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in

Colin M.E. (ed.), Ball B.V. (ed.), Kilani M. (ed.).
Bee disease diagnosis

Zaragoza : CIHEAM
Options Méditerranéennes : Série B. Etudes et Recherches; n. 25

1999
pages 91-97

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B. V. Ball
IACR-Rothamsted, Harpenden, Herts., AL5 2JQ, UK

Definition of the disease

Sacbrood is an infectious disease of honeybees caused by multiplication of sacbrood virus in larvae, propupae and adult bees. The specific symptoms of infection produced in propupae are a reliable means of diagnosis.

General epidemiology

Sacbrood was ably studied first by White (1917) in the USA and he established that the disease was caused by a filterable agent. He gave detailed descriptions of the symptoms and showed that it was readily distinguished from other diseases caused by bacteria, such as European foulbrood, with which it was frequently confused by inexperienced observers.

Although primarily a disease of larvae, sacbrood virus (SBV) also multiplies in young adult bees without causing obvious disease (Bailey, 1969) and this enables the virus to persist in bee colonies from year to year. Sacbrood is common in colonies, but few show a large percentage of diseased larvae because adult bees detect and remove most larvae in the early stages of infection and infected adult bees are prevented from transmitting much SBV by behavioural changes (Bailey and Fernando, 1972). Outbreaks of the disease most commonly occur in spring and early summer or when forage is limited, probably because when young bees are few, the usual division of labour according to age, is least well developed.

In Britain, over 80% of diseased larvae that were free of bacterial pathogens were infected with SBV and up to 30% of healthy colonies contained a few larvae killed by the virus (Bailey, 1967). In Australia, SBV is reported to be the most common honeybee virus and occasionally causes serious losses of brood (Dall, 1985); it was detected as an inapparent infection in more that 40% of healthy worker pupae (Anderson and Gibbs, 1988), as an overt infection in more than 90% of colonies (Anderson,1983) and in 10% of samples of dead queen larvae (Anderson, 1993). In Fiji, SBV caused some brood mortality in 30% of colonies in August (Simpson, 1983) and in 79% during October and November (Anderson, 1990), which suggests that in Oceania and elsewhere the seasonal incidence of SBV may follow a pattern similar to that seen in temperate regions.

SBV appears to be the most widely distributed of all the honeybee viruses, occurring in colonies of *Apis mellifera* on every continent. However, this may reflect the ease and reliability of field diagnosis by symptoms. Most records are of overt disease in brood, but in Poland (Topolska *et al.*, 1995) and Germany (Ball and Allen, 1988) SBV was detected in large amounts in dead adult bees from colonies infested with *Varroa jacobsoni*. The ability of the mite to transmit SBV from severely infected to healthy pupae has been demonstrated experimentally (Ball, 1989), but the possible role of the mite as a vector of SBV to adult bees is unclear.

A strain of sacbrood virus has been isolated from larvae of *Apis cerana* from Thailand (TSBV) (Bailey *et al.*, 1982). It is closely related serologically to SBV of *A. mellifera* and causes similar symptoms, but has distinctive properties.

Etiology

Pathogenic agent

The particles of sacbrood virus are icosahedral in shape and 30 nm in diameter. Unlike SBV particles, those of TSBV aggregate when exposed to buffers of low (about 0.01) molarity. In the absence of sodium ethylene diaminetetracetate (EDTA) TSBV is susceptible to penetration by neutral phosphotungstate stain, particles appearing empty when viewed by electron microscopy. Emptying is quicker when Mg^{2+} is added. SBV particles also appear empty in the presence of Mg^{2+} but are much less sensitive than those of TSBV.

TSBV sediments at 160 S in 0.1 M KCl but at only 150 S in 0.01 M phosphate. It produces three close but well-defined bands of protein of M_r 30, 34 and 39 x 10^3 on 5% SDS polyacrylamide gels. By contrast, SBV sediments at 160 S in 0.01 M phosphate and on 5% SDS polyacrylamide gels gives one broad band of M_r 29 to 34 x 10^3 which only separates into three proteins on gels of higher concentration. Both SBV and TSBV have the same buoyant density of 1.35 g cm^{-3} in CsCl and contain single stranded RNA.

Multiplication

In larvae

Each larva killed by sacbrood virus contains about 10^{12} particles, almost 1% of the body weight and the median lethal dose (LD_{50}) by feeding for young larvae, which are most susceptible, is between 10^5 and 10^6 particles (Bailey *et al.*, 1964). Much virus is in the fluid between the "sac" and the body of the larva. This fluid resembles the ecdysial fluid produced by healthy individuals but, unlike these, larvae with sacbrood cannot dissolve the thick endocuticle, so they cannot shed their final skin and the fluid accumulates abnormally beneath it.

Only about 100 particles of SBV are required to cause infection by injection into white eyed pupae and this is the most convenient means of propagating the virus in the laboratory. Mortality of pupae due to multiplication of SBV occasionally occurs in nature in colonies infested with *V. jacobsoni* because the mite can act as a virus vector (Ball, 1989).

In adult bees

SBV multiplies by injection into adult bees and more virus accumulates in the heads of infected bees than elsewhere in their bodies. Much is in the brain and in the hypopharyngeal glands and an extract of the head of an infected adult contains about 10^9 particles. There is some evidence that the fat body is also a site of virus multiplication (Lee and Furgala, 1967b; Bailey, 1968).

Adult bees less than 8 days old are readily infected by ingesting the virus, the infective dose for them by mouth being about 10^8 particles (Bailey, 1971). Curiously, much more SBV multiplies in infected male (drone) adult bees than in workers. This was unexpected as drones, unlike workers, have no developed glands in their heads such as hypopharyngeal glands in which much virus accumulates. About 0.1 mg of virus accumulates in the head of an infected drone, at least 100 times the amount in the head of an infected worker and most of it is in the brain.

Spread and transmission

The infectivity of SBV is lost after a few weeks in larval remains and experiments to spread the disease by placing combs containing many dead larvae into healthy colonies were unsuccessful (Hitchcock, 1966). Under natural circumstances sacbrood abates and usually disappears spontaneously during summer even though larvae are easily infected by feeding them the virus at any time of the year. This is probably because adult bees quickly detect and eject diseased larvae from the colony. Their action is probably responsible for the rapid disappearance of sacbrood in summer when bee colonies are reaching their maximum size and the ratio of larvae to adult bees is diminishing. It may well be

responsible also for the mechanical spread of infection causing epizootics when colonies are growing and the proportion of larvae to adult bees is large. However, sacbrood is perennial and common in spite of its virtual disappearance in summer, its failure to remain infective for long in larval remains and the absence of larvae in temperate regions in winter. The gap is almost certainly bridged in nature by adult bees, in which SBV multiplies without causing obvious disease.

The youngest workers are the most susceptible and probably become infected in nature mostly when they remove larvae killed by sacbrood. During this activity, they ingest liquid constituents of the larvae, especially ecdysial fluid, when they rupture them. Within a day after young adult bees ingest such material, much SBV begins to collect in their hypopharyngeal glands. Therefore, infected nurse bees probably transmit sacbrood when they feed larvae with secretions from these glands. However, infected adult bees cannot be very efficient vectors, or else they must usually be prevented from transmitting the virus because sacbrood subsides spontaneously in summer. Much evidence shows that bees are usually prevented from transmitting the virus by behavioural changes (Bailey and Fernando, 1972). Infected young bees cease to eat pollen, which is their only source of protein, and soon cease to feed and tend larvae. They fly and forage, but do so much earlier in life than usual and they almost all fail to collect pollen. The few that do collect pollen bring SBV back in their pollen loads, each load containing about a million virus particles, probably secreted by the bees from their glands into the liquid they add to pollen when they collect it. If many infected bees gathered pollen, which is quickly consumed by young susceptible individuals, much virus would soon reach and kill more larvae. SBV put into nectar is much diluted among the rest and is quickly and widely distributed within the bee colony, whereas pollen loads remain entirely within the cell of the comb where they are placed. Any virus in pollen would remain concentrated and more likely to infect a bee than virus in nectar. Studies in New Zealand (Anderson and Giacon, 1992) suggest that the collection of only nectar by adult bees infected with SBV may adversely affect the pollination of crop plants, particularly those that flower in early summer and produce only pollen.

Further effects of SBV which also decrease the chances of its spread, are a decreased metabolic rate and shortened life of infected adult bees; they become more susceptible to chilling and loss in the field or from the bee cluster, especially in winter. Overall, the effects of SBV infection in adult bees are similar to premature senility. The behavioural changes SBV infection induces in adult bees divert most of its potentially disastrous effects and ensure its own survival.

The only other obvious way that SBV might be transmitted is through the queen, but many attempts to show this have failed (Bailey, 1968, 1970). The virus was injected into laying queens or fed to young individuals, which successfully mated and produced larvae. None of the queens transmitted sacbrood, although infectivity and serological tests with extracts of their heads showed that sacbrood virus had multiplied in them.

Pathogenesis

Many particles, sometimes in crystalline array, have been seen in the cytoplasm of fat, muscle and tracheal-end cells of larvae that had ingested SBV under laboratory conditions (Lee and Furgala, 1967a). Since infected larvae fail to shed their final skin and pupate, infection may prevent the formation of chitinase. Much virus may multiply in the epidermis or the dermal moulting glands where the enzyme is normally secreted.

Similar particles have also been seen in the cytoplasm of the fat bodies of apparently healthy adult bees that had been injected with the virus (Lee and Furgala, 1967b).

Clinical diagnosis

Sacbrood can be reliably diagnosed in the field by the characteristic symptoms produced in developing brood of both *A. mellifera* and *A. cerana*. Healthy honeybee larvae pupate 4 days after they have been sealed in their cell, but larvae infected with SBV fail to pupate and remain, stretched on their backs with their head towards the cell capping. Fluid then accumulates between the body of a diseased larva and its tough unshed skin (Fig. 1b), and the body colour of the larva changes from a pearly white to a pale yellow. After it has died a few days later, it becomes dark brown. The head and thoracic regions darken first and at this stage the signs are most distinctive and specific (Fig. 1c). Finally, the larva dries

down to a flattened gondola shaped scale. This is easily removed and does not cling to the base of the cell, as do scales of larvae killed by American foulbrood.

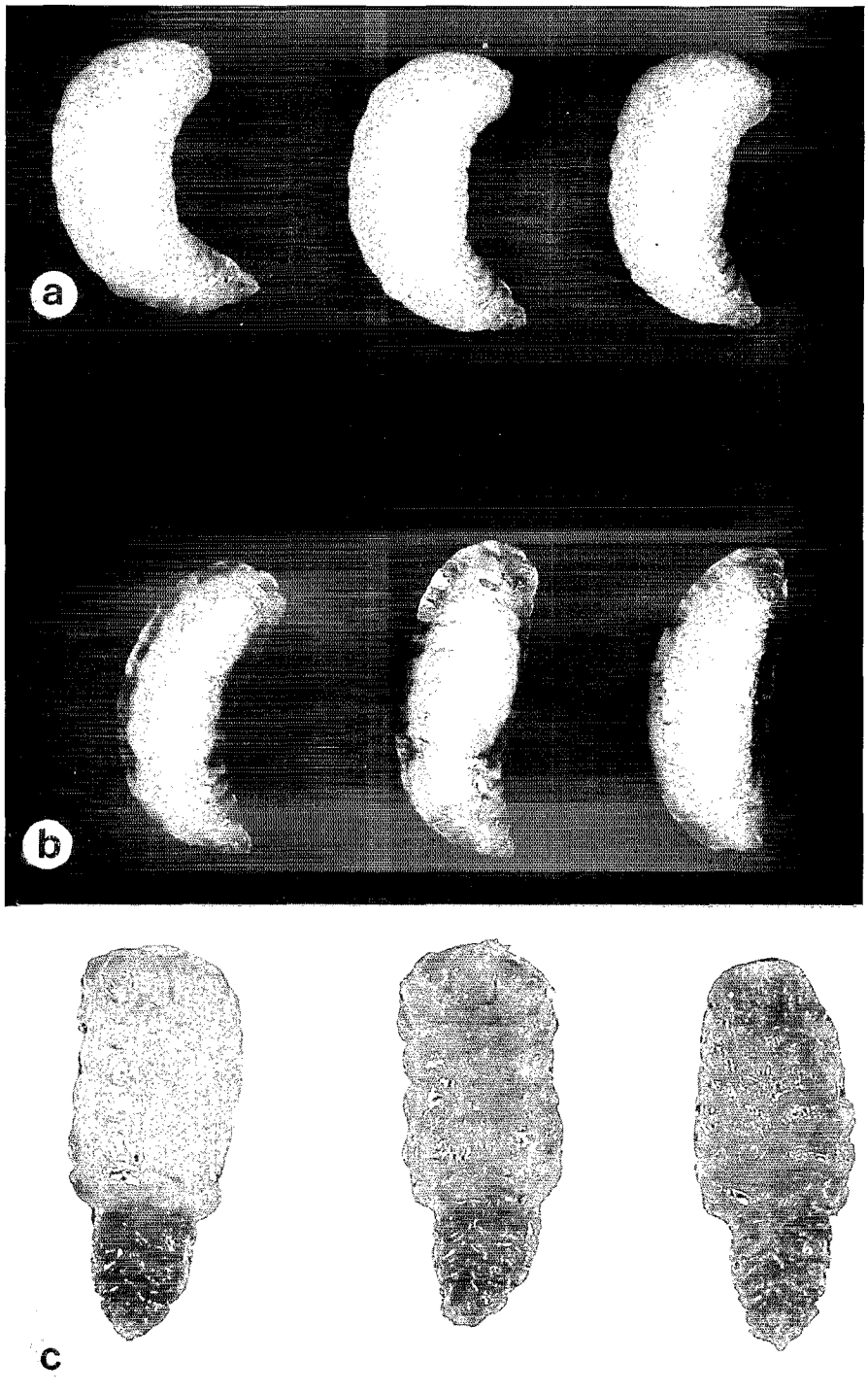

Fig. 1. Sacbrood: (a) healthy individuals; (b) sac-like appearance of diseased larvae showing the typical accumulation of fluid between the body of the larva and its unshed skin; (c) dead larvae start to darken from the head region and eventually dry down to a flattened gondola-shaped scale.

Sample collection, preservation and despatch to laboratory

Dried scales of infected larvae may be collected from the comb and placed in a stout, ventilated container for transportation. Fragile larvae at the "sac-like" stage may be picked out of the comb and smeared over a small area on a glass microscope slide or sheet of thin paper and allowed to air dry. The dried remains or smears may later be resuspended in a little saline or buffer and used for serological testing.

Naturally infected material is best preserved in the deep freeze at -20°C.

Laboratory diagnosis

Identification of the pathogen

Microscopical examination

Dried larval remains may be extracted in 0.5 ml potassium phosphate buffer (PB) of suitable molarity + 0.02% diethyldithiocarbamate (DIECA) and a nigrosin smear prepared for light microscopy to exclude the presence of pathogenic bacteria. A few drops of CCl_4 are then added and the extract cleared by centrifugation at 8000 g for 10 min. The supernatant can be negatively stained with sodium phosphotungstate and examined in the electron microscope for the presence of virus particles.

Immunological techniques

The most reliable and simplest method of laboratory diagnosis is serological. Immunodiffusion tests can be done with dried scales or smears, extracted as described above. The agar used for immunodiffusion is the same as that described for the testing of chronic paralysis virus.

Isolation and precise identification

SBV may be extracted from naturally infected material and purified in the same manner as described for chronic paralysis virus. Potassium phosphate buffer of 0.5 M containing EDTA is suitable for the extraction and purification of TSBV. Larval extracts contain much more proteinaceous material than those of adult bees and fewer larvae or a larger volume of extraction buffer will yield cleaner preparations. Virus yields from larvae are large and host protein contamination should be minimised to prevent substantial loss of SBV during the isolation and purification process.

The purified virus can be characterised by polyacrylamide gel electrophoresis, identified by the use of specific virus antiserum or by feeding to larvae, when the characteristic symptoms of infection should be produced.

Experimental inoculation

SBV may be propagated by inoculating the food of 2 day old larvae with a preparation containing 10^7 particles. However, the virus multiplies readily by injection into honeybee pupae at the white-eyed stage of development and these are more convenient for laboratory experiments. As few as 100 particles will cause infection by injection into pupae.

Routine diagnosis

Immunodiffusion using a specific antiserum is the quickest, simplest and least expensive means of routine diagnosis.

Treatments

At present, and in common with nearly all virus diseases of animals, there are no known direct treatments for virus infections of bees. However, as with chronic paralysis virus, there is some evidence that strains of bees differ in their susceptibility to sacbrood (Bailey, 1967). Colonies of bees headed by imported queens showed a significant increase in larval mortality due to SBV than local colonies maintained under the same conditions. Replacing the queen with a young vigorous individual may be beneficial.

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