Atypical viral and parasitic pattern in Algerian honey bee subspecies *Apis mellifera intermissa* and *A. m. sahariensis*

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Abstract

Unusually high losses of honey bee colonies are reported in many regions of the world, but little data is available concerning the status of honey bee stocks in Africa. However, the situation on this continent, where beekeeping is weakly developed and where the wild population of the pollinator remains large, can give us an insight on the causes of increased mortalities elsewhere. In this study, we evaluate the health status of *Apis mellifera intermissa* and *A. m. sahariensis* populations inhabiting the north and the south of Algeria, respectively. We report few colony losses associated with an atypical

pattern of prevalence for common honey bee parasites and pathogens. The presence or absence of these risk factors is discussed in relation to the occurrence of local and global colony losses to contribute to our understanding of how honey bee pathogens and parasite impact this pollinator's health.

Keywords

Apis mellifera intermissa
Apis mellifera sahariensis
virus
Nosema spp.
Varroa destructor
colony losses
Algeria

1. Introduction

Honey bees represent a well-studied group of social insects with both ecological and agricultural importance (Winston 1987; Breeze et al. 2014). It was estimated that one third of the food crop benefits for the service of pollinators, of which honey bees represent a large proportion (Delaplane and Mayer 2000; Gallai et al. 2008; Aizen and Harder 2009). Worldwide elevated mortality of honey bee colonies is therefore a worrying problem for beekeeping, and agriculture and, but also for ecosystem functioning. Although several factors may play a role in these declines (vanEngelsdorp et al. 2010; Cornman et al. 2012), most mortalities have been attributed to high loads of parasites and pathogens, such as high infestations by the ectoparasitic mite *Varroa destructor*, together with associated viruses (Cox-Foster et al. 2007; Berthoud et al. 2010; Dainat et al. 2012a, b; Dietemann et al. 2012; Nazzi et al. 2012; Francis et al. 2013). The microsporidian *Nosema ceranae* has also been

incriminated in colony mortality in southern Europe (Higes et al. 2008). The detection, biology, epidemiology of various parasites and pathogens and their relationships with mortality of are therefore studied in Europe, USA and Asia (e.g. Tentcheva et al. 2004; Chen and Siede 2007; Genersch and Aubert 2010; Cornman et al. 2012; Li et al. 2012; Francis et al. 2013; Yang et al. 2013). However little data on honeybee pathogens and health status is available from Africa (Dietemann et al. 2009; Adjlane et al. 2012a, b, c; Mumoki et al. 2014; Amakpe et al. 2015). Defining the relationship between honeybees and their pests and pathogens in various contexts, including those in which honey bee colonies are not declining, improves our understanding, to date still patchy, of the causes of the honey bee colony losses occurring globally. To contribute to the filling of this gap, we investigated the health status and pathogens occurring in the two honey bee subspecies endemic to Algeria, *Apis mellifera intermissa* and *A.m. sahariensis. A. m. sahariensis* predominates in the south of the country due to its adaptation to drought conditions, whereas *A. m. intermissa* occurs in the North. The presence of *N. ceranae*, SBV and DWV has already been reported (Matheson 1993; Higes et al. 2009a; Loucif-Ayad et al. 2013) for some localities in north and west Algeria in *A. m. intermissa*. To expand on these results, we measured the prevalence and infestation rates of the parasitic mite *V. destructor*, as well as the prevalence of deformed wing virus (DWV), Varroa destructor virus
(VDV-1), sac brood virus (SBV), black queen cell virus (BQCV), acute bee paralysis virus (ABPV), Kashmir bee virus (KBV), Israeli acute paralysis virus (IAPV), chronic bee paralysis virus
(CBPV), as well as of *N. apis* and *N. ceranae in the major beekeeping districts*. In addition, we determined the percentage of colony losses experienced by beekeepers with a questionnaire (van der Zee et al. 2012) and investigated the relation of parasite or pathogen occurrence to these losses. We compare the patterns observed in Algeria to the reports from other countries in Africa and worldwide in order to contribute to a more comprehensive understanding of the role of pathogens in honey bee health.

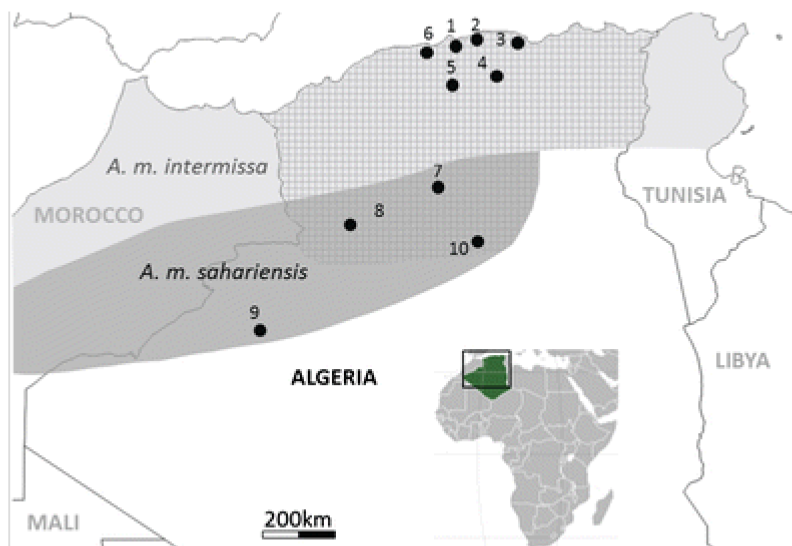
2. Materials and methods

2.1. Study areas

A. m. intermissa inhabits the north of Algeria from the coast, over the Tellian Atlas to the Saharian Atlas (Figure 1). The coast as well as the plains and high altitude plateaus between the mountains are rich in cultures, forests and low plants providing forage to the honeybees. The climate is of Mediterranean type, and brood production is interrupted shortly in December during winter when the temperature drop, as well as during the warmest month in summer. The oases south of the Saharan Atlas are home to *A. m. saharensis* (Figure 1). Given the more extreme temperatures in this desertic region, brood interruption in both winter and summer is longer. The subspecies can be differentiated by their distinct distribution areas on each side of the Saharan Atlas and by their differences in coloration. *A. m. intermissa* is black, whereas A. m. sahariensis is yellow. *A. m. intermissa* colonies in the south of Algeria were identified based on the beekeepers’ migration records and worker and worker body colour.

Figure 1.

Location of sampling areas for *Nosema spp*. and virus screening (*circles*) as well as *V. destructor* (*squared area*). Apiaries of *A. m. intermissa* are numbered *1–6* and apiaries of *A. m. sahariensis* are numbered *7–10*. Distribution ranges of the endemic honey bee subspecies are indicated in *shaded areas*.



2.2. Colony losses

Colony losses for the winter 2012–2013 were quantified with a questionnaire sent to beekeepers (Van der Zee et al. 2012). They were asked how many production colonies they had before winter and how many of these colonies were lost during winter (December to March). From these values, we calculated the percentage of colonies lost over winter. Out of the total 225 beekeepers who answered the questionnaire, 136 of the participating beekeepers kept A. m. intermissa, in the north of Algeria, while 20 beekeepers were known to migrate colonies of this subspecies to the South: In the South, nine beekeepers using the endemic A. m. sahariensis answered the questionnaire. The participating individuals represent 35 % of the beekeepers in their administrative districts.

2.3. Colonies used for parasite and pathogen screening

We collected samples from 130 apiaries within the administrative districts where beekeeping is practised (Figure 1) and belonging to beekeepers volunteering to take part to the study. Ninety apiaries located in northern Algeria and 28 in the South of the country hosted *A. m. intermissa*. In addition, 12 apiaries hosting *A. m.sahariensis* in southern Algeria were used. From each apiary, we randomly selected three apparently healthy colonies for sampling. These colonies showed no obvious clinical signs of varroasis, nosemosis, American or European foulbrood. Workers collected from brood frames were fixed in RNA later solution and placed in a refrigerated box for transportation to the laboratory. After a maximum of 2-h transport, the samples were frozen at −80 °C until shipping to Switzerland for molecular diagnosis (Dainat et al. 2011). After transport at room temperature between the countries, samples were frozen at −20 °C for a few days until analysis.

2.4. *Varroa destructor*

To determine the average *V. destructor* infestation rate of an adult honey bees per apiary (*N* = 130), 300 workers were collected from the brood frames of each colony sampled (N =360, 1-3 colonies per apiary). This was done between January and April 2012, a period between winter and beginning of spring in both north and south Algeria. The samples were stored at −18 °C until infestation rate measurement. For this measurement, we separated the mites from their host by placing the workers in jars filled with 70° ethanol and shaking for 3 min (Shimanuki and Knox 2000). To measure brood infestation rates, we obtained two frames with capped brood from each colony. A total of 250 cells were opened per colony to calculate the percentage of infested cells (see Dietemann et al. 2013 for methodology).

2.5. Virus and *Nosema* spp. screening

Six of the 90 *A. m. intermissa* and four of the 12 *A. m. sahariensis* apiaries were selected in the North and South regions, respectively (Figure 1) to be representative for both subspecies. Samples of 50 worker bees were collected once in spring, between May and July 2012. Virus screening and *Nosema spp.* species identification were performed on pools of these 50 workers. *V. destructor* functions as a vector and host for honeybee viruses (Rosenkranz et al. 2010). In order to determine whether these parasites might play a role in spreading viruses in Algerian honey bee populations, we also screened one to nine mites obtained from one to three colonies in five *A. m. intermissa* and three *A. m. sahariensis* apiaries (totalling 12 and six colonies, respectively). See supplementary material for technical details on molecular diagnosis of the pathogens.

2.6. Statistical analysis

A χ^2test with Yates correction was performed to compare virus prevalence between *A. m. intermissa* and *A. m. sahariensis* colonies. The same test was used to compare *Nosema spp*. prevalence between the subspecies. An ANOVA was used to compare the proportions of colony losses between the subspecies and the *V. destructor* infestation rates between the three population studied ($N = 130$ colonies). Percentage data were transformed with arcsine. Pairwise comparisons were performed with the post-hoc Scheffé test. Normal distribution was verified with the K-S (Lilliefors) test and homogeneity of variance with the Levene test. When one of these assumptions was not met, the results of the ANOVA were confirmed with a Kruskal-Wallis non-parametric test. Statistical analyses were performed with SYSTAT (version13.0).

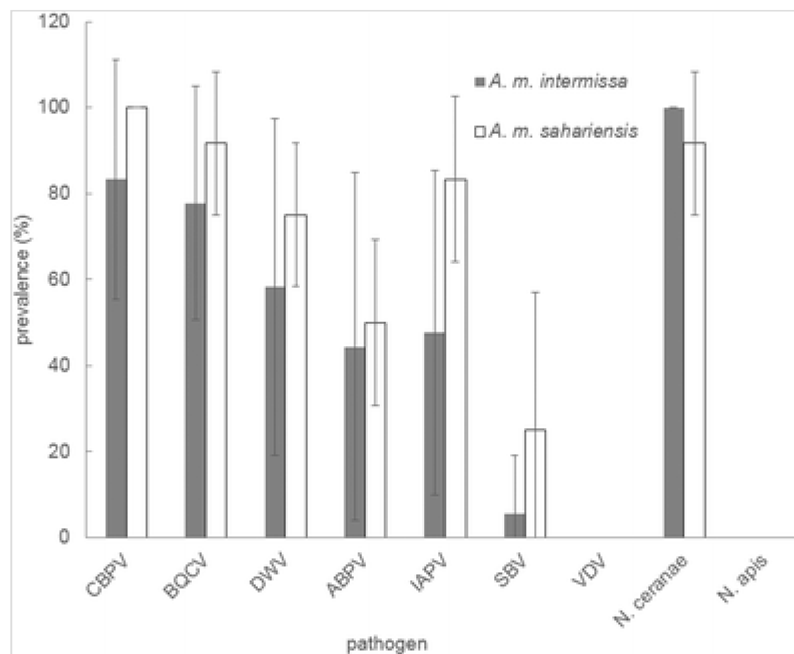
3. Results

3.1. Colony losses

The proportion of colony losses was significantly higher for *A. m. intermissa* in the North than for both subspecies in the South (ANOVA, F (2162) = 45.02, $P < 0.01$, Figure 2), where there was no significant difference between *A. m. intermissa* and *A. m. sahariensis* ($P = 0.98$).

Figure 2.

Percentage of colony losses over the winter 2012/2013 in the Algerian honey bee populations studied.

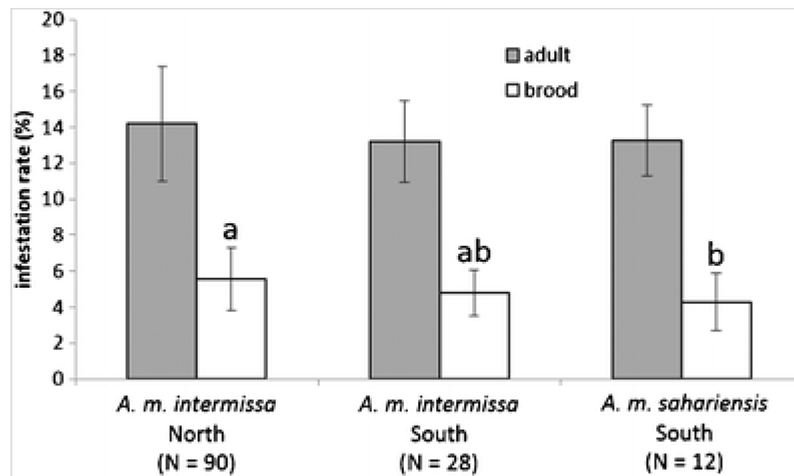


3.2. *Varroa destructor*

Varroa mites were found in 100 % of the 118 *A. m. intermissa* colonies (both from the north and south of Algeria) and in 100 % out of the 12 *A. m. sahariensis* colonies. Average infestation rates of adult bee populations varied in a narrow range (13.2 and 14.2 %) between the three populations (Figure 3). The same trend could be observed for average brood infestation rates, which ranged between 4.3 and 5.6 % between the three populations. Adult infestation rates were not significantly different between North and South populations of *A. m. intermissa* and between *A. m. intermissa* and *A. m. sahariensis* (ANOVA, F (2127) = 1.22, $P = 0.30$). The same was not true for the brood infestation rates, which were significantly different (ANOVA, F (2127) = 4.35, $P = 0.02$, Figure 3). Brood infestation rate of *A. m. intermissa* in the North was significantly higher than *A. m. sahariensis* ($P = 0.024$), but not than *A. m. intermissa* colonies kept in the South ($P = 0.35$).

Figure 3.

V. destructor infestation rates (±SD) of adult bees and brood in three populations of *A. m. intermissa* and *A. m. sahariensis* in Algeria. *Different letters* above the *bars* indicate *P* values below 0.05 with the Scheffé test.

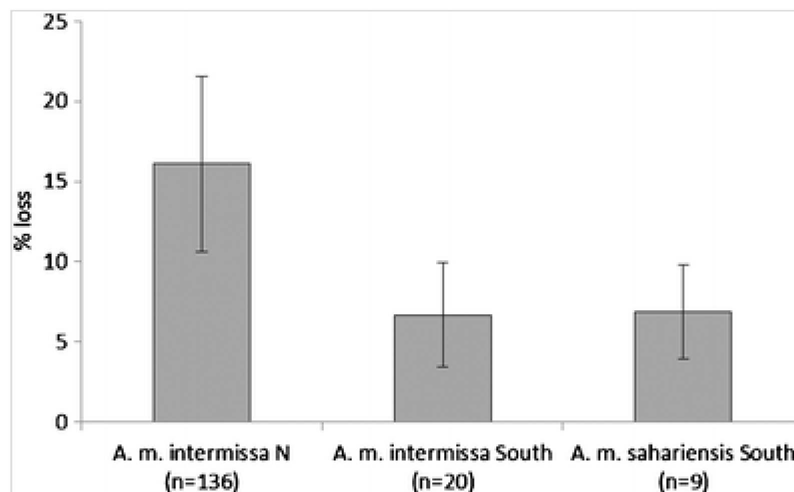


3.3. Viruses

Six of the seven viruses screened were detected at prevalence ranging from 6 to 100 %. There was no significant difference in prevalence at colony level of most viruses between the two subspecies ($\chi^2_{CBPV}(1, N = 29) = 0.24$, $P_{CBPV} < 0.62$; $\chi^2_{BQCV}(1, N = 29) = 0.32$, $P_{BQCV} < 0.57$; $\chi^2_{DWV}(1, N = 29) = 0.26$, $P_{DWV} < 0.61$; $\chi^2_{APBV}(1, N = 29) = 0.0$, $P_{ABPV} = 1.0$; χ^2_{SBV} $(1, N = 11) = 0.85$, $P_{SBV} = 0.36$). Of those, CBPV was the most prevalent, followed by BQCV, DWV, APBV and SBV (Figure 4). IAPV showed the largest difference between subspecies (Figure 4), but the difference was not significant (χ^2_{IAPV} $(1, N = 29) = 3.56$; $P_{IAPV} = 0.06$). IAPV came third in the decreasing order of prevalence in *A. m. sahariensis* and fifth in *A. m. intermissa*. VDV-1 was not found in this survey. The proportion of dual infections in the honeybee samples was 27 %, which of triple infections 16 % and 12 % for quadruple infections (Supplementary material Table S2).

Figure 4.

Average apiary pathogen prevalence in percent (±SD) for *A. m. intermissa* in north Algeria ($N = 17$ colonies in six apiaries) and *A. m. sahariensis* in south Algeria ($N = 12$ colonies in four apiaries).



In contrast to honey bees, ABPV was the most prevalent virus in *V. destructor* found on *A. m. intermissa* with 33 % of samples infected, followed by CBPV (20 %) and DWV (10 %). In *A. m. sahariensis*, only DWV was detected and found in 33 % of the mite samples. BQCV, SBV and IAPV were not detected in mites.

3.4. *Nosema* spp.

In both subspecies, most colonies (Figure 4) and all apiaries were found infected with *N. ceranae*. There was no significant difference in prevalence between the subspecies (χ^2 (1, $N = 29$) = 0.03, $P = 0.86$). *N. apis* was not detected.

4. Discussion

Colony losses were low in Algeria during winter 2012–2013, in comparison to most regions of Europe and North America at this time (van der Zee et al. 2012, 2014; vanEngelsdorp et al. 2012; Spleen et al. 2013; Steinhauer et al. 2014). In the south of Algeria, losses were approximately three times lower than apiaries located in the North, with no effect of honey bee subspecies or obvious link to pathogen or parasite prevalence. Colony management in Algeria is similar in the North and the South, irrespective of the subspecies used, and this parameter can a priori be excluded as an explanation for the differential mortality. Losses are therefore possibly affected by other factors linked with geographical or climatic factors, or use of agrochemicals (Adjlane et al. 2012a) in certain regions of the north of Algeria.

Remarkably, low losses occurred despite high prevalence of parasitic mites and pathogens in the colonies at the beginning of the year. *V. destructor* infestation rates of adults were similar in all regions and subspecies, and therefore do not explain the differences observed in morbidities. Brood infestation rates were not significantly different between the subspecies located in the South. The duration of the brood stops due to winter thus cannot explain the difference in infestation rate compared to *A. m. intermissa* in the North. Although a significant higher brood infestation rate in *A. m. intermissa* in the North could explain higher losses in this population, the range of infestation in comparison to the other population was similar and the effect on colony health expected to be weak. The high number of mites recorded both in the brood and on adult workers, in a majority of colonies during spring, was unexpected. Based on the damage thresholds identified in Europe and North America, most of these colonies should have collapsed during the previous winter (Genersch et al. 2010; Guzman-Novoa et al. 2010). The longer brood interruption in the South could limit the reproduction of *V. destructor* in the colonies (Moretto et al. 1991; Medina-Flores et al. 2011). In line with this hypothesis, mite treatments are less frequent in the South (one, rarely two treatments per year versus two to three in the North), which could result from a lower parasitic pressure at certain critical time of the year, a trend not visible based on a unique mite sampling as performed in our study. The monitoring of *V. destructor* population dynamics in these honeybee populations could help understand the relationship between the occurrence of the parasite and virus virulence and their effect on honeybee health.

Despite high number of mites recorded in the colonies in spring, the pattern of virus expected from surveys performed in Europe and North America (Tentcheva et al. 2004; vanEngelsdorp et al. 2009; Francis et al. 2013) was not observed. For instance, the high number of CBPV and BQCV positive colonies reported here (90–100 % and 72–92 % of colonies infected, respectively) was higher than expected, since these viruses are not usually associated with *V. destructor*. CBPV and BQCV occurrence has so far not been associated with colony losses. It is thus

unlikely that these viruses generate mortalities in Algeria. The absence of clinical signs reported in other African honey bee subspecies with high BQCV prevalence (Kajobe et al. 2010; Strauss et al. 2013; Muli et al. 2014) supports this idea.

In contrast, the DWV-VDV-1 as well as the ABPV-KBV-IAPV viral complexes is commonly found associated with *V. destructor* infestations and colony mortalities in Europe. DWV was detected in a large proportion of the colonies, but was not as ubiquitous as expected (de Miranda and Genersch 2010). It showed a lower, but not significantly different, prevalence in *A. m. intermissa* compared to *A. m. sahariensis* colonies (61 and 75 %, respectively), in spite of higher mortality records in northern Algeria. This virus is thus not likely to be responsible for the colony mortality observed in north Algeria. Although the prevalence values measured overlap with those recorded in Europe (e.g. Nguyen et al. 2010; Nielsen et al. 2008; Tentcheva et al. 2004), or in Africanized bee populations (Antùnez et al. 2006), it is worth mentioning that most surveys performed in other African honey bee populations have not, or only occasionally, detected this virus (Kajobe et al. 2010; Strauss et al. 2013; Muli et al. 2014; Amakpe et al. 2015). The absence of detection of VDV-1, a virus closely related to DWV (De Miranda and Genersch 2010), in both honey bee subspecies tested here also contrasts with other surveys performed in Europe or the Middle East, where this virus is frequently detected (De Miranda and Genersch 2010; Chejanovsky 2010). IAPV prevalence was also atypical since it was higher in Algeria compared to European as well as other African and Africanized honey bees (e.g. Ai et al. 2012; Blanchard et al. 2008; Cox-Foster et al. 2007; Strauss et al. 2013; Muli et al. 2014; Amakpe et al. 2015). This virus was more prevalent in *A. m. sahariensis* than in *A. m. intermissa* (39 and 83 %, respectively). This difference is inverse compared to the mortality trend observed between the subspecies. As a result, the implication of this virus in the higher mortalities observed in *A. m. intermissa* in north Algeria is unlikely. The marginally significant difference in prevalence of IAPV recorded between Algerian subspecies could be due to different intrinsic resistance levels, but requires further investigations. A discrepancy between honey bee subspecies was not observed for the closely related ABPV. Although ABPV was associated with colony

death in Europe (Genersch and Aubert 2010), in this case also, the prevalence and mortality patterns observed do not suggest a negative impact of this virus on Algerian colony survival. In contrast to the relatively high prevalence observed in this country, ABPV was absent or rare in surveys performed in Uganda, South Africa, Kenya and Benin (Kajobe et al. 2010; Strauss et al. 2013; Muli et al. 2014; Amakpe et al. 2015). As in other African or Africanized honey bee populations (Freiberg et al. 2012; Strauss et al. 2013; Muli et al. 2014), SBV was rarely detected in our samples, contrasting with studies in other parts of the world (Tentcheva et al. 2004; Ai et al. 2012; Chen et al. 2006; Antùnez et al. 2006; Reynaldi et al. 2010).

We found multiple virus infections in the colonies screened. Multiple infections are noted in other reports with values reaching 92 % of the colonies screened (Tentcheva et al. 2004; Antùnez et al. 2006; Forgach et al. 2008; Ai et al. 2012; Chen et al. 2004; Ryba et al. 2012). Their effect on honey bee health is yet to be determined. Overall, the high virus prevalence found in the honey bee populations screened in Algeria overlaps with the range associated with colony losses, more detailed surveys in the field are necessary to determine the sanitary status of both subspecies, and a better understanding of virus resistance in these subspecies is required.

The high prevalence of certain viruses in Algerian colonies, together with the high *V. destructor* infestation levels recorded in spring, was a reason to expect that mites act as vectors of these infections, as was demonstrated in many studies (Dainat et al. 2012a; vanEngelsdorp et al. 2009). However, few mites were found positive for viruses in this survey. With the exception of CBPV and BQCV, which were rarely detected in mite samples elsewhere (Celle et al. 2008; Locke et al. 2014), we expected a high frequency of DWV and IAPV-ABPV in Algerian mite samples. No mites were found positive for IAPV or SBV, while DWV and ABPV were only detected in mites of 10 and 16 % of the *A. m. intermissa* colonies, respectively. Only ABPV was found in mites of a third of the *A. m. sahariensis* colonies sampled. Concerning IAPV, our results are in line with those obtained from South Africa, where this virus was only occasionally detected in *V. destructor* (Strauss et al. 2013). Compared to the situation which prevails in

Europe, the pattern found in Algeria supports the idea that viruses such as DWV still display a low virulence in this country, and therefore do not have a strong impact on colony health, especially during winter time. However, more detailed surveys covering several seasons are needed to support this hypothesis. In addition, the occurrence of different evolutionary scenarios in the mite-viruses interaction in Algeria or Africa could affect their prevalence and virulence (Martin et al. 2012; Ryabov et al. 2014) and explain the atypical patterns observed on this continent. Such interactions deserve further investigation in mite-tolerant honey bee populations and might help us understand the evolution of this host-parasite relationship in time and ultimately help design methods to mitigate its negative impact on susceptible populations (Dietemann et al. 2012).

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In most of the world, *Nosema spp*. has been found at high prevalence at colony and apiary level reaching 95–100 % (e.g. Higes et al. 2010; Hong et al. 2011; Nabian et al. 2011). *N. ceranae* was found at higher prevalence than *N. apis* in the majority of samples (e.g. Strauss et al. 2013; Gisder et al. 2010; Higes et al. 2010; Nabian et al. 2011). Our survey of microsporidia infections in Algeria confirmed pure infections of *N. ceranae* in *A. m. intermissa* (Higes et al. 2009a) and showed for the first time its occurrence in *A. m. sahariensis*. In both subspecies, average prevalence at colony level was high with values of 100 and 90 %, respectively. No infection by *N. apis* could be detected. Our data thus correspond to the global trend of *N. ceranae* dominance and high prevalence with apparently low effects on colony health (Fries 2010). Since no historical data is available, we are not able to confirm or infirm the current hypothesis of a replacement of *N. apis* by *N. ceranae* (e.g. Paxton et al. 2007; Higes et al. 2013; Martín-Hernández et al. 2012). Given the total absence of *N. apis* in Algeria, it might have been completely replaced by *N. ceranae*. The resulting dominance of *N. ceranae* could reflect its better adaptation to warm climates (Fries and Forsgren 2009; Martín-Hernández et al. 2012). *N. apis* distribution is, based on cases for which species was identified, restricted to subequatorial Africa (Mumoki et al. 2015). Under the assumption that the endemic species in Africa is *N. apis*, this fact suggests that *N. ceranae* has not reached countries below the equator yet. Alternatively,

N. ceranae could be endemic to Northern Africa, and *N. apis* might never have been present in this region. A more precise picture might emerge after surveying more African countries.

5. Conclusion

We confirmed the presence of common honey bee virus infections in *A. m. intermissa* and *A. m. sahariensis* populations, as well as the wide distribution of the gut parasite *N. ceranae* in Algerian apiaries. Despite an atypical distribution of some of these infections compared to other parts of the world, our results showed few differences in prevalence of viruses and microsporidia or in infestation rates with *V. destructor* between these two subspecies of honey bees. The impact of these pathogens on colony health in Algeria remains unclear, but appears low overall. Our data suggest that the difference in mortalities observed in the two subspecies could be due to local parameters, such as those related to climate. A more comprehensive knowledge on the virulence and impact of honey bee pathogens is necessary to understand the factors affecting honey bee health and be able to improve beekeeping management to mitigate colony losses.

Profils viraux et parasitaires des sous-espèces algériennes d'abeilles, *Apis mellifera intermissa* et *A. m. sahariensis*

***Apis mellifera intermissa* / *Apis mellifera sahariensis* / virus / *Nosema* / *Varroa destructor* / perte de colonies / Algérie**

Atypische Viren- und Parasitenmuster bei Unterarten der Honigbiene, *Apis mellifera intermissa* und *A. m. sahariensis*, in Algerien

***Apis mellifera intermissa* / *Apis mellifera sahariensis* / virus / *Nosema* / *Varroa destructor* / Völkerverluste / Algerien**

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6. Electronic supplementary material

Below is the link to the electronic supplementary material.

ESM 1

(PDF 135 kb)

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Supplementary material

Nosema* sp. *and virus screening

Fifty frozen honey bees from each colony were pooled and crushed in 10 mL of 10 mM Tris pH 7.5; 400 mM NaCl (TN) buffer, using sterile disposable plastic tubes (gentleMACS™ Dissociator, Miltenyi Biotech). In parallel, eighteen pools of 1 to 9 varroa mites each were homogenized in 200 µL TN buffer using disposable sterile pestles (12 samples for A. *m. intermissa* and 6 samples for *A. m. sahariensis*). For *Nosema spp*. species identification, DNA was extracted from 50 µL of the homogenate using the NucleoSpin® DNA extraction kit (Macherey-Nagel). The DNA was eventually diluted in 100 µL of 5 mM Tris (pH 8.0), and stored at –20 °C until use for PCR analysis. For RNA virus detection, the homogenate was first clarified at 5,000 g for 5 min and 50 µL were used for total RNA extraction using the RNA II NucleoSpin® Kit (Macherey-Nagel), according to manufacturer's instructions. Total RNA was quantified by spectrophotometry (Nanodrop®) after elution in 50 µL of water. About 2 µg of total RNA was retro-transcribed at 25 °C for 10 min and at 37°C for one hour using M-MLV reverse transcriptase (Invitrogen) with random primers as anchor,

according to Dainat et al. (2011). The cDNA was eventually diluted 10-fold in water and stored at -20 °C until use in qualitative PCR assays. During the lysis step of the RNA extraction procedure, the samples were spiked with an external control reference gene (tobacco mosaic virus RNA) to ascertain the quality of the reverse transcription step, as described in Tentcheva et al. (2004).

The DNA and cDNA samples were diagnosed by PCR for the presence of *N. apis* and *N. ceranae* or for the detection of seven bee RNA viruses (ABPV, BQCV, CBPV, DWV, VDV-1, IAPV and SBV), respectively. The primers are described in Table 1 of the supplementary material. All PCR reactions were performed using the MyTaq™ DNA polymerase kit (Bioline), following supplier protocol. Briefly, the PCR mix was prepared in a final volume of 50 µL containing 5 µL of template DNA, 10 µL of 5X PCR buffer, 0.5 µM of each primer, 34 µL of water and 1.25 units of Taq DNA polymerase (MyTaq, Bioline). The cycling conditions consisted in a denaturation and DNA polymerase activation step at 95 °C for 2 min, followed by 35 cycles (95 °C denaturation for 20 s, 56 °C annealing for 20 s, and 72 °C extension for 30 s). Reactions were completed with a final extension step at 72 °C for 2 min. Negative (H_2O) and positive (previously identified positive samples) controls were included in each PCR run. Amplicons were separated by 1 % agarose gel electrophoresis and visualized under a UV transilluminator (Vilber Lourmat). The specificity of the PCR amplicons was assessed by Sanger sequencing (Fasteris Life Science Co., Geneva, Switzerland).

Table S1: Primers used for virus and pathogen diagnosis in this study

Primers	Product size (bp)	Source or reference
DWV Forward : CGGCCTATCAAAGAGTAC Reverse : CTTTTCTAATTCAACTTCACC	430	de Miranda and Fries 2008
VDV-1 Forward : GCCCTGTTCAAGAACATG Reverse : CTTTTCAATTCAACTTCACC	430	Gauthier et al. 2011
CBPV Forward : TCAGACACCGAATCTGATTATTG Reverse : TCTAATCTTAGCACGAAGCCGAG	1113	Blanchard et al. 2007
SBV Forward : GGATGAAAGGAAATTACCAG Reverse : CCACTAGGTGATCCACACT	426	Tentcheva et al. 2004
ABPV Forward : CATATTGGCGAGCCACTATG Reverse : CCACTTCCACACAACTATCG	398	Tentcheva et al. 2004
BQCV Forward : GGACGAAAGGAAGCCTAAAC Reverse : ACTAGGAAGAGACTTGCACC	424	Tentcheva et al. 2004
IAPV Forward : CCATGCCTGGCGATTCAC Reverse : CTGAATAATACTGTGCGTATC	203	de Miranda, unpublished
Nosema apis Forward : CCATTGCCGGATAAGAGAGT Reverse : CCACCAAAAACTCCCAAGAG	269	Chen et al. 2009
Nosema ceranae Forward : CGGATAAAAGAGTCCGTTACC Reverse : TGAGCAGGGTTCTAGGGAT	250	Chen et al. 2009

Supplementary material Table S2: Percentages of multiple virus infections in the *A. m. intermissa* and *A. m. sahariensis* honey bee populations sampled.

No. of virus	Virus identity	%
2	CBPV+BQCV	37.5
	CBPV+IAPV	29.7
	DWV+CBPV	28.1
	IAPV+BQCV	28.1
	DWV+BQCV	26.5
	ABPV+CBPV	20.5
	DWV+IAPV	18.7
	DWV+ABPV	16.2
	ABPV+IAPV	10.9
	CBPV+IAPV+BQCV	25.0
3	DWV+ABPV+BQCV	17.1
	DWV+ABPV+CBPV	17.1
	DWV+IAPV+BQCV	15.6
	ABPV+CBPV+IAPV	12.5
	ABPV+IAPV+BQCV	12.5
	DWV+ABPV+IAPV	10.9
4	DWV + ABPV + CBPV+BQCV	17.1
	DWV + ABPV + CBPV + IAPV	9.3
	ABPV+ CBPV+ IAPV+ BQCV	9.3