HAL
archives-ouvertes.fr

A scientific note: Survey for Nosema spp. in preserved Apis spp.

Brenna E. Traver, Richard D. Fell

To cite this version:

Brenna E. Traver, Richard D. Fell. A scientific note: Survey for Nosema spp. in preserved Apis spp.. Apidologie, Springer Verlag, 2015, 46 (2), pp.194-196. 10.1007/s13592-014-0306-1 . hal-01284429

HAL Id: hal-01284429

https://hal.archives-ouvertes.fr/hal-01284429

Submitted on 7 Mar 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Apidologie (2015) 46:194–196
© INRA, DIB and Springer-Verlag France, 2014
DOI: 10.1007/s13592-014-0306-1

Scientific note

A scientific note: Survey for *Nosema* spp. in preserved *Apis* spp.

Brenna E. Traver, Richard D. Fell

Department of Entomology, Virginia Tech, Blacksburg, VA 24061, USA

Received 17 April 2014 – Revised 8 June 2014 – Accepted 3 July 2014

***Nosema ceranae* / *Nosema apis* / honey bee**

1. INTRODUCTION

Historically, *Nosema apis* was thought to be the only species to infect *Apis mellifera*, but recent reports showed that *Nosema ceranae* is a second microsporidium capable of infecting *A. mellifera* (Higes et al. 2006; Huang et al. 2007). While *N. ceranae* was first described in 1996 in *Apis cerana* (Fries et al. 1996) and initially believed to be restricted to the natural host range of *A. cerana* (Fries 1997), *N. ceranae* has been found everywhere beekeeping is practiced. *N. ceranae* has been present in the USA since 1995 (Chen et al. 2008) and the earliest published report of *A. mellifera* samples containing *N. ceranae* dates back to 1990 in samples from Uruguay (Invernizzi et al. 2009). *N. ceranae* has also been found in other *Apis* spp. such as *Apis florea* and *Apis dorsata* colonies in Northern Thailand, but no *N. apis* was detected (Chaimanee et al. 2010; Suwannapong et al. 2011). Samples from the Solomon Islands revealed that *Apis koschevnikovi* were infected with *N. ceranae*, but only in areas where *A. cerana* had been sympatric and no *N. apis* was detected (Botías et al. 2012).

N. ceranae has also been found in species outside of the genus *Apis*. Three species of bumble bee collected from 2005–2008 in South America have been found to be naturally infected with *N. ceranae*: *Bombus atratus*, *Bombus morio*, and *Bombus bellicosus*, but not infected with *N. apis* or *Nosema bombi* (Plischuk et al. 2009). Interestingly, in *B. atratus*, *Nosema* spores were detected in a drone and a queen, and also in queens of *B. morio* (Plischuk et al. 2009).

Findings *of N. ceranae* in species other than *A. mellifera* and *A. cerana* and in *Bombus* spp. lead to the interesting question of how widespread *N. ceranae* infections are and whether *N. ceranae* is less host specific and more capable of adapting to other species (Chaimanee et al. 2010). Previous research compared the intergenic spacer region (IGS) of *N. ceranae* isolates from *A. mellifera* and *A. cerana* and found that there were minimal differences suggesting that there is no transmission barrier between the two species (Huang et al. 2008). Initially, it was thought that *N. ceranae* jumped hosts prior to 2003, but an exact time has been difficult to determine based on limited sample numbers prior to 2003 (Klee et al. 2007). *N. ceranae* infections from *A. cerana* could cross-infect other species of honey bees as they visit similar flower species, thus facilitating fecal–oral transmission (Suwannapong et al. 2011). Export of pollen from Asia to other parts of the world could be a potential route of transmission as pollen can contain infective *N. ceranae* spores (Higes et al. 2008). It has been speculated that *N. ceranae* may have originally jumped from *A. cerana* to *A. mellifera* from robbing colonies as the two bee species exhibit different behavior and nectar sources (Huang et al. 2008).

Determining when *N. ceranae* expanded its host range is difficult as analysis of older samples is limited because of the lack of preserved samples. In this study, we purchased preserved, well cataloged bee samples from a reputable insect supply company. The samples had been preserved in ethanol and detailed notes were taken regarding their collection date and location. Here, we report *N. ceranae* infections in samples dating from the 1960s in multiple *Apis* spp.

Corresponding author: B. E. Traver, traverb@vt.edu
Manuscript editor: Peter Rosenkranz

2. MATERIALS AND METHODS

2.1. Honey bee samples

Honey bee samples were purchased from BioQuip Products (Rancho Dominguez, CA, USA). These included *A. mellifera* white-eyed drones sampled in 1984 from Columbus, MO, USA.; *A. mellifera* drones sampled in 1993 from Columbus, OH, USA.; *A. mellifera* Africanized workers sampled in 1994 from Brownsville, TX, USA.; *A. mellifera* workers sampled in 1975 from Los Angeles County, CA, USA.; *A. dorsata* and *A. florea* sampled in 1968 from Cam Ranh Bay, Khanh Hoa Province, Vietnam. *A. cerana* (labeled *A. indica*) sampled in 1968 from Dong Ba Thin, Khanh Hoa Province, Vietnam.

2.2. Genomic DNA extraction and real-time PCR

DNA was extracted from individual honey bee abdomens using a Bender buffer lysis and a proteinase K incubation, followed by a phenol/chloroform extraction and isopropanol precipitation as previously described (Traver and Fell 2011). Following the first centrifugation in the phenol/chloroform DNA extraction, the organic phase was solubilized with 95 % ethanol so that a spore count could be performed for each bee (Mulholland et al. 2012). This modification was performed so that the genomic data for each bee could be compared to the spore count data of the same individual. qPCR was performed on individual honey bee abdomens as previously described (Traver and Fell 2011). For *A. florea* samples, DNA was extracted using a QIAGEN DNeasy kit (Valencia, CA) because of the small tissue size.

2.3. Spore counting

The number of spores contained in a sample of each lysate was determined through counts on a Bright-line hemocytometer (Hausser Scientific, Horsham, PA) as previously described (Cantwell 1970).

2.4. Statistics

A Wilcoxon signed rank test in JMP 11 (Cary, NC) was used to determine significant differences (α=0.05) in *N. ceranae* and *N. apis* levels between bees of the same species.

3. RESULTS

Apis mellifera workers from 1975 were co-infected with both *N. apis* and *N. ceranae* (Table I). The percent infected for both species was high: 80 % for *N. ceranae* and 73 % for *N. apis* (Table I), but *N. apis* levels were significantly higher than *N. ceranae* (S=147.5, N=30, P<0.01; Table I). Of the 30 bees examined, 70 % were co-infected with *N. ceranae* average copy number of $2\times10^4\pm5\times10^3$ and $5.62\times0^6\pm1.37\times10^6$ for *N. apis*, and 10 % only had *N. ceranae* infections with an average copy number of 358±109, while 20 % were not infected

Table I. *Nosema* spp. infection level and percent infected for various *Apis* spp.

Bee species	Year[a]	Number[b]	*Nosema ceranae*		*Nosema apis*	
			Avg. copy no.	% Infected	Avg. copy no.	% Infected
A. mellifera—white-eyed drone	1984	10	17.7±17.7	10	0.0	0
A. mellifera—drone	1983	10	0.0	0	0.0	0
A. mellifera—Africanized	1994	30	7. 0±4.9	13.3	0.0	0
A. mellifera	1975	30	$1.43\times10^4\pm3.9\times10^3$	80	$3.93\times10^6\pm1.07\times10^6$	73.3
A. dorsata	1968	15	65.7±56.9	13.3	0.0	0
A. florea	1968	15	0.0	0	0.0	0
A. cerana	1968	15	58.2±14.4	60	0.0	0

[a] Year bees were sampled

[b] Number of individual bees examined

with either species. Africanized *A. mellifera* from 1994 had very low levels of *N. ceranae* and a relatively low incidence, and no *N. apis* was detected. *A. mellifera* white-eyed drones from 1984 were found infected with *N. ceranae*, but normal drones from the prior year were not infected with either species. *A. dorsata* and *A. cerana* were both infected with *N. ceranae* with 13 and 60 % (Table I), respectively. *A. cerana* samples had significantly higher levels of *N. ceranae* compared to *N. apis* ($S=-27.5$, $N=15$, $P<0.01$). *A. florea* was not positive for either species. Spores were only found in *A. mellifera* samples. The counts were 12,500±7,683; 833±833; and 485,000±165,144 for white-eyed drones, Africanized workers, and workers, respectively.

4. DISCUSSION/CONCLUSIONS

Tracking when *N. ceranae* switched hosts and entered the USA is important to understand host range expansion. Here, we report the earliest known infections of *Apis* spp. with *N. ceranae. N. ceranae* was present in *A. cerana* and *A. dorsata* species dating back to 1968, while no *N. apis* was detected. In the USA, drones from 1984 and workers from 1975 were found infected.

ACKNOWLEDGMENTS

We thank Grace Mulholland and Sean Smith for technical assistance. Funding for BET was provided by the Agriculture and Food Research Initiative Competitive Grant no. 2012-67012-19924 from the US Department of Agriculture National Institute of Food and Agriculture.

Note scientifique sur une recherche de *Nosema* spp. dans des échantillons d'*Apis* spp., conservés en collection

Eine wissenschaftliche Notiz : Zum Vorkommen von *Nosema* spp. in konservierten *Apis* spp.

REFERENCES

Botías, C., Anderson, D.L., Meana, A., Garrido-Bailón, E., Martín-Hernández, R., Higes, M. (2012) Further evidence of an oriental origin for *Nosema ceranae* (Microsporidia: Nosematidae). J Invertebr Pathol **110**(1), 108–113

Cantwell, G.E. (1970) Standard methods for counting *Nosema* spores. Am Bee J **110**(6), 22–223

Chaimanee, V., Warrit, N., Chantawannakul, P. (2010) Infections of *Nosema ceranae* in four different honeybee species. J Invertebr Pathol **105**(2), 207–210

Chen, Y., Evans, J.D., Smith, I.B., Pettis, J.S. (2008) *Nosema ceranae* is a long-present and wide-spread microsporidian infection of the European honey bee (*Apis mellifera*) in the United States. J Invertebr Pathol **97**(2), 186–188

Fries, I. (1997) Protozoa. In: Morse, R.A., Flottum, K. (eds.) Honey Bee Pests, Predators, & Diseases, pp. 59–76. A.I. Root Company, Medina, OH

Fries, I., Feng, F., da Silva, A., Slemenda, S.B., Pieniazek, N.J. (1996) *Nosema ceranae* n. sp. (Microspora, Nosematidae), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). Eur J Protistol **32**, 356–365

Higes, M., Martín-Hernández, R., Garrido-Bailón, E., García-Palencia, P., Meana, A. (2008) Detection of infective *Nosema ceranae* (Microsporidia) spores in corbicular pollen of forager honeybees. J Invertebr Pathol **97**(1), 76–78

Higes, M., Martín, R., Meana, A. (2006) *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. J Invertebr Pathol **92**(2), 93–95

Huang, W.-F., Bocquet, M., Lee, K.-C., Sung, I.H., Jiang, J.-H., Chen, Y.W., Wang, C.H. (2008) The comparison of rDNA spacer regions of *Nosema ceranae* isolates from different hosts and locations. J Invertebr Pathol **97**(1), 9–13

Huang, W.-F., Jiang, J.-H., Chen, Y.-W., Wang, C.-H. (2007) A *Nosema ceranae* isolate from the honeybee *Apis mellifera*. Apidologie **38**(1), 30–37

Invernizzi, C., Abud, C., Tomasco, I.H., Harriet, J., Ramallo, G., Campa, J., Katz, H., Gardiol, G., Mendoza, Y. (2009) Presence of *Nosema ceranae* in honeybees (*Apis mellifera*) in Uruguay. J Invertebr Pathol **101**(2), 150–153

Klee, J., Besana, A.M., Genersch, E., Gisder, S., Nanetti, A., Tam, D.Q., Chinh, T.X., Puerta, F., Ruz, J.M., Kryger, P., et al. (2007) Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. J Invertebr Pathol **96**(1), 1–10

Mulholland, G., Traver, B., Johnson, N., Fell, R. (2012) Individual variability of *Nosema ceranae* infections in *Apis mellifera* colonies. Insects **3**(4), 1143–1155

Plischuk, S., Martín-Hernández, R., Prieto, L., Lucía, M., Botías, C., Meana, A., Abrahamovich, A.H., Lange, C., Higes, M. (2009) South American native bumblebees (Hymenoptera: Apidae) infected by *Nosema ceranae* (*Microsporidia*), an emerging pathogen of honeybees (*Apis mellifera*). Environ Microbiol Reports **1**(2), 131–135

Suwannapong, G., Yemor, T., Boonpakdee, C., Benbow, M.E. (2011) *Nosema ceranae*, a new parasite in Thai honeybees. J Invertebr Pathol **106**(2), 236–241

Traver, B.E., Fell, R.D. (2011) Prevalence and infection intensity of *Nosema* in honey bee (*Apis mellifera* L.) colonies in Virginia. J. Invertebr. Pathol **107**(1), 43–49