Paralysis

Ball B.V.

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Paralysis

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

Definition of the disease

Paralysis is a contagiously transmitted infection of adult bees caused by multiplication of chronic paralysis virus (CPV) primarily in the brain, nerve ganglia and in the hind-gut epithelium. It is the only common disease of adult bees that has striking, well-defined symptoms characterised by trembling, flightless individuals crawling on the ground beneath the colony entrance.

General epidemiology

Although paralysis was first described more than a hundred years ago, the cause was not identified until 1963 when Bailey *et al.* (1963) isolated and characterised the virus. Some twenty years earlier, before research on the small non-occluded viruses of insects had become established, Burnside (1945) showed that bacteria-free filtrates of bees with paralysis in the USA were infective. The infectivity was destroyed by heating and it seems likely that he was working with the same virus identified as the cause of paralysis in Britain, the USA and then throughout the rest of the world. CPV has been detected serologically in extracts of dead adult *Apis mellifera* from every continent except South America and its presence has not been confirmed in the Caribbean region. In the Canary Islands, CPV and its satellite virus, chronic paralysis virus associate (CPVA), were recently detected in more that 75% of samples of dead adult bees collected in spring and early summer. The cause of this high incidence of CPV is unknown, but it may be associated with high colony densities. Alternatively, it may be linked to hereditary factors as studies in the USA indicated that strains of honeybee can be selected that are more susceptible than normal to CPV (Kulincevic and Rothenbuhler, 1975; Rinderer *et al.*, 1975) and inbreeding amongst colonies that have paralysis maintains a high incidence of the disease. In some regions of mainland Europe outbreaks of paralysis seem to occur when colonies are taken to forested areas to forage on honey dew (Giauffret and Lambert, 1972).

The incidence of chronic paralysis virus has declined in Britain from about 8% of samples submitted by beekeepers when records began in 1947, to less than 2% by 1963 (Bailey *et al.*, 1983). The rate of decrease is very closely and significantly associated with the decline in the number of colonies of bees during the same period. Exactly the same significant regression on the numbers of colonies occurred during the same period with infestation by the tracheal mite *Acarapis woodi* (Bailey and Perry, 1982).

When there are too many colonies locally for the available forage, bees remain in their colonies more than usual. Under such circumstances pathogens such as CPV, which are transmitted by close contact between live bees, would tend to increase in incidence. It is significant that the incidence of paralysis remains high in the Black Forest region of Germany (Ball and Allen, 1988) where the population density of bee colonies is considerably greater than the already high national average. Spells of bad weather that restrict foraging, especially by curtailing major nectar flows unusually early, have a similar effect to that caused by placing too many colonies at one site and may also lead to outbreaks of CPV.

Just these type of conditions also favour the spread of *A. woodi*. The mite is dependent on close contact between old infested bees and young susceptible individuals. The fact that similar circumstances affect the prevalence of both of these pathogens has probably led to some confusion in the past; the symptoms of CPV infection have been attributed to the mite. The spectacular losses of bees in Britain between 1905 and 1919, which were first reported on the Isle of Wight, coincided with the discovery of *A. woodi* and the symptoms of crawling flightless bees have become inextricably linked with the parasite. However, investigations in the 1960's showed that in severely affected colonies in summer, crawling bees

and apparently healthy bees were about equally infested with mites, but the crawling bees were all infected with CPV (Bailey, 1969).

Etiology

Pathogenic agent

The particles of chronic paralysis virus are anisometric, mostly ellipsoidal in outline and often with a small irregular protuberance at one end. A wide range of shapes and sizes of particles occur, including rings, figure of eights, branching rods and lengths up to 640 nm. Three or four components are resolvable in sucrose gradients or by analytical centrifugation, with sedimentation coefficients of 82, 97 to 106, 110 to 124 and 125 to 136 S. These components contain particles that differ considerably in size but have a modal width of about 20 nm. Despite the diversity of particle sizes all have the same buoyant density of 1.33 g cm^{-3} in CsCl. The capsids are composed of a single coat polypeptide of M_r 23.5 x 10^3.

CPV has five single-stranded RNA components: two larger RNAs designated 1 (M_r 1.35 x 10^6; 4200 nucleotides) and 2 (M_r 0.9 x 10^6; 2800 nucleotides) and three smaller RNAs designated 3a, 3b and 3c, each with M_r 0.35 x 10^6 (1100 nucleotides) (Overton *et al.*, 1982). CPV adopts a flexible packaging arrangement in which individual RNA species may be packaged separately or in various combinations incorporated together.

Chronic paralysis virus associate (CPVA) has 17 nm isometric particles which are serologically unrelated to CPV but are frequently found associated with it in natural infections. The capsids have a single polypeptide of M_r 15 x 10^3 and particles have a buoyant density of 1.38 g cm^{-3} in CsCl. CPVA does not multiply when injected alone into bees and it interferes with the multiplication of CPV in individual bees, inhibiting particularly the relative amount of the longest, most infective particles (Ball *et al.*, 1985). It is more evident in queens than in workers (Bailey *et al.*, 1980) and may be of some significance in, or a reflection of, the defence mechanisms of individuals against paralysis. CPVA is the first well-documented example of a satellite virus in insects.

CPVA has three single-stranded RNA components designated A, B and C each with M_r of 0.35 x 10^6 (1100 nucleotides). Gel electrophoretic and oligonucleotide analyses have shown that RNAs 3a, 3b and 3c of CPV are very similar to and probably identical to RNAs A, B and C, respectively (i.e. these three RNAs appear to be encapsidated in both CPV and CPVA particles) (Overton *et al.*, 1982).

Multiplication

The LD_{50} of CPV injected into the haemolymph of adult bees is about 10^2 particles; when applied to bees as a spray in water it is between 10^9 and 10^{10} and when fed it is more than 10^{10} particles. The sprayed virus probably infects via the tracheae and the true LD_{50} is almost certainly fewer particles than those in the inoculum. Bees infected by any method become flightless and paralytic between 5 and 7 days later, when kept between 30°C and 35°C, and die a day or so later. At the lower temperature, the bees take longer to die, yet more virus multiplies in bees at 30°C than at 35°C, which is about the maximum temperature in a bee colony. Many millions of particles of CPV can be extracted from one bee with paralysis and the virus multiplies in many tissues although the brain, nerve ganglia and the hind-gut are primary sites of replication.

Spread and transmission

The distended honey sac of a paralytic bee contains up to about 10^{11} particles, but the virus is not transmitted in food sufficiently to cause disease readily. However, the pollen collected by apparently normal, but infected individuals of a colony suffering from paralysis, contains much CPV and this may serve as a source of infection for young individuals. The virus is probably added to the pollen in the gland secretions during collection and storage since CPV occurs commonly in the thoracic, hypopharyngeal and post-cerebral glands of adult bees of seemingly healthy colonies. Perhaps of greater significance is the fact that CPV occurs commonly in colonies that are accepted by beekeepers as healthy. Sensitive

infectivity tests have shown that apparently normal, live bees often contain some of the virus (Fig. 1) and it is evenly distributed between the heads and abdomens (Bailey *et al.*, 1981)

Very few particles are required to cause paralysis when the virus enters the body via a wound, probably because this permits access to vital tissues. In nature, this is most likely when adult bees are brushing close against each other, which breaks many hairs or bristles from the cuticle, to expose live tissue. This can be simulated in the laboratory by rubbing virus preparations on the freshly "shaved" body surface of bees or by keeping adult bees from seemingly healthy colonies crowded in cages at 35°C (Bailey *et al.*, 1983). The same circumstances can be expected to increase the likelihood that bees ingest broken hairs, which increases their chances of contracting paralysis when they also ingest the virus (Rinderer and Rothenbuhler, 1975; Rinderer and Green, 1976).

There is no particular time of year when paralysis, or the virus in seemingly healthy colonies, becomes most common, but the crowding of individuals during winter is probably unimportant for the transmission of paralysis because bees are then least active within the cluster and so least likely to damage each other. However, unseasonable weather in summer can cause unusual crowding of active individuals. This may explain the relationship between poor seasons and increased paralysis. Similarly, fluctuations in summer weather could well explain the unpredictability of local paralysis outbreaks. Kulincevic *et al.*, (1973) observed that paralysis symptoms occurred sooner in bees when they were deprived of their queen. The foraging activity of such bees decreases and they become agitated, perhaps suffering more physical damage than usual within the colony.

Factors affecting disease outbreaks

Like many of the other viruses of bees, CPV commonly persists in live individuals as an inapparent infection throughout the year (Bailey *et al.*, 1981). However, the incidence of disease outbreaks is low and erratic. This suggests that other factors, in addition to the presence of the virus in bee populations, lead to paralysis outbreaks.

There is some evidence that susceptibility to infection is closely limited by inherited factors (see Section on General Epidemiology) and inbreeding with colonies that have paralysis maintains a higher incidence of the disease than replacing the queen with one from elsewhere. Differences in defence mechanisms may also be genetically linked since the multiplication of the associate particle (CPVA), which interferes with the replication of CPV, inhibiting particularly the longest, most infective particles, is more evident in queens than in workers (Bailey *et al.*, 1980).

Environmental factors possibly play a part in disease outbreaks, as they seem to have done when strains of bee susceptible to paralysis showed more disease when kept in uncultivated "forest" regions of Germany than elsewhere (Drescher, 1964).

Pathogenesis

Many millions of particles of CPV can be extracted from one bee with paralysis and about half the number of particles are in the head, which is only about one tenth of the total body weight. However, the only particles resembling those of CPV seen in ultrathin sections of brain tissue were also seen in sections of brain from healthy bees and may have been microtubules or other components of normal nerve tissues (Bailey and Milne, 1969). Similar particles were seen in densely but randomly packed groups either free or within vesicles in the cytoplasm of thoracic and abdominal ganglia of paralysed bees, but not in the ganglia of healthy bees or in the cytoplasm of fat or muscle tissue (Lee and Furgala, 1965).

Light microscope sections of the hind-gut epithelium of paralytic bees show basophilic cytoplasmic bodies (Morison's cell inclusions, Morison, 1936) (Fig. 2), which seem specific to the disease. Giauffret *et al.* (1967) found similar basophilic granules in the cytoplasm of the cells of the thoracic ganglia of paralytic bees and determined histochemically that the granules were ribonucleic acid (RNA). The cellular lesions in nerve tissue associated with multiplication of the virus may explain the nervous symptoms of the disease.

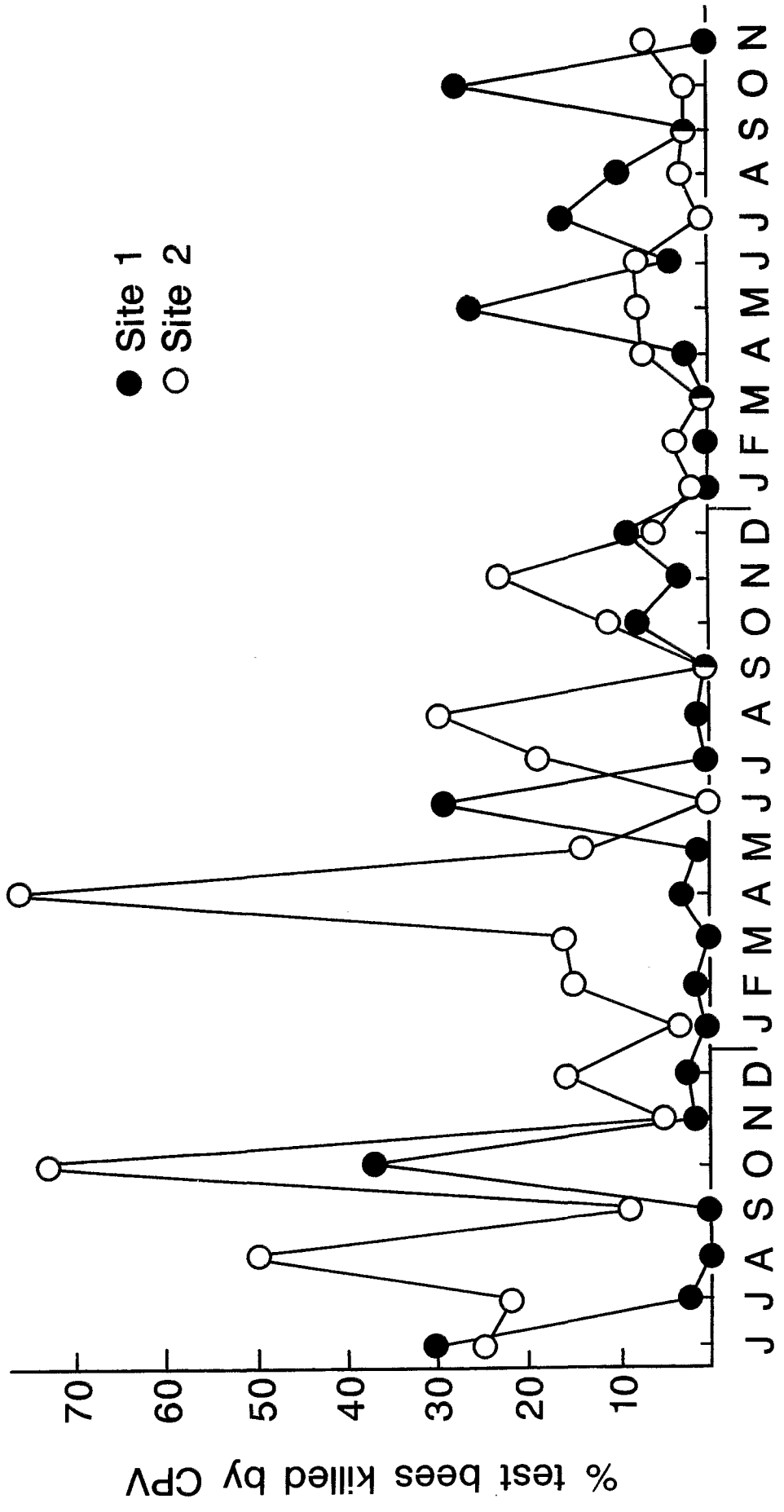

Fig. 1. The mean percentage of test bees killed by chronic paralysis virus when injected with extracts of 20 live, seemingly healthy adult bees from each of two normal colonies at site 1 (o) and two or three colonies at site 2 (o). (From Bailey *et al.*, 1981).

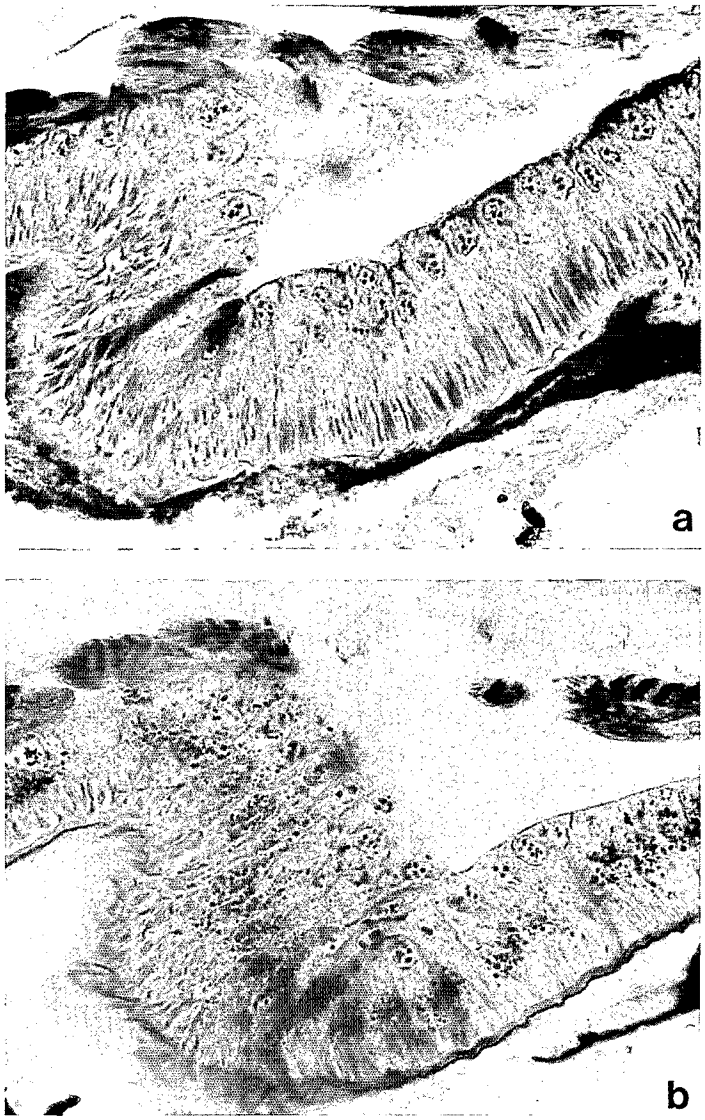

Fig. 2. Longitudinal sections of gut epithelium immediately posterior to the Malpighian tubules of (a) a healthy bee and (b) a bee infected with chronic paralysis virus, showing Morison's cell inclusions (Heidenhain's iron haematoxylin).

Clinical diagnosis

There are two distinct sets of symptoms or syndromes. One of these includes an abnormal trembling motion of the wings and bodies of the infected bees which are unable to fly but often crawl on the ground and up grass stems, sometimes in masses of thousands of individuals. Frequently they huddle together on top of the cluster in the hive. The sick bees have a bloated abdomen, which is caused by distension of the honey sac with liquid (Fig. 3b). Infected individuals die within a few days. Severely affected colonies suddenly collapse, often within a week and particularly at the height of summer, leaving the queen with a few bees on neglected combs. This kind of paralysis seems to correspond to the disease long known in Europe as Waldtrachkrankheit, so named because it often seems to be associated with nectar gathered from the forests.

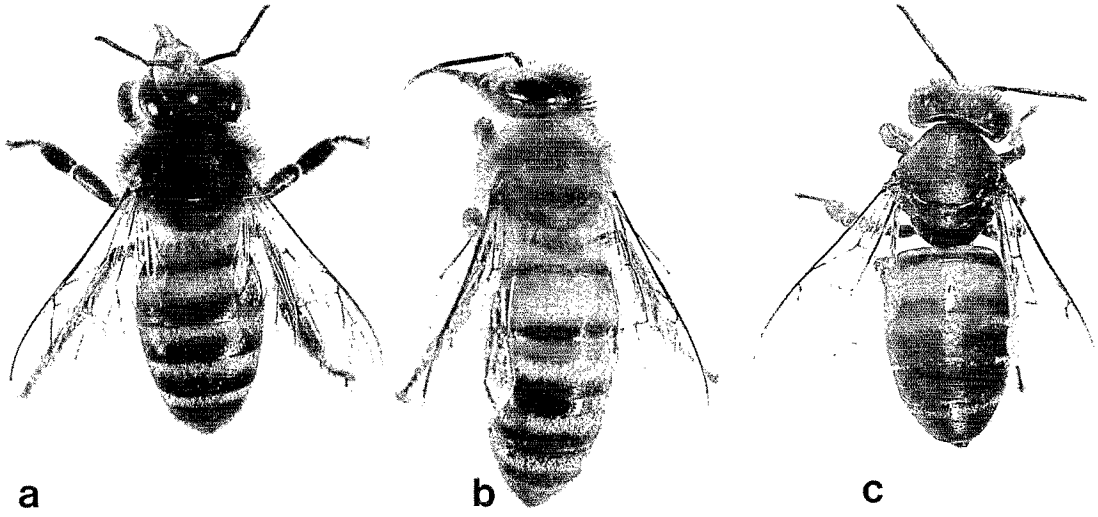

Fig. 3. (a) Healthy adult bee. (b) Bee infected with chronic paralysis virus, type 1 syndrome. The bloated honey-sac causes distension of the abdomen. (c) Bee infected with chronic paralysis virus, type 2 syndrome. The loss of cuticular hairs makes the bee appear black and shiny.

The other syndrome has been called a variety of names - "black robbers", or "little blacks" in Britain, Schwarzucht and mal noir or mal nero in continental Europe and "hairless black syndrome" in the USA. At first the affected bees can fly, but they become hairless, appearing dark or almost black and shiny which makes them seem smaller than usual (Fig. 3c). They suffer nibbling attacks by other bees in the colony, which may account for their hairlessness and they may be prevented from returning to the colony by guard bees, which makes them appear like robber bees. In a few days they become flightless, tremble and soon die. Both syndromes can occur in one colony, but one or the other soon predominates.

The difference between the syndromes is probably an expression of genetic differences between individual bees. There is considerable evidence that susceptibility to the multiplication of CPV is closely limited by several inherited qualities of bees and some variation of these qualities might well lead to variations in the symptoms.

Sample collection, preservation and despatch to laboratory

Adult bees showing the symptoms described above should be collected from beneath the entrances of affected colonies. Freshly dead individuals or bees exhibiting the "crawling" symptoms are best collected in a stout, ventilated container such as a matchbox and mailed to a diagnostic laboratory. Any live individuals may first be killed by freezing. Thirty to fifty bees are adequate for diagnostic purposes. It is essential that the sample is not wrapped in polythene or put into an air-tight container as rapid decomposition will take place. Material may be stored in the deep freeze (-20°C) until despatch.

Laboratory diagnosis

Identification of the pathogen

Microscopical examination

An extract of bees suspected to be infected with chronic paralysis virus may be examined in the electron microscope for the presence of the distinctive virus particles. However, semi- purified extracts

contain much bee protein which makes recognition of the irregularly shaped particles difficult and makes identification more than usually unreliable.

Immunological techniques

The most reliable and simplest method of diagnosis is serological. Immunodiffusion tests can be done with purified virus or with crude extracts made by grinding the head or abdomen of a bee in 0.05 ml of 0.85% saline or 0.01 M potassium phosphate buffer, pH 7.0 (PB) + a few drops of diethyl ether in a small conical tube. The agar for the test contains 0.05 M PB + 0.005 M sodium ethylene diaminetetra-acetate (EDTA) + 0.02% sodium azide.

Isolation and precise identification

The virus may be isolated from naturally infected material by grinding 30 bees in 30 ml of 0.01 M PB pH 7.0 + 0.02% diethyldithiocarbamate (DIECA) + 0.1 vol. diethyl ether followed by emulsification with 0.1 vol. carbon tetrachloride (CCl_4). After filtration through cotton or muslin to remove large particles of debris the extract is centrifuged at 8000 g for 10 min. The supernatant fluid is then centrifuged at 75,000 g for 3.5 h and the virus pellet resuspended in PB. After a further cycle of low and high speed centrifugation the virus may be purified by centrifugation down sucrose gradients. For the purification of virus to be used for antiserum production two cycles of centrifugation down sucrose followed by purification on caesium chloride gradients, are recommended.

The purified virus can be characterised by polyacrylamide gel electrophoresis when a single capsid protein of M_r 23.5 x 10^3 should be resolved.

Experimental inoculation

Chronic paralysis virus is readily propagated by injection into either adult bees or pupae at the white eyed stage of development. As few as 100 particles are required to cause infection by this route. A crude extract of the heads of a few paralytic bees may be used as an inoculum but to exclude other viruses which may be present it is always best to first inject a dilution series of the preparation. The greatest dilution of the original extract which causes 100% infection should be used for subsequent propagation.

Routine diagnosis

Immunodiffusion using a specific antiserum is the quickest, simplest and least expensive means of virus diagnosis (see Section on Immunological Techniques).

Treatments

There are no direct means of treating virus infections but as susceptibility to CPV seems to be influenced by hereditary factors replacing the queen of an affected colony with one from elsewhere rather than allowing colonies to constantly requeen themselves and become inbred, is good management practice.

Maintaining the number of colonies between the optimum and equilibrium densities appropriate to the available nectar of the region would also be expected to reduce the incidence of outbreaks of CPV. The optimum density produces most honey per colony without the need for the beekeeper to feed supplementary sugar. The equilibrium density is the maximum number of colonies that is self-sufficient in a region and they produce no honey for the beekeeper unless he replaces any that he takes, with sugar that he feeds. When the numbers of colonies exceed the equilibrium density they can be maintained only when sugar is supplied additional to that required to replace any honey that is harvested. In these circumstances, bees become underemployed and the ensuing increase in contact between individuals

within their colonies enables enzootic pests and diseases that are transmitted contagiously, like CPV, to spread and multiply more than is usual.

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