Veterinarski Glasnik 2024, 78(2), 155-167
UDC: 638.15
https://doi.org/10.2298/VETGL231129010D

Full Research Article

ZYMOGRAPHY OF PROTEASES IN HONEY BEES (*APIS MELLIFERA*) INFECTED WITH *NOSEMA CERANAE*

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Received 29 November 2023; Accepted 26 April 2024

Published online: 15 July 2024

How to cite: Mohammad Afrasiabi Doghuzlu, Sedigheh Nabian, Gholam Ali Nehzati Paghaleh, Mohammad Taheri, Zahra Asadollahi, Soheila Akhzari. Zymography of proteases in honey bees (*Apis mellifera*) infected with *Nosema ceranae*. Veterinarski Glasnik, 2024. 78(2): 155-167. https://doi.org/10.2298/VETGL231129010D

Abstract

Nosemosis is one of the most important honey bee diseases and is caused by two fungal species of the genus *Nosema*, i.e., *Nosema apis* and *Nosema ceranae*. Evidence suggests that in the coming years, *Nosema ceranae*, which is less sensitive to fumagillin, could replace *Nosema apis* as the predominant pathogenic *Nosema*. Identifying and developing drugs that target these microsporidia without having serious effects on host bee physiology is essential for controlling nosemosis, and this requires information about bee enzymes. Serine proteases, the major protease enzymes in insects, play an important role in the insect immune system. In the present study, some proteases of honey bees infected with *N. ceranae* were studied by zymography. PCR tests showed that all honey bee samples were infected with *N. ceranae*. Also, the protein pattern of infected honey bee tissue extracts was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Bands with molecular weights of 25, 35, 48, 63, 75, and 100 kDa were observed. Subsequently, the serine proteases in the bees' digestive tracts

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ranged in molecular weight between 23-35 kDa, and the molecular weight of cathepsins was 25 kDa by gelatin zymography. Investigation of bee proteases might be useful for acquiring more knowledge about the interaction between host and parasite and to access an effective and low-risk compound for nosemosis treatment.

Keywords: Nosemosis, *Nosema ceranae*, serine protease, cathepsin

INTRODUCTION

Nosemosis, a significant disease affecting honey bees, is caused by two microsporidian parasites: *Nosema apis* and *Nosema ceranae*. Although recently reclassified by Tokarev et al. (2020) to the genus Vairimorpha, these species remain widely known by their original names. Notably, in recent decades, *N. ceranae* has become the predominant species in many regions worldwide, as evidenced by Higes et al. (2013).

Evidence suggests that *N. apis* in many bee populations will be replaced by *N. ceranae* (Milbrath et al., 2015). Regardless, *Nosema* is an important agent of damage to bee populations. Many researchers are seeking ways to reduce the damage caused by these fungi (Higes et al., 2013; Nabian et al., 2011; Glavinic et al*.,* 2022).

N. ceranae appears to be seriously endangering the health of bees, which is characterized by inhibition of immune response, destruction of the midgut cells (stomach), and shortening the life span of the bees (Glavinic et al., 2022). Despite the abundance of information on the prevalence, spread, and pathology of this agent, little is known about the bee immunological mechanism against *N. ceranae* (Schüler, 2022).

Fumagillin is an antibiotic that inhibits the enzyme methionine aminopeptidase2 (MetAP2) in eukaryotic cells and interferes with protein modifications which are necessary for normal cell function. *N. ceranae* is less sensitive to fumagillin treatment than *N. apis* (Huang et al*.,* 2013), although this pesticide controls both species of *Nosema*. Since fumagillin is associated with a residual risk in honey, it should not be used during nectar flow and should be used seasonally (Huang et al., 2013). Many variables, such as population size, nectar flow, and other factors, can affect the concentration of fumagillin used in bee colonies (Huang et al., 2013). The studies reviewed (Yücel et al., 2005; Higes et al., 2008; Williams et al., 2011; Pajuelo et al., 2011) do not support the claim that fumagillin harms bee colonies, as none of the experiments showed damage to colonies after exposure to the drug. Also, some studies show that fumagillin prevents the reproduction of microsporidian parasites and is probably useful for protecting weak hives. Still, this antibiotic could have unwanted effects on bees, ultimately increasing the prevalence and pathogenicity of *N. ceranae* (Huang et al*.,* 2013). Identification and development of other drugs that target microsporidia without having serious effects on host physiology are necessary to control nosemosis. Therefore, more information about bee enzymes and body structure is needed to access an effective and low-risk compound for infected bees (Huang et al., 2013).

Insects typically use different types of digestive enzymes which are secreted by their midgut epithelial cells to digest food and control the homeostasis of the stomach

(Sharifi et al., 2012). Proteases are present in the digestive system and hemolymph of insects and play important roles including digestion, immune system, activation of phenol oxidase, and release of amino acids that are essential for growth (Shrifi et al., 2012). Haloi et al. (2023) found that proteolytic activity in honey bees infected with *Nosema* was reduced compared with that in healthy bees (Haloi et al., 2023).

Proteases are classified according to their catalytic mechanism, e.g., into serine proteases, cysteine proteases, aspartic proteases, and metalloproteases. Serine proteases and metalloproteases constitute a large part of the insect body's enzymes (Makedo and Freire, 2011). Serine proteases regulate several types of immune responses, including blood coagulation, antimicrobial peptide synthesis, and melanization of various pathogens. Performing these steps requires the presence of serine protease in the insect hemolymph (Gamal et al., 2023). Cathepsins are lysosomal proteases; some of them, such as cathepsin A and G, belong to the family of serine proteases and their major physiological role is the destruction of intracellular proteins and of extracellular proteins that enter the cell through endocytosis (Duran et al., 2023).

The innate immune system in insects consists of different pathways activated in response to external factors. One of the key elements in these responses is phenol oxidase, which produces indole groups that eventually polymerize into melanin. Phenol oxidase requires an activation and inhibitory system that includes different cell types, inhibitory enzymes, and signaling molecules (Cerenius and Söderhäll, 2021). Antunez et al. (2009) found that encapsulation is the most important defense response by hemocytes, which are responsible for protecting the insect's ventricle against fungal infections. Components of the prophenoloxidase system, including serine proteases, can stimulate the encapsulation. In this process, the invaders are compartmented into several cellular and capsule-like layers that have been melanized and are completely separate from the surrounding environment (Antunez et al., 2009). Thus, the quantity changes of serine proteases in an insect's body can affect the melanization process as an innate immune response.

In the present study, some proteases of honey bees infected with *N. ceranae* were studied by zymography.

MATERIALS AND METHODS

Identification of Nosema spores

The infected bees were selected from one of the apiaries around Tehran city. Then, 25 of the bee digestive tracts were removed by forceps in the laboratory. These sections were crushed and homogenized with 1 mL of distilled water in a Chinese mortar. Subsequently, the presence or absence of bee infection with *Nosema* spores was confirmed microscopically by the preparation of wet slides from these samples and the observation of *Nosema* spores. To determine the intensity of infection, the number

of *Nosema* spores was counted in five sections of a hemocytometer slide, and the mean spore number was determined and assigned as grades 1 to 3 as follows: 1 to 20 (grade 1), 20 to 50 (grade 2), and 50 to 100 (grade 3). Extracts from the abdominal contents of uninfected bees without spores were considered a negative control group. For species specific identification of *N. apis* and *N. ceranae*, PCR was performed using the 321 APIS, and 218 MITOC primers, and PCR products were analyzed on agarose gel.

Sample preparation for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

To obtain pure *Nosema* spores from the bees, the midgut was separated from the hindgut and crushed in 2 mL of sterile distilled water, then passed through a fine filter to remove any remaining coarse material. The filtered suspensions were centrifuged at 1500 rpm for 5 minutes. Then the supernatants were discarded and 1 mL of distilled water was added to each pellet. Subsequently, the suspended pellets were carefully layered on top of a discontinuous sucrose gradient formed with increasing concentrations of Percoll (25%, 50%, 75%, and 100%) from bottom (low concentration) to top (high concentration). This gradient was then centrifuged at 8,000 rpm for 20 minutes at 4°C. The supernatants were discarded, and the resulting pellet, enriched for spores, was further centrifuged at 8,000 rpm for 10 minutes.

To disrupt the spore cell walls and release their protein content, 250 μL (microliters) of distilled water and a small amount of acid-washed glass beads were added to the centrifuged spore pellet. This mixture was shaken on ice for 15 minutes at a speed of 150 rpm using a mechanical shaker (VDRL digital). The resulting suspension was then centrifuged at 4,000 rpm in a refrigerated centrifuge. The supernatant, containing the extracted proteins, was collected and stored at – 70°C.

SDS-PAGE analysis of the proteins in the supernatants was performed using a vertical electrophoresis apparatus (Bio-Rad) according to the method described by Laemmli (1970). Briefly, a 5% stacking gel and a 12% resolving gel were used to separate protein bands extracted from both infected and non-infected bees. The gels were then stained with Coomassie Brilliant Blue.

Electrophoresis of *Nosema ceranae* Spore Protein

Electrophoresis of *Nosema* spore proteins was performed similarly to the electrophoresis of bees' abdominal contents as described immediately above.

Zymography

Zymography is a SDS-PAGE-based electrophoresis method used to determine the enzymatic activity and to analyze protease components in biological samples. This study was performed using the method described by Beccaria et al. (2021). For electrophoresis, the samples were loaded on polyacrylamide gel (5% supernatant gel)

and 12% separating gel to which 0.1% gelatin was added, and electrophoresis was performed. The gel was then incubated for half an hour in solution containing 2.5% Triton X-100. After washing with distilled water, the gel was incubated overnight in incubation buffer at laboratory temperature. After washing with distilled water, the gel was stained with Coomassie brilliant blue and destained with acetic acid. Due to digestion of gelatin, protease bands were clearly visible on a blue background.

In order to discriminate whether the bands that formed on the gel belonged to metalloproteases or serine proteases, the gels were incubated in metalloprotease buffers (50 mM Tris buffer, pH 8, containing calcium chloride) and serine protease buffers (which contained EDTA). For the investigation of cathepsins, we used 10 mM acetate buffer, pH 4, containing 2 mM dithiothreitol (DTT) or the same buffer without 2 mM dithiothreitol.

RESULTS

A PCR molecular method was used to determine the species of *Nosema* in infected bees. *N. ceranae* was the only *Nosema* species in these infected bees. As shown in Fig. 1, positive samples showed a band of 217 bp size, while control groups without bands are shown in gel lanes 4 and 10.

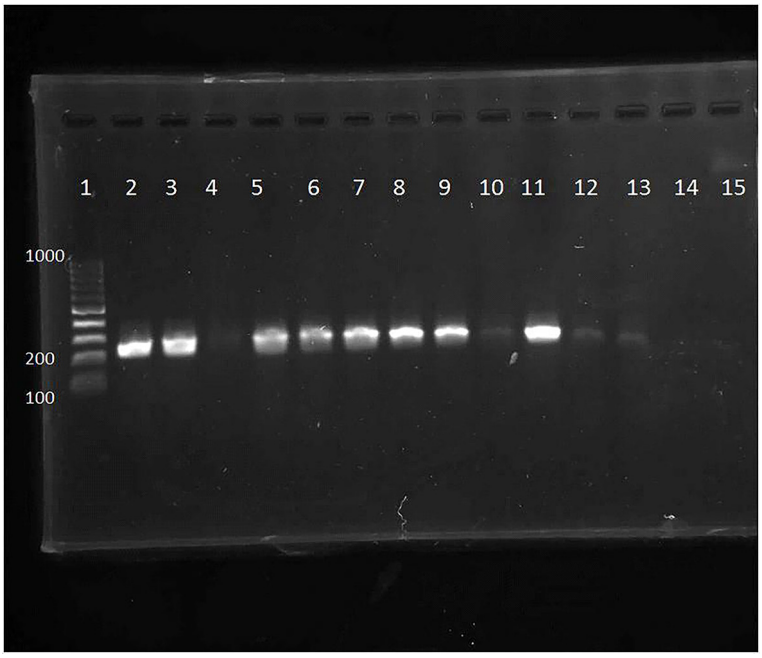

Figure 1: PCR test for bees infected with *Nosema ceranae*. Molecular weight marker in **L1**; bees negative for *Nosema* **L4** and **L10**.

In the second step of this study, SDS-PAGE of purified *Nosema* spore extract showed at least six protein bands with molecular weights of 25, 35, 48, 63, 75, and 100 kDa (Fig. 2).

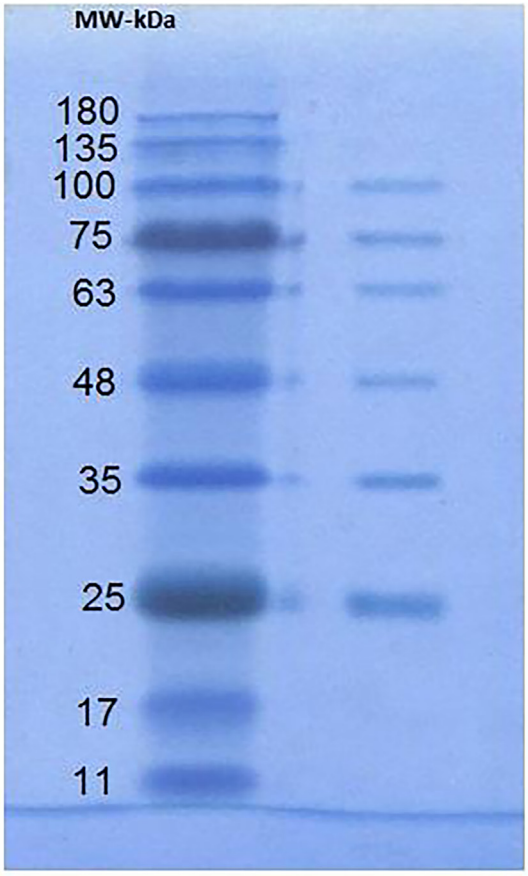

Figure 2: Protein pattern of pure *Nosema ceranae* spore extract

In the electrophoresis of abdominal content extracts of infected and non-infected bees, protein bands with molecular weights of 15, 25, 35, 48, 75, 100, 135, and above 180 kDa were observed (Fig. 3). Next, zymography was used to determine the presence of serine proteases in extracts of infected and non-infected honey bees. Protease activity was observed as transparent bands at a molecular weight of about 23-35 kDa, as shown in Fig. 4.

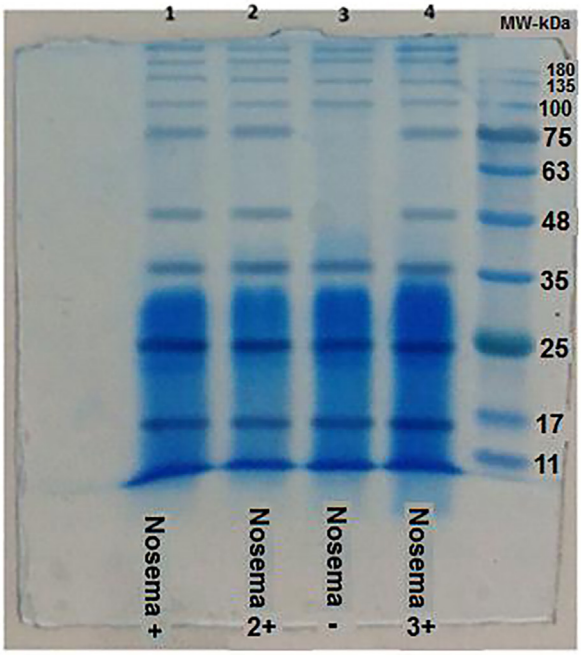

Figure 3: Protein pattern of midgut contents of bees infected and non-infected with *Nosema ceranae*

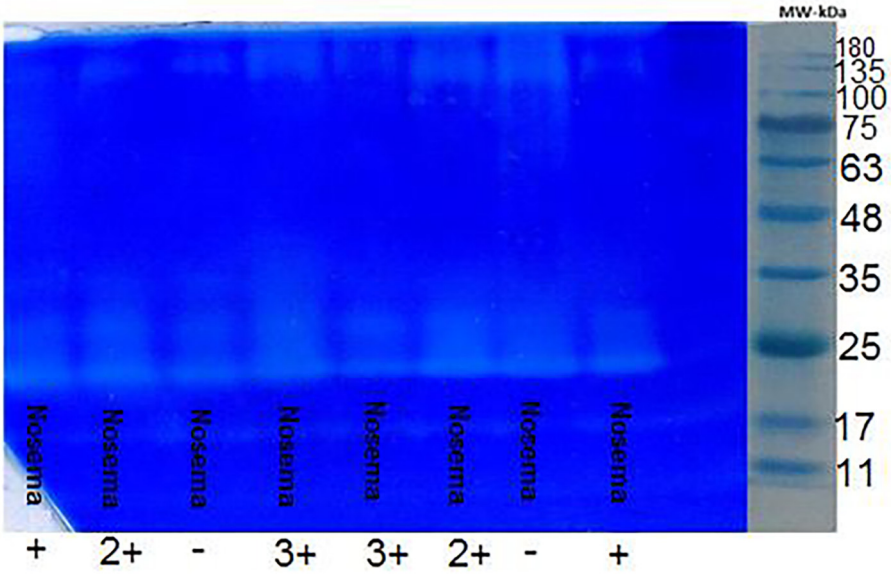

Figure 4: Zymography to study serine proteases

The presence of cathepsins in infected and non-infected honey bee extracts was observed, using zymography, as transparent bands with a molecular weight of about 23-35 kDa (Fig. 5).

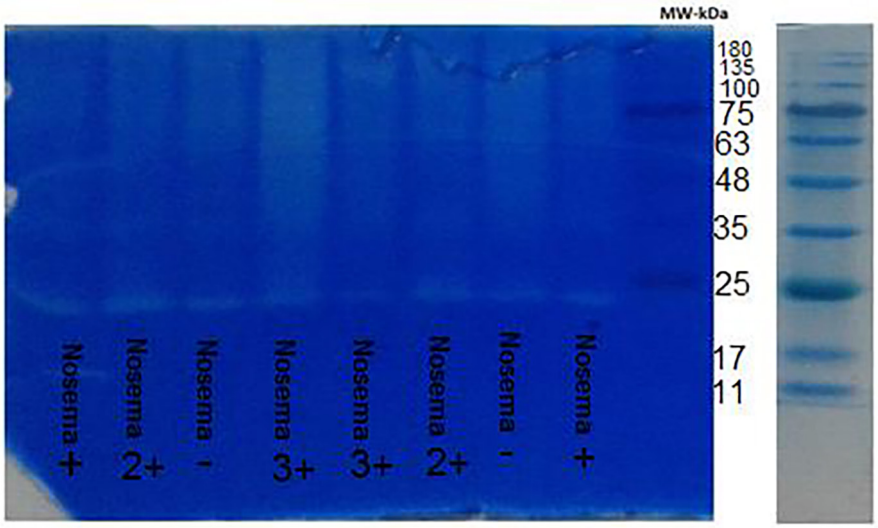

Figure 5: Zymography to study cathepsins

DISCUSSION

Nosemosis is one of the most important honey bee diseases caused by two fungal species of the genus *Nosema*, *N. apis* and *N. ceranae*. Several studies have confirmed that *N. ceranae* is the dominant species in recent years (Gisder et al., 2017; Klee et al., 2007; Chen et al., 2008; Williams et al., 2008; Invernizzi et al., 2009; Chen and Huang, 2010). In this study, a PCR method was used to determine the species of *Nosema* in infected bees. It was found that all infected bees were infected with *N. ceranae,* and according to previous reports, this supports the opinion that *N. ceranae* has become the dominant species in honey bees.

In the second step of this study, SDS-PAGE of purified *Nosema* spores was conducted, and the protein patterns from spores and from total abdominal extracts of *Nosema* infected and non-infected bees were compared. The protein patterns of pure *N. ceranae* spores showed at least six bands with molecular weights of 25, 35, 48, 63, 75, and 100 kDa, while electrophoresis of abdominal contents of *Nosema* infected and non-infected bees, as expected, showed these protein bands and other bands with molecular weights of 15, 135, and above 180 kDa. It seems these extra bands, which are not related to *Nosema* spores, are likely related to the presence of pollen grains of plants, cells, or other digestive tract contents. No differences were observed in the protein patterns of total abdominal extracts of bees infected with *Nosema* at different intensities.

To the best of our knowledge, there is no information in the published literature regarding the protein patterns of the two *Nosema* species in our study. Sironmani (1999), using polyacrylamide gel electrophoresis, analyzed the proteins of *Nosema bombycis* spores, a microorganism causing Pébrine disease in silkworms. He reported the presence of proteins with molecular weights of 17, 28, 31, 45, 68, and 94 kDa in the spore extracts (Sironmani, 1999). Wu et al., using SDS-PAGE, analyzed the proteome of the membrane from *Nosema* spores. They identified 14 proteins or peptides in the membrane of *N. bombycis* spores. The three major bands had molecular weights of 32.7, 30.4, and 25.3 kDa (Wu et al., 2008).

In the present study, zymography was used to determine the proteases of infected and non-infected honey bees. Transparent bands which belonged to protease activity were observed at the molecular weight of about 23-35 kDa. In order to identify the type of proteases, i.e., whether they were metalloprotease or serine protease, zymography gels were incubated in specific buffers. As EDTA is a calcium chelator and neutralizes metalloprotease activity, the presence of the mentioned bands in buffer both with or without calcium chloride proved that the bands belonged to serine proteases. In similar work, Guedes et al. (2007) purified serine proteases of *Leishmania braziliensis* and showed their bands by zymography. The molecular weights of related bands were estimated as 60, 45, 130, 83, 74, 30, 62, 59, 57, 49, and 35 kDa (Guedes et al., 2007).

In our study, the presence of cathepsins in the abdominal extracts of infected and uninfected bees was demonstrated by zymography using SDS-PAGE. Due to the presence of dithiothreitol in the incubation buffer, the protease activity appeared as a clear transparent band with a molecular weight of about 25 kDa which was related to cysteine proteases (cathepsins). The different molecular weights of cathepsins and serine proteases in different animal species can be explained by the process of evolution, whereby different isoforms of proteins can have different molecular weights. Several studies have shown there are about 650 proteases of different types in the genomes of higher animal species. Jayashnakar (2007) reported the molecular weights of the cathepsin family are between 20 and 35 kDa, with the exception of cathepsin C, an oligomeric enzyme with a molecular weight of 200 kDa (Jayashnakar, 2007). To the best of our knowledge, no similar research has been done to identify the molecular weight of cathepsins from honey bees.

In our study, no differences were observed in the protein patterns of honey bees infected with *Nosema* at different intensities. However, previous studies have reported a decrease in the activity of proteases in bees infected with *Nosema* compared to healthy bees (Malone and Gatehouse, 1998). Therefore, further investigations to properly identify bee proteases are necessary.

CONCLUSIONS

According to the results of this study, SDS-PAGE analysis of *Nosema* spores revealed multiple protein bands with varying molecular weights. Zymography further identified various protease bands, originating from both the parasite and the infected host bees. These findings suggest that some of the identified proteins might be involved in the parasite's lifecycle or its interaction with the host. This knowledge could contribute to a better understanding of Nosema infection. However, further genomic and proteomic studies are necessary to precisely identify and characterize these proteins for potential development of *Nosema* control strategies.

Ethical Statement

This research was conducted in accordance with all relevant regulations and institutional policies for the care and use of animals. Ethical review and approval were waived for this study, due to the study of invertebrates only.

Authors' contributions

All authors interpreted the data, critically revised the manuscript for important intellectual content, and approved the final version.

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REFERENCES

Antúnez, K., Martín-Hernández, R., Prieto, L., Meana, A., Zunino, P., & Higes, M. (2009). Immune suppression in the honey bee (*Apis mellifera*) following infection by *Nosema ceranae* (Microsporidia). Environmental microbiology, 11(9), 2284-2290.

Beccaria, C., Velázquez, N. S., Chervaz, V., Pirola, S. I., Baravalle, C., Renna, M. S., ... & Dallard, B. E. (2021). Involvement of matrix metalloproteinases and their inhibitors in Staphylococcus aureus chronically infected bovine mammary glands during active involution. Research in Veterinary Science, 137, 30-39.

Cerenius, L., & Söderhäll, K. (2021). Immune properties of invertebrate phenoloxidases. Developmental & Comparative Immunology, 122, 104098.

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. Journal of invertebrate pathology, 97(2), 186-188.

Chen, Y. P., & Huang, Z. Y. (2010). *Nosema ceranae*, a newly identified pathogen of *Apis mellifera* in the USA and Asia. Apidologie, 41(3), 364-374.

Duran Ş., Dede A.F.Ü, Dündar-Orhan Y And Arslanyolu M., (2023). Genome-wide identification and in-silico analysis of papain-family cysteine protease encoding genes in Tetrahymena thermophila. European Journal of Protistology, p.126033.

Gamal, E., Hassan, A. K., Abdel-Rahman, M. A., Hassan, M. K., & Tawfik, M. M. (2023). Immune Responses and Bioactive Peptides of Insect Hemolymph. Egyptian Academic Journal of Biological Sciences. A, Entomology, 16(3), 13-25.

Gisder, S., Schüler, V., Horchler, L. L., Groth, D., & Genersch, E. (2017). Long-term temporal trends of Nosema spp. infection prevalence in Northeast Germany: continuous spread of *Nosema ceranae*, an emerging pathogen of honey bees (*Apis mellifera*), but no general replacement of Nosema apis. Frontiers in cellular and infection microbiology, 7, 301.

Glavinic, U., Blagojevic, J., Ristanic, M., Stevanovic, J., Lakic, N., Mirilovic, M., & Stanimirovic, Z. (2022). Use of thymol in *Nosema ceranae* control and health improvement of infected honey bees. Insects, 13(7), 574.

Guedes, H. L., Rezende Neto, J. M., Fonseca, M. A., Salles, C. M., Rossi-Bergmann, B., & De-Simone, G. (2007). Identification of serine proteases from Leishmania braziliensis. Zeitschrift für Naturforschung C, 62(5-6), 373-381.

Haloi, K., Kalita, M. K., & Devi, D. (2023). Regulation and Characterization of Amylase Enzyme Secretion in the Digestive Tract of Antheraea assamensis Helfer. Indian Journal of Entomology, 01-07.

Huang, W. F., Solter, L. F., Yau, P. M., & Imai, B. S. (2013). *Nosema ceranae* escapes fumagillin control in honey bees. PLoS pathogens, 9(3), e1003185.

Higes, M., Meana, A., Bartolomé, C., Botías, C., & Martín-Hernández, R. (2013). N osema ceranae (Microsporidia), a controversial 21st century honey bee pathogen. Environmental microbiology reports, 5(1), 17-29.

Higes, M., Nozal, M. J., Alvaro, A., Barrios, L., Meana, A., Martín-Hernández, R., ... & Bernal, J. (2011). The stability and effectiveness of fumagillin in controlling *Nosema ceranae* (Microsporidia) infection in honey bees (*Apis mellifera*) under laboratory and field conditions. Apidologie, 42, 364-377.

Higes, M., Martín-Hernández, R., Botías, C., Bailón, E. G., González-Porto, A. V., Barrios, L., ... & Meana, A. (2008). How natural infection by *Nosema ceranae* causes honeybee colony collapse. Environmental microbiology, 10(10), 2659-2669.

Invernizzi, C., Abud, C., Tomasco, I. H., Harriet, J., Ramallo, G., Campá, J., ... & Mendoza, Y. (2009). Presence of *Nosema ceranae* in honeybees (*Apis mellifera*) in Uruguay. Journal of invertebrate pathology, 101(2), 150-153.

Klee, J., Besana, A. M., Genersch, E., Gisder, S., Nanetti, A., Tam, D. Q., ... & Paxton, R. J. (2007). Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the western honey bee, *Apis mellifera*. Journal of invertebrate pathology, 96(1), 1-10.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227(5259), 680-685.

Malone, L. A., & Gatehouse, H. S. (1998). Effects ofNosema apisInfection on Honey Bee (*Apis mellifera*) Digestive Proteolytic Enzyme Activity. Journal of Invertebrate Pathology, 71(2), 169-174.

Mussen, E. C., Furgala, B., & Hyser, R. A. (1975). Enzootic levels of nosema disease in the continental United States (1974).

Macedo, M. R., & Freire, M. D. G. M. (2011). Insect digestive enzymes as a target for pest control. Invertebrate Survival Journal, 8(2), 190-198.

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.

Nabian, S., Ahmadi, K., Shirazi, M. N., & Sadeghian, A. G. (2011). First detection of *Nosema ceranae*, a microsporidian protozoa of European honeybees (*Apis mellifera*) in Iran. Iranian journal of parasitology, 6(3), 89.

Pajuelo, A. G., Torres, C., & Bermejo, F. J. O. (2008). Colony losses: a double blind trial on the influence of supplementary protein nutrition and preventative treatment with fumagillin against *Nosema ceranae*. Journal of Apicultural Research, 47(1), 84-86.

Schüler, V. (2022). Biological effects of *Nosema ceranae* infections on honey bees (*Apis mellifera*) and bumblebees (Bombus terrestris) (Doctoral dissertation).

Sharifi, M ., Gholamzadeh, C M ., Ghadamyari, M., Ajamhasani, M. (2012). identification and characterization of midgut digestive proteases from the rosaceouse branch borer, Osphranteria Coerolescsn Redtenbacher (Coleopteran : Cerambycidae) . J , Biochem 49,1,33-47 .

Sironmani, T. A. (1999). Immunological characterization of spore proteins of the microsporidian Nosema bombycis. World Journal of Microbiology and Biotechnology, 15, 607-613.

Tokarev, Y. S., Huang, W. F., Solter, L. F., Malysh, J. M., Becnel, J. J., & Vossbrinck, C. R. (2020). A formal redefinition of the genera Nosema and Vairimorpha (Microsporidia: Nosematidae) and reassignment of species based on molecular phylogenetics. Journal of invertebrate pathology, 169, 107279.

Yücel, B., & Doğaroğlu, M. (2005). The impact of Nosema apis Z. infestation of honey bee (*Apis mellifera* L.) colonies after using different treatment methods and their effects on the population levels of workers and honey production on consecutive years.

Williams, G. R., Shutler, D., Little, C. M., Burgher-Maclellan, K. L., & Rogers, R. E. (2011). The microsporidian *Nosema ceranae*, the antibiotic Fumagilin-B®, and western honey bee (*Apis mellifera*) colony strength. Apidologie, 42, 15-22.

Wu, Z., Li, Y., Pan, G., Tan, X., Hu, J., Zhou, Z., & Xiang, Z. (2008). Proteomic analysis of spore wall proteins and identification of two spore wall proteins from Nosema bombycis (Microsporidia). Proteomics, 8(12), 2447-2461.

Wu Zhenghli, Li Y, Pan G, Tan X, Hu J, Zhou Z, Xiang Z.2008. Proteomic analysis of spore wall proteins and identification of two spore wall proteins from Nosema bombycis (Microsporidia). J.Proteomics,8(12):2447-61.

ISPITIVANJA PROTEAZA KOD MEDONOSNIH PČELA (*APIS MELLIFERA*) ZARAŽENIH SA *NOSEMA CERANAE* PRIMENOM ZIMOGAFIJE

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Kratak sadržaj

Nosemoza je jedna od najznačajnijih bolesti pčela koju izazivaju dve vrste mikrosporidija iz roda *Nosema* (*N. apis i N. ceranae*). Istraživanja pokazuju da *N. Ceranae*, koja je manje osetljiva na fumagilin, postaje predominantna vrsta u odnosu na *N. apis* poslednjih godina. Identifikacija i razvoj lekova za kontrolu ovih mikrosporidija, sa što manjim uticajem na fiziologiju domaćina, je od suštinskog značaja za kontrolu nozemoze. U tu svrhu značajne su informacije o enzimima pčela. Serinske proteaze, kao glavni proteazni enzimi kod insekata, igraju važnu ulogu u njihovom imunološkom sistemu. U ovom istraživanju, neke proteaze medonosnih pčela zaraženih sa *N.ceranae* su proučavane zimografijom. PCR analize su pokazale da je u svim uzorcima pčela bila prisutna vrsta *N.ceranae*. Osim toga određen je, proteinski profil ekstrakata zaraženih tkiva medonosne pčele pomoću SDS-PAGE. Uočene su određene trake sa molekularnom težinom od 25, 35, 48, 63, 75 i 100 kDa. Metodom želatinske zimografiije determinisana je molekulska težina serinskih proteaza u digestivnom traktu pčele u rasponu od 23-35 kDa, kao molekulska težina katepsina koja je bila 25 kDa. Smatramo da bi istraživanje proteaza moglo biti korisno za sticanje novih znanja o interakciji između domaćina i parazita, kao i za pronalazak efikasnih i bezbednih jedinjenja koja bi bila korišćena za lečenje nozemoze.

Ključne reči: Nozemoza, *Nosema ceranae*, serin proteaza, katepsin