Research Journal of Parasitology

ISSN 1816-4943

Academic Journals Inc.

www.academicjournals.com

Research Journal Parasitology 3 (4): 123-129, 2008
ISSN 1816-4943

The Comparative Evaluation of the Laboratory Methods of Separation Mite Varroa from the Mature Honeybee

[1]H.R. Azizi, [2]E. Sadeghi, [2]M. Taghdiri and [2]A.R.K. Vardanjani
[1]Department of Parasitology, Faculty of Veterinary Medicine,
Shahrekord University, Shahrekord, Iran
[2]Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

Abstract: In order to evaluate and compare the laboratory methods of separating and diagnosing of the mature honeybee's contamination to mite varroa, we used four hives, which were contaminated by this kind of mite. All of these samples were taken during the early days of autumn when most of the mites lived on the mature honeybees. In each sample, 250 honeybees were collected in a glass container for separating mite from the mature honeybees. For this sake, we used ethanol 70%, ether, gasoline, sugar, heat, hot water and hot water together with detergent. In sugar shake method, we used both commercial sugar and sugar powder. All of these methods were experienced three times and each time on new samples. Results indicated that ethanol 70%, with separating power 95.41% ±1.54 and sugar powder, with separating power 89.82±1.69%, were the most effective methods in separating mite varroa from the mature honeybees. By using the statistical software SPSS -13.0 and χ^2 test, the statistical comparison of these results indicated that there was no significant difference between ethanol and sugar powder shake method ($p > 0.05$). However, there was a significant difference between the ethanol roll method and other methods ($p \leq 0.05$). In addition, there was a significant difference between the sugar powder shake method and commercial using method ($p \leq 0.05$). Therefore, for the rapid and exact diagnose, we recommend to use ethanol 70% or sugar powder. Using sugar powder did not result to death of honeybee and had a high separating ability, no antivarroa drugs residue in hive, so it is advised for remedy of varroatosis.

Key words: Mite varroa, mature honeybee, separation, laboratory methods, sugar powder, diagnose

INTRODUCTION

Ectoparasitic mites of the genus Varroa are known from Asian honey bees, of which nine extant Apis species are recognized (Koeniger and Koeniger, 2000). This disease, which is one of the most serious ones in honeybee, is important in two respects; first, because it damages the hives suddenly and severely, destroys them if contamination is grave and second because of its outbreak and spread is worldwide. If varroa spreads in hives, other diseases will be disseminated because of the weakness, which it brings to the hives (Manuela *et al*., 2006). From the time of diagnosing mite varroa in 1904 until 2000, the only formalized species of this disease was *Acipenser transmontanus*. Anderson and Teroman (2000), based on Mitochondria DNA, citocrome Oxidise I, concatenation of gens, size and characterization of the sexual reproduction organs, indicated that *Varroa destructor* must be regarded as a new species. *Varroa destructor* had formerly been diagnosed and considered similar to *Acipenser transmontanus*. Mite *Acipenser transmontanus* lives harmlessly on the body of *Apis cerane* (Fakhimzadeh, 2000). Transferring *Apis mellifera* to Asia and contact of this mite with them, made mite vorroa a pathogenic parasite, which causes serious damages to *Apis mellifera* (Boot *et al*., 1997).

Corresponding Author: Hamidreaza Azizi, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

The reasons, which are reported for the tolerance level of Apis cerane to mite varroa, are as following: the short stage of its nymph stage in comparison with *Apis mellifera*, which causes that the sufficient time for growth and evolution and completion of the life cycle of this mite does not provide (Tewarson *et al*., 1992; Boot *et al*., 1997). The low amount of the youth hormone in *Apis cerane* in comparison with varroa. This hormone effects on the growth of female varroa ovaries (Fakhimzadeh, 2000). The cleaning behavior of *Apis cerane*, which causes mite to be separated from its body (Boecking and Spivak, 1999). Mite varroa sticks among head, chest, bottom of the chest and abdomen and abdomen joints of honeybee and diagnosing it is difficult during the early days of contamination and the clinical symptoms appear only when there are thousands of mites in colony (Ritter, 1981; Rere, 1998). If we do not find and cure the contaminated colony, it will be eradicated during 3-5 years (Bonney, 1990). Mite varroa feeds from the haemolymph of honeybee; this leads to create various wounds on different parts of honeybee's body, so that, numerous pathogenic germs can penetrate into the body of honeybee through these wounds (Ball, 1985). The post-feeding stage of the L5 larva and the prepupa are fed on by Varroa females, which puncture the host's still soft cuticle at various sites (Garrido *et al*., 2001). The adult female mite and progeny feed on the haemolymph of pupae from a single feeding site (Kanbar and Engels, 2003). Due to repeated feeding of the adult and nymphal mites, the healing of the perforation is prevented until scarring occurs prior to the imaginal moult. Various microbial organisms may be transferred through the punctures, although evidence for vector transfer by the Varroa mite is scarce (Bŕdsgaard *et al*., 2000). Colonies of various bacteria were found in the open wounds of about 15-30% of all inspected host pupae with an abundance depended on the level of host brood cell infestation by the mite (Kanbar and Engels, 2003). One type was identified as the European foulbrood agent *Melissococcus pluton* (Kanbar *et al*., 2002). Another pathogenic germs, which it is transferring through the wounds created by mite varroa became evident, are viruses. Shen *et al*. (2005) proposed that parasitisation by varroa suppresses the immunity of honeybees, leading to activation of persistent, latent viral infection. There are numerous reports about separating this virus from the contaminated hives. Tentcheva *et al*. (2004) reported the direct relationship between the rate of seasonal outbreak of six kinds of virus and mite varroa. Because the first step in controlling and remedy of varroatosis is the exact and rapid diagnose of contamination, beekeepers need a method, which can diagnose the rate of contamination exactly (Rere, 1998; Manuela *et al*., 2006). There are three methods for diagnosing of this contamination: Study of the cells of broods in hives and study the particles presented on the floor of hives separating mite from the mature honeybees via laboratory methods.

The first method is usable when the hives has many broods, especially male ones. This method is time consuming, boring and at least 200 cells must be studied (Manuela *et al*., 2006). The second method is effective when the amount of contamination is low although it is unable to indicate the exact amount of contamination and is not reliable. Some sources suggest the using antivarroa drugs after placing the plate in hives (Manuela *et al*., 2006).

There are various methods for separating varroa from the mature honeybee: shaking method, sugar shake methods, ether roll method and heating method.

In the first method, various materials are used which the most important of them are: ethanol 70% (Macedo and Ellis, 2000; Tentcheva *et al*., 2004), detergent solution, hot water, gasoline, sugar (Macedo and Ellis, 2000, 2002), hexane and diesel oil (De Jong *et al*., 1982). This research was done in order to determine the best method which has the least environmental effects and does not lead to death of the under permeated honeybee for separating mite varroa from mature honeybees and was done to compare the methods.

MATERIALS AND METHODS

In the middle of summer 2007, in Charmahal and Bakhtiyari province (south western of Iran) four hives contaminated with mite varroa were prepared the criterion for choosing these hives; in addition

to the beekeeper's remarks was the presence of some of contamination symptoms, which were produced by mite varroa, such as weakness of hives and presence of dead honeybees with abnormal wings at the front of the hive. To become sure about presence of contamination in the hives, we took sample from honeybees and then carried out the experiment of separating varroa with detergent solution and water. In addition, we chose the hives was which hadn't been cured during two recent years. Four hives were kept during 2 months in order to reach the favourite limit of contamination for experiment. In autumn season of each year, the rate of honeybee's procreation decrease and consequently the number of brood cells reduce, thus, most of the mites can be seen on the bodies of the mature honeybees. The best part of a colony for sampling and diagnosing of varroa is the place where honeybees live near the brood cells. Therefore, samples were taken from these parts. The ethanol 70% method has been advised as a standard method for separating varroa from the mature honeybee, thus, the efficiency degree of ether method has been compared to this method. Also, this ethanol 70% method is unable to separate all of mites from the mature honeybees. Therefore, in order to determine the efficiency of this method, we carried out the following experiments:

In a capped glass container containing 50 cc of ethanol 70%, we poured 250 honeybees and after closing the cap, shook it for ten min. Then we filtered its content through a lace, which its thickness was 3 mm and then, it was placed on a white china dish in order to count the mites inside it. After that, we put the entire aforementioned honeybees under the loop and then examined them one by one in order to separate and count the remained mite.

By adding these mites to the separated ones in the first stage, we calculated the total numbers of mite in the sample (Fakhimzadeh, 2000; Manuela *et al*., 2006). According to the following formula, the efficient (Ritter., 1981) CY of the ethanol roll method was calculated:

The number of the separated mites in the sample

$$X = \frac{\text{The No. of the separated mites in the sample}}{\text{The total No. of the present time in the sample}} \times 100$$

Also for carrying out this experiment exactly, we repeated each of these stage three times separately.

Ether Rolls Method

Around 250 honeybees were taken as samples and collected in a glassy container then; some drops of ether were added to it. Consequently, the honeybees immediately became anesthetized. After that, we closed the glass container cap and turned for one minute. As a result, a thin layer of moisture was formalized on the wall of the glass container and the separated mites stocked to this moisture in a manner that they were visible from behind of its wall. Then we counted these mites. Because most of sources considered the ethanol 70% method as the most effective method for separating (Fakhimzadeh, 2000; Macedo and Ellis, 2000), the honeybees were poured in another glass container and this time for departing the remained mite, the ethanol 70% was used. For calculating the total number of mites in samples, we added the mites, which were separated by ethanol and the ones, which were separated with ether. Ethanol cannot separate all the mites, so that, we added the percent which ethanol could not separate to the previous number in order to calculate all the mites in sample (Macedo and Ellis, 2000).

Sugars Shake Method

For this method, almost 250 honeybees were poured in a glass container like other methods. Instead of cap, we used a lace, which their pores diameters were 3 mm. Then we poured two spoons of sugar over the honeybees through the lace's pores and shacked it completely in order to take out all of the sugar and separate mites from the container, but not honeybees, then the separated mites were

counted (Macedo and Ellis, 2000, 2002). After that, we poured 50 cc ethanol 10% on the honeybees in the container in order to separate the remained mites and calculated them, like the pervious formula, we calculated the efficiency percent of sugar in separating mites from honeybees.

The Sugar Powder Method

We poured 200 g sugar in a mortar and pulverized it completely for 5 min in a manner that the average size of the sugar particles becomes 0.06-0.04 mm. We performed all the stages, which were, described in the Sugars shake method. At the end, the obtained results of various methods were compared with the ethanol roll method and with together and were analyzed by SPSS 13.0 software and Kay quadratic statistical test. The results of the ethanol roll method have been shown in Table 1. This method was repeated three times and each time on a new sample.

RESULTS

As shown in Table 2, that ethanol 70% was able to separate average of 95.41% of the present mites in sample. The results of other methods, which were tested like ethanol method, are as follow: Ether was able to separate the average of 57.65% of mites in the samples. The sugar powder method was better than using the commercial sugar in separating mite varroa. The commercial sugar was able to separate almost 41.51% of the mites while sugar powder separated 89.82% of these mites (Fig. 1).

Table 1: Numbers and percent of the mites separated by ethanol 70%

Methods	The percent of the separated mites	The total No. of the samples mites	The No. of separated mites by this method
Ethanol 70% (Experiment 1)	95.55	45	43
Ethanol 70% (Experiment 2)	92.00	50	49
Ethanol 70% (Experiment 3)	92.68	41	38

Table 2: The efficiency percent of the various methods for separating mite varroa (±is the standard deviation of the average) from the mature honeybees

Methods	Efficiency (%)
Ethanol 70 (%)	95.41±1.54
Ether	57.65±2.65
Sugar	41.51±2.29
Sugar powder	89.82±1.69
Heat	57.28±3.20
Gasoline	82.79±2.88
Hot water	69.35±4.06
Hot water with detergent	86.75±2.48

Fig. 1: The separated mites by sugar powder method

According to the obtained results, gasoline was able to separate 82.79%, heat was able to separate 82.79%, hot water was able to 10.56% and hot water with detergent was able to 86.75% of the total numbers of mites of the honeybees.

Among all of methods, there was no significant difference between the sugar powder shake method and ethanol70% method ($p>0.05$) but the difference between other methods and ethanol 70% method was significant ($p\leq 0.05$). There was no significant difference between sugar powder shake, ether and hot water with detergent method ($p>0.05$). In addition, ether roll method, heating method, gasoline method and hot water with detergent method did not show any significant difference ($p>0.05$) while the comparison of other methods with each other indicated significant difference ($p\leq 0.05$).

DISCUSSION

In this research, we chose the most important and commonest methods for diagnosing and separating mite varroa from the mature honeybees. Because the ethanol70 % method is considered as a standard method for separating this mite from honeybees, efficiency degrees of methods were compared with this method (Fakhimzadeh, 2000; Manuela *et al*., 2006). The result of this research indicated that ethanol 70% with separating power 95.41±1.54% and after that, sugar powder method with separating power 89.82±1.69% were the most effective methods for separating varroa from the mature honeybees. The use of ethanol 70% leads to death of the honeybees while the use of sugar powder leads to remain them alive after separating the mites and they can be returned to the colony. In addition, sugar powder can be used for remedy of the hives disease. Unlike many of chemical drugs, we can use sugar powder. The chemical drugs remain toxic dross in hives and are not usable in the season of producing honey. When commercial sugar was used in place of sugar powder, the separating power decreased remarkably in a manner that had a significant difference with the sugar powder. Varroa has sticky feet and stick to honeybee. Sugar powder prevents mite varroa to stick to the body of honeybees somehow. There have been two explanations for this power of sugar powder:

- Sugar powder provokes the cleaning behaviour of honeybee (when sugar is poured on the honeybee's body, it spend too much time on cleaning its body and this is useful for separating mite from the body of honeybee)
- Pouring sugar powder on the body of varroa incites it to release itself from sugar powder and this leads to separation of honeybee from mite (Macedo and Ellis, 2000). Some of beekeepers use hot water or water for diagnose while it is better to use hot water with a little detergent because it increases the separating power. This method can be used when ethanol and sugar powder are not available. In addition, there was no significant difference between this method and sugar powder shake method ($p>0.05$). The good thing about this method is its speed in a manner that it is performable in 1 min. Consequently, as a fast method, it has many usages especially in US. However this method has not a good separating power, especially when the degree of contamination is low, it is unable to determine the exact degree of contamination and most of time it is advised for discovering the presence and absence of contamination in colony. Furthermore, the use of ether in bee plain may provoke the defensive behaviour of honeybees

The heating method is time consuming and has no great efficiency. Up to now, various researches have been done for diagnosing varroa but so far no method has been reported better than ethanol 70% method for separating this mite form the mature honeybees and also no drug has been reported which can eradicate all of mites. Fakhimzadeh (2000), for diagnosing mite varroa in honeybee used the method of washing honeybee in detergent solution and centrifuge. He experimented four kinds of turning speed and two types of density of detergent solution for separating mite from the mature honeybees. He

concluded that the higher density of detergent and high speed led to separation of most of mite significantly but the time of centrifuge had no influence. Macedo *et al*. (2002) examined the effect of six kinds of powder in separating mite form honeybee. They used sugar, sugar powder, wheat flour, talcum powder, cornstarch and baking soda for separating mite form honeybee and after separation of this mite by these materials; they separated the remained mites by ethanol and then calculated the efficiency percent of each of these materials. They reported that among these materials, sugar powder had most influence on separating mite varroa and after that, talcum powder had the second rank. They also reported that in a sample containing 300 honeybees, presence of 1-5 mite varroa indicated the low degree of contamination and presence of mites more than 30 indicates high contamination. As it was said, most of time this disease is not diagnosed and many beekeepers don't diagnose the first year of contamination while with a simple experiment, we can discover the presence of contamination and by providing timely remedy, we can prevent damage causes by this contamination in second year easily. Because of excessive outbreak of this parasite bee plains and according to damage caused by it, it is advised that in addition to observing the rules of hygiene, specially leaving suitable spaces among bee plains, all of colonies should be controlled permanently. In addition, beekeepers should take sample from honeybees once every few months and by use of sugar powder determine the contamination percent of the colonies in order to remedy them timely and prevent from the probable damages.

REFERENCES

Anderson, D.L. and J.W.H, Trueman, 2000. *Acipenser transmontanus* (Acari: Varroidae) is more than one species. Exp. Applied Acarol., 24: 165-189.

Ball, B.V., 1985. Acute paralysis virus isolates from honeybee colonies infested with *Acipenser transmontanus*. J. Apicul. Res., 24: 115-119.

Boecking, O. and M. Spivak, 1999. Behavioural defences of honeybees against *Acipenser transmontanus* Oudmans. 30 141: 158-10.1051/apido:19990205.

Bonney, R.E., 1990. Hive Management: A Seasonal Guide for Beekeepers. 1st Edn. Garden Way Publishing, North Adams, Massachusetts, USA, ISBN-10: 0882666371, ISBN-13: 978-0882666372, pp: 166-179.

Boot, J.W., N.Q. Tan, P.C. Dien, L.V. Huan, N.V. Dung, L.T. Long and J. Beetsma, 1997. Reproductive success of *Acipenser transmontanus* in brood of its original host, *Apis cerana*, in comparison to that of its new host, *Apis mellifera* (Hymenoptera: Apidae). Bull. Entomol. Res., 87: 119-126.

Bŕdsgaard, C.J., W. Ritter, H. Hansen and H.F. Bŕdsgaard, 2000. Interactions among *Acipenser transmontanus* mites, acute paralysis virus and *Paenobacillus larvae larvae* and their influence on mortality of larval honeybees *in vitro*. Apidologie, 31: 543-554.

De Jong, D., D. De Andrea Roma and L.S. Goncalves, 1982. A comparative analysis of shaking solutions for the detection of *Acipenser transmontanus* on adult honeybees. Apidologie, 31: 543-554.

Fakhimzadeh, K., 2000. A comparative analysis of shaking solutions for the detection of *Acipenser transmontanus* on adult honeybees. Am. Bee J., 140: 736-739.

Garrido, C., R. Paxton and P. Rosenkranz, 2001. Varroa-reproduktion: Wirtsfaktorenoder genschaften des Parasiten? Apidologie, 32: 481-482.

Kanbar, G., W. Engels and G. Winkelmann, 2002. U¨ bertragen varroa-milben auch faulbrut (*Melissococcus pluton*)? Apidologie, 33: 487-488.

Kanbar, G. and W. Engels, 2003. Ultrastructure and bacterial infection of wounds in honeybee (*Apis mellifera*) pupae punctured by Varroa mites. Parasit. Res., 90: 349-354.

Koeniger, N. and G. Koeniger, 2000. Reproductive isolation among species of the genus Apis. Apidologie, 31: 313-339.

Macedo, P.A. and M.D. Ellis, 2000. Detecting and assessing *Varroa mite* infestations by using powdered sugar to dislodge mites. Am. Bee J., 40: 906-907.

Macedo, P.A. and M.D. Ellis, 2002. Using inert dusts to detect and access *Varroa infestations* in honeybee colonies. J. Apicul. Res., 40: 3-7.

Manuela, R.B., A.C.K. Neil and S.P. Robert, 2006. A comparative evaluation of sampling methods for *Varroa destructor* (Acari: Varroidae) population estimation. Apidologie, 37: 452-461.

Rere, S., 1998. Introduction to Bee Keeping. 1st Edn. Vikas Publishing House, Uttar Pradesh, India, ISBN: 81-259-0589-8, pp: 137-150.

Ritter, W.., 1981. Varroa disease of the honeybee *Apis mellifera*. Bee World, 62: 141-153.

Shen, M., X. Yang, D. Cox-Foster and L. Cui, 2005. The role of varroa mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honeybees. Viro., 342: 41-49.

Tentcheva, D., L. Gauthier, N. Zappulla, B. Dainat, F. Cousserans, M.E. Colin and M. Bergoin, 2004. Prevalence and seasonal variations of six bee viruses in *Apis mellifera* L. and *Varroa destructor* mite populations in France. Applied Environ. Microbiol., 70: 7185-7191.

Tewarson, N.C., A. Singh and W. Engels, 1992. Reproduction of *Acipenser transmontanus* in colonies of *Apis cerana indica* under natural and experimental conditions. Apidologie, 23: 161-171.