Microbial diseases of bees

Bees come under attack from a wide range of microbes. **Travis R. Glare** and **Maureen O'Callaghan** consider the role of bee diseases in the worldwide decline of these key ecosystem providers.

The humble bee has a special place in our lives. Essential for pollination of many plants, including food crops, the provider of honey and royal jelly and many other products, bees are important to the economies of countries and, as ecosystem service providers, have few equals among insects. There is a quote, often attributed to Einstein, suggesting that if all the bees disappeared then humans would follow within 4 years. While this is perhaps an overstatement, a recent estimate of the contribution of insect pollination, mainly by bees, to agriculture was €153 bn.

There are many threats to bee survival, including the risk of disease caused by micro-organisms. The vast majority of our knowledge of bee diseases focuses on the honey bee, *Apis mellifera,* although there are actually over 20,000 species, both stingless and stinging, from those with solitary lifestyles to complex societies such as honey bee hives.

Viruses, fungi, protozoa and bacteria are all known to cause infections in bees, sometimes leading to collapse of colonies, and causing serious threats to the bee-keeping industry. Bees have two distinct life forms, brood (egg, larva and pupal stages which develop within the hive) and adult. Most diseases are specific to just one of these life stages. While the list of diseases is quite long, only a few are of serious concern to apiculturists.

Major disease of bees

Various evocative names, based on the visual symptoms of diseased bees, are used to describe the most problematic diseases, for example foulbrood, sacbrood and chalkbrood.

American foulbrood (AFB) is caused by the spore-forming bacterium *Paenibacillus larvae.* The disease was first described in 1769. AFB is probably the most virulent disease of honey bee brood and is capable of causing the collapse of bee colonies. The name describes the symptoms of diseased brood; infected cells become discoloured, sunken and there is a characteristic smell. Adult bees are not affected. This disease has traditionally been treated with the antibiotic oxytetracycline, but some bacterial strains have developed resistance to this and the disease is now increasing in prevalence around the world. Although the disease was first described over 200 years ago, much is still unknown about this infection. A German research team has only recently discovered how the bacteria kill larvae, by building up to very high numbers in the gut before bursting into the haemocoel, causing death.

European foulbrood (EFB) is caused by the non-spore-forming bacterium *Melissococcus* (=*Streptococcus*) *plutonius*. Unlike AFB, EFB usually affects unsealed brood, and the recently dead larvae present as watery and yellowish brown cadavers twisted inside the cell. Despite the importance of EFB, the disease is poorly understood, but like AFB, has increased in prevalence in recent years.

Of the fungi known to infect bees, species of the fungus *Ascosphaera* are the most common. *Ascosphaera apis* is the causative agent of the well known chalkbrood disease in honey bees, so called because of the chalky appearance of infected brood. Chalkbrood is usually considered a minor disease of bees, as is stonebrood, caused by the fungus *Aspergillus.*

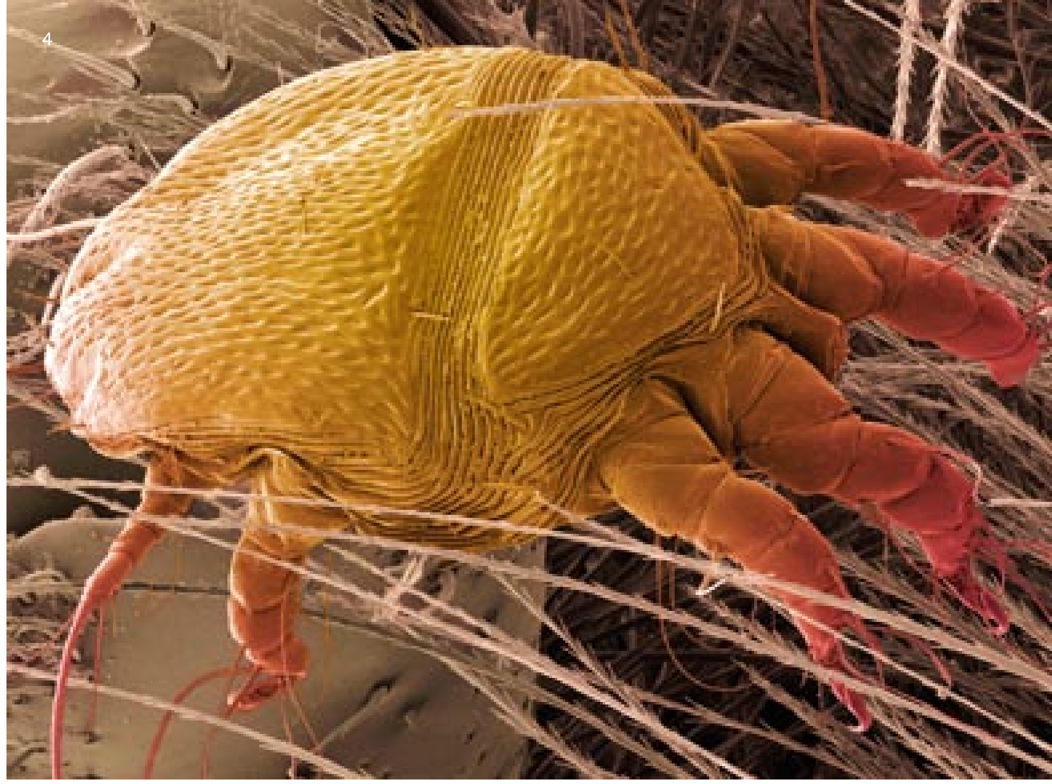
Viruses can also cause devastation in bee colonies. At least 18 types of viruses have been found infecting honey bees alone. Going by some delightfully descriptive names (e.g. deformed wing virus, chronic paralysis virus, acute bee paralysis, sacbrood virus and black queen cell virus), these viruses range from non-lethal to causing significant mortality in nests. One of the more interesting aspects of viral disease is that many infections cause no obvious symptoms much of

▲ 1. Sunken brood capping with holes suggests American foulbrood (AFB). *Zachary Huang, Michigan State University, USA*

2. A dead larva killed by AFB usually forms a 'false tougue' pointing upward. *M.V. Smith, University of Guelph, Canada*

3. Larvae showing typical European foulbrood (EFB) symptoms. These larvae show yellow streaks. *M.V. Smith, University of Guelph, Canada*

◀ A honey bee (*Apis mellifera*) feeding. *Dr John Brackenbury / Science Photo Library*



▲ 1. Chalkbrood, whereby the larvae become mouldy with white hyphae, then hardened to be similar to pieces of white chalk. This disease is mostly considered a stress disease, only occuring in weak, or in otherwise stressed colonies. *M.V. Smith, University of Guelph, Canada*

▲ 2. Close-up of the head of a larva killed by Sacbrood. *M.V. Smith, University of Guelph, Canada*

▶ 3. A honey bee (*Apis mellifera*) with two *Varroa jacobsoni* mites on its thorax. *Maryann Frazier / Science Photo Library*

▶ 4. Coloured SEM of a *Varroa* sp. honey bee mite. *Steve Gschmeissner / Science Photo Library*

the time. Kashmir bee virus can persist in bee populations causing no obvious symptoms, only to explode into lethal infections, possibly triggered by bee stress factors such as attack by the *Varroa* mite. *Varroa* mites are parasites on honey bees and have spread around most of the world, causing significant losses in hives as well acting as vectors for some viruses. Virus infections can be hard to detect and diagnose, as symptoms, if any, resemble other mortality causes.

Emerging diseases

Microbes are constantly evolving, leading to the emergence of new strains with novel pathogenic abilities. For example, some honey bee diseases appear to have widened their host range in recent years. Protozoa of the genus *Nosema* infect many invertebrates, and individual species are typically quite limited in their host range. *Nosema apis* has long been recognized as causing one of the most important diseases in adult honey bees, infecting the guts of adult bees. However, *Nosema ceranae*, thought to infect only the Asiatic or Eastern honey bee, *Apis cerana*, has recently been shown to infect the European honey bee, *A. mellifera*. Evidence is emerging of recent spread of *N. ceranae* in honey bee populations around the world since around 1998. There is ongoing risk that other highly virulent diseases of honey bees will emerge.

Bees under stress

There is still so much we don't know about how combinations of microbial diseases, parasites, pollution and urbanization are affecting bees. Colony Collapse Disorder (CCD) is the name given to the recent widespread mortality of worker (adult) honey bees on several continents, especially North America. The sometimes startlingly high mortality rates have not been attributed to a particular cause. Several recent studies suggest that some colony collapse is caused by a combination of disease and the parasitic attentions of *Varroa* mites. Various studies have found that prevalence of viral and protozoan diseases is higher in *Varroa*-infected hives and *Varroa* is thought to be capable of acting as a vector for pathogenic microbes. In some cases, viral diseases that do not usually cause high mortality are rampant in hives with *Varroa* or have been associated with CCD. Israeli Acute Paralysis Virus (IAPV) was recently found to be the most consistent indicator of CCD, as well as Kashmir bee virus and *Nosema* spp. However, no causal link has been made between IAPV and CCD. As with all living things, stress increases the susceptibility of the host to a pathogen, and if bees are under stress, disease can be more debilitating. Whether CCD is only caused by the interaction between a specific stress such as *Varroa* and some diseases, or widespread interaction between a number of stresses is unclear. Combinations of stresses could include multiple diseases. Using molecular techniques, several studies have shown multiple diseases infecting single bees. So diseases, some of which may not normally cause death, could act together to kill. Additionally, nutritional stress can exacerbate the incidence of pathogens.

How do diseases spread?

How diseases spread between individuals is still largely unknown. Both horizontal transmission (where viruses are transmitted among individuals of the same generation), and vertical transmission (where the disease is passed from queens to their offspring) are known. Modern molecular-based techniques have contributed significantly to our understanding, allowing investigation of whether pathogens are present inside eggs, and by establishing the relatedness of occurrences of disease in different hives. It is obvious how some diseases spread; the presence of large numbers of spores, whether fungal, bacterial or protozoan, inside a hive will contaminate brood and/or workers that come in contact. However, some more unusual routes have also been demonstrated. DNA from viral pathogens has been detected in the semen of honey bee drones, suggesting that mating may spread some disease both horizontally and vertically. Pathogens can be transmitted in bees and sometimes in bee products, prompting many countries to closely regulate the importation of bees and honey.

How do bees defend themselves from disease?

The high density populations and conditions within the bee colony (enclosed, moist, dark, poorly ventilated) are ideal for the outbreak and spread of disease. Fortunately, because bees are constantly exposed to pathogenic micro-organisms, they have evolved strategies to resist infection. The cuticle of bees acts as a barrier to penetration, and immune system-based defence can prevent infection of many minor pathogens. However, the recent completion of the honey bee genome sequence has shown that they have only about a third of the number of known immunologically related genes when compared to flies or mosquitoes, suggesting that bees rely less on individual immunity than most insects.

Bees, in common with a number of other social insects, have well developed behavioural responses to combat disease. These behavioural responses are collectively known as hygienic behaviour and include recognition and removal of diseased brood by worker bees. Bee species, and even different hives of the same species, differ in their ability to perform hygienic behaviours, with some colonies far superior to others. Some strains of bees are capable of recognizing diseased brood well before it is a threat to the hive, and remove diseased individuals. In some cases, the task of disposing of diseased insects falls to specialist 'undertaker bees' that appear to be old workers. Bees are also assisted in resisting disease by propolis, present in the plant resins collected by honey bees and used as a sealant in the hives. Propolis is known for its antimicrobial properties.

What hope is there for the future?

With increasing prevalence of disease, unexplained disappearance of bees on some continents and the emergence of new diseases, bee populations are under threat. Fortunately, increasing sophistication of research methods is allowing unprecedented understanding and insights into bee pathology by allowing detection of cryptic infections, generation of epidemiological data and detailed understanding of bee–pathogen interaction. With increasing understanding comes a better appreciation of the role of disease and methods for reducing impact. For example, the presence of Kashmir bee virus has been detected in the UK, despite never being identified as a cause of infection in UK bees based on visual symptoms. This suggests a potential non-lethal role for this virus. Detection of virus associated with CCD may also lead to a cure. Separating the various factors affecting bee colonies will allow the causal agents to be directly treated. Without understanding of the cause of CCD, no cure will be possible, but when the factors are known, many large and small mitigations can be used.

The risk is that with increasing pressure from civilization, bees could suffer increasingly from threats and stress, including increasing prevalence of disease. A better understanding of bee dynamics and the development of mitigations is urgently required.

Travis R. Glare & Maureen O'Callaghan
AgResearch, Lincoln, Private Bag 4749, Christchurch, New Zealand (t +64 3 321 8825; e travis.glare@agresearch.co.nz)

Further reading

Bailey, L. (1968). Honey bee pathology. *Annu Rev Entomol* 13, 191–212.

Chen, Y.P. & Siede, R. (2007). Honey bee viruses. *Adv Virus Res* 70, 33–80.

Cox-Foster, D.L. & others (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* 318, 283–287.

Evans, J.D. & others (2006). Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Mol Biol* 15, 645–656.

Paxton, R.J. & others (2007). *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. *Apidologie* 38, 558–565.

Wilkins, S. & others (2007). The incidence of honey bee pests and diseases in England and Wales. *Pest Manage Sci* 63, 1062–1068.