Journal of Animal and Veterinary Advances 9 (5): 879-882, 2010
ISSN: 1680-5593

A Survey of Nosema of Honey Bees (*Apis mellifera*) in East Azarbaijan Province of Iran

[1]Nasser Razmaraii and [2]Hamid Karimi
[1]Parasitology Laboratory, Razi Vaccine and Serum Research Institute, Northwest Branch, Marand, Iran
[2]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

Abstract: Microscopically examination for Nosema disease was studied in 101 apiary hives in East Azarbaijan Province of Iran. Colonies were situated in seventeen regions. One of the important beekeeping areas in Iran is the East Azarbaijan Province. Honey bee samples were collected from 17 different regions in April-October 2007. Results of examination for spores by light microscopy showed that 35.4, 59.8, 44.5, 1, 1 and 2% in April, May, June, July, August and September were respectively infected by *Nosema apis* in April, June and July level of humidity were the highest and in summers months humidity is lack. Prevalence of *Nosema apis* related to temperature and moist and spring in East Azarbaijan Province is the suitable season for *Nosema apis* than other season. And compared with Mediterranean climate summer in East Azarbaijan Province was so dry. In the experiment, we have found a significant relationship between the average number of spores per infected bee in the positive samples and rainy area.

Key words: *Nosema apis*, hives, light, honeybees, humidity, Iran

INTRODUCTION

East Azarbaijan Province is located in Northwest of Iran with mountainous climates with average rain high rate in spring and low rate in summer. This province is the most important beekeeping area in the Iran.

Nosema is one of the important disease of adult honeybees and in epidemics occur, Nosema may cause serious losses in colonies in spring. The microsporidian *Nosema apis* Zander is an endoparasitic protozoan of honey bees which causes considerable economic losses in the beekeeping industry.

Symptoms of the disease are not clear cut and sometimes, even at high levels of infestation are difficult to detect. They can include: unhooked wings, distended abdomens and what has been characterized as stupefied, disoriented or paralyzed behavior. Nosema leads to reduction in a honey crop as well as accelerating queen supersedure (Sanford, 2003). The disease evolves without producing any visible signs, meaning that in many cases no treatment is given. Other factors, such as mite infestation, with a consequent weakening of the colony, may influence the subsequent development of *N. apis* infection levels (Bermejo and Fernandez, 1997). Nosema disease is generally regarded as one of the most destructive diseases of adult bees, affecting workers, queens and drones alike. Seriously affected worker bees are unable to fly and may crawl about at the hive entrance or stand trembling on top of the frames (FAO, 2006). The spores develop exclusively within the epithelial cells of the ventriculus of the adult honey bee. The disease usually manifests itself in bees that are confined, so the heaviest infections are found in winter bees, package bees, bees from hives used for pollination in greenhouses and so on (Cantwell and Shimanuki, 1970).

The *N. apis* spores are big, regular oval bodies 4-6 μm long and 2-4 μm wide. Spores of *Nosema apis* nearly all germinate within 30 min after entering the ventriculus of the honeybee (Bailey, 1955). After entering the ventriculus of the honeybee. Once the spores are ingested and reach the ventricular lumen in the mid-gut they germinate in <12 h (Bailey, 1981). This does not mean however, they can be ignored. The most amaging adult disease appears to be Nosema, caused by a microsporidian that infects the digestive system. The incidence of Nosema very often is correlated with stress on a colony (Sanford, 2003). Differences in the seasonal incidence of infection depending on geographical location are well documented (Bailey, 1981). The parasite is ubiquitous and multiplies at a specific rate throughout

Corresponding Author: Nasser Razmaraii, Parasitology Laboratory, Razi Vaccine and Serum Research Institute, Northwest Branch, Marand East Azarbaijan Province, Iran

the year. Nosema levels generally increase when bees are confined, such as in the autumn and winter in colder climates when the amount of brood is decreasing and perhaps in the early spring when there is an increase in the brood (OIE, 2008). Such procedures come under the jurisdiction of national control authorities with protocols that vary from country to country. Disinfection can be carried out, for example by putting acetic acid solution into bowls or on to sponges that can soak up the liquid. Following disinfection after an outbreak, all combs should be well ventilated for at least 14 days prior to use. Suppression of Nosema disease can also be achieved by feeding an antibiotic, fumagillin, in sugar syrup to the colony (Cantwell and Shimanuki, 1970).

Controlling Nosema disease can be accomplished by one or a combination of practicing good management technique and feeding the antibiotic fumagillin (Sanford, 2003).

MATERIALS AND METHODS

Stocks of bees were obtained from seventeen apiaries area (Table 1) samples. One hundred honey bee samples were randomly chosen from apiaries that contained at least 300 colonies from 17 different regions of East Azarbaijan Province from April-October 2006. Adult researchers bees were taken from brood nests and the hive entrance.

This method makes a good representation of the hive's population. All samples were send to parasitology laboratory of Razi vaccine and serum research institute Northwest branch near the ice, then bees were fixed in 70% ethyl alcohol in order to prevent them from decomposing and to improve their reception and organization in the laboratory (OIE, 2008).

For quick qualitative examination, the abdomens from at least 10 bees were removed and placed in a dish with 10 mL of water and homogenized. This was purified by filtering through steel mesh (100 micrometer poor size) after three rounds of centrifuge by clean water, supernatants were decanted and the tubes are refilled to the 10 mL level.

The concentration of spores in this preparation was determined by a haemacytometer count: A drop of this solution was placed in a haemacytometer. The number of spores in each square is counted if One *Nosema apis* spore, observed in the haemocytometer's entire central square millimeter grid ($25\times16 = 400$ small squares) is equal to an average of 10,000 spores per bee.

Table 1: Sampling apiaries area

Areas	No. of samples
Osko	5
Ahar	7
Bostan abad	6
Bonab	4
Tabriz	8
Jolfa	4
Charimagh	4
Sarab	5
Shabestar	6
Kaleibar	7
Maragheh	9
Malekan	6
Marand	5
Mianeh	7
Warzeghan	7
Hashtrood	5
Heris	6
Total	101

RESULTS AND DISCUSSION

The results of the study in the three different seasons in the year 2008 are presented in Table 1. *Nosema apis* in East Azarbaijan Province in Northwest of Iran. Table 2 sets out the results of the sampling. *N. apis* is widely distributed, being diagnosed in 85% of the apiaries, with 51.55% of the colonies monitored in the study being infected. In total, 5000 bees were analyzed, of which 257 were infected, which represents 5.1.55%. These infected bees contained a mean of $7.5\times10^6\pm1.5\times10^6$ spores per bee (mean±SE). As shown in Table 2, we found that *N. apis* are most prevalent during spring.

In the experiment, we have found a significant relationship between the average number of spores per infected bee in the positive samples and colder area.

Nosema apis is an important pathogenic agent of hives which influenced by climatic conditions and managerial factors, deeply causing losses which are undetectable and usually insignificant to beekeepers (Bermejo and Fernandez, 1997).

Table 2: Prevalence of Nosemosis in different seasones in bee hives of East Azarbaijan Province

	No. of infection		
Area	Spring	Summer	Infection percentage
Osko	0	0	0.00
Ahar	5	1	50.00
Bostan abad	5	2	50.00
Bonab	0	0	0.00
Tabriz	3	1	22.20
Jolfa	2	0	20.00
Charimagh	4	1	45.40
Sarab	7	2	56.25
Shabestar	4	1	41.60
Kaleibar	5	1	40.00
Maragheh	3	0	20.00
Malekan	2	0	18.18
Marand	2	0	16.66
Mianeh	6	2	57.14
Warzeghan	4	1	45.45
Hashtrood	6	2	66.66
Heris	5	2	58.33
Total	63	16	-

Table 3: Result of survey of *Nosema apis* spore in adult researcher honeybees from East Azarbaijan Province of Iran carried out between march and Julay 2007 in 17 colonise

Locations	Altitude (m)	No. of samples analysed/positive	No. of total hive's samples	No. of hives sampled/infected
Osko	-	10/0	5	5/0
Ahar	1341	12/6	7	7/3
Bostan abad	1740	14/7	6	6/5
Bonab	1275	10/0	4	4/0
Tabriz	1366	18/4	8	8/3
Jolfa	-	10/2	4	4/2
Charimagh	-	11/5	4	4/2
Sarab	1680	16/9	5	5/4
Shabestar	1400	12/5	6	6/3
Kaleibar	1250	15/6	7	7/5
Maragheh	1450	15/3	9	9/2
Malekan	-	11/2	6	6/1
Marand	1334	12/2	5	5/1
Mianeh	1100	14/8	7	7/6
Warzeghan	-	11/5	7	7/6
Hashtrood	1660	12/8	5	5/4
Heris	1570	12/7	6	6/4
Total	-	215/79	101	101/47

Total infection rate in spring and summer were respectively 46 and 1.3%

The infection is transmitted by food when consuming the spores in the honey, water and faeces. In the bee's intestine, on transmission of the spore's content to the epithelium cell the breeding process of the parasite starts, this entails numerous cell divisions leading to the breakdown of the cell (Sokh *et al*., 2007). Cold winter and poor management of the colony, during the winter external factors such as coldness, bad diet, hives humidity can increase sensitivity of bees to nosema. In this study, the colonies with the highest range of infestation by *N. apis* are located in cold areas and beekeepers in theses area didn't have enough information to confront with this disease, so over than 15% of apiaries were died (Unpublished). Although, there is no cytogenic study on *Apis mellifera* biodiversity in Iran but consider to huge climatic diversity throughout the country someone can conclude, Iranian *Apis mellifera* is diverse in to different subspecies with different resistance to environmental condition and disease.

The results of the study in two seasons of the year 2007 are shown in Table 3. The infection rate of the honey bee colonies in the spring and summer were respectively, 46.6 and 1.3%. Humidity and temperature of spring in the study is suitable for Nosema appearance but Humidity and temperature are too low and high respectively in summer. In a research in Iran (Lotfi, 2009), the infection of the honey bee colonies in the spring was 59.5%, however the amount was considered to be low in the fall (0%) and in the summer (3.33%). In other study in Turkey Cakmak (2003) reported 24% of hives were infected with Nosema during spring. In 2006 Aydin *et al*. (2006) reported 23.8% of the 168 investigated colonies were infected with *Nosema apis*. Bermejo and Fernandez (1997) found that 20.37% of samples were infected by *N. apis* (n = 162). The study result are the same range by Lotfi (2009) report but east Azarbaijan Province is dry than arasbaran area and infection rate of Nosema are low but in spring results are near. In compare with studies in Turkey and Spain, different result maybe related to Mediterranean regions climate and rate of rains.

CONCLUSION

From this study, we can say that *N. apis* is more prevalent during spring (April-July), except in high mountainous regions (September). The Province is located in mountainous area with the cold climate in the winter it can make hives susceptible to disease especially Nosema in spring. where plentiful of pollen and nectar exist throughout the spring. Probably due to the fact that the bees which developed nosemosis defecate and die outside the hive. A series of circumstances exist which may mean that this study would offer different results if it were repeated. For example, during the sampling period, East Azarbaijan Province was suffering a severe freezing which had limited plants flowering. Climatic conditions are important and have meant that many studies carried out in the same region give different results (Bailey, 1981).

ACKNOWLEDGEMENTS

The most sincere thanks to the head Jahad-Keshawarzi and of Veterinary General Office in East Azarbaijan Province for their comments on some of the critical points in this research.

REFERENCES

Aydin, L., E. Gulegen, I. Cakmaki, O.G. Girisgin and W. Harrington, 2006. Relation between Nosema and Chalkbrood disease and its implication for an apiary management model. Bull. Vet. Inst. Pulawy, 50: 471-475.

Bailey, L., 1955. The Infection of the ventriculus of the adult honeybee by *Nosema apis* (Zander). Parasitology, 45: 86-94.

Bailey, L., 1981. Honey Bee Pathology. 1st Edn., Academic Press, London, UK., ISBN: 012073480X, pp: 48-51.

Bermejo, F.J.O. and P.G. Fernandez, 1997. Nosema disease in the honey bee (*Apis mellifera* L) infested with varroa mites in Southern Spain. Apidologie, 28: 105-112.

Cakmak, I., 2003. Honeybee pests and diseases in Southern Marmara region of Turkey. Uludag Bee Journal. 2003 No. 2.Cantwell, G.E. and H. Shimanuki, 1970. The use of heat to control Nosema and increase production for the commercial beekeeper. Am. Bee J., 110: 263-263.

FAO, 2006. Honey Bee Diseases and Pests: A Practical Guide. Food and Agriculture Organization of the United Nations, Rome, Itlay.

Lotfi, A.R., 2009. The prevalence of Nosemosis in honey bee colonies in Arasbaran region (Northwestern Iran). American-Eurasian J. Agric. Environ. Sci., 5: 255-257.

OIE, 2008. *Nosemosis* of honey bees. Chapter 2.2.4. OIE Terrestrial Manual 2008, pp: 410. http://www.oie.int/fr/normes/mmanual/2008/pdf/2.02.04_NOSEMOSIS.pdf.

Sanford, M.T., 2003. Diseases and Pests of the Honey. 2nd Edn., FAS Publications, Florida, USA., pp: 1-13.

Sokh, R., D. Molska and M. Siuda, 2007. The influence of the invasion of *Nosema apis* on the number of pollen seeds in bees' intestines. Polish J. Nat. Sci., 22: 150-156.