Honey bee pathogens in Ghana and the presence of contaminated beeswax

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Received 23 September 2016 – Revised 3 April 2017 – Accepted 11 May 2017

Abstract – A nationwide survey was performed to study the distribution of parasites, pathogens and pesticides in managed honey bee populations in Ghana. When 45 colonies were sampled and inspected for signs of disease, *Varroa destructor* was the most prevalent parasite (89%; $n = 40$), all mites corresponding to the Korean haplotype of this pathogen. *Aethina tumida* (42%; $n = 19$) and *Braula coeca* (7%; $n = 3$) were also detected, as were *Melissococcus plutonius* and trypanosomatids (7%). By contrast, *Nosema* spp., *Acarapis* spp., *Ascosphaera apis* and *Paenibacillus larvae* were not detected by molecular screening. Amitraz was the most widely distributed pesticide (75%; $n = 23$) followed by coumaphos (47%; $n = 15$), chlorpyrifos (34%; $n = 15$) and fluralinate (31%; $n = 10$). This survey lays the groundwork for further monitoring of honey bee populations in Ghana.

epidemiology / Ghana / *Varroa* / pesticides / honey bees

1. INTRODUCTION

Managed honey bees (*Apis mellifera* L.) are widely known for their importance as pollinators and honey producers (Allsopp et al. 2008; Strauss et al. 2013). Indeed, the productivity of many valuable crops is strongly tied to honey bee pollination services, reflecting a global value of 153 billion € and a value of 11.9 billion € in Africa

alone (Gallai et al. 2009). However, these values are based on services rendered only by managed honey bees, and thus, they underestimate the true value of pollination (Allsopp et al. 2008). Wild honey bee populations are an important characteristic of sub-Saharan African countries (Moritz et al. 2005), a distinctive feature that has been neglected in smallholding crop system ecosystem services research (Garibaldi et al. 2016) where beekeeping is mostly based on trapping swarms from the wild. This contrasts considerably with Europe or North America where apiculture is breeding-based, a feature that has affected the population structure of European honey bee sub-species (De la Rúa et al. 2009).

While the health of honey bees in Europe and North America has been an important topic of

Electronic supplementary material The online version of this article (doi:10.1007/s13592-017-0518-2) contains supplementary material, which is available to authorized users.

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Manuscript editor: Yves Le Conte

research in recent years (Genersch 2010), the health status of honey bees in Africa has been largely ignored (Dietemann et al. 2009; Frazier et al. 2010; Strauss et al. 2013; Pirk et al. 2016). Honey bee pathogens have been detected in some studies carried out in Africa, mainly in East Africa, although unlike other parts of the world no significant deleterious effects of these pathogens were evident (Dietemann et al. 2009; Rosenkranz et al. 2010). Nevertheless, recent reports of declining feral honey bee colonies in South Africa suggest that this region may be experiencing similar colony loss to other regions around the globe (Neumann and Carreck 2010; Goulson et al. 2015).

Still, African honey bees have been considered to be more resistant or better adapted to the effects of most common parasites (Moretto et al. 1991; DeJong et al. 1997; Vieira et al. 2000; Moretto and L.J. de M. 2001). Studies into the spread and distribution of *Varroa destructor* (Anderson and Trueman 2000) and other pathogens in South Africa (Strauss et al. 2013, 2014a, b, 2016; Mortensen et al. 2016), Kenya (Frazier et al. 2010), Uganda (Chemurot et al. 2016) or Benin (Paraiso et al. 2011) support this hypothesis. However, very few surveys have been undertaken in West African countries representing a significant gap in our understanding of the incidence, prevalence, diversity and geographic distribution of honey bee diseases on that continent (Muli et al. 2014; Mumoki et al. 2014). The presence of *V. destructor* has been reported in bee colonies from Ghana (Frazier et al. 2010; Mutsaers 2012, pers. comm.), yet there is no information available as to the prevalence and haplotype of *Varroa* in Ghana, nor on the presence of other agents such as *Nosema apis* (Zander 1909), *Nosema ceranae* (Fries 1996) or *Braula coeca* (Nitzsch 1818; reviewed by Pirk et al. 2016). By contrast, some pathogens have been described in nearby countries, such as *B. coeca* in Benin (Paraiso et al. 2011).

In July 2013, a nationwide survey was carried out to obtain comprehensive information about the distribution of parasites, pathogens (excluding viruses) and pesticides in honey bee populations throughout Ghana. Given the relatively recent report of *Varroa* in this region and the lack of knowledge about the health status of honey bee colonies, this survey lays the groundwork to

examine the factors affecting honey bee health in Ghana to further monitor the honey bee colony losses in this country.

2. MATERIALS AND METHODS

2.1. Sample collection

The colonies surveyed ($n = 45$) were distributed across 19 different geo-identified locations from the Upper East region of the country and covering seven of the ten regions in Ghana (Figure 1; Supplementary Table 1). Since there is no obligation to register as a beekeeper at any official establishment in Ghana, the list of beekeepers to be contacted was provided by Dr. Kwame Aidoo, professor at Cape Coast University (Ghana).

All samples were taken after sunset with the help of local beekeepers that manage the hives. House honey bees (>150) and a piece of brood comb were taken from each hive, and where possible, both comb-stored pollen and wax were also collected and kept chilled for further analysis.

2.2. Survey of beekeepers

Beekeepers at each sampling location filled out a questionnaire on beekeeping skills, apiary size and management, production and health status (see Supplementary material).

2.3. Molecular identification of honey bees and mites

2.3.1. DNA extraction

Total DNA from honey bees (three worker legs) and mites (whole individuals) was extracted following a modified Chelex-based method (Walsh et al. 1991; Muñoz et al. 2008).

2.3.2. Honey bee mtDNA analysis

The mitochondrial haplotype of 31 worker bees from 14 different locations was determined as described previously (Evans et al. 2013) and based on sequence variation in the intergenic *cox1*-tRNA^{leu} region (Gamery et al. 1993).

Figure 1. Map of Ghana showing the sampling sites (*dots*) and the names of the locations.

2.3.3. Mite mtDNA analysis

A total of 19 mites from 12 different locations were identified as described previously (Anderson and Fuchs 1998; Muñoz et al. 2008) and based on PCR-RFLP of a fragment of the mitochondrial *cox1* gene following digestion with the *Xho*I and *Sac*I enzymes.

2.4. Pathogen screening

Varroa mite detection was performed in situ as described previously (Higes et al. 2009; Garrido Bailón 2013). Adult bees were frozen for handling, while brood cells were opened directly to count the *Varroa* mites. Evidence of any other pests and any other findings were also noted. Mite infestation of adult bees and brood was calculated as the number of mites per 100 bees or cells, respectively. The adult bees and brood samples were then maintained in ethanol (70%) for PCR analyses. A broad screening of pathogens was then performed in Spain using previously published PCR assays for brood and adult pathogens (see below; Fig. 2).

2.4.1. DNA extraction

Samples were washed to remove the alcohol, and for each colony, 30 worker bees were macerated in 15 mL H₂O using sterile bags with a filter. The resulting tissue pellets were resuspended in 3 mL sterile water, and 400 µL was transferred to a 96-well plate (Qiagen) with glass beads (2 mm diameter: Sigma). Samples were then processed as described previously (Martín-Hernández et al. 2012).

2.4.2. PCR analyses

All PCR analyses were performed using previously published assays for brood pathogens, such as *Ascosphaera apis*, *Paenibacillus larvae* and *Melissococcus plutonius* (Garrido-Bailón et al. 2013); *N. apis* and *N. ceranae* (Martín-Hernández et al. 2012); *Acarapis* spp. (Garrido-Bailón et al. 2012); trypanosomatids and neogregarines (Meeus et al. 2010) and small hive beetle (SHB; Ward et al. 2007). Given that the 18S rDNA gene alone is unsuitable to define the trypanosomatid species infecting honey bee colonies, these were also studied as described elsewhere (Cepero et al.

Figure 2. Pathogen prevalence in the honey bees sampled in Ghana.

2014). All the sequences used for these analyses have been uploaded to GenBank.

2.5. Pesticide analysis on beeswax

Beeswax samples ($n = 32$; Table I) were analysed by Dr. Picó (Universitat de València) using a modified QuEChERS method (Krupke et al. 2012; Lambert et al. 2013) that is capable of detecting 58 compounds with a limit of detection $<10\text{ ng g}^{-1}$ in all cases.

2.6. Statistical analysis

Spearman correlation tests were performed to compare the different incidences of the pathogens and parasites detected in relation to altitude. Each sampling site was assigned an altitude value using GIS software ArcGis v.9.2 (ESRI, Redlands, CA, USA; Table I), and the statistical analysis was performed using SAS 9.4.

3. RESULTS

The questionnaire completed by the beekeepers showed that only 3 out of the 19 beekeepers

surveyed practiced beekeeping professionally (i.e. all their income came from beekeeping), while for the majority it was a complementary work. All their managed hives were “Top Bar” hives of different shapes and none used wax foundations. While they all produced and sold honey and beeswax, only 53% ($n = 10$) harvested propolis and 11% ($n = 2$) pollen. Most of the beekeepers surveyed (all but two) were neither aware of the presence of *V. destructor* mites nor had they observed any negative impact on the survival and/or productivity of their honey bees. In fact, while 42% of beekeepers ($n = 8$) had lost colonies in recent years, none had attributed this to poor health but rather to the absconding phenomena. SHB and the wax moth (*Galleria mellonella*) were best known among the beekeepers (74%; $n = 14$), yet none of the beekeepers surveyed apparently used any treatment against any pest or disease.

In terms of haplotype characterization, all the samples but one were of the African A_I sub-lineage and three haplotypes were detected: A_I ($n = 25$), A_4 ($n = 3$) and A_4' ($n = 2$). The other sub-lineage detected was the African sub-lineage with an Atlantic distribution A_{III} ; specifically, the A_{14} haplotype was detected in one colony.

Table I. Beeswax samples and pesticide analysis

	N x	N positives (%)	μ (ng/g)	sd	Max (ng/g)	Min (ng/g)
Amitraz ^a	32	24 (75)	5556.0	9730.8	45,890	194
Chlorfenvinphos	32	1 (3)	20.0		20	20
Chlorpyrifos	32	11 (34)	31.4	1.6	35	30
λ -Cialotrin	32	1 (3)	160.0		160	160
Coumaphos	32	15 (47)	588.2	2140.4	8320	5
Ethion	32	1 (3)	40.0		40	40
Fluvalinate	32	10 (31)	239.5	90.9	365	2
Imazalil	32	1 (3)	50.0		50	50
Methiocarb	32	1 (3)	20.0		20	20
Prochloraz	32	2 (6)	40.0	14.1	50	30
Pyriproxyfen	32	2 (6)	37.5	17.7	25	25
Thiabendazole	32	2 (6)	37.5	10.6	45	30
Terbutylazine-2-hydroxy	32	1 (3)	10.0		10	10

N number of analysed samples

^a Amitraz was detected as its metabolite *N*-(2,4-dimethylphenyl)formamide, and values converted using their molecular mass (1 mol of *N*-(2,4-dimethylphenyl)formamide, whose mass is 162.0, comes from 1 mol of Amitraz, whose mass is 293.4)

All the mites analysed showed 100% similarity to the Korean *V. destructor* haplotype described previously (GenBank AF106899; Anderson and Trueman 2000). In adult bees, *V. destructor* (phoretic mites) were found in 18 colonies (40%) with infestation levels ranging from 0.3 to 4.6% (mean 2.0%, s.d. 1.3). Significantly higher infestation was evident in brood cells, as it was seen in 40 out of the 45 colonies sampled and with values ranging from 1.7 to 105.0% (mean 15.9%, sd 22.4), representing a prevalence of 89%. Occasionally, mites caused double ($n = 32$) or triple ($n = 15$) infections in cells, although the most common finding was one mite per cell ($n = 352$, representing 58% of the observed prevalence). No correlation was detected between *Varroa* prevalence and the altitude (elevation) of the collection sites ($P = 0.4$).

Clinical inspection did not reveal any sign of varroosis, even though pests were readily detected in the colony. Indeed, 118 SHB were collected in 42% ($n = 19$) of the colonies when sampling adult bees, *B. coeca* was found in three colonies and wax moth (*G. mellonella*) and big beetles (*Oplostomus fuliginus*) were morphologically identified ($n = 2$). Pathogen screening failed to detect any of the following agents: *A. apis*, *P. larvae*, *N. apis*, *N. ceranae* and *Acarapis* spp. Conversely, the bacteria *M. plutonius* was present in three samples, as were trypanosomatids, yet no clinical signs of foul-brood disease were found in the samples.

Traces of 13 pesticides were found, and none of the samples were free of such residues. The number of residues found ranged from one to nine per sample. Amitraz was the most widely distributed pesticide (75%), and it was the pesticide detected in the highest amounts (194–45.9 ng/g), followed by coumaphos (47%; 5–8.3 ng/g), chlorpyrifos (34%; 30–35 ng/g) and fluvalinate (31%; 2–365 ng/g), all commonly used varroicides in Europe and America and also used in other branches of agriculture. The remaining chemicals detected were rarely present (3–6%), and they belonged to a wide range of agricultural or veterinary products that included thiabendazole, prochloraz or pyriproxyfen. In the questionnaire, none of the beekeepers recognized the use of any such treatments inside the hives.

4. DISCUSSION

There is considerable biodiversity among the *A. mellifera* subspecies on the African continent, yet beekeeping is not as fully developed there as it is in Europe. Indeed, 11 subspecies have been described on this continent (Ruttner 1988; Hepburn and Radloff 1998; Franck et al. 2000, 2001; Le Conte and Navajas 2008; Meixner et al. 2011): *A. m. intermissa* and *A. m. sahariensis* in northwest Africa; *A. m. lamarckii* in Egypt; *A. m. simensis* in Ethiopia; *A. m. jemenitica*, *A. m. adansonii*, *A. m. scutellata*, *A. m. litorea*, *A. m. monticola* and *A. m. capensis* in sub-Saharan Africa and *A. m. unicolor* in Madagascar, with *A. m. adansonii* being the subspecies thought to inhabit Ghana (Hepburn and Radloff 1998). In relation to the evolutionary lineages, *A. m. adansonii* colonies were shown to belong to two African sub-lineages, mainly A_I but also A_{III}, with A_I and A₄ being the most frequent haplotypes in this subspecies (Franck et al. 2001). Our results on the genetic composition of the honey bee colonies studied confirmed that it was described in other western African populations, and they corroborated the abundance of the A_I and A₄ haplotypes. The presence of the A₁₄ haplotype in these honey bees (described by De la Rúa et al. 1998 in honey bee colonies from the Canary Islands), albeit in a single unique colony, confirms the dispersion of the A_{III} sub-lineage along the Atlantic coast of Africa.

The honey bee mite *V. destructor* represents the greatest threat to apiculture, and it is found almost universally (Rosenkranz et al. 2010). The presence of *V. destructor* was first described in one region in Ghana (Fazier et al. 2010), while the present data shows it to be widely distributed in all the regions sampled, although the Korean haplotype was the only one found. This haplotype is considered to be more virulent than the Japanese haplotype (Anderson and Trueman 2000), and it has also been reported in other African countries (Allsopp et al. 1997; Dietemann et al. 2009; Fazier et al. 2010; Muli et al. 2014; Mumoki et al. 2014; Chemurot et al. 2016), supporting the hypothesis that this mite is spreading throughout the continent.

A relatively high mean *Varroa* prevalence was found in brood, higher than that reported

previously in Ghana (Fazier et al. 2010) and more similar to that reported for East Africa (Muli et al. 2014). Indeed, infestation rates are above the treatment threshold recognized for managed colonies in European subspecies of *A. mellifera* (Delaplane and Hood 1997). This high rate raises the question if indeed all colonies are tolerant, or if at current they are experiencing a selection process. Without underestimating the natural selection processes, it is important to note that no clinical signs of varroosis were detected on inspection (depopulation, deformed wings or brood abnormalities), supporting previous suggestions that African honeybees are more tolerant to *Varroa* mite infestation (Martin and Medina 2004; Dietemann et al. 2009; Muli et al. 2014; Strauss et al. 2014a). This tolerance might be related to the cell size, and the shorter capped time of both worker and drone cells (Martin and Medina 2004; Rosenkranz et al. 2010). These observations might also reflect the lower preference of the mites for worker cells in this honey bee species, explaining the fewer double or triple infestations found. This would also explain the low numbers of mites found on adult bees, and the relatively low levels observed in their brood in terms of prevalence and infection rates, similar to those reported in other east African countries (Muli et al. 2014; Chemurot et al. 2016; Mortensen et al. 2016).

Another consideration on the high infestation rates would be whether this represents an invasion wave or not. Allsopp (2006) observed very high mite levels before the development of mite tolerance, which took 3 to 5 years in the Cape honeybee (*Apis mellifera capensis*) and six to seven in the Savanna honeybee (*Apis mellifera scutellata*). According to Mutsaers (2012, pers. comm.), *Varroa* mites are present in Northern Ghana at least since early 2008, being more likely that infestation levels found are due to the local subspecies tolerance to the mite and not to an invasion wave.

Ghana is located in a tropical region between the Equator and the Tropic of Cancer, with big differences in climate across the country. The North has a typically longer dry season than the South, with rainfall over a shorter period. Latitude has been suggested to be linked to *Varroa* levels

in some areas due to the distinct environmental conditions, although infestation rates may vary for a number of reasons (Rosenkranz et al. 2010). Thus, a correlation with latitude might be due to some specific coincidences. In this study, no correlation was found between the *Varroa* levels in hives and the altitude of the sampling site, in accordance with other data from tropical climates (Chemurot et al. 2016; Mortensen et al. 2016).

Small hive beetle (*Aethina tumida*) is known to represent no threat in sub-Saharan Africa since it is endemic to this region (Dietemann et al. 2009) and honey bees have co-evolved with it. However, high infestation rates might be responsible for some deleterious effects both at the colony level and in terms of production. SHB was distributed extensively in the samples studied here, yet no clinical effects were attributed to its presence.

The identification of *B. coeca* represents the first time this parasite has been found in Ghanaian honey bee colonies. Given the high prevalence of pesticides found in wax, it is a surprising finding, as most of the compounds found are thought to act as both insecticides and acaricides. However, the *Braula* levels detected are very low, similar to those reported in the bordering Benin (Paraiso et al. 2011), and so it can be considered a sporadic finding. In Europe, the widespread use of pesticides to control *Varroa* has eliminated *Braula* from most European honeybee colonies, and a similar situation in Ghana (with high levels of pesticides in the hives) might explain this result.

The low levels of *O. fuliginis* found indicate that this pest currently does not suppose a serious health problem; even high levels can be problematic (Hepburn and Radloff 1998, reviewed by Pirk et al. 2016).

Molecular tools are very effective when identifying pathogens, although their detection should always be correlated to clinical signs in the host for a disease to be diagnosed. Detection of *M. plutonius* in Ghana is not new (reviewed by Pirk et al. 2016), yet its low levels of detection and the absence of signs of foulbrood disease are a good indicator of health. This is the first time trypanosomatids have been detected in an African country. While their true effect on honey bee health is still unknown, their

presence has recently been correlated with colony losses in the USA and Belgium, especially when detected in conjunction with *N. ceranae* (Cornman et al. 2012; Runckel et al. 2011; Ravoet et al. 2013), although *N. ceranae* was not detected here in any sample.

To date, there has been no evidence of pesticides in beeswax from Ghana and according to the beekeeper's survey, no treatment was applied to the colonies and beeswax is not reused in foundations. Acaricides, herbicides and other insecticides are the most prevalent chemical residues found in managed colonies in Europe and North America (Bernal et al. 2010; Johnson et al. 2010; Mullin et al. 2010; Lambert et al. 2013), which were also the most prevalent chemical families found in this study. All pesticide levels were low compared to those reported from studies in Europe (Vázquez et al. 2015; García et al. 2017), with the exception of two of them: Amitraz and coumaphos.

These results are worrisome due to the high prevalence found and because the route of exposure remains unclear. One hypothesis might be that beekeepers are pouring different commonly used pesticides for agriculture or tick control, in the areas surrounding the hives to avoid problems with ants or other common pests. Honey bee colonies seem to act as a sink for pesticides in the environment. While honey bees are sensible to toxic substances, their entry into the hive depends on particular circumstances of the colony and its individuals (Johnson 2015). Beeswax trade has in the past gone from Africa to Europe; therefore, it seems unlikely that Ghana had imported polluted wax, raising the need to clarify both the route of exposure and the health impact of these high levels of the different compounds.

This survey provides key data for both researchers and beekeepers in Ghana. The results show that *Varroa* has spread to Ghana and is well established, while beekeepers are unaware of this, suggesting that it is indeed not a problem for their bees. However, the beeswax contains several well-known varroacides, and how these may have come into the hives remains unknown.

This work lays the groundwork for further monitoring of bee populations in West Africa. The data presented here are an initial incursion into the examination of the factors affecting honey bee health in a West African country. It was noteworthy that few pathogens were detected, including some of those directly linked to the colony collapse phenomena in Europe and North America. Monitoring this health situation may help clarify if honey bee colony losses are also occurring in this geographical area and, if so, what is the cause.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the help and support received by beekeepers from all over Ghana, with special mention to the staff at the Animal Health and Production College at Pong-Tamale, Dr. Rosa Gálvez for her support using GIS software and Airbus Defence and Space for their funding. IM was supported by a postdoctoral fellowship from the University of Murcia (R-1017/2015). PDLR received support from the COST Action FA1307 (SUPER-B). Financial support for this research was also provided by the 19908-GERM-15 project of Regional Excellence from the Fundación Séneca (Gobierno Regional de Murcia, Spain).

AUTHORS' CONTRIBUTIONS

MLI-P wrote the paper and together with AM conceived and performed this research; KA, EOB and FP gave local support; MH and RM-H helped with analyses and PR and IM did the haplotype identification.

Pathogènes de l'abeille au Ghana et présence de cire contaminée

Epidémiologie / Afrique de l'Ouest / varroa / pesticides / *Apis mellifera*

Pathogene der Honigbienen in Ghana und das Vorhandensein von kontaminiertem Bienenwachs

Epidemiologie / Ghana / Varroa / Pestizide / *Apis mellifera*

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