Indian Farmer
Volume 11, Issue 07, 2024, Pp. 252-257
Available online at: www.indianfarmer.net
ISSN: 2394-1227 (Online)

Original Article

Reaction of Honey bees effected with *Nosema ceranae* and *Lotmaria passim*

Mr. Krishan Kant Saklani, Dr.Roop Kishor Sharma and Mr. Ramakant Mishra

Doon P.G College of Agriculture Science and Technology

Corresponding author: kn.saklani97@gmail.com

Received:11/07/2024 *Published:14/07/2024*

ABSTRACT

The trypanosome *Lotmaria passim* and the microsporidian *Nosema ceranae* are common parasites of the honey bee, *Apis mellifera*, intestine , but the nature of interactions between them is unknown. The frequency of single and double infections, estimate the parasite loads of single and double infections, and determined the type of correlation between both parasites in double infections. If interactions between both parasites are strong and antagonistic, single infections should be more frequent than double infections, double infections will have lower parasite loads than single infections, and double infections will present a negative correlation. The infection loads of individual bees were significantly higher for *L. passim* compared to *N. ceranae*, there were no significant differences in infection loads between single- and double-infected hosts for both parasites. These findings suggest that *N. ceranae* and *L. passim* pose threats to bee health, and that the beekeeping industry should monitor for both parasites in an effort correlate pathogen status with changes in colony-level productivity and survival. Overall, a total of 88 workers were infected with *N. ceranae*, 53 with *L. passim*, and eight with both parasites. Although both parasites were found in all three apiaries, there were significant differences among apiaries in the proportions of infected bees. The data show no significant differences between the expected and observed frequencies of single- and double-infected bees.

keywords: *Nosema ceranae, Lotmaria passim, Apis mellifera*, double infeaction, responsiveness, productivity, survival

INTRODUCTION

Host–pathogen interactions are a major driving force of evolution and have received considerable. Although hosts infected by more than one parasite are, further attention is required to address interactions among such parasites within one host. These parasite–parasite interactions in individual hosts can potentially range from competition to and may have tremendous effects. For example, a host may cope with a single infection, but succumb to multiple ones, depending on pathogen–

pathogen. This creates demand for better understanding the parasite–parasite interface in single hosts.

The health of western honey bees, *Apis mellifera*, has recently received considerable attention due to major losses of managed colonies at a global. Honey bee health is menaced by multiple stressors acting together or, with interactions among parasites likely to play a key role.

The microsporidian gut parasite *N. ceranae* switched hosts from Asian honey bees (*Apis cerana*) to *A. mellifera* and appears to have replaced the endemic European *Nosema apis* in many areas. Analyses of archived samples revealed that *N. ceranae* has been present in western honey bees for several decades, without replacing *N. apis*. First reports of both microsporidian gut parasites concurrently infecting a single host suggest competition between *N. apis* and *N. ceranae* , but no competitive advantage was affirmed for *N. ceranae* in mixed infections for either infectivity or spore growth.

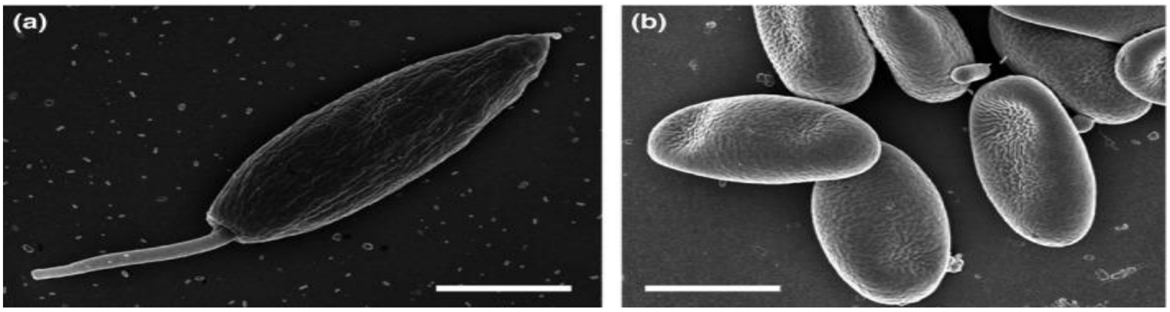

Figure 1: Scanning electron microscope images of the two honey bee parasites: (a) Lotmaria passim; lanceolate promastigote cell with anterior flagellum and caudate posterior extension visible (×10,000), (b) Nosema ceranae: ovocylindrical, straight to slightly curved spores (×10,000) Bars = 2 μm

Recently, *Lotmaria passim*, a trypanosome intestine parasite of honey bees, has received. Whereas the two trypanosomatids, *Crithidia mellificae* and *L. passim*, can both infect honey bee colonies, *L. passim* is currently the predominant trypanosomatid in *A. mellifera* host populations globally. Despite its global distribution, *L. passim* is poorly understood. However, it is known from laboratory experiments that mixed-species infections with *C. mellificae* and *N. ceranae* can significantly affect local and systemic immune gene transcription within honeybees. Such altered immune responses may also occur during mixed-species infections with *L. passim* and *N. ceranae*, but may differentially impact parasite populations.

As laboratory tests may not necessarily reflect field conditions, we took advantage of natural occurring infections of honey bee hosts with *N. ceranae* and *L. passim* in the field to test the following hypotheses: a) If the two parasites significantly interact with each other, we expect less or more individual hosts infected with both parasites compared to a random distribution. b) Infection loads of bees with one parasite alone should differ from those bees infected with both parasites. c) Positive or negative correlations between infection loads with both parasites are expected in double-infected hosts given that they interact with each other.

EXPERIMENTAL SETUP

I. DNA EXTRACTION,

All sampled workers were crushed individually in 2-ml microcentrifuge tubes containing 5 mm metal beads and 200 µl TN buffer (10 mmol/L Tris, 10 mmol/L NaCl; pH 7.6). The samples were homogenized with a Tissue Lyser for 30 s at 25 1/s frequency using a Qiagen Retsch MM 300 mixer mill. Then, the homogenates were centrifuged at 2500 rpm and 50 µl of the supernatant was used for DNA extraction using innuPREP DNA Mini Kit following the manufacturer's recommendations. After DNA extraction, a Spectrophotometer Thermo Scientific NanoDrop 2000 full-spectrum ultraviolet was used to quantify the DNA of each individual sample.

II. CLASSICAL AND QUANTITATIVE PCR,

PCR assays were performed to detect the occurrence of microsporidians (*N. apis* and *N. ceranae*) and trypanosomes (*C. mellificae* and *L. passim*). The PCR analyses were performed by using MyTaq kit(Bioline) with 2µl tenfold-diluted DNA. We followed manufacturer's recommendations by adding 5× reaction buffer, forward and reverse primers (final concentration of 0.4 µmol/L each) and 0.125µl (0.63 Units) of Taq polymerase in a total of 25µl final reaction volume. Three sets of species-specific primers available for *Nosema* species (multiplex PCR primer combination Mnapis-F, Mnceranae-F, and Mnuniv-R) and trypanosomes *C. mellificae* and *L. passim* (GAPDH-F/R and Cr ITS1-IR1/5.8R) were used. An independent PCR assay was performed for each set of primers. The qPCR cycling protocol was the same for each set of primers, which was as follows: Cycling profile for all runs consisted of a 2-min initial incubation at 95°C and 35 cycles of 20 s at 95°C for denaturation, 20s at 56°C for annealing, 30 s at 72°C for extension, and finally 2 min at 72°C before the samples remained at 4°C to cool. The PCR products were qualitatively analyzed with a 2% 2D agarose gel electrophoresis. Each gel contained negative and positive controls and an appropriate DNA size standard. The gels were run for 45 min in the electrophoresis chamber containing 0.5 TBE (Tris–boric acid–EDTA) buffer. Subsequently to the electrophoresis, each gel was placed for 20 min in a GelRed bath for staining and visualization under ultraviolet light.

table 1, Primers used for PCR diagnosis of microsporidian and trypanosome parasites in an individual honey workers, *Apis mellifera*

TARGET	PRIMER	SEQUENCE(5’-3’)	SIZE(bp)
Nosema apis	Mnapis-F Mnuniv-R	GCA TGT CTT TGA CGT ACT ATG GAC TTA GTA GCC GTC TCT C	224

Nosema ceranae	Mnceranae-F	CGT TAA AGT GTA GAT AAG ATG TT	143
	Mnuniv-R	GAC TTA GTA GCC GTC TCT C	
Crithidia mellificae / Lotmaria passim	GAPDH-F	GTG CTC GTG GTG AAC GGC CA	402
	GAPDH-R	GTC CTT GAG CGA CAC GCC GT	
	Cr-ITS1-IR1	GCT GTA GGT GAA CCT GCA GCA GCT GGA TCA TT	
	Cr-ITS1-5.8R	GGA AGC CAA GTC ATC CAT C	~1,200-1,500

The detected parasites were quantified by independent qPCR using KAPA SYBR FAST qPCR Master Mix (Kapa Biosystems) with 10ng extracted DNA, 0.24µl of forward and reverse specific primers (10µmol/L) and 6µl of 2× reaction buffer in a total of 12µl final reaction volume. The qPCR cycling profile was the same for each set of primers and consisted of 3-min incubation at 95°C and 40 cycles of 30 s at 95°C for denaturation, 30s at 57°C for annealing, and a final extension at 50°C before cooling down to 4°C. Purified PCR products of known concentrations were used as standard curves on each individual plate, along with nontemplate controls and β-Actin as reference gene.

table 2, Primers used for qPCR of *N. ceranae* and *L. passim* in individual honey bee workers, *Apis mellifera.*

TARGET	PRIMER	SEQUENCE(5’-3’)	SIZE(bp)
Nosema ceranae	Ncer bour F	AAG AGT GAG ACC TAT CAG CTA GTT G	104
	Ncer bour R	CCG TCT CTC AGG CTC CTT CTC	
Lotmaria passim	qCrFw1	TCC ACT CTG CAA ACG ATG AC	153
	qCrRev1	GGG CCG AAT GGA AAA GAT AC	
A. m. Actin	q92F	CGT TGT CCC GAG GCT CTT T	66
	q157	TGT CTC ATG AAT ACC GCA AGC T	

III. SEQUENCING,

Use of primers mentioned above requires subsequent sequencing of PCR products if the trypanosomatid species should be identified. Therefore, PCR products from selected samples were sequenced using Cr-ITS1-IR1/Cr-ITS1-5.8R primers. Additionally, selected PCR products from *N. ceranae* assays were also sequenced using Mnceranae-F/Mnuniv-R primers. Both species were confirmed as the parasites present in our samples using reference sequences deposited in Gene Bank.

IV. DATA ANALYSES,

L. passim and *N. ceranae* parasites/bee were calculated by comparing experimental Cq-values with those of the standard curve. The calculation was performed by conversion factors from copies/µl to spore or cell equivalents/bee as follows:

$$\frac{\text{Number individual parasite}}{\text{bee}} = \frac{\left(\frac{\alpha \text{ parasite copies}}{\mu\text{l PCR}}\right) \times \left(\frac{25\ \mu\text{l}}{0.05\ \text{bee}}\right)}{\beta \text{ copies per genome}}$$

where α = copy number from real-time PCR, β = 1 or 10 copies per genome for *L. passim* or *N. ceranae*, respectively, and 0.05 bee represents the 50 µl supernatant taken from the homogenate.

CONCLUSION

According to the result of this article that is not suggest any strong interactions between the two honey bee parasites, which may be explained by spatial separation in the host. These studies illustrates the negative effects of single *L. passim* and mixed *L. passim* and *N. ceranae* infections on honey bee survival. We recommend that beekeepers continue to monitor their colonies for *N. ceranae*, and begin to routinely monitor for *L. passim* in an effort to improve honey bee health by correlating parasite diagnosis with colony-level changes that could affect survival and productivity.

REFERENCES

- Olive, A. J., & Sassetti, C. M.(2016). Metabolic crosstalk between host and pathogen: Sensing, adapting and competing. *Nature Reviews Microbiology*, 14, 221–234.
- Bordes, F., & Morand, S.(2011). The impact of multiple infections on wild animal hosts: A review. *Infection Ecology & Epidemiology*, 1, 1–10.
- Alizon, S., & Lion, S.(2011). Within-host parasite cooperation and the evolution of virulence. *Proceedings of the Royal Society of London B: Biological Sciences*, 278, 3738–3747.
- Neumann, P., Yañez, O., Fries, I., & de Miranda, J. R. (2012). Varroa invasion and virus adaptation. *Trends in Parasitology*, 28, 353–354.
- Neumann, P., & Carreck, N.(2010). Honey bee colony losses. *Journal of Apicultural Research*, 49, 1–6.

- Fries, I.(2010). *N.ceranae* in European honeybees (*Apis mellifera*). *Journal of Invertebrate Pathology*, 103, 73–79.

- Fries, I., Chauzat, M.P., Chen, Y.P., Doublet, V., Genersch, E., Webster, T.C., …Williams, G.R. (2013). Standard methods for nosema research. *Journal of Apicultural Research*, 52, 1–28.

- Chen, Y.P., Evans, J.D., Murphy, C., Gutell, R., Zuker, M., Gundensen-Rindal, D., & Pettis, J.S. (2009). Morphological, molecular, and phylogenetic characterization of *Nosema ceranae*, a microsporidian parasite isolated from the European honey bee, *Apis mellifera* . *Journal of Eukaryotic Microbiology*, 56, 142–147.
- Fernández, J.M., Puerta, F., Cousinou, M., Dios-Palomares, R., Campano, F., & Redondo, L.(2012). Asymptomatic presence of *Nosema* spp. in Spanish commercial apiaries. *Journal of Invertebrate Pathology*, 111, 106–110.
- Natsopoulou, M.E., Mcmahon, D.P., Doublet, V., Bryden, J., & Paxton, R.J. (2015). Interspecific competition in honeybee intracellular gut parasites is asymmetric and favours the spread of an emerging infectious disease. *Proceedings of the Royal Society of London. Series B*, 282, 20141896.
- Milbrath, M. O., van Tran, T., Huang, W.F., Solter, L.F., Tarpy, D.R., Lawrence, F., & Huang, Z.Y. (2015). Comparative virulence and competition between *Nosema apis* and *Nosema ceranae* in honey bees (*Apis mellifera*). *Journal of Invertebrate Pathology*, 125, 9–15.
- Ravoet, J., Schwarz, R.S., Descamps, T., Yañez, O., Tozkar, O.O., Martin-Hernande, R., … de Graaf, D.C. (2015). Differential diagnosis of the honey bee trypanosomatids *Crithidia mellificae* and *Lotmaria passim* . *Journal of Invertebrate Pathology*, 130, 21–27.
- Retschnig, G., Williams, G.R., Odemer, R., Boltin, J., di Poto, C., Rosenkranz, P., … Neumann, P. (2015). Effects, but no interactions, of ubiquitous pesticide and parasite stressors on honey bee (*Apis mellifera*) lifespan and behaviour in a colony environment. *Environmental Microbiology*, 17, 4322–4331.
- Dainat, B., Evans, J. D., Chen, Y.P., & Neumann, P.(2011). Sampling and RNA quality for diagnosis of honey bee viruses using quantitative PCR. *Journal of Virological Methods*, 174, 150–152.
- Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J.D., Williams, G.R., … Zheng, H.Q.. (2013). Miscellaneous standard methods for *Apis mellifera* research. *Journal of Apicultural Research*, 52, 1–56.
- Evans, J.D., Schwarz, R.S., Chen, Y.P., Budge, G. Cornman, R.S., Muñoz, I., … Pinto, M.A. (2013). Standard methods for molecular research in *Apis mellifera* . *Journal of Apicultural Research*, 52, 1–54.
- de Miranda, J.R., Bailey, L., Ball, B.V., Blanchard, P., Budge, G.E. , de Smet, L. … van der Steen, J. J. M . (2013). Standard methods for virus research in *Apis mellifera* . *Journal of Apicultural Research*, 52, 1–56.
- Scharlaken, B., de Graaf, D.C., Goossens, K., Brunain, M., Peelman, L.J., & Jacobs, F.J.(2008). Reference gene selection for insect expression studies using quantitative real-time PCR: The head of the honeybee, *Apis mellifera*, after a bacterial challenge. *Journal of Insect Science*, 8, 1–10.
- Bourgeois, L., Rinderer, T.E., Beaman, L.D., & Danka, R.G.(2010). Genetic detection and quantification of *Nosema apis* and *N. ceranae* in the honey bee. *Journal of Invertebrate Pathology*, 103, 53–58.