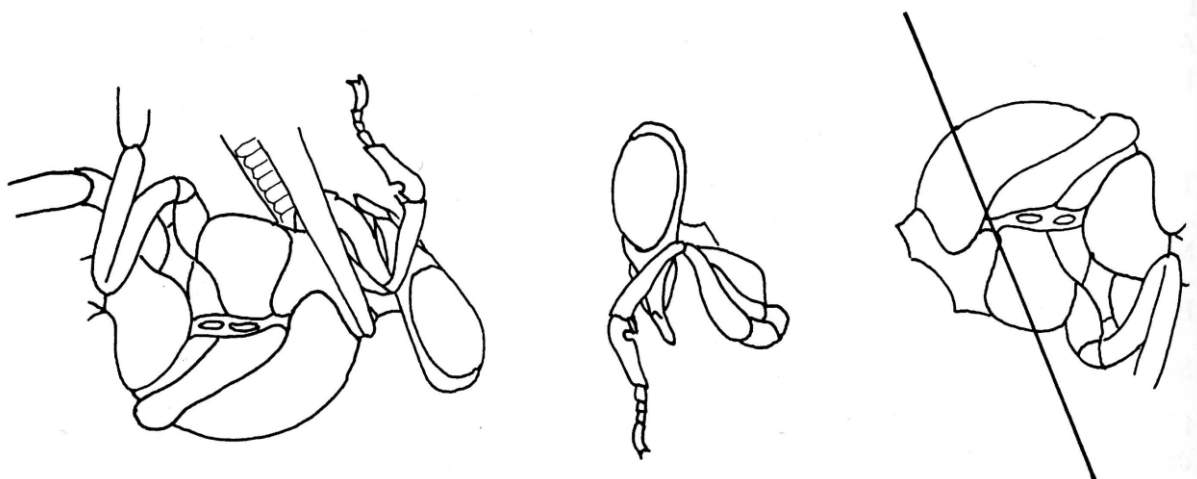
Acarapisosis can be detected only in the laboratory using microscopic examination or an enzyme-linked immunosorbent assay (ELISA). There is no reliable method for detection of very low levels of infection. The number of bees sampled determines the detection threshold of the method. It has been shown that a 1 to 2% rate of infection can be detected by sampling 50 bees. Sequential sampling data are available (Frazier *et al.*, 2000; Tomasko *et al.*, 1993). The best time to take bee samples is in the early spring or late autumn (Northern hemisphere), when *Acarapis* populations are high. Visualisation of mites is easier in older bees, which have more mites. Samples of queens, drones or workers can be used, but *Acarapis* prefer drones.

#### 1.1. Dissection (Giordani, 1974)

A sample of 50 bees (see above) is collected at random from the suspected colony. These are mainly bees crawling and unable to fly, found within about 3 metres of the front of the hive. This is preferable to random collection from within the colony. The bees may be living, dying, or dead. Live bees must first be killed with ethyl alcohol or in a deep freezer (–20°C); bees must not have been dead for over 2–3 days unless kept at 4°C for up to 4 weeks or –20°C for several months. They may be preserved indefinitely in a preservative such as Oudemann solution: glacial acetic acid (80 ml); glycerol (50 ml); 70% ethanol (870 ml).

##### 1.1.1. Test procedure: direct preparation (Ritter, 1996; Wilson *et al.*, 1997)

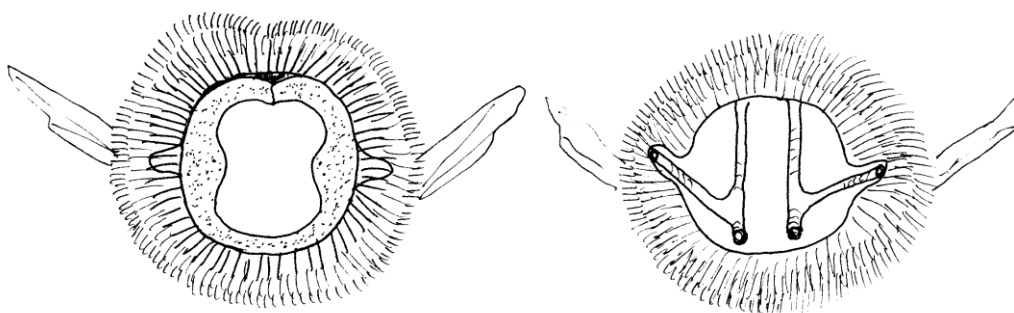
- i) Remove the abdomen at the thorax of the bees (see Figure 3).
- ii) Pick up the thorax with the beginning of the head and examine it under the binocular magnifying glass at 20–30-fold magnification.
- iii) Remove the pleural sclerite of the first thoracic segment with the first pair of legs, by means of a pair of tweezers. In the circular opening the main strains of the thoracic tracheae and the branches of the head tracheae can be seen.
- iv) By means of a fine pair of tweezers, remove the thoracic tergite of the first thoracic segment and part of the second thoracic tergite. After removing the overlying musculature, the two thoracic tracheae are exposed. Positive diagnosis consists of either the presence of melanisation of one or both tracheae or, in light infestation, of the presence of oval translucent bodies (eggs etc.) easily seen within the tracheae.
- v) For further microscopic examination (e.g. confirmation of light infestation), remove the tracheae and put them onto a slide, with a drop of water. Under the microscope at 100-fold magnification the adult mites as well as their individual stages of development can be recognised.



**Fig. 3.** Preparation of bees to reveal *Acarapis woodi* in the first thoracic pair of tracheae.

#### 1.1.2. Test procedure: maceration (Ritter, 1996)

- i) Lay and secure bees on their backs or hold with thumb and first finger.
- ii) Remove the heads and forelegs using a small forceps and remove the collar surrounding the neck opening to expose the tracheae (Figure 4). Check the tracheae nearest to the spiracle (as mites enter through the spiracle) to see light infestations. Heavy infestations are easily visible as shadows or dark objects in clear to dark brown tracheae. Old and heavy infestations will make the tracheae brown to black.
- iii) Cut through the thorax in front of the middle pair of legs and the base of the forewings with a sharp razorblade. These thin disks can be further treated to clear muscle tissue.
- iv) Macerate either by gentle heating in an 8% solution of potassium hydroxide for approximately 20 minutes or by leaving them to stand overnight without heating.
- v) Examine the first pair of tracheae, which are covered by muscle tissue, under a dissecting microscope at a magnification of  $\times 18$ – $20$ , or transfer the tracheae to another slide, add glycerin or water and observe at higher magnification.
- vi) Mites are easily seen through the transparent wall as small, oval bodies.



**Fig. 4.** Left: front view of bee thorax with head removed and collar intact.  
Right: Collar removed and tracheae exposed to spiracle openings.

This is the simplest and most reliable technique for the laboratory diagnosis of acarapiosis, allowing the detection of early infections and enabling the infection rate to be established. Even light infections can be detected by using a dissecting microscope with this technique. Only in very exceptional instances will it be necessary to employ higher magnifications in order to make a diagnosis. However, this is a demanding technique, especially when a large number of acarapiosis diagnoses have to be made. If it is necessary only to distinguish between heavily

infected and lightly or non-infected colonies, dissection can be stopped at step ii and the colour of the tracheae observed.

### 1.2. Grinding (Colin *et al.*, 1979)

A sample of about 200 bees is collected at random from the suspect colony. The wings and legs of each bee are removed from the thorax, and the bodies are pooled in a 100 ml container that has been one-quarter filled with water. This suspension is homogenised three times, each time for several seconds, in a homogeniser at 10,000 rpm with the addition of more water. The resulting suspension is strained through a sieve (mesh 0.8 mm) and the sieve is rinsed with water to a final volume of approximately 50 ml. The filtrate is centrifuged at 1500 **g** for 5 minutes and the supernatant fluid is discarded. A few drops of undiluted lactic acid solution are added to the debris of the deposit, which will contain the mites. This is left for 10 minutes to allow the muscle fibres to dissolve, and is then mounted under a cover-slip for microscopic examination. This technique is quicker than dissection, but may be less accurate. External mites *A. externus*, *A. vagans* and *A. dorsalis*, all of which are morphologically similar to *A. woodi* (Table 1). It seems, however, that they do not cause any serious threat to bees or beekeeping. This method should therefore only be chosen if all that is required is a rough estimation of the degree of infection in a region. It is not suitable for determining a first outbreak.

**Table 1.** Differential diagnosis of *Acarapis* species (Ritter, 1996)

Character	<i>A. dorsalis</i>	<i>A. externus</i>	<i>A. woodi</i>
Notch of the coxal plate	Deep	Short	Flat
Space between stigmata	16.7 µm	16.8 µm	13.9 µm
Length of tarsal limb (IV leg pair)	7.6 µm	11.4 µm	7.5 µm

### 1.3. Staining (Peng & Nasr, 1985)

The mites and trachea can be stained specifically, rendering them easily visible by microscopy.

#### 1.3.1. Test procedure 1

- i) Remove the head and forelegs.
- ii) Make a transverse cut through the membranous areas behind the forelegs.
- iii) Make a second transverse cut in front of the middle pair of legs at the base of the forewings.
- iv) To clear the sections (1–1.5 mm thick), place them in an 8% solution of potassium hydroxide.
- v) Stir gently and heat near to boiling point for approximately 10 minutes until the soft internal tissues are dissolved and cleared, leaving the chitinous tissues intact.
- vi) Retrieve sections by filtration and wash with tap water.
- vii) Stain and mount the sections.
- viii) Examine for mites by low-power microscopy.

Permanent mounts are prepared by the usual histological techniques.

Cationic stains are the most suitable and specific as they stain the mites intensely but the tracheae only weakly. A solution of 1% aqueous methylene blue is the most suitable, prepared by dissolving the methylene blue first and then adding sodium chloride to make a 0.85% NaCl solution.

#### 1.3.2. Test procedure 2

- i) Stain in 1% aqueous methylene blue.
- ii) Differentiate sections in distilled water for 2–5 minutes.
- iii) Rinse the sections in 70% alcohol.

When kept in 95% ethanol, the mites will retain the stain for 6 hours (Bancroft & Stevens, 1982). It is essential with this technique to macerate the tissues effectively in the potassium hydroxide solution. Using this method, it is possible to process a large number of samples rapidly and conveniently.

#### 1.4. Enzyme-linked immunosorbent assay

An ELISA for trachea mites has been developed (Grant *et al.*, 1993; Ragsdale & Furgala, 1987; Ragsdale & Kjer, 1989). This test may produce false-positive results, and is therefore only recommended for survey examinations. Another method is the visualisation of guanine, a nitrogenous waste product of mites (Mozes-Koch & Gerson, 1997).

### C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

There are no biological products available. Menthol crystals (50 g for a two story colony) control mites if left in the colony for 28 days, providing the ambient temperature is at least 18°C. The optimum temperature range for the vapours to work is 27–29°C. Small cakes made with vegetable shortening (e.g. margarine, not animal fat) and white granulated sugar will keep mite levels to 10%. The cake (about 100 g in weight) should be placed on the top bars of the frames in the brood nest in the autumn and early spring (Sammataro & Needham, 1996). Formic acid may be used to treat infected colonies (Hood & McCreddie, 2001).

Some races of bees, such as Buckfast bees (Brother, 1968) and some hygienic strains, are less susceptible to attack by *Acarapis*.

### ACKNOWLEDGEMENTS

Illustrations by Diana Sammataro and Wolfgang Ritter are reproduced with their permission.

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[http://www.fao.org/WAICENT/faoINFO/AGRICULT/ags/subjects/en/industFoodAg/pdf/AGST\\_techrep\\_4.pdf](http://www.fao.org/WAICENT/faoINFO/AGRICULT/ags/subjects/en/industFoodAg/pdf/AGST_techrep_4.pdf)

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**NB:** There are OIE Reference Laboratories for Bee diseases  
(see Table in Part 4 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list:  
<http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/> ).  
Please contact the OIE Reference Laboratories for any further information on  
diagnostic tests and reagents for bee diseases