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Sección Control / Control

Surveillance and genotyping of *Varroa destructor* parasitizing *Apis mellifera jemenitica* in Saudi Arabia

Vigilancia y análisis genotípico del ácaro *Varroa destructor* parásito de *Apis mellifera jemenitica* en Arabia Saudí

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Abstract: A total of 72 honeybee samples, *Apis melllifera jemenitica*, were collected from eight different provinces within Saudi Arabia. Samples consisted of adult worker bees and capped brood cells. All samples were analyzed to estimate *Varroa destructor* mite infestation levels and isolated *V. destructor* mites (n = 90) were subjected to DNA analysis. Results revealed that all colonies sampled in this survey were infested with *V. destructor* mite. The overall average infestation levels were 5.5 ± 4.8% and 13.1 ± 10.6% on adult worker bees and in capped brood cells, respectively. Differences in worker infestation levels were not significant among sampling locations (F = 1.5; P = 0.19), however, infestation levels in capped brood cells were highly significant among locations (F = 5.2, P < 0,001). Moreover, results indicated that all *V. destructor* mites from *A. m. jemenitica* were recognized as Korean haplotype of *V. destructor*. These results should assist forthcoming mite management efforts in Saudi Arabia.

Key words: *Varroa* mite. Infestation levels. Yemeni honeybee. RFLP, Genotype.

Resumen: Un total de 72 muestras de abejas, *Apis melllifera jemenitica*, se obtuvieron de ocho diferentes provincias de Arabia Saudita. Las muestras consistieron en abejas obreras adultas y celdas de cría selladas. Se analizaron todas las muestras para estimar niveles de infestación con el ácaro *Varroa destructor*. Estos ácaros aislados (n = 90) fueron sometidos a un análisis de ADN. Los resultados revelaron que todas las colonias muestreadas en este estudio estaban infestadas de *V. destructor*. Los niveles de infestación promedio general fueron 5,5 ± 4,8% y 13,1 ± 10,6% en abejas obreras adultas y en las celdas de cría selladas, respectivamente. Las diferencias en los niveles de infestación de las obreras no fueron significativas entre los lugares de muestreo (F = 1,5; P = 0,19), sin embargo, los niveles de infestación en celdas de cría selladas fueron altamente significativas entre localidades (F = 5,2; P < 0,001). Por otra parte, los resultados indicaron que todos los ácaros *V. destructor* de *A. m. jemenitica* fueron reconocidos como haplotipo coreano de esta especie. Estos resultados servirán para esfuerzos futuros en el manejo de ácaros en Arabia Saudita.

Palabras clave: Ácaro de abejas. Niveles de infestación. Abejas Yemeni: RFLP. Genotipo.

Introduction

The mite, *V. destructor* is one of the most destructive pests of the honeybee (*Apis mellifera*) worldwide (Rosenkranz *et al.* 2010). *Varroa* mite has two haplotypes that are able to reproduce successfully on *A. mellifera*; the Japan-type is relatively non-virulent whereas the Korea-type, is extremely virulent (Anderson and Trueman 1998; Anderson 2000). In Saudi Arabia, the mite was first reported in 1987 (AlGhamdi 1990), perhaps through a shipment of introduced honeybees from Egypt, the usual package bee supplier of Saudi Arabia. Later, several studies reported on the occurrence and impact of *Varroa* mite throughout the country (Al Ghamdi 1990; Al Ghamdi 2002). Yet, general infestation levels and genotyping of *V. destructor* in Saudi Arabia is still needed.

Beekeeping in Saudi Arabia is based on native as well as on the exotic honeybee subspecies, *A. mellifera* (Alattal *et al.* 2014 and Alqarni *et al.* 2011). About 70 % of these colonies are kept in log hives colonized by native honeybee, *A. m. jemenitica* (Al-Ghamdi *et al.* 2013). Disease monitoring is nominal or absent and control of *Varroa* mite species is set up on calendar bases with almost consistent high colony losses. In addition to *Varroa* mite species, adverse climatic conditions in Saudi Arabia are the most obvious causes of colony losses in the country (Al Ghamdi *et al.* 2013; Alqarni *et al.* 2011).

In temperate areas, infestation levels are critical and honeybee colonies with no annual treatment against *Varroa* mite will collapse within 2-3 years (Rosenkranz *et al.* 2010), but in the majority of tropical climates the infestation levels remain under the injury threshold (Ritter and De Jong 1984). Apparently, it is not well explained whether the bee types of the tropics or climate contribute more to the documented stable honeybee-*Varroa* species relationships (Rosenkranz 1999; Carneiro *et al*. 2007; Rosenkranz *et al*. 2010).

Ruttner (1988) described the native honeybee of Saudi Arabia as the smallest and the most heat and drought tolerant honeybee subspecies of *A. mellifera*. Drought and high average ambient temperatures are dominant in Saudi Arabia. Consequently, the infestation levels and buildup of *Varroa* species population density under Saudi Arabia conditions may be different than that in the temperate regions where abundant flows of nectar and pollen lead to profoundly populated colonies causing an exponential population growth of the parasite (Renz 2003). This study focuses on mite haplotyping and on assessment of general infestation levels of *Varroa* mite species in Saudi Arabia.

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Materials and methods

Determination of infestation levels. Samples were taken from eight locations representing beekeeping areas within Saudi Arabia. Samples were collected shortly after the summer season during September and October 2013. Samples colonies were last treated with acaricides eight months before sample collection. Some related metrological parameters of the sampled locations are shown in Table 1. *Varroa* species infestation levels of adult worker bees was anticipated by sampling 100 adult workers from brood combs using plastic jars (450 cm^3) and were then stored at -20 °C. To determine the number of adult *Varroa* mite species in each sample, the jars were filled with water and detergent and shaken for 2-3 min. and were then given into a double-layer honey sieve where adult bees were separated from *Varroa* mite species by a jet of water and the infestation level was calculated as no. of *Varroa* mites/ no. of adult bees and expressed in percentage.

Brood infestation rates were estimated by inspecting about 100 capped worker brood cells. After being frozen, the capping's of the frozen brood samples were cut and the brood with the *Varroa* mite were rinsed with water in the double-layer honey sieve to separate *Varroa* mites that were then counted to determine brood infestation. Then, relative infestation ratio = brood infestation rates/worker infestation rates (Renz 2003) was calculated.

Separations of means were computed based on ANOVA and LSM performed on data after transformation using arc-sin function (SAS 1989).

***V. destructor* haplotype identification.** A number of female adult mites isolated from worker or brood samples were collected (n = 10 mites per apiary). Collected mites were placed in 1.5 micro-centrifuge tubes containing 96% ethanol, labeled and stored at -20ºC until DNA was extracted. Total genomic DNA was extracted from individual female mites by chelex resin™ according to Cano *et al.* (1993). After that extracted DNA was used to amplify the cytochrome oxidase subunit one (CO-I) region of the mtDNA with the primers (COXF) 5′-GG(A/G)GG(A/T)GA(C/T)CC(A/T)ATT(C/T)T(A/T)TATCAAC-3′ and (COXRa) 5′-CCTGT(A/T)A(A/T)AATA GCAAATAC-3′ using PCR (Saiki *et al*. 1988) following the method described by Anderson and Fuchs (1998). PCR products were then electrophoresed, stained with ethidium bromide, visualized with UV light (260 nm) and photographed. Amplified CO-I region was then used as a template for the RFLP reaction. The restriction enzymes Sac-I, which distinguish the G/AGCTC sequence and Xol-I which distinguish the C/TCGAG sequence were added separately each to 12.5 µl of the PCR product as described by Anderson and Fuchs (1998). The mixture was then incubated at 37ºC for about one hour and was then run in a 1.5% agarose gel. The resulting bands were then visualized with UV light and photographed. One amplified CO-I region from each apiary were then sequenced using an auto- mated 96 capillary ABI 3730XI DNA genetic analyzer (Applied Biosystem). Sequences were manually checked and assembled using GeneBank database (NCBI). Sequences were then exposed to two procedures. First, sequences were aligned using CLUSTALW software (Thompson *et al*. 1994), BLASTed and compared with other sequences available on the GenBank database (NCBI). Sequences were finally submitted to Genebank data base (NCBI).

Results

Results revealed that all colonies sampled in this survey (n = 72) were infested with *Varroa* mite species. The general average infestation levels were 5.5 ± 4.8% and 13.1 ± 10.6% on adult worker bees and in capped brood cells, respectively. Differences in infestation levels of adult worker bees were not significant among sampling locations (F = 1.5, P = 0.19, Figure 1), however, infestation levels in capped brood cells were highly significant among locations (F = 5.2, P < 0.001, Figure 2) the highest brood infestation levels were in Jazan then Abha. Generally, relative infestation of capped worker brood was about 4.7 ± 6.3 times higher than the infestation on adult bees, and differences between locations were significant (P < 0.001, Figure 3). The highest value being in Jazan.

The banding patter of the RFLP reaction and sequence analysis of the mtDNA revealed that all mite samples were of *V. destructor* Korean haplotype. Fragment digestion occurred only when using Xol-I and resulted bands had a fragment size between 200 and 300 bp. Sequences were 99 to 100% identical to that of Korean type of *V. destructor* previously described by Anderson and Trueman (2000) (GeneBank accession number AF106899). In all *Varroa* mite samples analysed, two

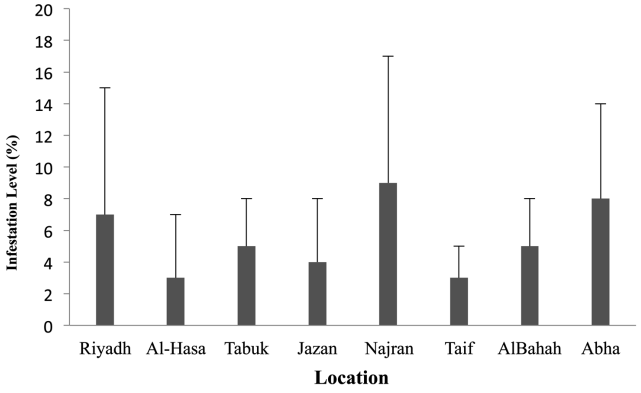

Figure 1. *Varroa destructor* infestation levels of adult worker honeybees at different survey locations. Mean separation based on ANOVA and LSM, DF = 7 and P < 0.05, F = 1.5, P = 0.19.

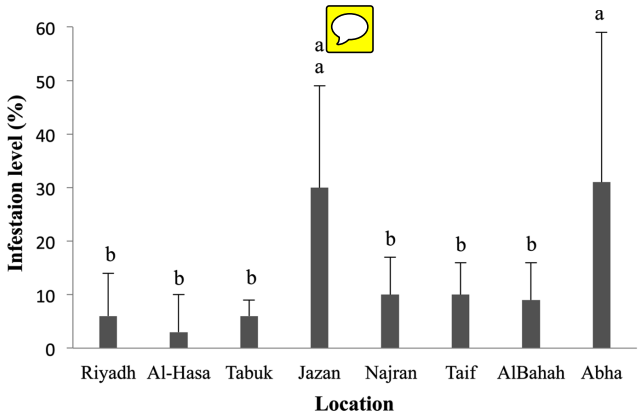

Figure 2. *Varroa destructor* infestation levels of capped brood cells of the honeybee at different survey locations. Mean separation based on ANOVA and LSM, DF = 7 and P < 0.05, F = 5.2, P < 0.001. Means sharing same letters are not significantly different.

Table 1. Name, latitude, climatic zone, annual maximum and minimum temperatures and average annual precipitation of sampled locations (PoMIP, 2007).

Location	Climatic zone*	Latitude	Average Annual Temp.(°C)** Min.	Max.	Rainfall (mm)**
Riyadh	Arid	24.63ºN, 46.71ºE	15	35.5	10
Al-Hasa	Arid	25º25'N, 49º37'E	10	42	65
Tabuk	Arid	28°23'N 36°34'E	-6	30.6	46
Jazan	Semi-Arid	16°53'N 42°33'E	9	48	23
Najran	Semi-Arid	17°29'N 44°7'E	14.6	30.9	83
Taif	Semi-Arid	21°16'N 40°25'E	13.7	30.9	208
AlBahah	Semi-Arid	20°00'N 41°27'E	12	23	250
Abha	Dry -Med	18°13'N 42°30'E	1	40	600

(* Köppen climate classification *BWh*), ** (PoMEP, 2007).

genotypes were detected with more than 99% similarity. The genotypes were deposited in the NBCI GenBank under the accession numbers KJ507740 and KJ507741.

Discussion

The survey demonstrated that *V. destructor* Korean haplotype is distributed in all beekeeping areas within Saudi Arabia; results also indicated that relative infestation levels may reach higher levels within few months without *Varroa* treatment. However these infestation levels are lower than what was described for other *V. destructor-Apis mellifera* systems with different subspecies such as *A. m. carnica* in central Europe (Rosenkranz *et al.* 2010), *A. m. syriaca* system in Jordan (Alattal *et al.* 2005) or *A. m. lamarckii* in Egypt (Ismail *et al*. 2006). This epidemic spread of the mite within Saudi Arabia could be mainly related to mass importation of *Varroa* species infested package bees from neighboring countries mainly Egypt. In addition to poor management practices in *Varroa* mite control, an un-controlled migration of bee colonies among locations increases the contact between highly infested colonies, where *Varroa* mite species can build up more quickly such as Jazan and Abha, and colonies with low infestation. When highly infested colonies die, its remaining bees with the *Varroa* mites will enter new colonies. Even that damage and colony losses were not visible during the assessment; increased colony mortality due to *Varrroa* associated viruses is probable after the end of spring and till the end of winter. Although, several locations showed relative infestation levels of adult bees below 15%, a proposed economic threshold on adult worker bees in temperate regions (Toscano 1996), we believe that the impact of *Varroa* infestation under arid conditions with very limited nectar and pollen resources, such as Saudi Arabia, would be also dramatic if colonies were not treated.

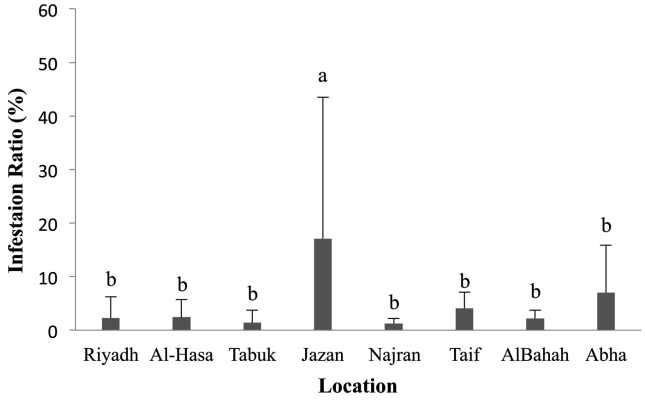

Figure 3. *Varroa* mite infestation ratio (brood infestation/worker infestation) of the honey bee at different survey locations. Mean separation based on ANOVA and LSM, DF = 7 and $P < 0.05$, $F = 5.2$, $P < 0.001$. Means sharing same letters are not significantly different.

Although the significant differences in the infestation of worker brood cells among different locations might indicates real spatial differences in the course of colony development in these areas, it was not possible to classify locations according to their climatic zones or climatic parameters in predicting high or low infestation rates.

This demonstrates that spatial variations in infestation levels are rather a result of interaction among various factors including bee type, *Varroa* mite species genotype, and other ecological factors. Since only the highly virulent Korean genotype has been reported in all sampling areas within Saudi Arabia, variation in infestation levels may be related to other factors. On the other hand, nearby colonies in the same apiary may have clearly uneven relative infestation rates, which might be related to the variations in the patterns of colony build-up and colony population growth rate during the season. Consequently, *Varroa* mite population increased rapidly in some colonies than in others.

The infestation ratio is a sign of the relative number of reproducing mites in the honeybee colony. The maximum ratio took place in Abha and Jazan, which demonstrates that the increase in *Varroa* mite population is higher in these locations compared to other locations of this study. Consequently, colony losses due to higher infestation levels of adult workers at the time the brood area is reduced could be high. For that reason, monitoring of infestation levels by sampling of brood cells and adult bees is an essential practice to prevent late treatment or multiple treatments.

On the other hand, results indicated an extremely low divergence in the CO-I of *Varroa* mite in an extensive geographical area and all of these *Varroa* mite species were of a Korean haplotype identity, this may also suggest the presence of the same genotype in neighboring countries.

The *Varroa* mite species may have a common origin in the investigated populations (as also mentioned by Solignac *et al*. (2005) for the European *Varroa* mites).

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