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Review article

An inventory of documented diseases of African honeybees

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Current trends in global honeybee population changes have been linked to drastic declines in honeybee populations caused by complex interactions between pathogens, arthropod pests such as *Varroa*, pesticides, honeybee stress and habitat loss. Although substantial information exists for this sudden decline in honeybee populations in Europe and North America, in Africa the effect of this threat continues to draw debate. Despite recent reports showing the presence of *V. destructor* mites across the continent, knowledge on pathogens associated with bees and this mite in various parts of the continent is scanty. This review provides a comprehensive update on the documented diversity and geographic distribution of honeybee pathogens and points to the need of further information on these constraints of honeybee health.

Key words: pathogens, *Nosema*, chalkbrood, stonebrood, *Apis mellifera*, viruses, *Varroa*, Africa.

INTRODUCTION

Through pollination of cultivated and wild flowering crops, honeybees, *Apis mellifera* L., provide essential ecosystem services (Kremen *et al.* 2007) that ensure sustained food production, ecosystem stability and opportunities for income generation and habitat conservation for rural poor communities through sale of bee products such as honey, pollen, propolis and wax (Sande *et al.* 2009; Raina *et al.* 2011). The value of pollination to food production is estimated at C153 billion globally and C11.9 billion in Africa (Gallai *et al.* 2009). These values are based on the services rendered by managed honeybee colonies (responsible for 80–85 % of pollination in commercial farm plots), and as a result, underestimates the real value of honeybees (Allsopp *et al.* 2008).

The unexplained sudden loss of adult worker bees in managed honeybee colonies in the United States of America (Oldroyd 2007; van Engelsdorp *et al.* 2009; Evans & Schwarz 2011) and some European countries (Dainat *et al.* 2012b) was first described as 'Colony Collapse Disorder' (CCD), but currently referred to as 'decline in honeybee populations'. This syndrome is unique in that the dead adult bees cannot be found in the vicinity of the hives, implying that the bees go missing and

food resources left by the missing worker bees remain intact without attacks from robbing bees or pests (Cox-Foster *et al.* 2007; Oldroyd 2007; van Engelsdorp *et al.* 2009). CCD and the decline in honeybee populations have received global attention, primarily because of the solid link between pollination and food security. Intensive use of pesticides and fungicides in agriculture (Frazier *et al.* 2008; Mullin *et al.* 2010), forest destruction and fragmentation, pests (mites, beetles, moths, birds, frogs, bears) and pathogens (fungi, bacteria, viruses and protozoans) are well known to negatively affect the health of honeybees (Genersch *et al.* 2010). An interaction of these factors has been suggested to cause CCD and the decline in honeybee populations (Oldroyd 2007).

Africa is home to a great diversity of honeybee races (Kajobe & Roubik 2006; Moritz *et al.* 2007; De la Rúa *et al.* 2009; Dietemann *et al.* 2009) and together with the continent of Asia, it has been considered a potential region of origin of *Apis mellifera* (Whitfield *et al.* 2006; Han *et al.* 2012). A review by Dietemann *et al.* (2009) showed that Africa was free of sudden honeybee losses, which warrants stringent preventive conservation measures to avert the losses experienced in other parts of the world. However, despite discussing

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principal biotic and abiotic threats to honeybee colonies on the continent and suggesting ways to ensure their conservation, the review did not delve into the finer details on the exact nature of these threats and their geographic occurrence across the continent and made no mention of pesticides and fungicides as potential threats, contrary to the fact that pesticide use is well documented in tree and vegetable cropping systems in Africa (Crawford *et al.* 2003; Gunnell & Eddleston 2003; Fatoki & Awofolu 2004; Williamson *et al.* 2008). Although presumed free of sudden honeybee losses, pests and diseases associated with this disorder have been reported on the continent over the last three decades (Hussein 2000; Frazier *et al.* 2010; Kajobe *et al.* 2010; Strauss *et al.* 2013), suggesting that a closer examination of the possible existence of CCD in Africa is warranted. Despite claims of a decline in honeybee populations on the continent (Neumann & Carreck 2010; Kluser *et al.* 2011), these changes appear inconspicuous compared to those in Europe and North America (Neumann & Carreck 2010). This scenario has been attributed to greater resilience of African honeybees towards pests and diseases compared to their European counterparts (Tapy 2003) and paucity of information through insufficient surveys (Dietemann *et al.* 2009). The observation of colony decimation and death due to *Varroa* mites and diseases on the island of Madagascar (OIE 2010; Rasolofoarivao *et al.* 2013) points to the probable existence of isolated and undocumented cases of CCD on the continent. More so, presence of *Varroa* has been confirmed in many countries in Africa (Dietemann *et al.* 2009; Frazier *et al.* 2010; Rasolofoarivao *et al.* 2013), clearly suggestive that the health status of the continent's main pollination resource is under threat and therefore urgent and extensive health surveys are needed.

This review provides an update on the diversity, distribution and management of honeybee diseases in Africa. It is anticipated that this paper will spur increased interest in African honeybee research and engender future efforts to increase knowledge of threats and potential mitigation tools and strategies.

HONEYBEE DISEASES

Like most living organisms, honeybees are afflicted by a myriad of diseases whose causative agents could be bacterial, viral or fungal in origin

(Genersch *et al.* 2010). Factors such as the eusocial life of honeybees, abundant stores of resources such as pollen and the haplodiploidy nature of honeybees seem to make honeybees more susceptible to colony-wide destruction in the case of a disease epidemic (Schmid-Hempel 1998). However, other aspects of this super-organism's way of life, including its grooming, hygienic (Spivak & Reuter 2001), and absconding behaviours (Fries & Raina 2003) have been reported to enable it to avoid complete decimation in the case of a disease or pest outbreak. In addition to group defence mechanisms, honeybees possess individual immune responses in the form of cellular and humoral immune responses (encoded by genes belonging to at least 17 gene families involved in insect immunity) which ensure maintenance of their health status (Evans *et al.* 2006; Weinstock *et al.* 2006). Despite the existence of both colony and individual levels of immunity, honeybees around the world remain plagued by diseases and pests that threaten to destroy this super-resource.

Diseases caused by viruses

There are approximately 18 viruses known to affect honeybees (Allen & Ball 1996) at different stages of their life cycle (Chen & Siede 2007). The viruses mostly belong to the taxonomic order Picornavirales which contains five families, two of which (Ifilaviridae and Dicistroviridae) contain common honeybee viruses (ICTV 2013).

Virus infections could occur as single, double or multiple infections (Chen *et al.* 2004; Chen *et al.* 2005a) or in combination with other known pathogens (Bailey *et al.* 1983). In addition, they can be maintained in the body of the honeybee for long periods of time, sometimes resulting in undetectable, acute or chronic infections. Acute and chronic infections elicit dramatic changes in the insect, such as shrivelled wings, loss of hair, 'shivering' and even paralysis (Bailey *et al.* 1963; Bailey & Gibbs 1964). Among the 18 viruses, seven have been implicated as major contributors in the decrease of honeybee populations globally (Cox-Foster *et al.* 2007; Dainat *et al.* 2012b).

Five honeybee viruses – Acute Bee Paralysis Virus (ABPV), Israeli Acute Paralysis Virus (IAPV), Sac Brood Virus (SBV), Black Queen Cell Virus (BQCV), and Deformed Wing Virus (DWV) – have so far been identified in Africa. This assessment is most probably an underestimation of the actual disease prevalence due to the fact that most viral

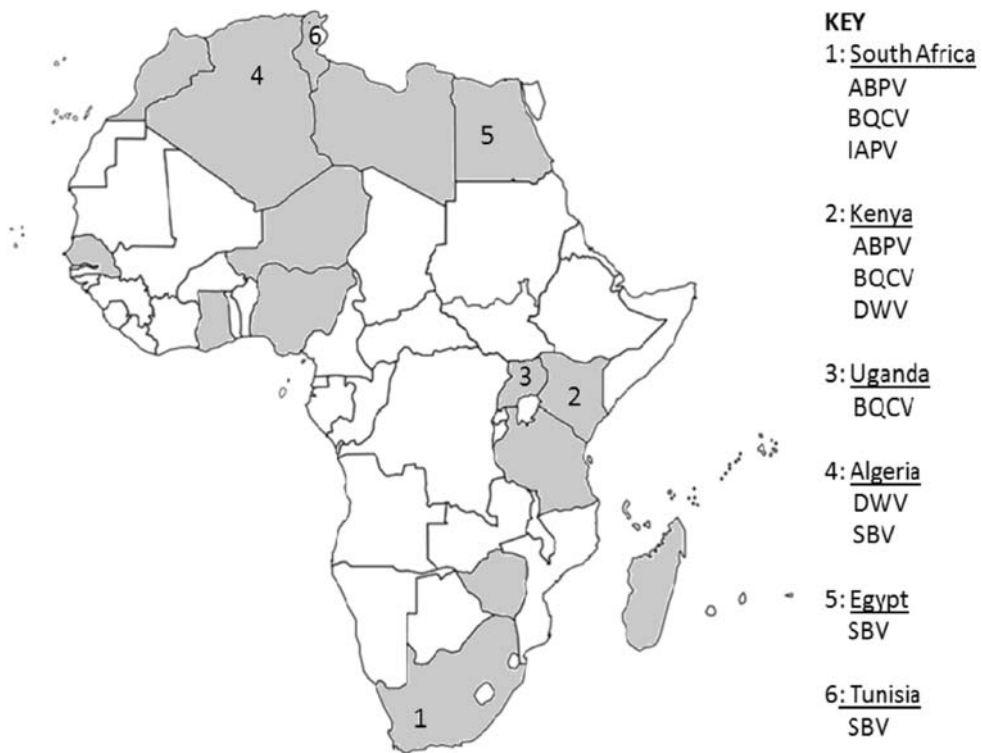


Fig. 1. Detection of honeybee viruses in Africa. Countries shaded in grey have confirmed presence of *Varroa destructor*, currently described in 20 out of the 54 countries on the continent (about 37.5 % of Africa). With the exception of Kenya, Uganda and South Africa, there is lack of an extensive investigation of viral diseases of honeybees. In non-shaded countries similar information is lacking and the disease status is unknown.

diseases are asymptomatic in honeybee adults and for that reason, more accurate tools of detection of the pathogen genome should be applied. Such tools include RT-PCR (Grabensteiner *et al.* 2001; Chen *et al.* 2004; Yue & Genersch 2005; Chen *et al.* 2006; Yue *et al.* 2006), qPCR (Chen *et al.* 2005b; Chantawannakul *et al.* 2006) and ELISA (Anderson 1984). Next Generation Sequencing (NGS) technologies have also been used in studying known viruses and are currently being explored for virus discovery (Mardis 2008; Barzon *et al.* 2011). The inability to observe viral infections *via* non-genetic methods has made their timely detection and management virtually impossible.

Acute bee paralysis

This disease is caused by the Acute Bee Paralysis Virus (ABPV) and affects both brood and adult honeybees in a colony. It differs from Chronic bee paralysis (CBP) in that bees infected with the former show signs of 'flightlessness' and die more quickly than those infected by CBPV (Bailey *et al.* 1963;

Bailey & Gibbs 1964). Acute bee paralysis disease is present on all continents except Australia (de Miranda *et al.* 2010). In Africa, it has been reported in South Africa (Govan *et al.* 2000; Allsopp 2001) and in Kenya (Muli *et al.* 2014), as shown in Fig. 1.

ABPV is an RNA virus belonging to the family Dicistroviridae and genus *Aparavirus* (ICTV 2013). It is closely related to two other bee viruses namely IAPV and Kashmir Bee Virus (KBV), and all three are sometimes considered to be a complex of related viral species (Chen & Siede 2007). The virus is spread among the honeybees *via* salivary gland secretions exchanged during trophallactic feeding between workers, brood and the queen. Recent studies have shown that the mite *Varroa destructor* can vector APBV (Bakonyi *et al.* 2002; Siede *et al.* 2008), supposedly acting as an activator for the virus as well. However, the mite's role as a virus activator has been disputed following the observation of viral replication in honeybees in the absence of any mites (Bakonyi *et al.* 2002; Tentcheva *et al.* 2004).

Israeli acute paralysis disease

This disease, which is a close relative to ABPV and the Kashmir Bee Virus, is caused by Israeli Acute Paralysis Virus which was first described in 2004 from dead honeybees originating from Israel (Maori *et al.* 2007). In Africa detection reports have so far been limited to South Africa (Strauss *et al.* 2013) (Fig. 1).

IAPV belongs to the Dicistroviridae family of viruses (Sabath *et al.* 2009) and genus *Aparavirus* (ICTV 2013). It is considered one of the markers of CCD (Cox-Foster *et al.* 2007) with signs similar to those caused by ABPV which includes shivering wings that progress to paralysis and eventually death a few days post-infection (Maori *et al.* 2007). Owing to the close similarity of IAPV, ABPV and CBPV infection syndromes, the most accurate method of detection of IAPV is through molecular detection of the viral genome using techniques such as qPCR and RT-PCR (Francis & Kryger 2012).

While little is known about the transmission of IAPV among honeybees, *V. destructor* has been experimentally shown to serve as an effective vessel for viral replication and transmission inside the colony (Di Prisco *et al.* 2011). Globally, the virus has been recorded in Australia, Israel, U.S.A., Spain, France (Blanchard *et al.* 2008) and Poland (Pohorecka *et al.* 2011).

Sacbrood disease

This disease was first described in 1913 (White 1913) but it was not until 1964 that SBV, its causative agent, was described (Bailey *et al.* 1964). It belongs to the Iflavirus family which contains one genus, *Iflavirus* (ICTV 2013). In Africa, the pathogen has been identified in South Africa using the reverse-transcription PCR (Grabensteiner *et al.* 2001) and in North Africa (Algeria, Egypt and Tunisia) using field diagnostics involving observation of affected larvae (Hussein 2000) (Fig. 1).

Sacbrood disease is easily detected in the field due to the characteristic sac-like appearance of diseased larvae. Infected larvae do not pupate, as the outer larval skin sags, becomes sac-like and later accumulates fluid (White 1913).

Possible routes of transmission of this virus include horizontal spread *via* vectoring by *V. destructor* (Tentcheva *et al.* 2004) and *Aethina tumida* (Eyer *et al.* 2008, 2009) and spread from foragers to other colony nest mates through shared food resources such as pollen, honey and royal jelly (Shen *et al.* 2005a). Vertical transmission of the virus from

infected queens to their eggs has also been documented (Chen *et al.* 2006). While infection may persist in adult bees, huge populations of larvae infected by SBV are rarely detected in colonies as honeybee workers are very effective in detecting and removing infected brood, through their hygienic behavioural practices (Chen & Siede 2007).

Black queen cell disease

This disease is caused by the Black Queen Cell Virus (BQCV), which is an RNA virus from the Dicistroviridae family (Leat *et al.* 2000) and genus *Cripavirus* (ICTV 2013). It has been detected in South Africa (Allsopp 2001; Strauss *et al.* 2013), Uganda (Kajobe *et al.* 2010) and Kenya (Muli *et al.* 2014) (Fig. 1).

First isolated from field-collected dead samples of queen larvae and pre-pupae found in darkened rearing cells, the virus mostly affects the pre-adult stage of honeybee development with early stages of infection similar to those of sacbrood disease. Also, it has been detected in adult worker bees, especially those tending the queen and in worker pupae (Chen & Siede 2007). Infection in the adult bees is asymptomatic and only detectable by molecular analysis of the viral genome (Chen *et al.* 2004; Tentcheva *et al.* 2004).

Development of infection with BQCV has often been associated with infection with *Nosema apis*, under both field and laboratory conditions (Leat *et al.* 2000; Tentcheva *et al.* 2004). A possible reason for such co-occurrence (or symbiosis) is a microsporidian-facilitated access to host gut cells. However, the exact mechanism behind this symbiosis remains unclear. Transmission of the virus has been shown to occur vertically from the queen to her offspring or through the oral-faecal route (Chen *et al.* 2006; Singh *et al.* 2010). In Europe, the prevalence of BQCV is highest in spring and early summer (Tentcheva *et al.* 2004).

Deformed wing disease

Deformed wing disease is caused by Deformed Wing Virus (DWV), a pathogen first discovered in adult honeybees sampled from colonies heavily infested with *V. destructor* in Japan (Ball 1983). The virus belongs to the order Picornavirales, family Inflaviridae and genus *Iflavirus* (ICTV 2013) and is commonly associated with the mite *V. destructor* (Tentcheva *et al.* 2004; Tentcheva *et al.* 2006; Chen & Siede 2007). In Africa, DWV has been detected in

Algeria (Loucif-Ayad *et al.* 2013), Kenya (Muli *et al.* 2014) and Benin (Paraíso *et al.* 2012) (Fig. 1). It has been named one of the predictors of CCD (Cox-Foster *et al.* 2007; Dainat *et al.* 2012a; Francis *et al.* 2013).

Symptoms of DWV infection include poorly developed wings (deformed wings) in honeybees infected pre-emergence, reduced size of emerging adult, swollen and shortened abdomen, discoloration and pupal/adult death (Yue & Genersch 2005; Miranda & Genersch 2010). The main route of spread of the virus appears to be horizontal transmission through feeding of *Varroa* on larval and pupal haemolymph (Bowen-Walker *et al.* 1999). This virus has been shown to undergo replication (Gisder *et al.* 2009) and activation (Shen *et al.* 2005b) in the mite which also possesses the capacity to transmit the virus to other honeybees (Tentcheva *et al.* 2004; Martin *et al.* 2012; Francis *et al.* 2013). Adult bees infected post-emergence are usually asymptomatic, as are other life stages infected with DWV in the absence of *Varroa* which is the main vector of the virus, hence the need for molecular diagnosis of DWV in honeybee adults (Yue & Genersch 2005). Other routes of transmission include vertical transmission *via* infected queens (Chen *et al.* 2006) and drones (Yue *et al.* 2006), and through the faecal-cannibal-oral route and ingestion of contaminated food resources including royal jelly and pollen (Yue & Genersch 2005).

Mycoses in honeybees

Extensive research has been done on the honeybee mycoflora from different castes (Gilliam *et al.* 1977), in healthy and sickly bees (Prest *et al.* 1974), and from both managed and feral colonies (Gilliam & Taber 1991). These studies have led to the identification of main genera of fungi that cause mycoses in honeybees namely *Ascospaera*, *Nosema* and *Aspergillus*. In contrast to diseases caused by viruses, fungal diseases seem to be greatly influenced by climatic conditions (Gilliam *et al.* 1988; Martín-Hernández *et al.* 2007; Tapaszti *et al.* 2009; Fries 2010) and are mainly introduced to healthy colonies through consumption of contaminated food resources (De Jong 1976; Higes *et al.* 2008b). The ease of field diagnosis of most honeybee mycoses has led apiculturists to employ synthetic chemicals such as fumagillin to control the diseases (Williams *et al.* 2008; Higes *et al.* 2011). Owing to the increasing risks associated with

overuse of these chemicals (Desneux *et al.* 2007; Frazier *et al.* 2008; Wu *et al.* 2011; Stokstad 2012), current management efforts are directed at improving the genetic stock of the honeybee colonies (Gilliam *et al.* 1983; Spivak & Gilliam 1998; Swanson *et al.* 2009).

Chalkbrood disease

Chalkbrood disease is caused by the fungus *Ascospaera apis* (Maassen 1913), with the genus *Ascospaera* known to contain both saprophytic and parasitic fungi (Spiltoir 1955). In Africa, chalkbrood disease has been reported in Ethiopia (Hussein 2000; Gebeya & Genet 2006), Tunisia (Heath 1985; Hussein 2000), South Africa (Allsopp 2001; Strauss *et al.* 2013), Egypt (Hussein 2000; Sanad & Mohanny 2011) and Nigeria (Ajao & Babatunde 2013; Akinwande *et al.* 2013) (Fig. 2).

The fungus is morphologically heterothallic (sexually dimorphic). The female reproductive organ is the ascogonium which contains a receptive hypha known as the trichogyne. During sexual reproduction the trichogyne fuses with the nutricytes (inflated ascogonia) leading to the formation of asci and ascospores. It is thought that each ascus produces eight infective spores (Spiltoir 1955). In honeybees, *A. apis* causes a severe and invasive mycosis fatal to the brood of all castes (Aronstein & Murray 2010) with larvae less than 5 days old being the most susceptible (De Jong 1976).

Infection of larvae by *A. apis* has been well demonstrated to occur solely through ingestion of spores in contaminated honey or pollen (De Jong 1976; Flores *et al.* 2005a) obtained from foraging, robbing or spore-contaminated foundation wax (Koenig *et al.* 1986; Flores *et al.* 2005a; Flores *et al.* 2005b).

Once consumed, the spores move to the gut of the honeybee larva where germination occurs, a process thought to be activated by carbon dioxide found in the larva's gut (Heath & Gaze 1987). Germinated spores penetrate the host body cavity, causing death due to mechanical, enzymatic and toxicological damage (Glinski & Buczek 2003), leading to a hard mummified larva with a white or black colouration (Aronstein & Murray 2010). Such mummified larvae may contain up to 10^9 *A. apis* ascospores (Hornitzky 2001). Infected larvae have been reported to produce volatile compounds such as phenylethyl acetate, 2-phenylethanol, and benzyl alcohol which probably induce hygienic

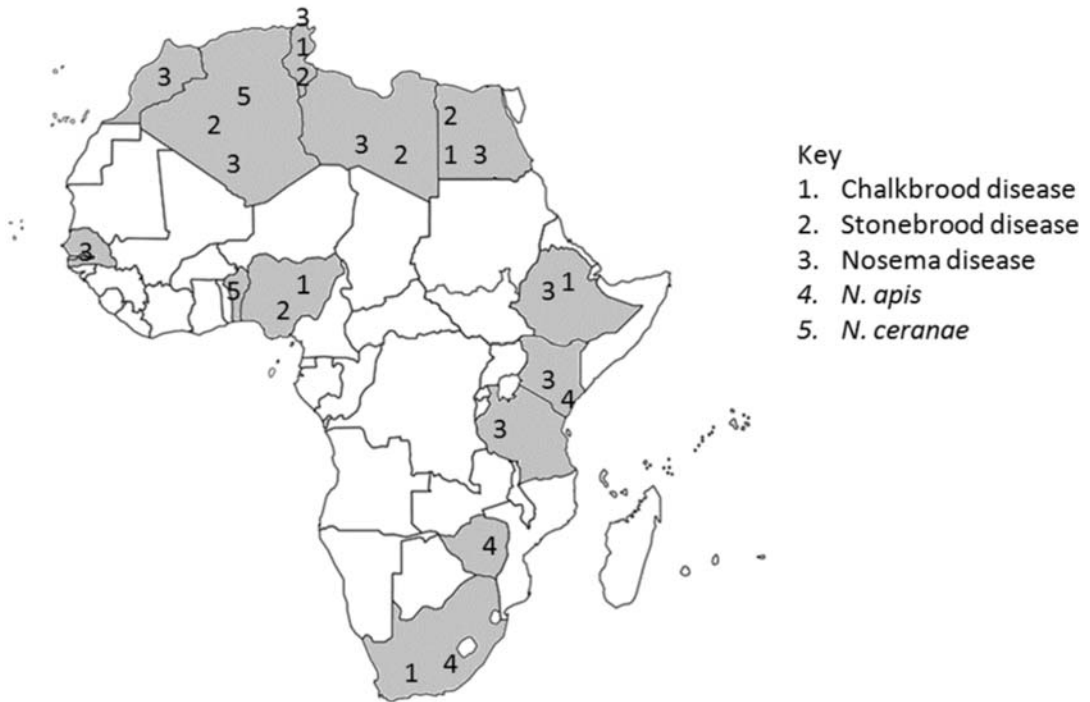


Fig. 2. Detection of chalkbrood, stonebrood and Nosema diseases of honeybees in Africa. In non-shaded countries the status of honeybee mycoses is unknown.

behaviour in the colony's worker bees, against the diseased larvae (Swanson *et al.* 2009; Boucias *et al.* 2012).

The disease is most prevalent when humidity is high and conditions are chilly, with sudden environmental changes in temperature and humidity thought to play a crucial role in its development (Flores *et al.* 1996). Honeybee colonies with poor hygienic behaviour have a higher susceptibility to chalkbrood disease (Gilliam *et al.* 1983; Spivak & Gilliam 1998), with tolerance and susceptibility shown to differ among honeybee subspecies (Jensen *et al.* 2009) and with pollen and nectar flow cycles (Flores *et al.* 2005a).

Stonebrood disease

Stonebrood disease was first described in honeybees in 1906 by Massen in Germany (Shoreit & Bagy 1995). It is caused by a variety of fungal pathogens, mostly of the saprophytic soil fungal genus *Aspergillus* (Gilliam & Vandenberg 1997; Vojvodic *et al.* 2011). In Africa, this disease has been reported in Algeria, Egypt, Libya, Tunisia (Hussein 2000) and Nigeria (Shoreit & Bagy 1995; Hussein 2000) (Fig. 2).

Signs of stonebrood disease are similar to that of infection with *Ascosphaera apis* in chalkbrood disease, with the main difference being that the 'mummified' brood in chalkbrood disease are sponge-like while that of stonebrood disease is hard and compact (like little stones), hence its name. Transmission of this disease is *via* oral and cuticular routes (Burnside 1930), and by the parasite *V. destructor* (Benoit *et al.* 2004). Stonebrood disease is an opportunistic infection which often appears alongside other honeybee stressors and affects all life stages of the honeybee.

Nosema disease

In honeybees, *Apis mellifera*, Nosema disease is caused by two pathogens of the genus *Nosema* which belong to the kingdom Fungi and phylum Microspora. This phylum consists of spore-forming unicellular parasites whose main mode of reproduction is *via* heat-resistant spore production and germination (Sprague *et al.* 1992). Examples of microsporidian parasites that infect honeybees are *Nosema apis* and *Nosema ceranae*, with the latter being more virulent (Paxton *et al.* 2007; Martín-Hernández *et al.* 2012) although this viewpoint has

been disputed by other researchers (Forsgren & Fries 2010). *Nosema ceranae* has been listed as one of the diagnostic pathogens of colony collapse disorder in the U.S.A. and Europe (Cox-Foster *et al.* 2007; Higes *et al.* 2008a; Dainat *et al.* 2012a) and has been identified in all colonies that collapsed due to this disorder. In addition to differences in virulence between the two species, *N. ceranae* shows less host specificity (Plischuk *et al.* 2009; Chaimanee *et al.* 2010; Suwannapong *et al.* 2010) and less seasonality than *N. apis* (Martín-Hernández *et al.* 2007; Paxton *et al.* 2007; Martín-Hernández *et al.* 2012; Traver *et al.* 2012) and has been purported to be displacing *N. apis* in its colonization of *A. mellifera* (Fries 2010; Martín-Hernández *et al.* 2012), a view disputed by other authors (Forsgren & Fries 2013). Phylogenetic analyses show that *N. ceranae* is a sister species of *N. bombi*, while *N. apis* is a basal member of that clade (Shafer *et al.* 2009).

On the African continent, *N. apis* has been identified in Zimbabwe (Fries *et al.* 2003), in South Africa where it is reported to be widespread (Swart 2003; Strauss *et al.* 2013) and Kenya (Muli *et al.* 2014). Similarly, *N. ceranae* has been identified in Algeria (Higes *et al.* 2009b) with reports of the same in Benin (Paraiso *et al.* 2012). Nosema disease, not distinguished as being caused by either *N. apis* or *N. ceranae*, has been reported in Algeria, Egypt, Libya, Morocco, Tunisia, Kenya, Tanzania, Ethiopia and Senegal (Hussein 2000) (Fig. 2).

Nosema ceranae is mostly prevalent in warmer climates (Martín-Hernández *et al.* 2007; Tapaszti *et al.* 2009), while *N. apis* is more prevalent in cooler climates (Fries 2010). The infective stage of the pathogen is the non-germinated spore which is ingested in contaminated pollen or honey (Higes *et al.* 2008b).

Nosema is mainly transmitted horizontally through the oral-faecal route from workers to queens (Higes *et al.* 2009a) and brood (Smith 2012). Other non-oral-faecal routes such as transmission of *Nosema* to/from queens to drones during mating (Traver & Fell 2011) and to eggs laid (Traver & Fell 2012) have been described using molecular techniques but are yet to be validated using histological evidence.

Heavy infection by *N. apis* can be detected by faecal marks of diarrhoea on the combs and the sides of the hive (Hertig 1923). This contamination on the hive is a main source of infection to honeybees as the spores contained in honeybee faecal waste are viable and capable of maintaining their

viability for over a year (Bailey 1962). In contrast to *N. apis*, *N. ceranae* infection has not been observed to be associated with diarrhoea (Higes *et al.* 2007).

Aside from the use of infection signs for diagnosis, more accurate diagnosis of nosemosis can be done through light microscopy by checking for *Nosema* spores in preparations of the ventricular tissue of honeybees (Giersch *et al.* 2009; Nabian *et al.* 2011). The use of light microscopy is limited in that spores of both species cannot be clearly separated from one another (Martín-Hernández *et al.* 2007; Nabian *et al.* 2011), the most accurate method of diagnosis capable of delimiting both species is molecular diagnosis (Chen *et al.* 2009; Forsgren & Fries 2010). This technique is highly sensitive and permits *N. apis* and *N. ceranae* detection from a diverse array of organic matter including hive bottom board scraps and frass (Copley *et al.* 2012). Different synthetic chemicals have been used against the *Nosema* disease, the most popular being fumagillin (Katznelson & Jamieson 1952; Williams *et al.* 2008; Higes *et al.* 2011).

Diseases caused by bacteria

Two of the most globally prevalent honeybee infections caused by bacteria include American foulbrood (AFB) and European foulbrood (EFB) diseases caused by the bacteria *Paenibacillus larvae* (Genersch *et al.* 2006) and *Melissococcus plutonius* (Bailey 1983), respectively. The former, AFB, is more widespread geographically and more acute of the two diseases. Both EFB and AFB are highly contagious diseases and are by OIE standards, notifiable (Lewbart 2011; OIE 2013).

European foulbrood disease

European foulbrood disease is caused by the Gram-positive bacterium *Melissococcus plutonius*, initially identified as *Streptococcus pluton* (Bailey 1957), then as *Melissococcus pluton* in 1982 (Bailey 1983) and finally as *M. plutonius* (Trüper & de'Clari 1998). The disease has been reported in South Africa (Govan *et al.* 1998; Davison *et al.* 1999), Algeria, Libya, Morocco, Tunisia, Tanzania, Senegal and Guinea-Bissau (Hussein 2000) (Fig. 3).

Melissococcus plutonius mostly affects larvae, especially those just about to be capped, and eventually kills larvae when they are about four to five days old (Forsgren *et al.* 2005). Characteristics of infection include larvae dying in a stretched-out (instead of typically coiled) position, change in colour from white to yellow then brown and even-

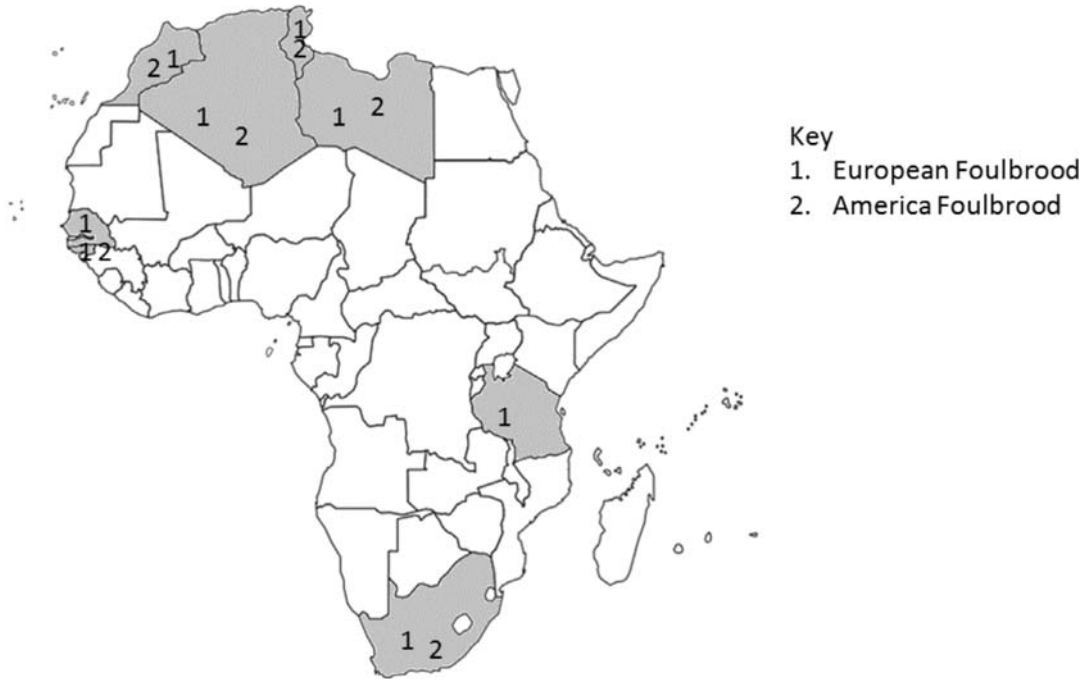


Fig. 3. Countries shaded in grey have confirmed presence of foulbrood disease. In non-shaded countries the status of the disease is unknown.

tually grey, and in cases where a large proportion of the brood in a hive are infected, a foul smell is emitted (Forsgren 2010). The pathogen mainly infects the gut of the honeybees, after gaining entry through contaminated food. The actual mechanism by which the pathogen kills the larva remains unclear. Adults infected by European foulbrood disease are asymptomatic and aid in the spread of the disease from one colony to another through foraging activities such as honey robbing.

The bacterium has been detected in larvae, pupae and honey using conventional and quantitative PCR (Forsgren *et al.* 2005; Forsgren *et al.* 2013) and new nano-particle-based techniques (Saleh *et al.* 2012). Field detection is done smelling the characteristic 'foul' odour, indicative of EFB infection. In Europe and North America, EFB is mostly experienced in spring and early summer.

Control of EFB in few European countries has been carried out through the application of the bacteriostatic antibiotic oxotetracycline hydrochloride (OTC) although use of this and other antibiotics in the hive has been banned in most countries around the world (Forsgren 2010).

American foulbrood disease

American foulbrood disease is caused by the Gram-positive bacterium *Paenibacillus larvae* formally known as *Bacillus larvae* (Heyndrickx *et al.* 1996; Genersch *et al.* 2006). Previously, AFB was thought to have a worldwide distribution, except in sub-Saharan Africa (Matheson 1996; Fries & Raina 2003). However, more recent surveys have shown the presence AFB in the Western Cape region of South Africa (Human *et al.* 2011) and Guinea Bissau (Hussein 2000; Hansen *et al.* 2003). This disease has also been identified in honey originating from Tunisia (Matheson 1993; Hussein 2000; Fries & Raina 2003; Hamdi *et al.* 2013), Algeria, Libya and Morocco (Hussein 2000) (Fig. 3).

The bacterium almost exclusively infects honeybees at the larval stage, as early as 12 hours after hatching (Genersch 2010). The infective stage of the pathogen is the endospore, which gains entry into the larva *via* the oral route and makes its way into the gut, where it degrades the chitinous peritrophic lining of the larva resulting in massive infection of the underlying epithelial cells (Garcia-Gonzalez & Genersch 2013).

The main routes of transmission are horizontal through inter-colony robbing activities (Lindström *et al.* 2008) and vertical *via* swarming of strong but clinically infected colonies (Fries & Camazine 2001; Fries *et al.* 2006). This pathogen can be vectored by *Aethina tumida* (Schäfer *et al.* 2010). Recently, researchers have developed models to simulate the spread and impact that different control strategies could have in case of an AFB epidemic (Zuur *et al.* 2009; Datta *et al.* 2013), although these models are still at the experimental stage.

The commonly used methods of control include burning of the clinically infected honeybee colonies and contaminated hive materials (Waite *et al.* 2003), shake swarm method where adult honeybees are shaken and let loose into a new, hygienically cleaned hive, without brood, and brood from the old hive destroyed (Pernal *et al.* 2008), and breeding of colonies with improved hygienic behaviour towards AFB infections (Spivak & Reuter 2001).

CONCLUSION

This survey of existing literature shows significant knowledge gaps on the incidence, prevalence, diversity, and geographic occurrence of honeybee diseases in Africa, emphasizing the need for continent-wide bee epidermiological surveys. Although it is clear that honeybee pathogens have so far had a much less adverse impact on African honeybees compared to honeybees in Europe and U.S.A., reports of declining feral colonies of bees in some parts of Africa suggest a trend similar to other affected regions around the world (Neumann & Carreck 2010; Kluser *et al.* 2011). Despite the high genetic diversity and numerous feral bee colonies

that Africa possesses (Kajobe & Roubik 2006; Moritz *et al.* 2007; De la Rúa *et al.* 2009; Dietemann *et al.* 2009), knowledge on bee diseases indigenous to the African continent and the true origin of several already described diseases is lacking. The paucity of information concerning risk factors associated with these diseases and pathogens implies that there is still much to be done to fill these knowledge gaps. Specifically, we need to understand the diversity and epidemiology of bee diseases and the effects that apicultural practices unique to this continent have on feral and managed honeybee populations in Africa. Also, there is an urgency to understand how honeybee pathogens and pests are spread across the continent, influenced by both anthropological (such as trade and modern beekeeping practices), biotic factors (such as honeybee migration and hybridization) and abiotic (such as climatic and environmental) factors. While it has been noted that many African countries have developed policies and regulations in a bid to conserve wildlife, few countries have regulations specific to honeybee conservation and fewer still understand the threats faced by honeybees within their borders. Addressing these issues will bring us closer to identifying the threats to bee health on the African continent and develop/adopt appropriate and cross-border policies to ensure their conservation and continued resource to food, feed and the broader ecosystem.

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