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Research Artcile

Hatay İli Arıcılık İşletmelerinde *Nosema* ve *Amoeba* Enfeksiyonlarının Yaygınlığı

Aykut ZEREK[1]*

[1]Hatay Mustafa Kemal Üniversitesi, Veteriner Fakültesi, Klinik Öncesi Bilimler Bölümü, Veterinerlik Parazitolojisi Anabilim Dalı, Hatay

*Sorumlu Yazar: aykutzerek@mku.edu.tr

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Öz

Nosemosis ergin bal arılarının sindirim sistemini etkileyen, arı kayıplarına neden olan, Dünya'da ve Türkiye'de yaygın görülen tehlikeli ve bulaşıcı bir hastalıktır. *Malpighamoeba mellificae* etkeninin sebep olduğu amoebiasis güçlü kolonilerde tek başına ciddi problemlere yol açmaz. Ancak nosemosis ile birlikte seyrettiğinde hastalığın virülansını ve mortalitesini artırarak önemli kayıplara neden olabilir. Bu çalışmada, Hatay ili arıcılık işletmelerinde mikroskobik yöntemle nosemosis ve amoebiasis hastalıklarının yaygınlıklarının araştırılması amaçlanmıştır. Örneklerin çoğunluğunu gezgin arıcılık işletmelerinin oluşturduğu çalışma, 2021 ilkbahar aylarında yapıldı. Çalışmada, Hatay ilinin dört ilçesindeki 62 arıcılık işletmesinde 343 kovandan ergin işçi arı örnekleri toplandı. Mikroskobik inceleme sonuçlarına göre arıcılık işletmelerinde *Nosema* spp., *M. mellificae* ve miks enfeksiyon oranları sırasıyla %21, %15 ve %29; bu işletmelerdeki kovanlarda ise sırasıyla %17, %5 ve %8 olarak belirlendi. Sonuç olarak, Hatay ilindeki işletmelerin %65'inde, bu işletmelerdeki kovanların ise %30'unda nosemosis ve amoebiasis hastalıkları tespit edilmiştir. Bu çalışmada *Nosema* spp. ve *M. mellificae* enfeksiyonları birlikte görülmesine rağmen, önemli klinik semptomların ve arı kayıplarının olmaması, dirençli kolonilerde ciddi bir risk oluşturmayacağı sonucunu doğurmuştur.

Anahtar kelimeler: Hatay ili, Nosemosis, Amoebiasis, *Nosema* spp., *Malpighamoeba mellificae*

Prevalence of *Nosema* and *Amoeba* Infections in Beekeeping Farms in Hatay Province

Abstract

Nosemosis is a dangerous and contagious disease that affects the digestive system of adult honey bees, causes bee losses, and is common in the world and in Turkey. Amoebiasis caused by *Malpighamoeba mellificae* does not lead to serious problems in strong colonies alone. However, when it progresses with nosemosis, it may cause significant losses by increasing the virulence and mortality of the disease. In this study, it was aimed to investigate the prevalence of nosemosis and amoebiasis diseases by microscopic method in beekeeping enterprises in Hatay province. The study, in which the majority of the samples were composed of mobile beekeeping enterprises, was carried out in the spring of 2021. In the study, adult worker bee samples were collected from 343 hives in 62 beekeeping enterprises in 4 districts of Hatay province. According to the results of the microscopic examination, the rates of *Nosema* spp., *M. mellificae* and mixed infections in beekeeping enterprises were 21%, 15% and 29%, respectively; and they were determined as 17%, 5% and 8% in the hives in these enterprises, respectively. As a result, nosemosis and amoebiasis diseases were detected in 65% of the enterprises in Hatay, in 30% of the hives of these enterprises. Although *Nosema* spp. and *M. mellificae* infections, were observed together in this study, the absence of significant clinical symptoms and bee losses, led to the conclusion that it would not pose a serious risk in resistant colonies.

Key words: Hatay province, Nosemosis, Amoebiasis, *Nosema* spp., *Malpighamoeba mellificae*

Introduction

Honey bees are under the threat of many disease agents and pests in the world and in Turkey. Varroosis, American foulbrood and nosemosis, which are commonly found in bees, negatively affect the development and production of beekeeping when accompanied by other viral and parasitic diseases (Uygur and Girişgin, 2008; Balkaya et al., 2016; Muz and Muz 2017). Nosemosis caused by *Nosema apis* and *Nosema ceranae*, which are microsporidial agents, is reported as a highly contagious and dangerous disease that affects the digestive system of adult honey bees and causes significant losses in bees from time to time (Ütük et al., 2010; Whitaker et al., 2011). Despite not being proven, *Nosema* infections, which are accompanied by *Varroa* and virus infections, are thought to be one of the causes of colony losses in recent years (Muz and Muz 2017; Özüiçli and Aydın, 2018).

Amoebiasis, caused by the protozoan *Malpighamoeba mellificae*, which settles in the malpighian tubes of adult bees, does not cause serious problems in strong colonies other than diarrhea, if it creates an infection alone (Bailey, 1968; Uygur and Girişgin, 2008; Aydın et al., 2017). However, it is reported that when it progresses together with nosemosis, it can cause significant losses by increasing the virulence and mortality of the disease, as in other opportunistic pathogens living in the intestinal flora of bees (Aydın et al., 2017; Özüiçli and Aydın, 2018).

Bee diseases spread through the trade of bees, bee products and beekeeping materials between countries in parallel with rapid transportation. In Turkey, this situation is mostly due to migratory beekeeping activities (Uygur and Girişgin, 2008). The presence of nosemosis and amoebiasis, which is reported to increase in the spring months (Aydın et al., 2017), has been announced in various studies in the world (Bailey, 1968; Varis et al., 1992; Plischuk and Lange, 2010; Mengistu 2017) and Turkey (Aydin et al., 2005; Aydin et al., 2006), yet no detailed study has been found in Hatay, a study conducted study on 28 queens (Muz and Muz, 2009).

In this study, it was aimed to investigate the prevalence and risks of nosemosis and amoebiasis diseases by microscopic method in honey bee samples collected from the colonies of the enterprises mostly composed of mobile beekeepers in Antakya, Arsuz, Dörtyol and Samandağ districts in Hatay province.

Material and Methods

Determination of study areas

TUIK data in 2020 were used to determine the number of beekeeping enterprises to be studied in Hatay province. According to these data, the total number of beekeeping enterprises operating in Hatay province is 710 (TUIK, 2021).

The number of enterprises required to be taken as a sample in the province of Hatay was determined as 62 with the help of the sampling method (Can and Yalçın, 2015) used for screening studies where the population volume is known. In the study, mainly Dörtyol (33) and Antakya (15), which constitute approximately 1/3 (231/710) of the enterprises in Hatay province, and Samandağ (8) and Arsuz (6) districts were selected (Table 1).

Collection of samples

The enterprises outside Antakya, where sampling was carried out, consist of mobile beekeepers who come to Hatay in winter and spring. Nosemosis and amoebiasis are seen in the spring months when the bees are released after wintering. For this reason, the study was carried out in the spring of 2021. The selection of hives from which samples were taken was random in beekeeping establishments registered by Hatay Provincial Beekeepers Association. The feces on the top covers, flight boards and frame surfaces of the selected hives were examined for diarrhea cases. Flightless, crawling or dead bees were investigated in and around the hive entrances. The owners of the enterprises were asked whether they had encountered colony losses in the past years. A total of 100 samples were collected from hive entrances into sterile falcon tubes, four to six hives from each of the enterprises and 15-20 live or dead adult worker bees from each of the hives. A total of 6200 adult worker bee samples were taken from 343 hives in 62 enterprises included in the study.

Microscopic method

The digestive systems of the bees which were brought to the laboratory were examined macroscopically for the signs of nosemosis, after separating the abdomen from the thorax. Then, it was homogenized by crushing in 3-5 ml of distilled water and a mixture was obtained. A few drops of this mixture were taken between the slide and the cover slip then *Nosema* spp. spores and *M. mellificae* cysts were examined under a light microscope on a 40X objective with the help of the relevant literatures (Plischuk and Lange, 2010; Aydın et al., 2017). The safranin-methylene blue staining method was used to distinguish *Nosema* spores from *M. mellificae* cysts and other fungi (Aydın et al., 2017). In this staining method, it was determined that *M. mellificae* cysts and other

fungal spores were stained blue with methylene blue, while *Nosema* spores were stained pink or red with safranin dye (Figure 1).

Results and Discussion

Except for a few colonies, beekeepers have not had any complaints about colony losses in the past years. Flightless, crawling or dead bees were found at the hive entrances yet it was not significant. Apart from a few suspected cases (3/343), no diarrhea symptoms were observed on the top covers, flight boards and frame surfaces of the selected hives. In the macroscopic examination, the nodes of the digestive systems of the bees infected with *Nosema* spores were unclear and they were pale.

As a result of the microscopic examinations, *Nosema* spp. and *M. mellificae* infections were detected in all sampled districts. In a total of 62 enterprises sampled, 13 (21%) *Nosema* spp., 9 (15%) *M. mellificae* and 18 (29%) mixed infections were detected. In addition, 59 (17%) *Nosema* spp., 17 (5%) *M. mellificae* and 28 (8%) mixed infections were detected in 343 hives in these enterprises (Table 1).

As a result, nosemosis and amoebiasis diseases were detected in 40 (65%) of 62 enterprises and 104 (30%) of 343 hives in these enterprises in Hatay (Table 1). In addition, a large number of mummified adult bees (Chalkbrood disease) were also encountered at the entrance of one hive with mixed infection in Samandağ district. *Nosema apis* was thought to be the cause of nosemosis cases that previously occurred in *Apis mellifera* in Europe, America and Asia continents including Turkey. When *N. ceranae* was detected in *Apis cerana* in China in 1994 (Fries et al., 1996), it was understood that many other cases were actually caused by *N. cerenae* (Higes et al., 2006; Huang et al., 2007; Klee et al., 2007; Paxton et al., 2007; Ütük et al., 2010). In the following years, *N. ceranae* has been shown to replace *N. apis* worldwide, depending on the increasing international trade (Ansari et al., 2017; Aydın et al., 2017). In recent studies in Turkey, over 90% of the dominant species have been found to be *N. ceranae* (Ütük et al., 2016). In the Hatay region, *N. ceranae* was reported in 89% and *N. apis* in 11% of 85 hives with colony losses between 2007-2009 (Muz et al., 2010). In another study conducted with the molecular method in 2019 (Zerek et al., 2022), *N. ceranae* was seen in 20% of the examined colonies, while *N. apis* was not found at all. In this study, *Nosema* spp. and *M. mellificae* were found alone in 59 (17%) and 17 (5%) of 343 hives, respectively, and as mixed infections in 28 (8%) hives. Considering the previous studies in Hatay and the absence of serious
diarrhea cases in the examined colonies, we believe that may be *N. ceranae* that the spores of *Nosema* spp. The reason is that *N. ceranae* is a more dominant species than *N. apis* in the world and in Turkey. It has also been reported that there is an increase in the rate of amoeba infection in the presence of *Nosema* infection (Fries, 1993). In this study, the detection of mixed infections at both the enterprise (15%/29%) and hive (5%/8%) levels at higher rates than the infections in which *M. mellificae* was detected alone supports this view.

Contrary to *N. apis*, which develops in the digestive system and causes acute diarrhea, *N. ceranae* is associated with colony loss syndrome since it is more pathogenic and asymptomatic, and increases susceptibility to viruses and other pathogens by creating nutritional stress (Higes et al., 2006; Higes et al., 2007; Mayack and Naug, 2009; Muz and Muz, 2017). Contrary to this view, it is stated that there has not been a large-scale colony loss due to this pathogen throughout Europe since 1998 and that the claims on this subject cannot constitute statistical evidence (Paxton, 2010). In the history of the selected enterprises in this study, no colony loss was reported, except for a few hives, and no significant diarrhea and bee deaths were encountered. Considering that no serious risk has been encountered in colonies where nosemosis and ameobiasis cases are seen alone or even together, it can be concluded that these diseases will not cause serious bee losses in strong and resistant colonies.

There are few studies in the world investigating the prevalence of nosemosis and amoebiasis diseases together. Only cases of nosemosis have been reported in Finland (Varis et al., 1992), while cases of nosemosis and amoebiasis have been reported in Ethiopia (Mengistu, 2017) and Argentina (Plischuk and Lange, 2010). Few samples were examined in these studies and were not conducted systematically, so these studies were not considered in the discussion. In a microscopic study conducted in different parts of England between 1954-1958, it was reported that infections with *N. apis*, *M.mellificae* and mix were detected as 39.92%, 6.12% and 4.8%, respectively (Bailey, 1968). In this study, *Nosema* spp. infection was found about twice as much as our results, while mixed infections were detected at a rate of about half. This situation may have arisen depending on the type of the agent, the care and feeding conditions of the bees, and environmental differences such as climate and humidity.

Only a few studies have been found in Turkey in which the prevalence of nosemosis and amoebiasis diseases was investigated together. *Nosema apis* was detected in 21 (14%) and mixed infections (*N. apis* and *M. mellificae*) were detected in 11 (5%) of 230 colonies in different regions of Turkey (Aydin et al., 2005). In another study conducted in Bursa, Balıkesir and Çanakkale, *N. apis* and mixed infections were reported in 28 (17%) and 12 (7%) out of 168 hives, respectively (Aydin et al., 2006). In this study conducted in Hatay, *Nosema* spp. were found in 59 (17%) of 343 colonies and 28 (8%) of 343 colonies with mixed infections. The aforementioned studies are similar in that the percentages of infection are close to each other and nosemosis infections approximately 2-3 times more than mixed infections. Nosemosis was observed in all 28 queen bees (100%) and mixed infection with amoebiasis was observed in 14% of the enterprises in the Hatay region where colony loss was observed by microscopic method (Muz and Muz, 2009).

Conclusion

According to the results of this study, the rates of *Nosema* spp., *M. mellificae* and mixed infections in beekeeping enterprises were 21%, 15% and 29%, respectively; and it were determined as 17%, 5% and 8% in the hives in these enterprises, respectively. The reason for the proportional difference between our study and the other study conducted in Hatay may be the fact that our study was carried out on adult worker bees, while the other study was carried out on enterprises with colony loss and on queen bees. As a result, nosemosis and amoebiasis diseases were detected in 65% of the enterprises in Hatay and 30% of the hives in these enterprises in 2021, in which no serious colony losses were reported in the past. In this study, in which nosemosis and amoebiasis cases were seen together, thought to cause colony collapse, the absence of any serious clinical signs and bee losses led to the conclusion that clinical disease would not occur in resistant colonies.

Table 1. Numbers of enterprises and hives which infected with *Nosema* spp. and *M. mellificae* in Hatay

		Positive enterprises					Positive hives			
Districts	Number of enterprises	N	Mm	Mix	Total	Number of hives	N	Mm	Mix	Total
Antakya	15	-	4	5	9	85	9	4	12	25
Arsuz	6	1	3	-	4	28	3	4	-	7
Dörtyol	33	9	2	10	21	194	35	7	13	55
Samandağ	8	3	-	3	6	36	12	2	3	17
Total	62	13	9	18	40	343	59	17	28	104

N- *Nosema* spp.; Mm- *M. mellificae*

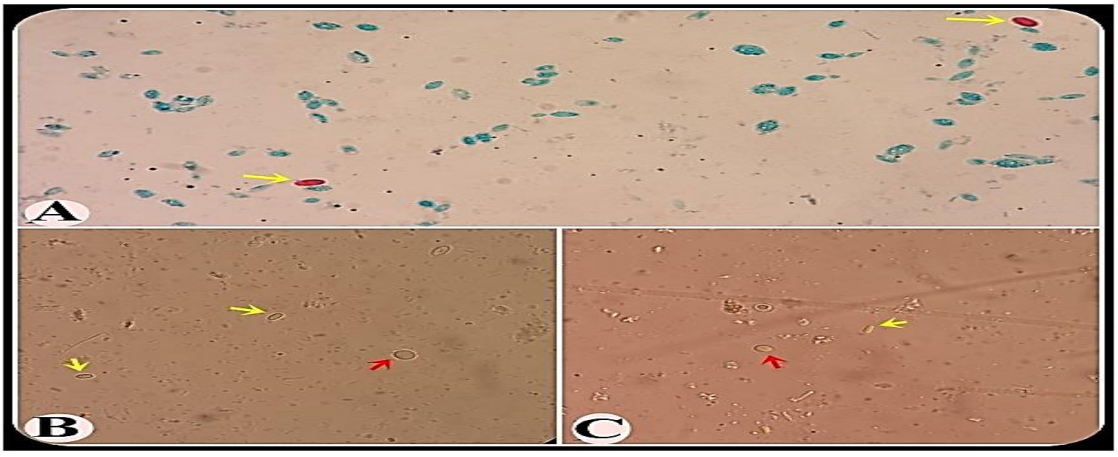

Figure 1. In the light microscope; A) *Nosema* spores were stained with safranin dye, B-C) *Nosema* spores and *M. mellificae* cysts (*Nosema* spores were marked with yellow arrows, *M. mellificae* cysts were marked with red arrows)

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