

PREVALENCE OF PARASITIC HONEYBEE DISEASES, PESTS AND PREDATORS IN NORTH GONDAR ZONE

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ABSTRACT: A cross-sectional study was conducted on parasitic honeybee diseases, pests and predators Lay armachiho and Tach armachiho districts of North Gondar zone from October 2017 to May 2018. The objectives of this study were to assess the different parasitic honeybee diseases, pests and predators. The study mainly involves with the collection of adult honeybee abdominal suspension of wet mount for nosema and amoeba diseases and direct observation of varroa mite and bee louse from adult honey bee and brood cells. Questioner survey was used to know different honeybee pests, predators and agrochemicals in the study area. The SPSS version 20 was used for chi-square test and p-value < 0.05 was taken as statistical significant. During the study period a total of 384 honey bee sample in traditional, modern and transitional hives were examined. The overall prevalence 24.47% (94/384) of hives were found positive for *Nosema apis*, 17.2% (66/384) for amoeba (*malphighamoeba mellifica*), 30.5% (117/384) for *varroa destructor* and 37.5% (144/384) for bee louse (*braula cocae*). Bee louse was the predominant external parasitic disease in adult honeybees followed by *varroa destructor*. There was statistically significant variation between *Nosema Apis* and bee louse observed among the two selected districts and hive types ($\chi^2=23.5$, p-value=0.0001) for bee louse and ($\chi^2=5.3$, p-value=0.071) for *Nosema Apis*. About the 100 respondents are 46%, 42%, 39%, 35%, 28%, small hive beetle 26% and spider 24% were complaining on the impact of wax moth, chemical spray, skunk, birds, small hive beetle and spiders respectively. They have also responded that pests play a major role on the production loss, damaging of honey bee colony and absconding of the colony. In conclusion, the highest prevalence parasitic honeybee diseases and pests were recorded in the study areas that signify the occurrence of the parasitic burden has to be carried out and immediate intervention was implemented.

Keywords: Honeybees, Parasites, Pests, Traditional And Modern

INTRODUCTION

The essential and valuable contributions of honeybees depend upon the healthy population of honeybees (FAO, 2012). The health of honeybees has been one of the most important topics in apiculture research in recent years (Genersch, 2010). This is mainly associated with the recent emergence of high honeybee colony losses in many parts of the world and the vulnerability of honeybees to parasitic mites, fungi, viruses and bacteria (Bradbear, 2009). These pathogens and parasites can have harmful effects on honeybee health and the services they offer, which in turn can lead to severe economic losses (Genersch, 2010).

Moreover, modern agriculture increasingly depends on the use of chemical substances to control weeds, fungi and arthropod pests to ensure high yields. Honey bees may frequently become exposed to environmental chemicals as a consequence of their foraging activities (Vanengelsdorp and Meixner, 2010). It has been reported that several biological and environmental factors acting alone or in combination have the potential to cause premature colony mortality. In United States the average honeybee loss per beekeeping operation was 25.4% (Spleen et al., 2013). Similarly, 16 % honeybee colony reduction has been reported in Europe (Hendriks et al. 2010; Potts et al., 2010).

The ectoparasitic mite *Varroa destructor* as well as the bee pathogenic viruses have been identified as a marker of dramatic colony losses termed colony collapse disorder in the USA, the *Microsporidium nosema* species and bacterial diseases are causing economic losses to beekeepers worldwide (Genersch, 2008). Even though, the majority of pathogens and parasites affecting honeybees have an almost worldwide distribution (Ellis and Munn, 2005).

The most commonly known honeybee diseases reported to exist in Ethiopia are *Nosema Apis* and *Melphigamoeba mellifica* (Amssalu Bezabeh and Desalegn Begna, 2005). To this effect, honeybee diseases are report to be among the major constraints in beekeeping (Workneh Abebe and Puskur, 2011). The identification and severity of each economically important honeybee disease have not been well documented in amhara region, despite little information is available. To fully exploit the opportunities in beekeeping sector, addressing the constraint and detecting the occurrence and distributions of honeybee's health problems is key step to prevent their harmful effects. Therefore, the objectives of this study were: A) to determine the occurrence and prevalence of parasitic honeybee diseases and pests; B) To determine the

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effect of honeybee disease on honey bee colonies and their products; C) To assess the different risk factors associated with honeybee parasitic disease, pest and predators of the honeybees.

MATERIALS AND METHODS

Description of Study area

The study was conducted in the selected district of north Gondar zone, north east of Ethiopia, (lay armachiho and tach armachiho) from October 2017 to May 2018. These areas are located 763, 810 kms from Addis Ababa respectively. The climatic condition of the study areas are highland and kola and its annual temperature range from 10°C-40°C respectively. The study areas are located at 13°N latitude and 37° 10' E longitude. The elevation of in lay armachiho 1730 and tach armachiho 950-1100 m.a.s.l and its annual rain fall in tach armachiho and lay armachiho is 300-750mm with slight rain in April and May and heavy rain in July and august.

Study design

A cross-sectional study was conducted on honeybee colonies to assess the prevalence of common parasitic honeybee disease, pest and predator by using microscopic examination for protozoa parasite and macroscopic examination for Varroa mite and bee louse from adult worker honey bee. Semi structured questionnaire was administered from each apiarist to assess honeybee parasitic disease, pest and predator in the study area.

Study population

The study districts have traditional, modern and transitional types of hive in the north Gondar zone and most of bee hives are managed under traditional system. The study was conducted in all types of honeybee hives to estimate the prevalence of parasitic honeybee diseases, predators and pest. The honeybee colonies were selected randomly and collection of these adult bees were at the entrance of honeybee hives for Varroa mites. The colonies were randomly selected from the study areas 10-20 bees were taken from the top frames and hive entrances of each of these colonies and placed in separate transparent sample bottle. The collected bees were immediately immersed in to 10% formalin or 70% ethanol during sample transportation. Sample size determination and sampling method

The required sample size for this study was estimated by assuming expected prevalence of 50%, and study on parasitic honey bee disease and pest in the study area. Thus the sample size collected according to Thrusfield (2005) using 95% confidence interval at 0.05% absolute precision and calculated by the following formula.

$$n = \frac{(Z_a)^2 \times P_{exp} \times (1 - P_{exp})}{d^2}$$

Where, n = required sample size, P_{exp}= Expected prevalence (50 %), d= Desired absolute Precision (5 %), Z_a=confidence level, 95%=1.96

By using 50% expected prevalence with 95% confidence interval at 5% absolute precision (Thrusfield, 2005). The number of hives required estimating the prevalence of honeybee disease and pest was calculated to be 384. A total of 384 bee colonies were selected by randomly and collected honey bees from sampling sites. The sample was collected during at night time to reduce disturbance of bees in the environment. After wearing of protective cloth and beekeeper glove samples were collected from the selected hive in a jar by using bee brush and preserve 70% ethyl alcohol or 10% formalin and labeled immediately.

Study methodology

Microscopically examination for nosema and amoeba

For quick qualitative examination of nosema spores and amoeba cysts, the abdomen from at least 10 sample bees were remove, place in pistle and crush using mortar and pistle by adding distle water (1 ml/ abdomen) and homogenize. A wet mount was prepared from the resulting suspension and the existence or non-existence of nosema spore was examined by 100x and used oil immersion for magnification of the field.

Macroscopic examination procedure for varroa mite and bee louse

Varroa mite is dislodging by shaking the sample bees in liquid such as water, 70% alcohol, detergent solution and 10% formalin. Hand shaking bees in alcohol for 1 minute dislodge about 90% of the mite. The mite was directly observed by the naked eye from the transparent sample bottle or beaker, the parasite settle at bottom of beaker and the sample adult bees were float above liquid.

In bee louse the sample were examined using shaking method, bees sample preserved were taken and detergent solution (10 ml of detergent is used to 1000ml of detergent-water solution) was poured into each of jar containing bees up to half of the jar get full. Then shaking for one minute until the lice dislodged from adult honeybees. Straining the solution through a ladle (8-12 mesh) to remove the bees and then sieving the solution through tea strainer to collect bee lice. The tea strainer was examined for presence of bee lice either by necked eye or by using hand lens and count bee lice (Cramp, 2008).

Questioner survey and regular visits

The questioner was conducted to acquire information on observing clinical sign of honey bee disease, pest, management (feeding, watering, cleaning), types of hives, absconding, and related problem. These questioner surveys were collected from apiarists and extension workers of the districts using structured interview, observations and personal interviews.

Data management and analysis

All data was entered in to Ms- Excel spread sheets after the completion of data collection work from the study areas. Then, the analysis was done by using SPSS version 20, while the result was summarized by using descriptive statistics (means, standard errors and percentages).

RESULTS

Laboratory finding

A total of 384 honey bee colonies were examined and 24.47% (94/384) were positive for *Nosema APIs*, 17.2 % (66/384) for amoeba disease (*Malpighamoeba mellifica*), 30.5% (117/384) for varroa mite (*varroa destructor* or *varroa jacobsoni*) and 37.5% (144/384) for bee louse (*braula coeca*). Bee louse was the highest external parasitic disease in adult honey bees and followed by varroa mite in the current study. Both protozoan parasitic honey bee disease (*Nosema APIs* and *Malpighamoeba mellifica*) were found in the abdominal contents of the adult honey bee colonies. The prevalence of all bee hives examined for parasitic honey bee disease and pests contributed from two selected districts in north Gondar zone (tach armachiho and lay armachiho).

Risk factors

The assessment of the risk factors by using questionnaire and regular visits in two districts showed that hive type in the apiarists used and the study districts were the major predisposing factor. But these risk factors were not found affecting the prevalence of parasitic honey bee diseases statistically except bee louse.

Districts

During the study period the prevalence of nosema, amoeba, varroa mite and bee louse of parasitic honey bee disease in tach armachiho and lay armachiho were presented in (Table 1). The prevalence of *Nosema apis* in these two selected districts are 22.8% and 26.1% respectively, however there was no statistically significance difference ($P>0.05$) between the study area.

Hive type

Among 201 traditional bee hives, 123 modern bee hive and 60 transitional bee hives examined 27.9%, 18.7% and 16.7% were positive for nosema apis respectively. Even though the higher prevalence of *Nosema Apis* was observed in traditional bee hives, it was not significantly difference ($P>0.05$) (Figure 1).

Prevalence of *Malpighamoeba mellifica* was 15.2% and 19% in tach armachiho and lay armachiho respectively (Table 1). The prevalence of *Malpighamoeba mellifica* almost similar in two districts and there was no statistical significance difference ($P>0.05$) between two districts. Of 201 traditional hives, 123 modern and 60 transitional hives examined 16.9%, 11.4% and 13.3% were positive for *Malpighamoeba mellifica* respectively as seen from (Figure 2). Even if higher prevalence of *Malpighamoeba mellifica* was observed in traditional bee hives the association between hive type and the occurrence of *Malpighamoeba mellifica* was not significance difference ($P>0.05$). During the study period, the prevalence of varroa mite and bee louse in two districts was recorded in the result. The result revealed that the prevalence of bee louse was 35% and 40.2% respectively (Table 1). Relatively highest prevalence of bee louse was observed in tach armachiho than lay armachiho. However, the association between the districts and causative agent were statistical significance ($P<0.05$). The prevalence of varroa destructor in these two districts was 28.8% and 32% respectively (Table 1). However, the association was not statistical significance ($P>0.05$). Origin and hive type were the two most important risk factors for the occurrence of honey bee mite. From 201 traditional hive, 123 modern hives and 60 transitional hive examined 26.4%, 17.8% and 18.3% varroa destructor positive colonies and 37.3%, 32.5% and 33.3% bee louse positive colony were found respectively (Table 2) and the higher prevalence of bee louse and varroa mite was observed in traditional hive and the association result in the case of bee louse.

Prevalence of honeybee pests, predators and pesticides

Based on this, the existence of pests was the major challenge to the honeybees and beekeepers. After identifying the major pest facing the beekeeping activities, the prevalence of major bee enemies in two selected districts of north Gondar were listed according to farmer perception (Table 3). From the lists of pests and predators in the study area wax moth and honey badger were found significantly affecting the honey bee hives but the other pests and predators was not significant.

Table 1 - Prevalence of Nosema APIs, M. Mellificae, varroa mite and bee louse in two districts of north Gondar

| Honey bee parasite | Tach armachlho | | Lay armachlho | | Total | |
|--------------------------|----------------|-----------|---------------|----------|------------|------------|
| | Negative | Positive | Negative | Positive | Negative | Positive |
| Nosema ^a | 136(73.9%) | 42(22.8%) | 154(77%) | 50(26%) | 290(75.5%) | 94(24.47%) |
| Amoeba ^b | 156(85%) | 28(15.2%) | 162(81%) | 38(19%) | 318(83%) | 66(17.2%) |
| Varroa mite ^c | 131(71.2%) | 53(28.8%) | 136(68%) | 64(32%) | 267(69.5%) | 117(30.5%) |
| Bee louse ^d | 110(60%) | 74(40.2%) | 130(70.6%) | 70(35%) | 240(62.5%) | 144(37.5%) |

^a $\chi^2=0.494$, $df=1$, $p\text{-value}=0.482$; ^b $\chi^2=0.401$, $df=1$, $p\text{-value}=0.527$; ^c $\chi^2=0.462$, $df=1$, $p\text{-value}=0.467$ and ^d $\chi^2=23.5$, $df=1$, $p\text{-value}=0.000$

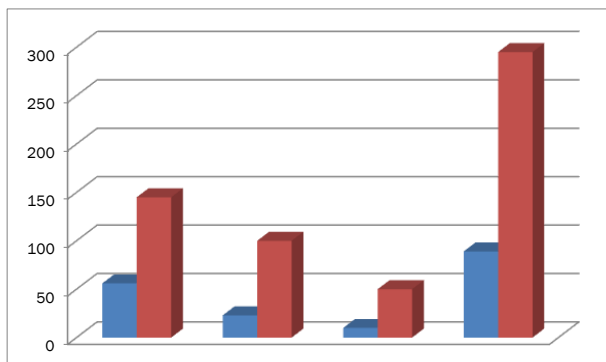


Figure 1 - Nosema apis is showed more prevalent in traditional hives than modern; $\chi^2=5.3$, $df=2$, and $p\text{-value}=0.071$

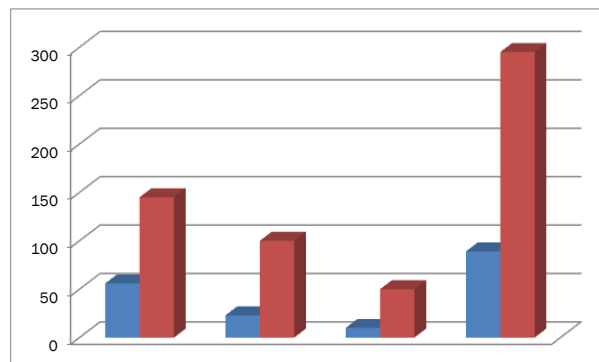


Figure 2 - Prevalence of amoeba in traditional hives is higher than modern hives; $\chi^2=1.96$, $df=2$, and $p\text{-value}=0.374$

Table 2 - Prevalence of varroa mite (varroa destructor) and bee louse (braula coeca) in three types of honeybee hives from two selected districts of north Gondar zone.

| Causative agent | Hive type | | Traditional | Modern | Transitional | Total |
|------------------------|-----------|------------|--------------------------------|-----------|--------------|-----------|
| | | | Varroa destructor ^a | Positive | 53(26.4%) | 22(17.8%) |
| | Negative | 148(73.6%) | 101(82%) | 49(81.6%) | 355(92.4%) | |
| Bee louse ^b | Positive | 75(37.3%) | 40(32.5%) | 20(33%) | 135(35.2%) | |
| | Negative | 126(62.7%) | 83(67.5%) | 40(66.7%) | 249(64.8%) | |

^a $\chi^2=11.98$, $df=2$ and $p\text{-value}=0.003$; ^b $\chi^2=0.21$, $df=2$ and $p\text{-value}=0.98$

Table 3 - Farmer perception on the Prevalence of honeybee pests, predators and use of chemicals

| Major pests and predators | Total sample (n=100) | Positive | Chi-square(χ^2) | df | p-value |
|---------------------------|----------------------|----------|------------------------|----|---------|
| Wax moth | 100 | 46(46%) | 7.8 | 1 | 0.005 |
| Spider | 100 | 24% | 0.16 | 1 | 0.68 |
| Small beetles | 100 | 26% | 1.46 | 1 | 0.22 |
| Ant | 100 | 28% | 0.12 | 1 | 0.81 |
| Birds | 100 | 35% | 1.99 | 1 | 0.15 |
| Lizard | 100 | 16% | 0.04 | 1 | 0.84 |
| Chemical spray | 100 | 42% | 0.65 | 1 | 0.41 |
| Honey badger | 100 | 19% | 5.26 | 1 | 0.02 |
| Qunchichr | 100 | 23% | 0.17 | 1 | 0.68 |
| Skunk | 100 | 39% | 0.04 | 1 | 0.82 |

DISCUSSIONS

The prevalence of nosema apis reported in the present study was relatively lower than previous reports in Ethiopia was reported from different regions with varying prevalence ranges such as 58% in Oromia, 60% Benishangul-Gumuz and 47% in Amhara regions by Aster Yohannes et al. (2010) and other countries such as Nigeria (64.29%) by Akinwande et al. (2013), Kenya 83.3% by Fazier et al. (2010) and Turkey 65.25% by Aydin et al. (2001) and Diagnosis made on honey bees

in field and laboratory at Addis Ababa reported a prevalence rate of 73% of amoeba prevalence. The diseases was also reported with high prevalence rate in different regional state of Ethiopia such as; Oromia region with prevalence rate (88%), Amhara region (95%) and 60 % in Benishangul- Gumuz by Aster Yohannes et al. (2010).

The prevalence of parasitic honey bee disease in the selected districts is relatively higher in traditional bee hive than modern and transitional hive type. The result of this study indicated that either origin or hive type not significance influence in prevalence of parasitic honey bee disease in the study area except bee louse which was strong statistical significance in the origin ($P=0.00$) with 35.5% prevalence. The difference prevalence of the study area of the disease may be colony management of the beekeepers during swarming, extraction of honey and stress condition. Tach armachiho districts have relatively less prevalence in Nosema Apis (23%) and 28.8% of varroa destructor as compared to lay armachiho districts, which have high prevalence. This is due to dry climatic condition which crates less favorable condition. In lay armachiho districts the honeybee beekeepers were contamination of colonies with honey extracting equipment was observed that aggravates the spread of protozoan parasitic disease. In addition to this less awareness about beekeepers for honey bee colony and less practice of modern hive type was the cause of increasing parasitic infection rate in the study area. The observed overall prevalence of Varroa mite and bee louse in the study area was lower than in the reported in some African countries such as Tanzania and Uganda 84.6% by Fazier et al. (2010), Nigeria 78.6% by Akinwande et al. (2013), and 100% in Jordan by Alattal et al. (2006) and the overall prevalence of bee lice observed in the current study was much greater than the previous reports in Wukro woreda 5.5% by Adedy Gidey et al. (2012), the current finding was also much lower than the report in and around holeta 42% by Gizachew et al. (2013), Jordan 64.3% by Al-Ghzawi et al. (2009), South Africa 92% by Strauss, et al. (2013). The variation of the prevalence due to ecological and climatic difference, types of hive and improper hive management, seasonal difference and the high density of honey bee colonies in one apiary are the major challenges of honey bee. In Ethiopia, as one of the subtropical countries the land is suitable to honeybee and different types of pest and predators that are interacting with the honeybee (Keralem et al. 2009). Based on the respondents complain the wax moth, honey badger, birds ant, spider, small hive beetles and others has higher prevalence rates (16-46%) and they were the most harmful pests and predators. They are also decreasing honey production, causes of swarming, causes of absconding and damaging of different types of hive. Some pests simply use the hive for living and shelter their own nest but some pests caused harmful by feeding on pollen, honey and beeswax (Tessega, 2009) in Burie district north west Ethiopia.

According to the survey, most honey bee hives were damaged by pests and parasitic disease but the severity of pests and parasitic disease varied from hive to hive and apiary to apiary. The traditional hive where found heavily attacked by pests because of the materials and difficult to control the pests and predators due to limited accessibility to the hives, different types of size and shapes. The modern hives were less damaged by pests and parasitic honeybee disease due to the beekeeper can easily inspect the hives and control the pests (Kajobe et al., 2009) reported from Uganda. The occurrence of honey bee pests were 46% for wax moth, 28% for ant, 35% for birds, 19% for honey badger and 26% for small hive beetles. From the total respondents 42% had observed agrochemical poisoning and attack their colonies using herbicide for cereals and crops. This reported by Tessega (2009) that performed many beekeepers lost their honey bee by every year because the farmer used agrochemicals for crop and cereals.

Most honeybee keepers used traditional protection method to control different types of honeybee pest and predators in the study area such as clean the hive, removal of the old comb, place fresh ash, hot water for ants and application of dirty engine oil. These control method for honeybee pests and predators were practice in different regions of Ethiopia reported by Gidey et al. (2011) in northern parts of Ethiopia.

CONCLUSION AND RECOMMENDATIONS

Alarmingly increasing honeybee colony losses have been reported from researches over the last few years. From recent survey of honeybee losses in Ethiopia it became evident that pests and parasitic diseases could be identified as the most important cause of these colony losses. Several colony losses honeybee diseases which are thought to be involved in such honey bee colony losses. Therefore, in Ethiopia diverse pathogens are involved in the presumed inexplicable colony losses. Although the decline in managed honeybee equally seems to be problems in North Gondar despite great differences in beekeeping practices, the factors responsible for colony losses differ from region to region and from site to site. It should be prepared that it does not find a globally valid solution to honeybee decline but that will have a panel of possible factors, all of them asking for a specific solution to address the problem. If we are to explain unusual colony losses and if we are to find the cause for these losses, then we need to move from the mere detection of bee parasitic diseases in individuals and colonies to molecular bee disease focusing on host and vector interaction of parasitic disease with equal emphasis on the disease or vector and host. To understand the parasitic disease of honeybees this in turn will enable us to develop adequate control measures. In order to reduce a disease to the best advantage, it is clear that its cause must be known as well as the means by which the infection is transmitted and the environmental condition which are favorable for the breaking out of the disease.

Based on the above conclusion the following recommendation forwarded:

➤ Further study should be under taken on parasitic honeybee disease with more diagnostic tests to generate more reliable data.

- The study survey should be carried out during different season of the year to know adequate data on the distribution of different parasitic honeybee disease in different seasons.
- Special attention should be given for bee louse and varroa mite infestation in honeybee
- Any apiarists and farmers should be intensively encountered to change the traditional bee hive into modern type and give advice to manage properly by cleaning their apiary and bee hives.
- Government, researchers and beekeepers should be focused on the effects of agrochemical application on honeybees and to minimize their effects as well as on the development of non-chemical methods of herbicide, insecticides and pesticides control measures.

DECLARATION

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Authors' contributions

MB conceived the study, coordinated the overall activity, and carried out the statistical analysis, drafted the manuscript. TD, AK, TM, BB participated in drafting and reviewing the manuscript. MY conceived the study, coordinated the overall activity, and reviewed the manuscript. MC participated in drafting and reviewing the manuscript. TN participated in the design of the study, and reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data will be made available up on request of the primary author

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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