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Definition of tracheal acariosis

Acariosis is a parasitic, contagious disease of the respiratory apparatus of the adult honeybee due to the mite *Acarapis woodi* Rennie. It is a cosmopolitan disease, ranging as an epizooty in North America and as an enzooty elsewhere.

General epidemiology

In 1919, British researchers, while investigating dramatic honeybee (*Apis mellifera* L.) colony losses occurring on the Isle of Wight, discovered the bee parasite named *Tarsonemus woodi* by Rennie in 1921, later reclassified as *Acarapis woodi*. Over the next 30 years, it was identified in numerous European countries (Switzerland, Russia, Scotland, Czechoslovakia, France, Spain). In Spain, the disease was found for the first time in 1949 in the eastern Mediterranean region and its low incidence in this country was recently studied by Orantes *et al.* (1997a,b).

The mite then spread to the American continent, first to Argentina in 1968. In 1980, it was found in Colombia and Mexico. More recently, the mites have been found in the USA by Delfinado-Baker (1984) where the honeybees proved to be more susceptible than European honeybees.

Outside the European and American continents, *A. woodi* has been identified in *Apis mellifera adansoni* in Zaire (Benoit, 1959) and Egypt. Shigh (1957) found the mite in India. It is usually assumed that the mite was introduced into Africa and Asia by *A. mellifera* imported from Europe. In recent reviews of the status of world bee health, including parasitic mites (tracheal mite and Varroa), Matheson (1993, 1996) reported that only the Australian continent remains free of this parasite.

Although always considered a disease, the pathological effects of the mite are the subject of much controversy. According to Bailey and Ball (1991), no conclusive experiments were undertaken to measure the effect of the mite until Bailey (1958, 1961), showed that overwintered infested bees died sooner than uninfested individuals and that the difference became statistically significant from about March onwards. Field experiments have shown that infested bees in summer die only a little sooner than uninfested individuals, but they seemed normal until they died (Bailey and Lee, 1959). Gary and Page (1989) concluded from their experiments that "there is a strong indication that tracheal mite infestations do not have a detectable economic effect on honeybee colonies when brood-rearing is active and honey production is under way".

In contrast, Morgenthaler (1931) suggested that high infestation levels eventually cause colony death. Giordani (1965, 1977) observed a shortened life-span in infested honeybees and a loss of infested colonies in spring if 30% or more of individuals were infested. Thus, it is apparent that overwintering bees will die sooner if high levels of infestation occur, as has recently been demonstrated by Eischen *et al.* (1989) and Otis and Scott-Dupree (1992). A three year survey of the incidence of Varroa and the tracheal mite in colonies in Wisconsin, conducted between 1992 and 1995, concluded that the number of colonies infested with these mites remained fairly constant, with a very high overwintering mortality rate (between 10-45%) (Phibbs, 1996).

A fact that is now evident, is a general decline in the prevalence of *A. woodi* in bees in Europe and Asia. Bailey and Perry (1982) indicate a progressive decrease in the frequency of parasitisation of

colonies in England and Wales from 1925 to 1980. The degree of infestation by this mite has also continued to decrease progressively over the last few years, even before the widespread use of treatments against the Varroa mite. However, the importance of the disease is not the same all over the world, because acariosis represents a serious problem in some areas, such as North America. The first outbreaks were reported there in 1980 and 1984 and the mite populations recorded in these initial infestations were greater than those reported later in areas where the mites had been present for a longer period of time (Otis *et al.*, 1988; Otis, 1990). The colonies seem more susceptible than European ones. Royce and Rossignol (1990) suggest that persistent parasitism overcomes a colony's ability to compensate for losses, leading to its sudden decline and death. Parasitism is, almost by definition, associated with morbidity and mortality of the host, and these authors conclude that mortality occurs at every level of infestation and increases with density. However, this point of view is open to debate.

Etiology

Pathogenic agent

Classification

Acarapis spp. are included in:

Order: Acariforms
Suborder: Actinedida (Prostigmata; Krantz, 1978; Lindquist, 1986)
Family: Tarsonemidae
Genus: *Acarapis*
Species:
A. woodi Rennie, 1921
A. dorsalis Morgenthaler, 1931
A. externus Morgenthaler, 1931

Characteristics of the Acariforms order

Mites without a visible stigma posterior to coxae II, with an idiosomal gland complex next to palps, coxae often fused into the ventral body wall, forming coxisternal regions delimited by epimera; number of legs occasionally reduced.

Characteristics of the Actinedida suborder

The Actinedida is a large and complex group of terrestrial, aquatic and marine predators, phytophagous, saprophagous, and parasitic mites. These mites are weakly or incompletely sclerotised forms. When an internal respiratory system exists, it may open out through paired stigmata at or near the bases of the chelicerae. The genital apparatus and its opening are often close to or contiguous to the venter of the opisthosoma. Sexual dimorphism is rarely pronounced, minor differences in genital valve size or in internal genital structure often provide the only clues in differentiating males from females.

The ontogenic development of these mites includes a unique hexapod larva, the structural development in successive stages (nymphs) tends to be gradual and subtle. Additional characteristics which apply to Actinedida are that the coxae of the legs are distinguished by a coxal field or they are joined to the venter, with specialized sensilla (= trichobotria) often present on the back. The Actinedidae are cosmopolitan mites and are virtually unlimited in habitat, their remarkable morphological variety is reflected in the great number of families and superfamilies with numerous species (Krantz, 1978).

The characteristics of the Tarsonemidae family

The Tarsonemids are considered mites associated with insects, other mites, and plants. They are grouped in over a dozen genera. We refer only to the mites associated with insects and exclusively to the Acarapis genus with three recognized species, all parasites of bees (*Apis* spp.) (Krantz, 1978).

Three species were distinguished in the *Acarapis* genus: *Acarapis woodi* (Rennie) [originally described as *Tarsonemus woodi* (Rennie *et al.*, 1921)], *A. externus* and *A. dorsalis*.

Rennie found *A. woodi* in the prothoracic spiracles and occasionally in the abdominal and thoracic air sacs of bees. It is the causal agent of tracheal acariosis. The remaining two species of *Acarapis* live externally on honeybees and are not considered as serious pests (De Jong *et al.*, 1982).

Anatomy

The *A. woodi* female (Fig. 1c,d) is 120-180 µm in length whilst the male (Fig. 1a,b) is 96-102 µm. The body is oval, widest between the second and third pair of legs, and is whitish or shiny pearly white, with a smooth cuticle; a few long hairs are present on the body and legs (Sammataro and Needham, 1996). The morphology of the gnathosoma consists of two cheliceral stylet digits that are grooved medially along their entire length and can be brought tightly together to form a tube-like structure. The cheliceral stylets and labrum may be clamped together during feeding forming a canal allowing the haemolymph to be sucked up. *Acarapis woodi* is an intermittent feeder and, as such, haemolymph and particulate matter would tend to dry and stick to or clog the stylets following feeding (Bruce and Kethley, 1993).

Biology and life cycle

Acarapis woodi lives in the first thoracic tracheae, opening through the prothoracic spiracles and occasionally in the abdominal and thoracic air sacs of bees. Mated female mites leave the trachea in which they developed and migrate to the tip of the bee's body hair, awaiting the hair contact of another passing bee. The most susceptible bees are the youngest. It is generally believed that after a bee is nine days old, the spiracular opening becomes impassable, preventing the entry of mites. However, it also seems that bees less than 4 days old, called callow bees, are chemically more attractive to female mites because of the presence of certain hydrocarbons. The female mite moves immediately to the spiracles and into the trachea. A single female mite lays 5-7 eggs after 2 days. The eggs hatch after 3 or 4 days into a hexapod larval stage, lasting 6 to 7 days (Fig. 1e,f). A nymphal stage follows. The males mature 11-12 days after hatching and females after 14-15 days (De Jong *et al.*, 1982; Royce *et al.*, 1988; Bailey and Ball, 1991; Sammataro and Needham, 1996). The total duration of the development cycle (at least 21 days) may explain the reduced incidence of severe infestations in summer bees, as these have a life span of approximately one month. In contrast, overwintering bees may live 5 or 6 months and more than 6 mite generations may be possible in the tracheae, which represents some hundreds of individuals.

Spread and transmission

All stages (eggs, larvae, nymphs and adults) live exclusively in the tracheae, except mated females, which can leave them to contaminate another bee. However, the survival of these free females is of a few hours duration, depending on the temperature, relative humidity or state of nourishment of the mite (Sammataro and Needham, 1996). Consequently, the dissemination of mites inside and outside the colony, is only due to direct contact between bees.

The spread of the tracheal mite is presumably caused by migratory beekeepers and the sale of infested packages of bees and queens, causing a rapid dispersal of the mite, as occurred recently in the USA and Canada. Within apiaries, infestation of one colony can result in the infestation of other neighbouring colonies, presumably via drifting bees. It is known that queens, workers, and drones are all susceptible to infestation by *A. woodi.* The mite has a certain preference for drones, and this favours dissemination to other colonies and mite population growth within the colony. A greater number of migratory female mites and a greater total number of all mite stages are found in drones in comparison to worker bees (Dawicke *et al.*, 1992). It can be said that *A. woodi* behaves like a parasitic disease of an enzootic nature (Bailey and Lee, 1959), except in North America, where it spreads as an epizooty.

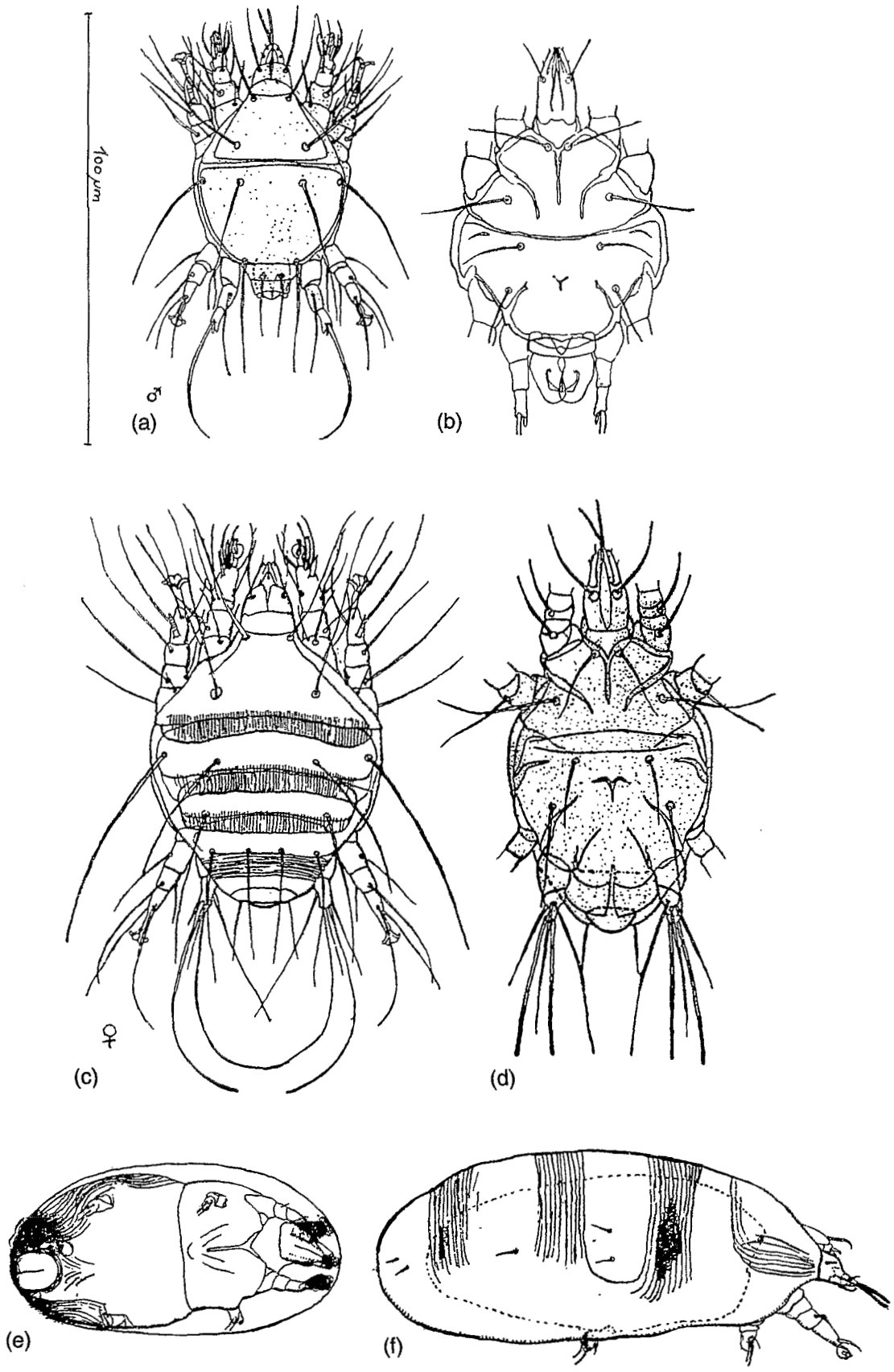

Fig. 1 *Acarapis woodi* Rennie. (a) Male dorsal face. (b) Male ventral face. (c) Female dorsal face. (d) Female ventral face. (e) Larva within its eggshell. (f) Larva.

Pathogenesis

A shortened lifespan of bees is generally related to the unilateral or bilateral infestation of the tracheae. Royce and Rossignol (1990) showed that the parasite load of bees is influenced by stress, the age of the bees, the strength of the colony and the nectar flow, the intensity of which influences the life span of the foragers.

Several factors may contribute to the pathogenic action of *A. woodi:* (i) as an endoparasitic mite it withdraws haemolymph from its host; (ii) mites crowding together may block the first pair of thoracic tracheae reducing the diffusion of oxygen to the flight muscles and to the brain; and (iii) because of the vectoring of pathogens, such as bacteria and viruses (Scott-Dupree *et al.*, 1995). Bees parasitised by the mite are more susceptible to infection with other diseases such as septicaemia (*Pseudomonas apiseptica*) and viruses such as chronic bee paralysis virus (CPV) (Bailey, 1965). More recently, *Varroa jacobsoni* and *A. woodi* have been implicated as potential vectors of different bee viruses. The ability of *A. woodi* to transmit bee viruses has not been demonstrated but the presence of the mites may be associated with an increase in the incidence of certain viruses such as acute paralysis virus (APV), CPV or Kashmir Bee Virus (KBV). *Acarapis woodi* could be an opportunist, as in weakened colonies, without a queen, more severe infestations appear. Climate also seems to influence mite population dynamics. In cold climates such as England, Switzerland, etc., young bees born at the end of summer can readily become infested, because they often are confined to the hive.

Clinical diagnosis, differential diagnosis

There are no specific clinical symptoms of acariosis, meaning that infested bees appear very similar to healthy ones. Infested queens can live for many years (Fyg, 1964). During summer, indications attributed to infestation are that some bees are incapable of flying and remain at the hive entrance, some have dislocated wings, and others crawl around in the surrounding vegetation. Generally, *A. woodi* causes economic losses in colonies when the level of infestation exceeds 25-30% of bees, causing a reduction of the brood area, an increase in the consumption of honey stores, and increased colony mortality (Eischen *et al.*, 1988). During winter, acariosis may be suspected when many dead bees are observed in or near the hive, several months after cluster formation.

The other species of *Acarapis* (*A. externus* and *A. dorsalis*) may be distinguished from each other, although they both live externally on the thorax. These three closely related mites are morphologically distinct species. Each one attaches itself to a specific, restricted part of the bee. *Acarapis externus,* known as "the neck mite", is found only in the area where the head and thorax join. *Acarapis dorsalis* lives on the thorax in a groove between the mesoscutum and mesoscutellum. It may also be found at the bases of the wings and the forepart of the abdomen. A third species of external mite, *A. vagans* has been proposed, but this species has been relegated to a synonym of *A. externus* (Bailey, 1963). These two external *Acarapis* species have been virtually ignored as regards their life cycle and pathogenic status (Burgett *et al.*, 1989). These authors reported the concurrence of the *Acarapis* species complex at colony and individual host levels, comparing worker and queen infestation rates in honeybees in North West America.

Sample collection

Generally, 25 carefully selected sick bees are enough to ensure diagnosis. However, the size of the sample should be larger for an epidemiological survey in suspected apiaries.

Laboratory diagnosis

The early stages of infestation probably go undetected, but as the mite population in the tracheae increases, these become darker in colour because of the accumulation of mite faeces and feeding scars. This discolouration means that mite population is detectable without the aid of a microscope. Although mites may sometimes be found in air sacs in the thorax and abdomen, diagnosis can usually be made by checking only the prothoracic tracheae. All stages of development, eggs, larvae and adults may be found in a trachea at one time (De Jong *et al.*, 1982).

The standard method of detecting *A. woodi* in an individual bee from a sick colony is to remove the prothoracic trunk by dissection to expose the two largest tracheae of the body. The analysis is based on a pooled sample of prothoracic trunks from 25-50 sick bees. To estimate the percentage of individuals infested, bees may be collected from the surfaces of 2 randomly selected combs inside the hive (Calderone and Shimanuki, 1992).

A variant of this technique is a thoracic disk dissection or slicing/KOH (Shimanuki and Knox, 1991), where tracheal mite infestation levels are determined using a thoracic disk obtained after individual dissection. Between 25-50 thoracic disks are placed in 10% KOH in small vials and incubated at 35°C for two days. Once the discs have become clear, the material is mounted between a slide and cover slip in an alcohol/glycerine mixture and observed under the microscope. The mite infestation level is expressed as the number of bees, out of a sample of 25, that are infested.

A variation on the standard method of dissection is rapid differential staining, which distinguishes the live mites from the dead. Liu (1995) uses thiazoly blue tetrazolium (MIT solution: 5 mg of stain / 5 ml distilled water) to perfuse the tracheae mounted on a glass slide, over which a cover slip is placed. The staining reaction takes place immediately, or after a few seconds, precipitating at the site where there is enzymatic activity. The live mites take on a bright purple colour because they imbibe the stain solution, whilst the dead mites turn a greenish yellow.

A bulk extraction technique can be used as a general screen for the diagnosis of acarine disease (Colin, 1979). In this method the wings and the legs are removed from the thoraces, which are placed in an homogeniser and ground 3 times for several seconds at 10,000 rpm. A large number of honeybee samples, comprising 100-200 bees, can readily be examined. The triturate is strained through a 0.8 mm mesh sieve, which is rinsed through with water, the final volume being about 50 ml. The suspension is then centrifuged at about 1500 rpm for 5 minutes, the supernatant discarded and the sediment observed. A few drops of pure lactic acid are added to the preparation and left for 10 min, causing the lysis of muscle cells. The mites can be seen and counted under a microscope. The extraction technique is not only rapid (15 min), making it possible to process a large number of samples of bees, but it is also much more sensitive than the dissection technique, revealing the presence of weak infestations and it could be used in epidemiological surveys.

More recently other methods have been used in the diagnosis of *A. woodi*.:

(i) A practical enzyme-linked immunosorbent assay (ELISA) has been developed by Gordon *et al.* (1993) using bee samples stored in saturated sodium chloride solution (>30% pickling salt). The results of the ELISA showed a good correlation with the dissection method in samples that had over 5% mite infestation (number of infested bees per 100 bees). This method can analyse large numbers of apiary samples with approximately one tenth of the labour required by the conventional dissection method. Moreover, the costs are lower and there is more specificity and sensitivity of results.

(ii) Mozes-Koch and Gerson (1997) described a method based on the visualization of the large amounts of guanine found in the mite faeces. After grinding the bee thoraces and cell lysis, the extract is centrifuged and the supernatant deposited on a TLC plate. Guanine spots are visualized under UV light. The sensitivity of the method is similar to that of dissection.

Treatment and prophylaxis

The methods of control used against *A. woodi* are those classically used against mites; acaricides are very effective. However, the application of these products has some disadvantages, such as the cost, the risk of contaminating stored honey, the risk to bees and the risk of the development of mites resistant to the acaricides.

In Europe between 1930 and 1940, nitrobenzene fumigating mixtures were widely used by beekeepers, until their efficacy and safety came to be questioned. In 1960 tests were carried out with numerous acaricide products; of these, menthol appeared to be the most effective, with the additional advantage of being a fairly harmless product to bees and one which does not contaminate honey.

The spread of *A. woodi* in North America in 1984 has led to a renewed interest in the application of menthol. Best results are obtained in spring time, with doses of 50 g enclosed in a porous plastic screen packet being placed over the cluster. This method was adopted by the USA Environmental Protection Agency in 1989 and by the Agriculture Ministry of Canada in 1992 (Delaplane, 1996).

The use of alternative products against *Varroa jacobsoni* such as formic acid and menthol (Nelson, 1994; Nelson *et al.*, 1994) or vegetable oils and menthol (Delaplane, 1992) has been successful against *A. woodi*. The formula recommended for applying formic acid is 30 ml of 65% acid in three applications at weekly intervals during the spring (Nelson *et al.*, 1994). Sammataro *et al.* (1994) proved the efficiency of oil patties (solid vegetable oil and white sugar) administered in 300 g amounts per colony.

The different susceptibilities of bees to tracheal acariosis, which presents fairly stabilised infestations in Europe and Asia, in contrast to the USA and Canada, means that programmes of selection and improvement in the tolerance of bees to the disease, like those implemented against varroosis, have yet to be established.

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