# *TERRESTRIAL MANUAL* REVISION: ADVICE FOR MEMBER COUNTRY COMMENTS

# Chapter Number and Title: Chapter *3*.2.1. Acarapisosis of honey bees(infestation of honey bees with *Acarapis woodi*)

Country making the comments:

Date:

It would be appreciated if the following guidance is followed when making a reply:

1. Comments may be general or specific, but specific comments are more valuable. General comments should be such that some conclusion and action can be taken in response to them. For example, instead of stating “This test is no longer used in our laboratory”, indicate the reasons the test is no longer used and what test is used instead.

2. Specific comments should be identified by indicating the line number in the text, to facilitate the editorial process.

3. Highlighting typing or technical errors is welcome, but the correct word or figure should be indicated in its place. For example, instead of indicating simply “0.8 M is too high”, the preferred value should also be indicated.

4. Bear in mind that the introductory chapters (Part 1 of the *Terrestrial Manual*) set general standards for the management of veterinary diagnostic laboratories and vaccine facilities and are not intended to be exhaustive, and indeed none of the chapters can give a completely comprehensive cover of the subject, otherwise the *Terrestrial Manual* would be too long. However, assistance in indicating priorities is always helpful.

5. The *Terrestrial Manual* is intended for world-wide use. The chapters need to reflect the development of new technology, while maintaining the established methods, usually requiring less sophisticated apparatus. New technology should not be described in detail until it has gained wide acceptance as a reliable method.

6. We recommend that if you have no specific comments, please respond to the OIE to that effect.

7. Any comments, proposed changes or revisions should be supported by clear evidence (the scientific rationale) such that some conclusion and action can be taken in response to them.

*Your participation in the OIE Standard-setting process is valued. Thank you for your engagement in the process!*

*General Comments*

*Specific Comments* (*add continuation sheets if required*)

*line:*

Chapter 3.2.1.

acarapisosis of honey bees  
(infestation of honey bees   
with *Acarapis woodi*)

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 tracheae 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~~ mites within the trachea ~~and are attributable~~. The mites can cause 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~~ mite population increases, the tracheal walls, normally white and translucent, become opaque and discoloured with blotchy black areas, probably due to melanin crusts.

Some strains of bees are less susceptible to A. woodi infestation. The mortality rate in infected bees 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~~ mite spreads by direct contact. Generally, ~~only newly hatched bees under 10 (~~adult bees less than 4 days ~~old~~ post-emergence~~)~~ are more susceptible. Reproduction occurs within the tracheae of adult bees, where female mites may lay ~~8–~~up to 20 eggs. There are ~~2–4 times as many~~ usually more females ~~as~~ than males, though the ratio can vary. Development takes 11–12 days for males and 14–15 days for females.

**Identification of the agent:** The ~~parasites~~ mites are demonstrated only by laboratory methods ~~and under~~ either by microscopy, or molecular detection.

For microscopy, 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 tracheae. Each trachea is examined under a dissecting microscope (×~~18–20~~ 40–60), 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~~ mites may be stained by histological techniques so that they can be observed within the bee trachea. The tracheae are separated out, cleared with ~~8~~ 5–10% potassium hydroxide, and stained with 1% methylene blue. This is the best method for large numbers of samples.

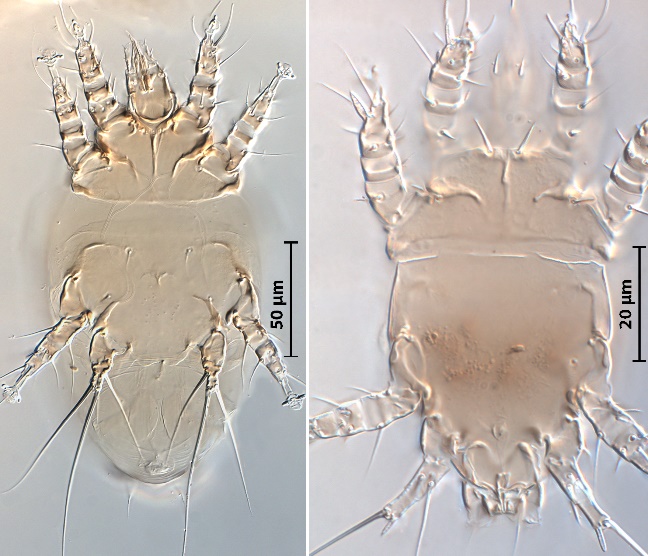
For molecular detection, both conventional and real-time polymerase chain reaction (PCR) methods that detect the cytochrome I oxidase gene of Acarapis woodi can be used. Amplicons from the conventional PCR must be sequenced to provide confidence in the detection of A. woodi, as distinct from the related mites A. dorsalis and A. externus. A sample of 105 bees is obtained from a colony and their abdomens removed and discarded. Seven separate DNA extractions are performed, with 15 bees each, where bees are homogenised in lysis buffer and the extracted DNA subjected to PCR. The real-time PCR is useful when large numbers of samples are processed. False-positive detections of A. externus and A. dorsalis may be possible, and confirmatory testing by microscopy is required.

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

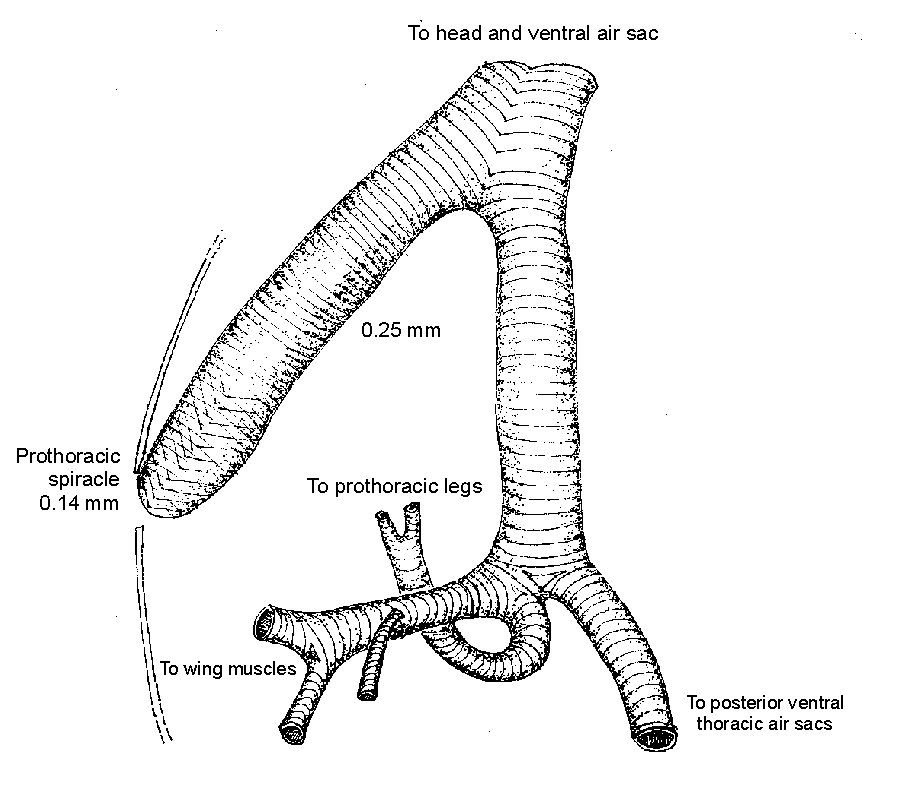
**Requirements for vaccines ~~and diagnostic biologicals~~:** There are no vaccines ~~biological products~~ available. ~~Menthol crystals or oil patties made with vegetable oil (not animal fat) and white granulated sugar will keep mite levels under control.~~

A. introduction

Acarapisosis (syn. acariosisor acarine disease) 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 (Figure 1) 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*). *Ventral views of adult female* (*left*) *and adult male.   
~~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 depends on the numbers of ~~parasites~~ mites 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~~ Colony loss rates may ~~range from moderate to~~ vary, but these losses can be very high~~.~~ when the mite first establishes in bee populations with no prior exposure. Early signs of infection ~~normally~~ may 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 in~~to~~ the longer-living winter bees. ~~This applies mainly to the Northern Hemisphere where there are seasonal variations in the reproduction of bees.~~ Some races of bees, such as Buckfast bees (Brother, 1968) and some hygienic strains, are less susceptible to attack by *A. woodi*. ~~Infection spreads~~ Mites spread from one bee to another by direct contact. Generally, only ~~newly hatched~~ young adult bees (under ~~10~~ 4 days ~~old,~~ post-emergence), are susceptible. Attempts to rear *A. woodi* artificially ~~on artificial and synthetic diets~~ have ~~been unsuccessful, while culturing them on~~ had limited success (Bruce *et al.,* 1991). Controlled infestation of immature ~~stages of the honey bee itself has been only partially successful~~ bees can be achieved (Giordani, 1970)~~. The~~ and has allowed for the determination of mite life ~~span~~ cycle, host preference and host resistance, and the effect of the mites ~~in dead~~ on adult 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

Table 1. Test methods available and their purpose

| Method | **Purpose** | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| Population freedom from infection | Individual animal freedom from infection prior to movement | Contribute to eradication policies | Confirmation of clinical cases | Prevalence of infection – surveillance | Immune status in individual animals or populations post-vaccination |
| **Agent identification** | | | | | | |
| **Microscopy – bee dissection** | +++ | – | +++ | +++ | ++ | – |
| **Microscopy – bulk sample** | + | – | + | ++ | + | – |
| **Conventional PCR** | + | – | + | ++ | + | – |
| **Real-time PCR** | ++ | – | ++ | ++ | ++ | – |

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

PCR = polymerase chain reaction.

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~~ by molecular detection. 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~~ any caste can be used, but *Acarapis* ~~prefer~~ *woodi* has a higher abundance in drones.

1.1. Microscopy – dissection of individual bees ~~(Giordani, 1974)~~

Microscopy provides the simplest and most reliable technique for the laboratory diagnosis of acarapisosis, allowing for the detection of early infestations and enabling the infestation rate to be established. Even light infestations can be detected using a dissecting microscope (40–60×). Only in very exceptional instances will it be necessary to employ higher magnifications to make a diagnosis. However, detection methods using microscopy are demanding techniques and require lots of time, especially when a large number of samples is to be processed.

A sample of 50 bees (see above) ~~is~~ should be 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~~ Oudeman’s solution: glacial acetic acid (80 ml); glycerol (50 ml); 70% ethanol (870 ml).

1.1.1. Test procedure: direct preparation (Milne, 1948; Lorenzen & Gary, 1986 ~~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- to 30-fold magnification.~~

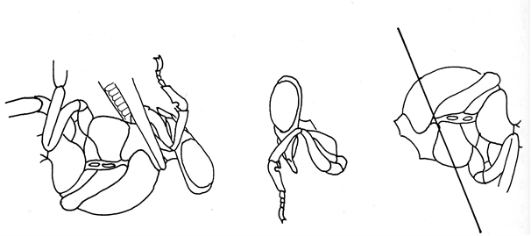
~~Iii) Remove the pleural sclerite of the first thoracic segment with the first pair of legs, by means of a pair of tweezers. In the circular opening the main strains of the thoracic tracheae and the branches of the head tracheae can be seen.~~

i) Place bee under a dissection microscope on their backs and hold using forceps or insect pins, then remove the head and first pair of legs from the thorax using a blade.

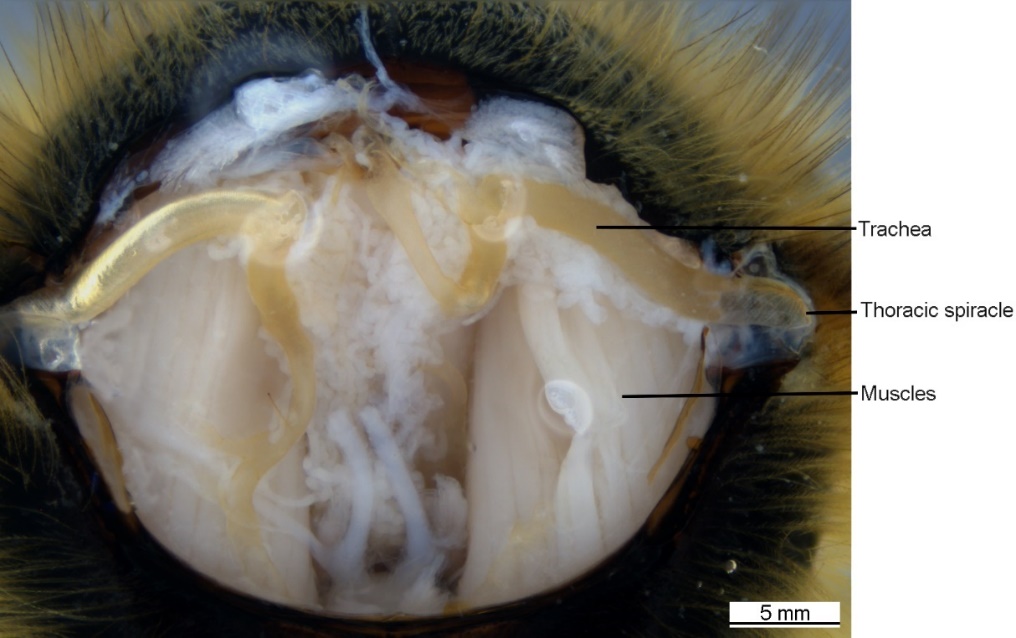
ii) Remove the prothoracic sclerite (collar) using forceps.

~~iv)~~iii) ~~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~~ tracheal trunks in the mesothorax are exposed (Figure 2). 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.

iv) For further microscopic examination ~~(e.g.~~, especially for confirmation of light infestation~~)~~, remove the tracheae and put them onto a slide, with a drop of ~~water.~~ Hoyer’s medium: distilled water (50 mm), chloral hydrate (200 g), glycerine (20 ml) and crystalline gum arabic (30 g). Under the microscope at ~~100-fold~~100× 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.~~*



***Fig. 2****. The first thoracic pair of tracheae exposed in the mesothorax of a bee*(Acarapis woodi *are not present in this specimen*).

1.1.2. Test procedure: ~~maceration (Ritter, 1996)~~ thoracic disc method (Peng & Nasr, 1985; Sammataro *et al.*, 2013)

i) Lay ~~and secure~~ bees on their backs ~~or~~ and hold with ~~thumb and first finger~~ forceps.

ii) Remove the heads and forelegs using a ~~small~~ second pair of forceps ~~and remove the collar surrounding the neck opening to expose~~. This exposes 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~~ the mesothorax.

iii) Using a sharp scalpel or razorblade, 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~~ to create a 1–1.5 mm thin thoracic section (disc). Many thoracic discs can be prepared in this way and kept chilled at 4°C, or frozen, before further ~~treated to clear muscle tissue~~ preparation.

iv) ~~Macerate either~~ Muscle is cleared from the thoracic disc by ~~gentle~~ heating at 60°C in ~~an 8%~~ a 5–10% solution of potassium hydroxide (KOH) for 2 hours ~~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 ×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.~~

v) Debris is removed by rinsing the sections under running water in a fine sieve.

vi) The sections are returned to a 5–10% solution of potassium hydroxide and heated to 60°C for 1 hour. The sections will become transparent in the centre leaving only the sclerotised tergites and main tracheal trunks.

vii) The sections are washed gently under running water in a fine sieve to remove any residual debris.

viii) Sections are transferred to a petri dish containing distilled water and a few drops of 1% aqueous methylene blue; staining for 5 minutes.

ix) When staining is complete, remove the sections from the staining solution and transfer them to a dissecting microscope for visual assessment. Examine at 20–40× magnification, with illumination from below. Mites are easily seen through the transparent wall as small, oval bodies (Figure 3).

x) The sclerotised tergites can be torn away using forceps to make the trachea more visible. Trachea can then be mounted on a glass slide and transferred to a compound microscope for more detailed visualisation of mites (*ca.* 100× magnification; Figure 3C).

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

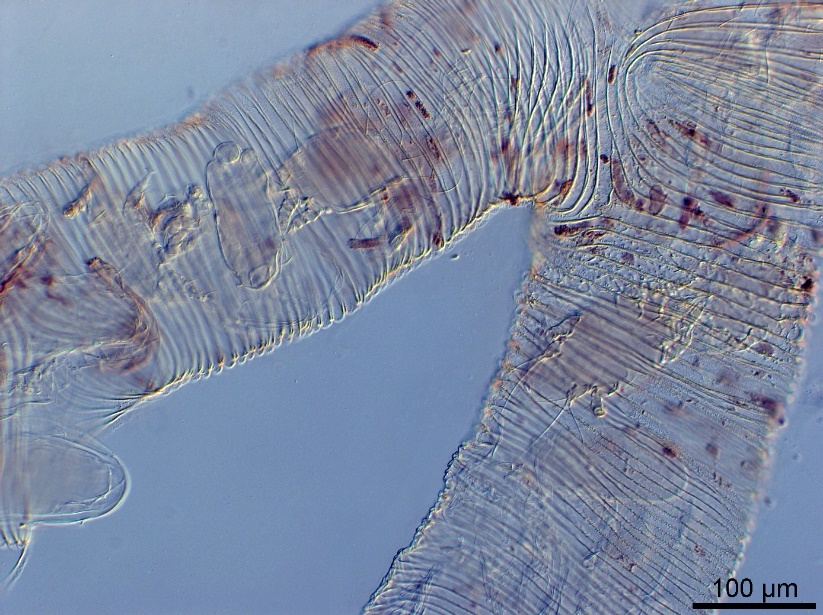


**a**

**b**



**c**



***Fig. 3****.* (*a*) *Presence of* Acarapis woodi *in tracheae of honey bee, revealed using the thoracic disc method   
and a dissecting microscope;* (*b*) *under higher magnification;* (*c*) *presence of* Acarapis woodi *in tracheae  
 viewed using a compound microscope.*

~~This is the simplest and most reliable technique for the laboratory diagnosis of acarapisosis, 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 acarapisosis 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. Microscopy – bulk sample preparation and screening based on mite morphology (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~~ is less accurate. External mites *A. externus~~, A. vagans~~* and *A.* *dorsalis*, ~~all~~ both of which are morphologically similar to *A.* *woodi*, are often found on the neck and thorax of healthy bees and can very easily be mistaken for *A.* *woodi* (~~Table 1~~ Delfinado-Baker *et al.*, 1982). 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~~ infestation in a region. It is not suitable for ~~determining~~ detection of an incursion into a ~~first outbreak~~ region.

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

|  |  |  |  |
| --- | --- | --- | --- |
| **~~Character~~** | ***~~A. dorsalis~~*** | ***~~A. externus~~*** | ***~~A. woodi~~*** |
| ~~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. Molecular detection of *Acarapis woodi* infestation of *Apis mellifera*

Detection of *Acarapis woodi* infestation in bee colonies using PCR methods is faster and more efficient than microscopy, and may be more sensitive. However, caution must be exercised when interpreting the results of PCR testing due to the genomic similarities between *A. woodi* and the close-relatives *A. dorsalis* and *A. externus*, which for certain rare genotypes may lead to false-positive detections of *A. woodi*. Positive detections require confirmation by microscopy.

1.3.1. Extraction of nucleic acid from *Apis mellifera* for detection of *Acarapis woodi* (Delmiglio *et al.*, 2016)

A conservative sample size of 105 bees per colony should be used, although smaller sample sizes can be used if it is expected that high mite infestations are present. A maximum number of 15 bees can be used in a single DNA extraction, ensuring that a single bee with a low-level of infestation (<10 mites) can be detected. A minimum of seven DNA extractions are required to test a colony sample of 105 bees.

i) 105 bees are shaken in warm water on an orbital mixer for 20 minutes to dislodge external mites.

ii) The abdomen of each bee is removed using a clean scalpel and the heads and thoraxes are placed into filter-mesh grinding bags to separate exoskeleton fragments after maceration. 0.5 ml of a nucleic acid lysis buffer is added per bee; commercial nucleic acid extraction buffers that contain chaotropic salts e.g. guanidine thiocyanate, are available for DNA extraction.

iii) Bees are macerated in the lysis buffer using a grinder or paddle blender, and 600 µl of lysate is placed into a clean reaction tube with 30 µl proteinase K (concentration) and incubated at 65°C for 30 minutes with mixing

iv) The lysate is then subjected to centrifugation at 8000 ***g*** for 1 minute.

v) The resulting supernatant is aspirated and subjected to DNA extraction; commercial DNA extraction kits are available and selected kits should be validated for diagnostic purposes before use e.g. magnetic-bead particle separation methods, or affinity column-based separation.

1.3.2. Conventional PCR (Evans *et al.*, 2007; Kojima *et al.*, 2011; Navajas *et al.* 1996)

Conventional PCR approaches for the detection of *Acarapis woodi* are available but require confirmatory sequencing of the amplicons to provide confidence of detection. Nucleic acid extraction can be performed using the method described in Section B.1.3.1, but alternative DNA extraction techniques have been used for the conventional PCR approaches, including extraction of DNA from individual *Acarapis* sp. mites.

Evans *et al.* (2007) use amplification of the mitochondrial cytochrome oxidase I gene (Navajas *et al.,* 1996) to detect *Acarapis* sp., with sequencing of the amplicon to provide species-level determination of *A. woodi, A. externus* and *A. dorsalis*. Nested-PCR primers are also available that may enhance the sensitivity of detection.

*Table 2. PCR primer sequences*

|  |  |  |  |
| --- | --- | --- | --- |
| Primer/Probe | Sequence (5’-3’) | Amplicon length\* | Region |
| MitCOI.F | AGT-TTT-AGC-AGG-AGC-AAT-TAC-TAT | 559 bp\* | Cytochrome oxidase I |
| MitCOI.R | TAC-AGC-TCC-TAT-AGA-TAA-AA |  |  |
| AcwdCOI.F | TCA-ATT-TCA-GCC-TTT-TAT-TCA-AGA | 377 bp\* | Cytochrome oxidase I |
| AcwdCOI.R | AAA-ACA-TAA-TGA-AAA-TGA-GCT-ACA-ACA |  |  |
| \*Inferred from primer alignment with genbank accessions, KX790788 and LC512730. | | | |

If using a commercial PCR kit, the required reagents may already be included. Check and follow the manufacturer’s instructions.

PCR reactions using MitCOI primers (Evans *et al.,* 2007; Navajas *et al.,* 1996) are set up in a total volume of 25 μl, as follows:

i) 1–5 μl template DNA (see Section B.1.3.1);

ii) 0.2 μM forward (MitCOI.F) and reverse primer (MitCOI.R);

iii) 1 mM dNTPs;

iv) 2 mM MgCl2;

v) 1 U of Taq polymerase in the appropriate PCR buffer

Using the following thermocycling conditions: 30 cycles of 94°C (1 minute), 52°C (1 minute), and 72°C (1 minute); and a final cycle of 72°C (5 minutes).

Subsequent nested-PCR on the amplicon using the same reaction conditions but with internal forward (AcwdCOI.F) and reverse (AcwdCOI.R) primers may provide greater sensitivity for detection, where Evans *et al.* (2007) report this nested-PCR approach can be used to determine the *Acarapis* species of a single isolated mite. The molecular weights of the amplicons can be determined by electrophoresis in a 0.8% agarose gel and staining with a DNA-intercalating dye. Amplicon size is not sufficient for *Acarapis* species-level assignment, and sequencing of the amplicon with comparison to reference sequences on genetic databases is required to be confident when assigning a detection to one of the three *Acarapis* species.

Amplicons can be purified using a commercially-available method, such as resin-binding or enzymatic-digestion of <100 bp fragments, and then amplicons sequenced using Sanger-method, or an alternative sequencing approach. Amplicon sequence should be aligned with *Acarapis* sp. sequences from genetic databases and a phylogenetic tree constructed to determine the closest relative.

An alternative series of primers is available for the detection of *Acarapis* sp. (Kojima *et al.,* 2011) but they have not been trialled against *A. dorsalis* and therefore have unknown utility in distinguishing *A. dorsalis* from *A. woodi*.

~~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).~~

1.3.3. Real-time PCR (Delmiglio *et al.*, 2016)

Specific detection of *A. woodi* using real-time PCR can be achieved by amplification of a 113 nt single variable region within the mitochondrial cytochrome oxidase I gene (COI) (Delmiglio *et al.,* 2016). A small proportion of genetically different *A. externus* have been reported to cross-react with this assay. Therefore, if a real-time PCR positive is recorded in a population of bees where *A. woodi* has not been reported before, then a confirmatory test using microscopy should be performed.

*Table 3. Primer sequences for real-time PCR*

|  |  |  |  |
| --- | --- | --- | --- |
| Primer/Probe | Sequence (5’-3’) | Amplicon length | Region |
| aw\_F1-flap | AAT-AAA-TCA-TAA-TGA-TAT-CCC-AAT-TAT-CTG-AGT-AAT-G | 113 bp | Cytochrome oxidase I |
| aw\_R3 | AAT-ATC-TGT-CAT-GAA-GAA-TAA-TGT-C |  |  |
| aw\_LNAprobe | 6-FAM-ACC[+T]GT[+C]AA[+T]CC[+A]CCTAC-BHQ1 |  |  |
| \*[+] locked nucleic acid bases | | | |

If using a commercial PCR kit the required reagents may already be included. Check and follow the manufacturer’s instructions.

PCR reactions (modified from Delmiglio *et al.,* 2016) are set up in a total volume of 10 μl, as follows:

i) 1 μl template DNA (see Section B.1.3.1);

ii) 0.3 μM forward (aw\_F1-flap) and reverse primer (aw\_R3);

iii) 0.1 μM probe (aw\_LNAprobe);

iv) 1 mM dNTPs;

v) 3.5 mM MgCl2;

vi) 0.3 μg bovine serum albumin

vii) 1 U of Taq polymerase in the appropriate PCR buffer

Using the following thermocycling conditions: 95°C (2 minutes), 35 cycles of 95°C (10 seconds), 59°C (45 seconds).

c. REQUIREMENTS FOR VACCINES ~~AND DIAGNOSTIC BIOLOGICALS~~

There are no ~~biological products~~ vaccines 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 & McCreadie, 2001).~~

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~~Illustrations by Diana Sammataro~~ Photographs courtesy of Qing Hai Fan ~~and Wolfgang Ritter~~ are reproduced with their permission.

~~An FAO publication,~~ *~~Honey Bee Diseases and Pests: a Practical Guide~~*~~, W. Ritter & P. Akratanakul (eds). Agricultural and Food Engineering Technical Report No. 4. FAO, Rome, Italy, 42 pp. ISSN 1814-1137 TC/D/A0849/E, is available free of charge at:~~

[~~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 is an OIE Reference Laboratory for Infestation of honey bees with *Acarapis woodi*  
(see OIE Web site for the most up-to-date list:  
<https://www.oie.int/fr/ce-que-nous-proposons/reseau-dexpertise/laboratoires-de-reference/#ui-id-3>).   
Please contact the OIE Reference Laboratory for any further information on   
diagnostic tests and reagents for acarapisosis of honey bees.

**NB:** First adopted in 1989 as Acariasis of honey bees; Most recent updates adopted in 2008.