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MINI-REVIEW

Honey bee pathology: current threats to honey bees and beekeeping

Elke Genersch

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Abstract Managed honey bees are the most important commercial pollinators of those crops which depend on animal pollination for reproduction and which account for 35% of the global food production. Hence, they are vital for an economic, sustainable agriculture and for food security. In addition, honey bees also pollinate a variety of wild flowers and, therefore, contribute to the biodiversity of many ecosystems. Honey and other hive products are, at least economically and ecologically rather, by-products of beekeeping. Due to this outstanding role of honey bees, severe and inexplicable honey bee colony losses, which have been reported recently to be steadily increasing, have attracted much attention and stimulated many research activities. Although the phenomenon "decline of honey bees" is far from being finally solved, consensus exists that pests and pathogens are the single most important cause of otherwise inexplicable colony losses. This review will focus on selected bee pathogens and parasites which have been demonstrated to be involved in colony losses in different regions of the world and which, therefore, are considered current threats to honey bees and beekeeping.

Keywords Honey bees · Varroa · Virus · Nosema · European Foulbrood · Colony losses

Introduction

Honey bees and honey bee health have become a major topic recently due to the important role honey bees play in pollination and food production. Although it is often suggested that mankind will not survive for long once honeybees are gone, this is rather exaggerating the role of honey bees for human nutrition since primary food production, and especially our staple foods, is independent of animal (insects, birds, bats) pollination. Our staple foods (e.g. wheat, corn, rice, potatoes) are wind- or passively self-pollinated or are vegetatively propagated, meaning that their production does not increase with animal pollinators (Ghazoul 2005a; Klein et al. 2007; Richards 2001). Yet, the production of many fruits, vegetables and stimulant crops contributing to a healthy diet depends on animal pollination. Therefore, thinking beyond caloric intake, for a balanced and nutritionally valuable human diet, animal pollination is essential (Steffan-Dewenter et al. 2005), leading us back to the unquestionable importance of honey bees for food production. To roughly put it into figures: Crops which are independent of animal pollination account for ~65% of global food production, leaving as much as ~35% depending on pollinating animals (Klein et al. 2007). Of commercial pollination, 90% is performed by managed honey bees (*Apis mellifera*): Therefore, although mankind will not die if honey bees go extinct, they are still the most important commercial pollinators worldwide, and the human diet would be greatly impoverished if honey bee populations decline or disappear (Steffan-Dewenter et al. 2005).

Due to this link between honey bees and global food security, the decline of managed honey bees and the loss of wild pollinators are of increasing concern. Although there is an ongoing discussion whether or not we are really facing a "global pollinator crisis," there is no question that many solitary and social bees are declining (Allsopp et al. 2008; Ghazoul 2005a, b; Steffan-Dewenter et al. 2005). A recent metastudy revealed that although the global number of managed honey bee colonies increased by 45% over the last

E. Genersch (✉)
Institute for Bee Research,
Friedrich-Engels-Str. 32,
16540 Hohen Neuendorf, Germany
e-mail: elke.genersch@rz.hu-berlin.de

five decades, there is a marked decrease of such colonies in Europe and North America at the same time (Aizen and Harder 2009). Since crop pollination in North America and Europe is highly and increasingly dependent on honey bees (Aizen et al. 2008), this development is alarming, although not all countries are equally affected. In Europe, for instance, Austria, Germany, Sweden and Switzerland are facing a critical decrease in the number of managed honey bee colonies, while other European countries like Greece, Italy, Portugal, and Spain even report a considerable increase (vanEngelsdorp and Meixner 2010).

Many factors may account for the rise and fall in the apicultural sector, and socio-economic factors for sure do not play an underpart. The decline in the number of honey bee colonies observed during the 1990s in Europe can be related to the political and economic upheaval in Eastern Europe caused by the Soviet collapse (Aizen and Harder 2009). In many countries of the Soviet bloc, honey had served as a second currency, and, thus, many people had been motivated to keep bees. When the economic system changed in the early 1990s, honey lost its relevance, and people who had kept their bees for economic reasons gave up beekeeping or reduced the number of managed bee hives (vanEngelsdorp and Meixner 2010). The influence of the profitability of beekeeping on the managed colony populations can also be observed in the USA where changes in the honey price and the beekeepers' income from renting colonies for pollination are significantly related to changes in national colony numbers (Sumner and Boriss 2006; vanEngelsdorp and Meixner 2010). These economic links imply that sustainably increasing the economic benefit for beekeepers or the profitability of beekeeping operations should contribute to a lasting stabilization or even an increase in the number of managed bee hives in those countries currently facing a decline in honey bee populations.

However, although the long-term (positive and negative) development of colony numbers over the last five decades may have been influenced by economic factors, in recent years we are confronted by a steady decline in honey bee populations and/or catastrophic winter losses in some regions of the world which elude this explanation (Genersch et al. 2010; vanEngelsdorp et al. 2007, 2008, 2009). Honey bees are susceptible to a variety of diseases and environmental threats, some of which have increased significantly over the last 5 to 10 years. While it is impossible to identify a single factor which on its own can account for all colony losses in all regions of the world over a given time period, it is clear that several biological and environmental factors acting alone or in combination have the potential to cause premature colony mortality by adversely affecting colony health and lifespan. Among these factors, certain honey bee diseases and parasites have been shown to play a significant role in increased honey bee colony mortality and in the described colony losses. The ectoparasitic mite *Varroa destructor* as well as the bee pathogenic viruses acute bee paralysis virus (ABPV) and deformed wing virus (DWV) are implicated in winter losses in Germany (Genersch et al. 2010); Israeli acute paralysis virus (IAPV) has been identified as a marker of dramatic colony losses termed colony collapse disorder (CCD) in the USA (Cox-Foster et al. 2007; vanEngelsdorp et al. 2009); the microsporidium *Nosema ceranae* is causing severe colony mortality in Spain (Higes et al. 2006, 2008a); *Melissococcus plutonius*, the etiological agent of European Foulbrood, is of increasing concern in Switzerland and the UK (Roetschi et al. 2008; Wilkins et al. 2007); *Paenibacillus larvae*, causing American Foulbrood, is causing economic losses to beekeepers worldwide (Genersch 2008). This review will focus on these honey bee pathogens and their corresponding diseases (Table 1) because of their role in honey bee collapse (Ratnieks and Carreck 2010) and their impact on beekeeping rather than giving a general overview on bee pathology.

Table 1 Honey bee pathogens shown to be involved in severe colony losses in different regions of the world

Pathogen	Region	Reference
Viruses		
ABPV	Germany	(Genersch et al. 2010)
DWV	Germany	(Genersch et al. 2010)
IAPV	USA	(Cox-Foster et al. 2007)
Bacteria		
Melissococcus plutonius	Switzerland, UK	(Roetschi et al. 2008; Wilkins et al. 2007)
Fungi		
Nosema ceranae	Spain	(Higes et al. 2008a)
Metazoan parasites		
Varroa destructor	Germany, Canada and elsewhere	(Genersch et al. 2010) (Guzmán-Novoa et al. 2010)

Parasites: *V. destructor*

The ectoparasitic mite *V. destructor* impairs both brood and adult bees causing a non-uniform disease pattern called varroosis or parasitic mite syndrome and including a specific form of brood damage termed "snotty brood" (Shimanuki et al. 1994). The symptoms of varroosis are dependent on the rate of mite infestation of a given colony and on viral infections vectored to individual bees by the parasitizing mites (see below). Infested colonies in temperate climates will eventually die within around 2 years after the initial infestation if left untreated (Boecking and Genersch 2008). Therefore, varroa control strategies have had to become an integral part of the beekeeping practice in order to keep infestation levels below the damage threshold (Delaplane and Hood 1999) for reducing colony losses caused by this parasite. Still, varroosis inflicts much greater damage and higher economic costs than all other known bee diseases.

V. destructor belongs to the genus *Varroa* which is currently represented by at least four species of obligate ectoparasitic mites of honey bees: (1) *Varroa jacobsoni* Oudemans (Anderson and Trueman 2000; Oudemans 1904), which is a natural ectoparasitic mite of the Eastern honey bee *Apis cerana*; (2) *Varroa underwoodi*, which is also a parasite of *A. cerana* (Delfinado-Baker and Aggarwal 1987); (3) *Varroa rindereri*, which was found parasitizing *Apis koschevnikovi* in Borneo (De Guzman and Delfinado-Baker 1996); and (4) *V. destructor*, which was erroneously classified as *V. jacobsoni* until it turned out to be a separate species (Anderson 2000; Anderson and Trueman 2000) [Cave: Due to this classification problem, *V. destructor* is called *V. jacobsoni* in many pre-2000 articles!]. The original host of *V. destructor* is *A. cerana*. Several mitochondrial haplotypes of *V. destructor* exist, but only two of them, the Korean type and the Japanese/Thailand type, are able to reproduce on *A. mellifera*. Microsatellite analyses found almost no polymorphism within these two haplotypes, indicating a quasi clonal population structure (Solignac et al. 2003, 2005) and suggesting that most likely two independent switches from the original host *A. cerana* to the new host *A. mellifera*, at two different times and from two different populations in Asia, occurred. From there on, *V. destructor* started to conquer the Western honey bee around the world in the second half of the past century. Today, *V. destructor* can be found worldwide wherever *A. mellifera* colonies are kept, and it is hardly possible to find a mite-free colony any longer. The only exception is Australia, which still considers *V. destructor* an exotic bee mite since it has not become established there so far (AQIS, Australian Government: http://www.daff.gov.au/aqis/quarantine/pests-diseases/honeybees, visited Jan 4, 2010).

The ectoparasite *V. destructor* is intimately linked to its host, the honey bee. All life stages of the mites take place on bees and/or in the colony, and no free-living, bee-independent stages do exist.

The biology and reproduction of *V. destructor* has been recently reviewed (Rosenkranz et al. 2010) and, therefore, will not be detailed here. The pathology of *V. destructor* at the individual insect level is in particular determined by (1) the feeding activities of the mites (i.e. injuring the cuticle of pupae and adults, sucking substantial amounts of haemolymph) and (2) vectored viruses. The loss of haemolymph during the honey bee pupal development significantly reduces the size and the weight of the hatching bee (De Jong et al. 1982; Duay et al. 2003). For drones, it has been demonstrated that such a reduced weight led to decreased flight performance and sperm production (Duay et al. 2002). Parasitized foragers showed a reduced capability of non-associative learning, and their orientation and homing ability was impaired, i.e. infested bees needed longer time to return or even did not return at all to the colony (Kralj et al. 2007; Kralj and Fuchs 2006).

Recent studies revealed that mite infestation during pupal development might also have an effect on the immune capacity of the parasitized pupae and even on the adult bees. Injection bioassays performed with adult bees and *Escherichia coli* provided correlative evidence for a partially impaired immune response towards microbial challenge in adult bees which suffered as pupae from mite parasitism (Yang and Cox-Foster 2005). However, when transcript levels for genes encoding antimicrobial peptides (abaecin and defensin) in pupae which differed in the number of parasitizing mites were analyzed, a differential regulation of these immune effectors in relation to the level of parasitization could be demonstrated (Gregory et al. 2005). Only pupae with low mite abundances showed the proposed decrease in immune response, while heavily parasitized pupae showed increased transcript levels for abaecin and defensin. Accordingly, a recent analysis of honey bee immune-gene activity in *V. destructor* parasitized pupae using microarray analyses (Evans 2006) did not reveal any decrease in transcript abundance of immune pathway members found on this array (Navajas et al. 2008). These three different studies (Gregory et al. 2005; Navajas et al. 2008; Yang and Cox-Foster 2005) show that *V. destructor* mites have impacts on the bee immune response and, thereby, most likely on the susceptibility of honey bees towards various pathogens, but we are still far from understanding the interplay between the parasites and their hosts' immune system.

In addition to these direct effects of *V. destructor* on the performance and health of individual bees, there are also indirect effects caused by viruses vectored through the mite.

So far, 18 different viruses have been isolated from honey bees (Chen and Siede 2007) and for Kashmir bee virus (KBV), sacbrood virus, acute bee paralysis virus, Israel acute paralysis virus and deformed wing virus, it has been proven that they can be vectored by *V. destructor* [see references in Boecking and Genersch (2008)]. In the absence of *V. destructor*, these viruses cause covert infections (Hails et al. 2007); therefore, they have been considered a minor problem to honey bee health until the arrival of the mite changed the picture (Allen et al. 1986; Bailey and Ball 1991; Ball 1983, 1989, 1996; Bowen-Walker et al. 1999). While feeding on covertly infected bees, the mites obviously acquire viral particles which can then be vectorially transmitted to the next parasitized bee. The virulence of the aforementioned viruses had been tested and confirmed in the laboratory by injection bio-assays using pupae or adult bees (Bailey 1964, 1967; Bailey and Ball 1991). Hence, it is not surprising that these viruses, when "injected" into the haemocoel of pupae and adult bees in the course the mites' feeding activities, induce overt disease outbreaks and exhibit all their potential virulence. In addition, evidence exists that *V. destructor* can cause activation of endogenous virus infection also leading to overt disease outbreaks as a consequence of the immune suppression in pupae and adult bees which are/were parasitized during their ontogenetic development (Yang and Cox-Foster 2005).

Although it has long been known that the haemophagous honey bee mite *V. destructor* is able to induce colony losses especially in combination with virus infections (Ball 1983, 1989; Ball and Allen 1988; Delaplane and Hood 1999; Fries et al. 1994; Hung et al. 1995; Martin 2001; Todd et al. 2007), the mite did not come into focus when inexplicable overwintering losses and seasonal losses were reported from different regions in the world in the recent past. One explanation used to exculpate *V. destructor* was that the mite has been around now for nearly 40 years and has spread around the world during this period, but increased and inexplicable colony losses—like CCD in the USA (vanEngelsdorp et al. 2007, 2008, 2009)—have been reported only recently. Therefore, many efforts have been made to identify *the* cause of these losses which was expected to be newly introduced or to have emerged recently. One of these efforts has been a long-term bee-monitoring program initiated in Germany in the winter 2004/2005. Analysis of the first period of 4 years revealed that, at least in Germany and over the study period, *V. destructor* still has been the main cause of winter losses (Genersch et al. 2010), confirming that no other pathogen has a comparable impact on beekeeping. In addition, two honey bee pathogenic viruses known to be associated with *V. destructor*, DWV and ABPV, were significantly related to the observed winter losses (Genersch et al. 2010).

Viral pathogens: DWV, ABPV and IAPV

The existence of honey bee viruses has been known since 1963 when chronic bee paralysis virus (CBPV) and ABPV were first isolated (Bailey et al. 1963). Most of the 18 known honey bee viruses may exist and even co-exist in honey bee individuals or colonies without causing any symptomatic infection and, hence, without causing any obvious problems for apiculture (Gauthier et al. 2007; Tentcheva et al. 2004). However, in the wake of *V. destructor*, two viruses became of increasing concern: DWV (Iflaviridae) and ABPV (Dicistroviridae).

Soon after *V. destructor* arrived in the *A. mellifera* population of the Western World, emerging bees with deformed or atrophied wings were increasingly observed. As the occurrence of these deformed wings was clearly related to mite infestation of the developing pupae, these deformities were first considered a consequence of the haemolymph deprivation of pupae by the parasitizing mites (De Jong et al. 1982; Koch and Ritter 1991; Marcangeli et al. 1992). However, since 1989, evidence has been accumulating that the deformed wing symptom was rather related to the simultaneous infection of infested bees by a virus which was then called deformed wing virus (Bailey and Ball 1991; Ball 1989, 1993).

Like all other bee viruses, DWV is a rather benign virus mainly causing covert, symptomless infections (Hails et al. 2007), as long as it is transmitted vertically (through drones and queens) or horizontally (through larval food) (de Miranda and Fries 2008; Yue and Genersch 2005; Yue et al. 2006, 2007). Vectorial transmission of DWV to pupae through *V. destructor* is a prerequisite for the manifestation of overt DWV infections characterized by deformed wings, shortened and bloated abdomen and miscolouring (Ball and Allen 1988; Bowen-Walker et al. 1999; Yang and Cox-Foster 2007; Yue and Genersch 2005). Bees with deformed wings are not viable and die within less than 67 h after emergence (Yang and Cox-Foster 2007). Hence, overt infections induced by the mite acting as virus vector can cause considerable damage to colonies. The degree of virus-induced damage is related to the proportion of overtly DWV-infected and, hence, non-viable bees. This again is related to the varying proportion of mites which actually act as virus vector in the colony (Yue and Genersch 2005). In addition, recent studies have shown that replication of the virus in the mites prior to transmission and a high enough DWV titre in the mites are necessary preconditions for the induction of an overt DWV infection in the developing bee (Gisder et al. 2009; Yue and Genersch 2005). These results indicate that the mite can act not only as mechanical but also as biological vector for DWV and that it is the latter function which is related to overt DWV infections. Therefore, the more mites in a colony transmit the virus

and the more of these mites support replication of the virus prior to transmission, the higher the chances that developing pupae will develop a fatal DWV infection and that the colony will eventually collapse [for a recent detailed review: de Miranda and Genersch (2010)]. Furthermore, the above-discussed manipulation of the bees' immune system by parasitizing mites also seems to play a role in the development of overt DWV infections. Recent studies suggested that *V. destructor* might actively contribute to the activation of endogenous DWV infections by immunosuppressing the host (Navajas et al. 2008; Yang and Cox-Foster 2005).

In conclusion, in association with *V. destructor*, DWV can be considered an emerging viral disease of honey bees with detrimental effects not only for individual bees but also for entire colonies. The negative impact of DWV on winter survival of honey bee colonies has been confirmed recently by a 4-year bee-monitoring program in Germany. The detection of DWV viral sequences in the brains of otherwise symptomless honey bee workers, which had been shown to be of diagnostic relevance (Yue and Genersch 2005; Yue et al. 2007), was significantly related to winter mortality of the respective colonies (Genersch et al. 2010).

ABPV and IAPV are closely related members of the family Dicistroviridae. ABPV had been discovered inadvertently during laboratory work on CBPV (Bailey et al. 1963). In contrast to CBPV, which causes natural outbreaks of bee paralysis [for a recent review: Ribière et al. (2010)], ABPV can be found at similar concentrations in naturally paralyzed (caused by CBPV) and apparently healthy bees (Bailey et al. 1963). However, in injection bioassays, ABPV turned out to be highly virulent causing death of injected adult bees within 3–5 days (Bailey et al. 1963). This contrasted sharply to the observations in the field at that time, where ABPV had null or low impact on infected bees and colonies suggesting that ABPV in contrast to CBPV caused covert infections (presence of the virus in the absence of disease symptoms). ABPV has a geographical distribution similar to that of *A. mellifera* and, therefore, has been isolated from healthy adult bees from most regions of the world (Allen and Ball 1996; Anderson 1988; Bailey 1965, 1975; Bailey et al. 1981; Hung et al. 1996a; Tentcheva et al. 2004). The apparent harmlessness of ABPV infections dramatically changed with the advent of *V. destructor* in Europe. In severely mite-infested colonies, brood and adult bees were obviously dying from ABPV infection (Ball 1983, 1985; Ball and Allen 1988; Bekesi et al. 1999; Berenyi et al. 2006; Hung et al. 1996c; Nordström et al. 1999; Ritter et al. 1984). Considering the extreme virulence of ABPV when injected into the bee haemolymph, it is not surprising that this virus started to cause problems when *V. destructor* entered the stage, became established as ABPV vector and began to inject the virus into pupae and adult bees (Ball 1989). For ABPV, *V. destructor* acts solely as mechanical vector (Ball 1989; Tentcheva et al. 2004; Wiegers 1988); in contrast to vectorial DWV transmission, there is no evidence that *V. destructor* supports or allows ABPV replication and hence can act as biological vector of ABPV.

Since ABPV can frequently be detected in apparently healthy as well as in dead bees and, accordingly, likewise in bees from healthy and collapsing colonies (Bekesi et al. 1999; Berenyi et al. 2006; Tentcheva et al. 2004), it is difficult to assess the impact of ABPV on colony mortality. A recent 2-year study on ABPV and winter losses in Germany by Siede et al. (2008) demonstrated a significant relation between pre-winter ABPV infection and winter mortality for the 2005/2006 season but not for the 2004/2005 season. A much broader German study performed over 4 years and involving more than 1,200 colonies from more than 120 apiaries, however, revealed a highly significant relation between ABPV infection in autumn and colony collapse in the following winter season (Genersch et al. 2010).

In summary, while ABPV had originally been considered an economically irrelevant viral pathogen of honey bees, it developed an alarming virulence in association with *V. destructor*. Similar to DWV, it now adds to the damage inflicted to honey bee colonies by *V. destructor* parasitism and became a key player in the parasitic mite syndrome (Hung et al. 1995, 1996b; Shimanuki et al. 1994). The exact role of the mite in the increase in ABPV virulence still remains elusive.

IAPV has recently been identified when the homogenate of a single dead bee collected in the course of studies related to severe colony mortality in Israel was inoculated into healthy-looking bee larvae (Maori et al. 2007). Subsequent studies revealed that IAPV has been around for quite some time (Chen and Evans 2007; Palacios et al. 2008) and may have been mistaken for KBV in past studies due to its close genetic relationship with KBV, which makes it difficult to discriminate between these two viruses via conventional reverse transcription polymerase chain reaction (PCR) protocols (de Miranda et al. 2010). IAPV, KBV and ABPV form a complex of genetically and biologically related viruses within the family Dicistroviridae. IAPV is extremely virulent when injected into pupae or adult bees (Maori et al. 2007). Hence, it can be assumed that *V. destructor* plays a role in the virulence of IAPV as it does for DWV and ABPV. So far, little is known about the transmission and pathomechanisms of IAPV since it came into the focus of bee virologists only quite recently in the context of colony collapse disorder, a condition described mainly in the USA and leading to severe colony losses (vanEngelsdorp et al. 2007, 2009). However, the potential virulence of IAPV for bees and colonies is unquestioned as

it has been identified as a marker or secondary agent of CCD (Cox-Foster and VanEngelsdorp 2009; Cox-Foster et al. 2007), and anti-viral treatment using IAPV-specific RNAi was able to silence IAPV and to reduce the symptoms of CCD (Maori et al. 2009). These results suggest that IAPV is at least in part responsible for the described symptoms and colony mortality in the course of CCD. IAPV is prevalent in the Middle East, Australia and the USA but less frequently found in Europe (de Miranda et al. 2010). This could explain why IAPV is implicated in colony losses in the USA (Cox-Foster et al. 2007) but so far not in Europe (Genersch et al. 2010).

Bacterial pathogens: *M. plutonius* and *P. larvae*

Only two bacterial pathogens are known from honey bees, and both are pathogenic for honey bee larvae but not for adult bees: *M. plutonius*, causing European Foulbrood (EFB) (Bailey 1956, 1983) and *P. larvae*, causing American Foulbrood (AFB) (Genersch et al. 2006). AFB is not implicated in any inexplicable colony losses since this brood disease is easily diagnosed and, as a notifiable disease, well controlled by the authorities. However, it is not at all a rare disease but occurs rather frequently (about 5–10% of the colonies in Germany are infected although not yet clinically AFB diseased; own unpublished data from nearly 10 years of monitoring *P. larvae* incidence) and causes considerable economic losses to beekeepers all over the world. Therefore, it can be considered one of the major threats to honey bee health. Despite its relevance for apiculture, AFB will not be covered in this review since this disease and its etiological agent, *P. larvae*, have been reviewed in great detail recently (Ashiralieva and Genersch 2006; Genersch 2007, 2008, 2010).

M. plutonius, the causative agent of EFB, is a Gram-positive, lanceolate coccus with a close phylogenetic relationship to the genus *Enterococcus* (Cai and Collins 1994). The vegetative form is occurring singly, in pairs or in chains of varying length. The identification of *M. plutonius* as causative agent of EFB has long been hampered by the fact that *M. plutonius* is very fastidious and, therefore, hardly culturable from diseased larvae. It took quite some time until the original hypothesis of White (1912) that EFB is caused by a unique organism named *Bacillus pluton* had been substantiated (Bailey 1983). The classification of *M. plutonius* has not been easy and, hence, it can be found in the literature as *B. pluton* (White 1912), *Streptococcus pluton* (Bailey 1957), *Melissococcus pluton* (Bailey and Collins 1982; Cai and Collins 1994) and finally *M. plutonius* (Truper and dé Clari 1998).

Infection of larvae occurs when larvae ingest food contaminated with *M. plutonius*. Larvae are susceptible at any stage before cell capping, but their susceptibility decreases with increasing age. Bacteria proliferate in the larval midgut assimilating much of the larval food. It is assumed that infected larvae die from starvation (Bailey 1983) and are then decomposed by secondary invaders like *Paenibacillus alvei* and *Enterococcus faecalis*, two saprophytic bacteria frequently found associated with EFB. Dead larvae are not found in their normal coiled position at the cell bottom, but instead they are twisted around the walls or stretched out in the cell (Fig. 1). Usually, larvae die when they are 4 or 5 days old, but infected larvae may also survive and pupate after discharging bacterially contaminated faeces which is deposited on the walls of the brood cells (Bailey and Ball 1991). These faecal remains are infective, indicating that the durable encapsulated stages of *M. plutonius* are also infectious. Surviving infected larvae produce pupae and adults of subnormal weight (Bailey 1960). Nothing is described about the role of these infected (?) adults in the intra-colonial spread of the disease and the persistence of the pathogen within the bee population [see Forsgren (2010) for a recent review].

Many aspects of the pathogenesis, transmission and control of *M. plutonius* are poorly understood and still remain elusive. A literature survey will reveal that most of the work concerning EFB has been conducted decades ago, and molecular work so far mostly concentrated on developing and applying PCR techniques for the detection of *M. plutonius* (Djordjevic et al. 1998; Forsgren et al. 2005; Govan et al. 1998; Roetschi et al. 2008). This was for sure influenced by the fact that for a long time EFB did not create much of a problem in apiculture since many infected and diseased colonies spontaneously recovered from the disease (Bailey and Ball 1991). This situation changed at least in some regions of Europe, like Switzerland and the

Fig. 1 Honey bee larvae succumbed to EFB (picture taken by Pia Aumeier)

UK, where EFB has become a major problem for apiculture recently (Roetschi et al. 2008; Wilkins et al. 2007). In Switzerland, the infection and re-infection rates have been dramatically increasing since 2002, and the situation now is nearly out of control with 796 official outbreaks in 2009 (Fig. 2). Sanitizing measures formerly successfully applied seem to be ineffective now. There is an urge to find a way to effectively combat the disease and to prevent further spread of *M. plutonius*. But the lack of knowledge about this pathogen is hampering the development of a sustainable cure or control method against EFB at the time being. The situation exemplifies that understanding a disease is an imperative prerequisite for developing a cure.

Fungal pathogens: *Nosema* spp.

Nosema spp. belongs to the phylum Microsporidia which comprises more than 160 genera and almost 1,300 species isolated from insects and other invertebrates but also known from vertebrates including humans (Becnel and Andreadis 1999; Canning and Lom 1986; Weber et al. 1994). Microsporidia are obligate intracellular, fungal parasites which exist outside the host cell only as metabolically inactive spores. These environmental spores are the infectious form of all microsporidia driving disease transmission between individuals. Infection of the host cell involves germination of the spores leading to mechanical injection of the extruded polar tube into the host cell followed by the transmission of the sporoplasm through the polar tube into the host cell's cytoplasm (Bigliardi and Sacchi 2001; Franzen 2005). Two species of this phylum, *Nosema apis* and *N. ceranae*, are pathogenic for adult honeybees. The original assumption was that *N. apis* specifically infects the European honey bee, *A. mellifera*, causing nosemosis (Zander 1909), and *N. ceranae* is a specific pathogen of the Asian honey bee, *A. cerana* (Fries et al. 1996). Recently, it became evident that *N. ceranae* is also widespread in the *A. mellifera* population throughout the world (Chen et al. 2008; Giersch et al. 2009; Higes et al. 2006; Huang et al. 2007; Invernizzi et al. 2009; Klee et al. 2007; Paxton et al. 2007).

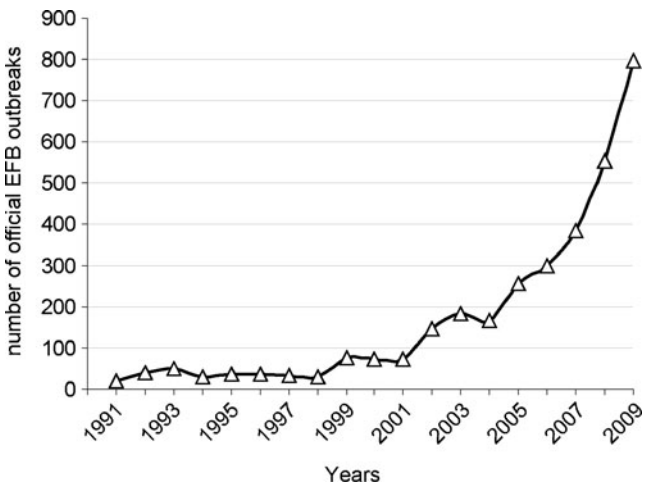

Fig. 2 Official data on the development of diagnosed EFB outbreaks in Switzerland between 1991 and 2009 (Source: Bundesamt für Veterinärwesen BVET, Switzerland)

Adult bees become infected by ingesting *Nosema* spores which are present in faeces but can also be found in pollen (Higes et al. 2008b). Spores germinate in the midgut and infect the cells of the midgut epithelium where they vigorously proliferate to produce new environmental spores which are released into the gut lumen (Fries 2010). Nosemosis, i.e. the clinical outbreak of *Nosema* infection caused by *N. apis*, is characterized mainly by dysentery, whereas *N. ceranae* is described to cause death of individuals and colonies not preceded by any visible symptoms (Higes et al. 2008a; Martin-Hernandez et al. 2007). *N. apis* infection is restricted to the midgut epithelium (Fries 1988), whereas *N. ceranae* has also been detected in other bee tissues like malpighian tubules and hypopharyngeal glands although so far only via highly sensitive, molecular methods (Chen et al. 2009).

Reports on the impact of *N. ceranae* infections on honey bee health and colony survival are contradictory. In Spain, *N. ceranae* causes an unusual form of nosemosis eventually leading to colony collapse (Higes et al. 2008a; Martin-Hernandez et al. 2007). Accordingly, in laboratory infection assays, Spanish *N. ceranae* isolates were found to be highly virulent (Higes et al. 2007). However, this extreme in vitro virulence of *N. ceranae* could not be confirmed by others (Mayack and Naug 2009; Paxton et al. 2007), which might be due to different isolates used. Likewise, the worldwide distribution of *N. ceranae* (Chauzat et al. 2007; Chen et al. 2008; Cox-Foster et al. 2007; Fries et al. 2006; Higes et al. 2006; Invernizzi et al. 2009; Klee et al. 2007; Paxton et al. 2007; Tapaszti et al. 2009; Williams et al. 2008), which is not inevitably accompanied by the symptoms described by Higes et al. (2008a), also suggest that *N. ceranae* killing honey bee colonies might be a regional problem rather than a global phenomenon. The observed virulence of *N. ceranae* in Spain has been explained by the better adaptation to elevated temperatures of *N. ceranae* compared to *N. apis* (Fenoy et al. 2009; Martin-Hernandez et al. 2009). However, it has also been reported that *N. ceranae* spores were susceptible to freezing in laboratory experiments (Fries 2010) and that the virulence of *N. ceranae* might be influenced by climatic conditions (Gisder et al. 2010). This is an especially intriguing thought since changes in disease prevalence and pathogen virulence due to climatic change is a hot topic nowadays.

Summary

Alarmingly increasing honey bee colony losses have been frequently reported in the media over the past few years and attracted much attention in non-scientific and scientific communities. From recent surveys of honey bee losses in North America and Europe, it became evident that pests and pathogens could be identified as the single most important cause of these colony losses so far (Genersch et al. 2010; vanEngelsdorp et al. 2008; vanEngelsdorp and Meixner 2009). We here introduced several bee pathogens which are thought to be involved in such honey bee colony losses. These examples show that, even within Europe, diverse pathogens are involved in the presumed "inexplicable" colony losses. Therefore, although the decline in managed honey bees equally seems to be a problem in the USA, Europe and Japan (Oldroyd 2007) despite great differences in beekeeping practice (Ratnieks and Carreck 2010), the factors responsible for colony losses differ from continent to continent and from region to region. We should be prepared that we will not find a globally valid solution to honey bee decline but that we will have a panel of possible factors, all of them asking for a specific solution to address the problem properly. If we are to explain unusual colony losses and if we are to find the cause for these losses, then we need to move from the mere detection of bee pathogens in individuals and colonies to molecular beepathology focussing on host–(vector–)pathogen interactions with equal emphasis on the pathogen (the vector) and the host. Only then will we understand the diseases and the pathogens of honey bees which in turn will enable us to develop adequate control measures.

I will close this review with a quotation from George F. White, who identified *Bacillus larvae* [later reclassified as *P. larvae* (Genersch et al. 2006)], the etiological agent of American foulbrood, a quotation that did not lose its timeliness over the past hundred years:

> In order to combat a disease to the best advantage, it is clear that its cause must be known, as well as the means by which the infection is transmitted and the environmental conditions which are favourable for the breaking out of an epidemic (White 1906).

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