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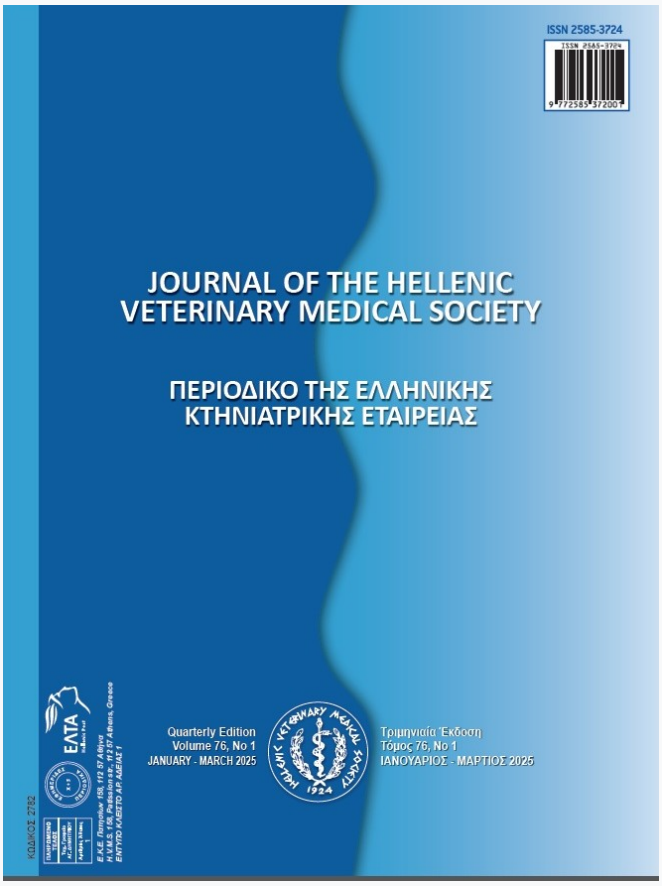

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Research article
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Prevalence of Varroa and *Acarapis woodi* mites and Nosema in honey bee colonies of Kermanshah Province, Iran

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ABSTRACT : Varroa and Acarapis woodi mites and Nosema are the most critical bee pathogens and cause much damage to the apiculture industry. This study aimed to investigate the infection of Varroa, Acarapis woodi mites and Nosema among bee colonies in Kermanshah. For this purpose, 25 apiaries with at least 50 colonies were randomly selected, and eight from each apiary were randomly selected for sampling (200 colonies total). From each colony, 100 adult bees were collected from around and inside the hive and transferred to the laboratory. Also, the bottom debris of each colony was collected separately in unique bags. A 5x5cm piece of comb, containing worker cells with nymphs was also cut from each colony. Adult bees and brood cells were examined for Varroa infection in the laboratory. Experiments were performed on adult bees to determine infection with Acarapis and Nosema. The results showed that 88% of studied apiaries and 32.5% of colonies were infected with Varroa. Also, 16% of apiaries and 7% of colonies showed Acarapis woodi infection, while 76% of apiaries and 34% of colonies were infected with Nosema. Most Varroa infections were found in adult bees. The results show that high infection with bee parasites, especially Varroa and Nosema, in the apiaries of Kermanshah, requires structural reform, management, and training of beekeepers to reduce these risks

***Keywords*:** Honey bee; Kermanshah; Varroa*; Acarapis woodi;* Nosema

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INTRODUCTION

The issue has been proven so far is that the bee was born and lived on earth long before humans. The first sign of human presence on the planet was discovered by geologists back about 600,000 years ago. At the same time, it has been proven in the scientific explorations carried out in the depths of the earth that the bee existed nearly 150 million years ago and was engaged in activity and reproduction.

The only difference is the lack of social life at that time. Like any other living creature, the bee is involved in many infectious organisms, pests, and non-infectious disorders. Sometimes these factors cause many problems for the beekeeping industry and even cause death in some cases (Ribani et al., 2020). The living environment of bees is suitable for parasites to live, and the presence of honey, wax, larvae, pupae, and other substances in the hive also attracts some organisms. So far, more than 150 species of mites have been reported concerning bees, the most dangerous of which are *Varroa*, Tracheal, and *Tropilaelaps clareae*. The giant Asian honey bee, *Apis dorsata*, is Tropilaelaps' primary host. However Tropilaelaps may also be found in the colonies of other Asian honey bee species including *Apis cerana* and *Apis florae* (Hristov et al., 2020). *Varroa,* like unknown diseases of bees, was imported to Iran by the queen (Mendoza et al., 2020; Pourelmi et al., 2010). Varroa has been a major concern among beekeepers worldwide since the late 1970s (Mendoza et al., 2020). Varroa mite is a parasite with a significant economic impact on the apiculture industry and is one of the most important pests of bees. It spreads very quickly and causes severe damage to its host colonies, which reproduce inside the brood cells with a cap and are protected from pesticides. Therefore, if the Varroa mite is not controlled, it severely threatens the apiculture industry. The lifespan of mites in the larval or adult stage of bees depends on temperature and humidity. Under practical conditions, the lifetime may vary from a few days to several months (Smoliński et al., 2021). So far, about 18 different viruses have been isolated from bees, and many of them can be transmitted by Varroa mites (Rosenkranz et al., 2010). The condition is caused by *Acarapis woodi* mite, an internal parasite of the respiratory system. Its life cycle and reproduction are mainly in the anterior trachea of bees. Still, they are sometimes found in the head, chest, and abdominal air sacs. The mite feeds on the host hemolymph (Durán et al., 2019; Frazier et al., 2000). According to the Food and Agriculture Organization (FAO), the mite is also seen in Iran, often in the respiratory system trachea that branch off from the first pair of respiratory spiracle of thorax.

Acarine attacks the respiratory system of all three adult bees (queen, worker, and drone) and is a major cause of winter deaths (Durán et al., 2019; Mossadegh and Bahreini, 1994). The pathogenic effects in infected bees depend on the number of parasites in the trachea, and mechanical damage and physiological disorders result from airway obstruction, lesions in the tracheal wall, and hemolymph reduction (Frazier et al., 2000). The causes of nosemosis in bees are *Nosema apis* and *Nosema ceranae*. These two fungi belong to the phylum Microspora and generally infect insects, but they can also infect some vertebrates such as fish, reptiles, amphibians, and some mammals (Klee et al., 2007; Sulborska et al., 2019). The disease is related to humid areas or is prevalent during the year when the humidity is high, i.e., late winter and early spring. The condition is highly contagious, severely weakening bees and killing them all. (Emsen et al., 2020; Webster et al., 2004). Nosema causes host gastrointestinal disorders, malnutrition, and premature death, reducing the lifespan of infected bees by 22-44%. The hypopharyngeal glands of infected adult bees are also weak and dry, with less protein, fat, and carbohydrates in their hemolymph. The growth of the wax-secreting glands is limited, and the queen's ovaries shrink. Another symptom of this disease included the weakening of winter, late winter, and early spring populations. The infected bees usually have a swollen and fluid-filled abdomen and cannot fly. On the body and door of the hive, in front of the flight hole, the ground, and the plants around the colony, brown and foul-smelling waste can be seen (Sulborska et al., 2019). Kermanshah province is also known as the country's central pole of bee breeding and honey production, with a long history of beekeeping and many bee colonies. Sahne city is considered one of the most important cities in this province in the field of beekeeping due to favorable weather conditions. Therefore, considering this city's important position in beekeeping and the importance of parasitic diseases in the amount of production and activity of honey bees, it was investigated the significant parasitic infections, including *Varroa* infestation, *Acarapis woodi*, and Nosema sp. among the apiaries of this city. Considering the role of management factors, health, and individual training in the field of beekeeping and control of parasitic diseases, by preparing and completing questionnaires from beekeepers, it was inves-

tigated the relationship between management factors and parasitic infections. It is hoped that by finding the weak points, valuable suggestions can be made to reduce the parasitic problems in the apiaries of this city

MATERIALS AND METHODS

For the research project, the apiary population of Kermanshah was selected as the target population. After receiving information and addresses of beekeepers in this city with the help of the veterinary department, 25 apiaries with a population of at least 50 colonies were randomly selected for sampling. Then, by referring to beekeepers, we tried to gain their trust and participation in cooperating with this study. The information preparation and sampling stages occured in spring and summer, from mid-May to late July. First, some information was obtained based on the designed questionnaire by visiting the apiary in person. A separate questionnaire was recorded for each apiary containing information such as the name and surname of the beekeeper, exact location of the apiary, number of colonies in the apiary, type of hive, history of various diseases, history of drug use, type of feeding (use of manual feeding) over-wintering conditions, migration location, bee breed, annual harvest, and apiculture history. Eight colonies were randomly selected from each apiary for sampling (200 colonies from all studied apiaries). A total of 100 worker bees were selected from each colony, from the front of the hive's flying hole and insides of hives. Also, a few days before sampling, materials, and debris spilt on the bottom of the hive were collected by placing cartons on the bottom. The collected samples were placed separately in plastic bags, and the required information was recorded on each bag. In order to sample the worker bees, the area around the hive was first carefully examined. In the case of crawling bees with weak flight, some of them were collected and placed in plastic bags, and other required bees were selected from the population of workers inside the hive and between combs and transferred to the given plastic bag. Also, a 5x5cm piece of comb, containing worker cells with nymphs, especially in parts of the comb with drone cells, was taken using a knife and transferred to a separate bag. The samples were transferred to the freezer at -20 ° C immediately after transfer to the laboratory. Freezing bees, were considered essential for not damaging their anatomical structure. The worker bees from each colony were divided into three groups to be tested for infection with different parasites. First, 50 bees were isolated to determine the infection of Varroa mite. The remaining 50 bees were divided into two n= 25 groups to determine the infection of Acarapis woodi mites and Nosema. The samples that were taken from the hive bottom and wax-containing comb larval cells were examined for Varroa mites.

To determine the host of infection with Varroa, samples taken from brood cells, hive floor debris, and bees collected from around the hive were tested. Debris collected from the bottom of the hive, which is stored in separate bags, was individually poured into laboratory Petri dishes and carefully examined using a hand magnifier and a stereomicroscope (loupe). The waxes cut from combs containing bee nymphs were also placed in a petri dish after being removed from the freezer at -20 ºC and reaching the ambient temperature. Carefully, the cap of the emerged bee was removed by scalpel and forceps, and the inside of the cells was carefully observed with the help of a hand magnifier.

In the next step, the number of 50 worker bees, which were taken from around and inside the hive as samples, were transferred to a plastic container with a lid containing 70% alcohol. After adding a few drops of dishwashing liquid, the glass was shaken well. After a few minutes, the mites separated from the bee's body and gathered at the bottom of the container. Finally, the samples collected for each colony were morphologically examined separately by light microscope and loupe. Based on the parasitological diagnosis keys (Bailly and Ball, 1991), the results were recorded after ensuring the determination of the genus and species. From each colony, twenty-five bees were selected to investigate the infection of the *Acarapis woodi* mite, which lives inside the respiratory tracts of bees. First, the bees were placed on a slide from the dorsal surface. Then, using a scalpel blade, a transverse incision was made on the back of the first legs of the bee body so that the head and the first pair of legs were separated, and then the next incision was made on the back of the legs of the second pair. This way, small pieces of the bee's chest were obtained, containing the first pair of chips. These parts were placed in a glass petri dish containing 8% potassium hydroxide solution for 30 minutes to digest muscles and extra material and examined using a 40 lens. To detect Nosema spores' contamination, the abdominal ~~rings~~ of 25 bees from each colony were separated with a scalpel and poured into a porcelain mortar. For each bee, 2-3 ml of physiological serum was added. After grinding the abdominal rings, a wet slide was

prepared from the obtained solution and examined with *400 magnification by a light microscope.For the required statistical analysis, SPSS software version 22 was used. The Chi-square test was used to compare the studied parameters at $P < 0.05$.

RESULTS

In addition to the presence of the diseases studied in this research, including Varroa, Nosema, and Acarapis, according to the beekeepers, the presence of American foulbrood (*Paenibacillus larvae*) in 12 apiaries (48%), as well as chalk brood (*Ascosphaera apis*) in 5 apiaries (10%), was titled. Regarding the use of different drugs in the apiary, all the beekeepers stated that they use different drugs in their apiary, which were often chosen based on their own or others' experiences, and mostly they did not consult with the veterinarian about drug treatment. All beekeepers used anti-Varroa drug for example Apistan strips (tau-flyvalinate. In 10 apiaries (about 40%), they used specific Nosemosis drugs (Fumagilin). In 10 apiaries (about 40%), they used specific Nosemosis drugs (Fumagilin), and in 12 apiaries (48%), different antibiotics such as Tetracycline, Neomycin, and Erythromycin that were used to control diseases in their colonies (Most of these drugs are used by the beekeeper arbitrarily).

Also, 11 apiaries (44%) used traditional treatments to treat and prevent diseases in their apiaries.Another point concerns the time of using various drugs, especially anti-Varroa drugs, that Only 12 beekeepers (48% of the colonies) observed the appropriate time of using this drug (during wintering and in the non-honey production season). Others needed to learn about the importance of medicinal residues in honey. The information related to the number of colonies of the studied apiaries, the history of beekeeping, and the range of their honey production is given in Tables 4-1 to 4-3. This study showed that 24 of the studied apiaries (94%) were infected with at least one Varroa, Acarapis, or Nosema parasite. Out of 25 apiaries studied, a total of 22 colonies (88%) were infected with Varroa mite, four apiaries (16%) were infected with *Acarapis woodi*, and 19 apiaries (76%) were infected with Nosema sp.

Out of the total of 200 colonies examined in the studied apiaries, 65 colonies (32.5%), 14 colonies (7%), and 18 colonies (34%) showed infection with Varroa, Acarapis, and Nosema sp., respectively. Also, in the studies conducted on Varroa contamination, *Braula coeca* flies were isolated from 6 apiaries (24%) and 21 colonies (11.5%), and the range of contamination was between 1 and 4 flies per colony.Among Varroa-infected apiaries, at least one and at most five colonies were infected. This number was at least one and at most five colonies for *Acarapis woodi* and at least one and at most seven colonies for Nosema sp.

In a part of the results, samples such as worker bees, beehive floor debris, and nymphs were examined for Varroa contamination. The highest contamination was on bees collected from around and inside the hive (21 apiaries and 53 colonies), and the lowest was inside the drones (14 apiaries and 29 colonies). Although the start site of the Varro*a* mite life cycle is in drone cells, due to the limited sampling in our survey (only one 5 * 5 cm piece of wax from each colony), the lower infection of this parasite can be justified**.**

Table 4-4 shows the level of infection with the studied parasites in different apiaries. Table 4-5 also shows the level of Varroa mite infection in different samples prepared in apiaries.

The parasitological studies showed no significant difference between Varroa and Nosema infection in apiaries and colonies. However, infection with *Acarapis woodi* mite was significantly lower than the other two parasites studied. Also, examining different samples for infection with Varroa mite showed no significant difference was observed between infestation with Varroa in the hive bottom debris and on the body the worker bees examined in this study.

However, bees were somewhat more likely to be infected. However, the comb samples showed significantly lower infection, possibly due to sampling problems, inadequate sample size, and the sampling season. The best way to examine Varroa infection in colonies was to test worker bees taken from the hive population. No Nosema infection was found in 2 apiaries that did not overwinter (9%). But both of these apiaries were infested with Varroa mite and one with *Acarapis woodi* mite. Among six apiaries producing less than 20 kg of honey per colony, all six were infected with Varroa mite and Nosema. While in the product range of 20-30 kg of honey per colony, seven apiaries were infected with Nosema, and eight apiaries were infected with Varroa (among nine apiaries).

There is probably a correlation between the small

Table 1. Information on the number of colonies in each apiary in the studied farms

Total	600-1000 colonies	300-600 colonies	100-300 colonies	50-100 colonies	
25	6	4	9	6	Number of apiaries
100%	24%	16%	36%	24%	Percent of total studied apiaries

Table 2. Information on the apiculture history of beekeepers in the studied farms

Total	20-30 year	10-20 year	Less than 10 years	
25	2	15	8	Number of apiaries
100%	8%	60%	32%	Percent of total studied apiaries

Table 3. Information on honey production in the studied apiaries

Total	More than 30 kg per colony	20-30 kg per colony	Less than 20 kg per colony	
25	10	9	6	Number of apiaries
100%	40%	36%	24%	Percent of total studied apiaries

Table 4. Comparison of infection of different parasites in the studied apiaries

Minimum and maximum infected colonies in each apiary	Number of infected colonies (%)	Number of infected apiaries (%)	The name of the parasite
1-5	65(33.5%)	22(88%)	Varroa
1-5	14(7%)	4(16%)	Acarapis woodi
1-7	68(34%)	19(76%)	Nosema

Table 5. *Varroa* mite infection rate in different samples prepared from the studied apiaries

Minimum and maximum parasites isolated from each colony	Minimum and maximum infected colonies in each apiary	Number of infected colonies (%)	Number of infected apiaries (%)	Sample type
1-20	1-5	44(22%)	18(72%)	Debrid of the hive bottom
1-25	1-5	53(26.5%)	21(84%)	Worker bees
2-12	1-4	29(14.5%)	14(56%)	Inside larval cells (5 by 5 cm)
1-45	1-5	65(32.5%)	22(88%)	Total

honey production, the infection's severity, and the number of colonies infected with Varroa and Nosem. For the *Acarapis woodi* mite, among four infected apiaries, two apiaries produced less than 20 kg of honey per colony, and two apiaries produced 20-30 kg of honey per colony. No significant relationship was between the number of colonies in the apiary and the level and severity of infection with different parasites. Also, the history of apiculture did not affect the level of infection. All 12 apiaries with a history of American foulbrood also showed Varroa infection. Also, ten apiaries were infected with Nosema. Also, all five apiaries with a history of Chalk Brood (ascosphaerosis) were infected with Varroa, and three apiaries also showed Nosema infection. In all the cases where *Braula coeca* was isolated (in 6 apiaries), it was accompanied by Varroa contamination. Out of 4 apiaries infected with *Acarapis woodi*, two were simultaneously infected with Varroa and Nosema and had a history of being infected with American foulbrood. One apiary was infected with Varroa and had a history of American foulbrood. One apiary had a simultaneous infection with Varroa and Nosema and a history of chalk brood (ascosphaerosis). Forty eight colonies (24% of total colonies) were infected with Varroa and Nosema simultaneously, and nine colonies (4.5% of whole colonies) were infected with Varroa, Nosema, and *Acarapis woodi* at the same time. Also, four colonies were infected with *Acarapis woodi* and Nosema simultaneously, and only one colony (0.5% of the total colonies) had only *Acarapis woodi* infection.

DISCUSSION

What is certain is that prevalence of infectious and parasitic diseases in bees is directly related to management issues and climatic conditions in the region. In recent years, the breeding and development of beekeeping in the world have become an academic science. Like many other sciences, many types of research have been conducted on increasing production and disease control methods. Nevertheless, unfortunately, beekeeping is still carried out in most of Iran's regions, in the old way and based on individual experiences (Bailey and Ball, 1991; Hristov et al., 2020).

One of the most essential and well-known parasites of bees is *the* Varroa mite. This mite was first reported in 1904 on Indian bees in Java, Indonesia, and in less than 50 years, it spread to all parts of the world and there were a few reports from all continents and most countries (Mortazavi Ardestani et al., 2005). The first varroosis was reported in Iran in 1978 when this mite was officially registered in Iranian apiaries (Mohammadian et al., 2019). In the following years, there were few reports of contamination of honey bees by Varroa mite in different parts of Iran. Maghsoud et al. (2012) reported Varroa infection in the apiaries of Alborz Province (Maghsood et al., 2012). In the northeastern regions of Iran, Moshaverinia et al. (2013) found the rate of Varroa mite infection in 31.5% of apiaries and 7.4% of colonies (Moshaverinia et al., 2013). Also, in four seasons, Jamshidi et al. (2009) investigated the prevalence of Varroa mite in apiaries of East Azerbaijan province. They showed the highest prevalence (37.33%) in winter and then in autumn (25.33%), summer (23.17%), and spring (7.72%) (Jamshidi et al., 2009). Also, Rahimi et al. (2014) in different cities of Kurdistan Province (neighbouring Kermanshah Province) investigated the prevalence of Varroa mite in two seasons of summer and autumn; the highest infection was in Saqez (32%), and the lowest infection was in Marivan (5%) (Rahimi et al., 2014). The results of the present study showed that 32.5% of the colonies in Kermanshah were infected with Varroa, similar to the contamination level of bee colonies in Saqez city, Kurdistan province. Still, the level of contamination reported by Moshaverinia et al. (2013) is more than the northeast of Iran and other cities of Kurdistan province.), On the other hand, it is consistent with the findings of Jamshidi et al. (2009) in northwest Iran. (Jamshidi et al., 2009; Moshaverinia et al., 2013). A study on African bee colonies in Tanzania showed that 92% of apiaries and 48% of colonies were infected with this parasite. This study showed that bee colonies were compatible with Varroa mite (Mumbi et al., 2014). Another parasite studied in this study was *Acarapis woodi* mite, a parasite of bee respiratory trachea whichfed bee hemolymph. It causes disruption in growth and shortens the life of bees, as well as weakness in flight and decreases their performance. Acarapis mite infection in the present study in was 16% of apiaries, and 7% of colonies studied. Thirty years after this finding, infection in several European countries such as Switzerland, Russia, Scotland, France, and Spain was reported. It arrived in the Americas in 1968 after arriving in Argentina. It was seen in Colombia and Mexico in 1980 and then in the United States in 1984 (Bailey and Ball, 1991). For the first time in 1994, Bahreini and Mossadegh reported infection with *Acarapis woodi* in 7 provinces of Iran (Mossadegh and Bahreini, 1994). After that, significant efforts to find this parasite in different parts of Iran were unsuccessful. In other parts of the world, there have been reports of bee colonies infected with *Acarapis woodi* (Straus et al., 2014; Khezri and Moharam, 2017). This parasite has also been reported in Australia, New Zealand, Scandinavia, and Canada (Webster et al., 2004) Lotfi et al. (2009) in Arasbaran, northwestern Iran, reported 3.33-59.5% of *Nosema* infections in different months. Also, in this study, 24.5% of the total colonies were infected with Nosema (Lotfi et al., 2009). Maghsood et al. (2012) also reported this parasite in their studies in the apiaries of Alborz Province (Maghsood et al., 2012). Moharrami and Mansouri. (2014) showed that all collected samples of Nosema from Iranian bees belong to *Nosema ceranae* (Moharrami and Mansouri, 2014). Nabian et al. (2011) and Paykari et al. (2013) reported *Nosema ceranae* in the apiaries of Mazandaran and East Azarbaijan Provinces, respectively (Nabian et al., 2011; Paykari et al., 2013). According to this study, the possibility that the infection of the apiaries of Kermanshah is also related to *Nosema ceranae* species is not far from expected, and detailed genetic studies are needed. In a study in South Australia, Daull et al. (1961) found that *Nosema apis* was present in all colonies at all times of the year (Doull, 1961). Langridge (1961) reported that colonies in the Australian state of Victoria had at least 1% of Nosema-infected bees in the summer and that this parasite was probably the most common disease in adult bees (Langridge, 1961). In Europe, the level of Nosema infection was reported as less than 2%, and in Australia, more than 60% of the German Black Forest (Bailey and Ball, 1991). In studies by Aydin et al. (2000), 23.8% of the studied colonies were infected with No-

sema (Aydin et al., 2006). Also, during 2005-2007, 14.1% of Nosema infection was reported in the spring in Spain, 7.2% of Nosema infection was reported in Germany, 2.8% of Nosema infection was reported in Switzerland, and 0% of Nosema infection was recorded in France in the colonies(Martín-Hernández et al., 2007). Nosemosis can have devastating effects on bee performance, leading to colony collapse. Also, pathogenicity and drug resistance differ between *Nosema apis* and *Nosema ceranae*. It was estimated that bee mortality in *Nosema ceranae* infection was significantly higher than in *Nosema apis* alone or in *Nosema apis* and *Nosema ceranae* simultaneously (Williams et al., 2014). Also, bees in colonies infected with *Nosema apis* collect less pollen than healthy bees (Anderson and Giacon, 1992). The bees infected with Nosema do not develop sufficient submandibular glands (these glands produce royal jelly that is used to feed larvae and queens), causing up to 15% of the eggs in infected colonies to not evolve in early summer. In another study, *Nosema ceranae* was reported in all samples of colonies with CCD and 80.9% without CCD, indicating a relationship between Nosema and CCD. Although drugs such as Fumagillin have been used effectively for many years to treat Nosemosis with *Nosema apis*, recent studies have shown that these drugs are not helpful in the long-term treatment of *Nosema ceranae* and should be replaced by alternative medications such as Nosevit (Gajger et al., 2009; Giacobino et al., 2016). In the present study, at least 40% of apiaries acknowledged their continued use of Fumagillin, suggesting that treatment failure may be due to drug resistance, especially in the case of *Nosema ceranae.*

CONCLUSION

In general, the results of the present study show that the apiaries of Kermanshah are not in a good situation regarding parasitic infections. Despite the widespread use of various antiparasitic drugs, the prevalence of these pests in the colonies is still high. Despite the use of modern hives in all apiaries, the low honey production per colony can be due to infections such as varroosis and nosemosis. Teaching new control methods to beekeepers, being careful in choosing the location of the apiary and staying away from contaminated apiaries, as well as biological control instead of chemical control due to drug resistance and drug residues, and not using contaminated beekeeping tools and equipment can reduce this pollution and increase production in apiaries. Consultation with expert veterinarians and periodic tests of bees can also help in this critical matter.

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CONFLICT OF INTEREST

The authors of this paper have declared that no competing interests exist.

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