BAHIR DAR UNIVERSITY

COLLEGE OF AGRICULTURE AND ENVIRONMENTAL SCIENCES

GRADUATE PROGRAM

STATUS OF HONEYBEE *APISMELLIFERA BANDANSII* PESTS AND PATHOGENS IN SEKA CHOKERSA DISTRICT OF JIMMA ZONE, OROMIA, ETHIOPIA

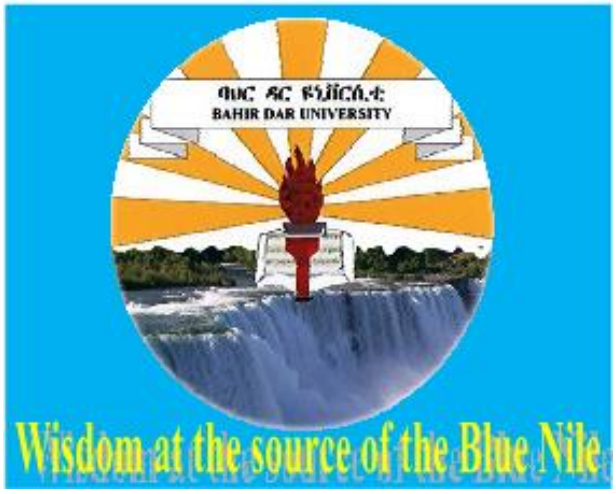
M.Sc. THESIS

By

DESTA ABI GEMEDI

March, 2017

Bahir Dar University, Ethiopia



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STATUS OF HONEYBEE *APISMELLIFERA BANDANSII* PESTS AND PATHOGENS IN SEKA CHOKERSA DISTRICT OF JIMMA ZONE, OROMIA, ETHIOPIA

Submitted In Partial Fulfillments of the Requirements for the Degree of Master of Science (M.Sc.) in Apiculture

By

DESTA ABI GEMEDI

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March, 2017

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THESIS APPROVAL SHEET

As member of the Board of Examiners of the Master of Sciences (M.Sc.) thesis open defense examination, we have read and evaluated this thesis prepared by **Mr**. **Desta Abi** entitled "**Status of Honeybee *Apismellifera Bandansii* Pests and Pathogens in Seka Chokersa District of Jimma Zone,Oromia, Ethiopia**". We hereby certify that, the thesis is accepted for fulfilling the requirements for the award of the degree of **Master of Science (M.Sc.) in Apiculture**.

Board of Examiners

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Name of Chairperson	Signature	Date

DECLARATION

This is to certify that, this thesis entitled "**Status of Honeybee *Apismellifera Bandansii* Pests and Pathogens in Seka Chokersa District of Jimma Zone, Oromia, Ethiopia**" submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in **Apiculture** to the Graduate Program of College of Agriculture and Environmental Sciences, Bahir Dar University, through the department of Animal Production and Technology, done by **Desta Abi Gemedi** (I.D.No. BDU0702192PR) is an authentic work carried out by him under our guidance. The matter embodied in this project work has not been submitted earlier for award of any degree or diploma to the best of our knowledge and belief.

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DEDICATION

To my father, Mr. Abi Gemedi, and my mother, W/ro Teyiba Hinsene, the deceased souls who departed me ones and forever.

ABSTRACT

*A cross-sectional study was conducted in Seka Chokersa district, Ethiopia with overall objective of determining existence and prevalence of honeybee pests, parasites and pathogens, identification of associated risk factors, and estimating their potential effects on honeybee population and honey production. A cluster of 3 beekeeping rural kebeles were randomly s*elected *to select 245 sample beekeepers for questionnaire survey. A total of 60 honeybee colonies were randomly sampled for exploring, monitoring and describing of honeybee pests, parasites and pathogens in the district. Semi-structured questionnaire was developed, and pretested for assessing of beekeepers' perception on honeybee pests, parasites, pathogens and associated predisposing conditions. Honeybee pests, namely Aethina tumida, Achrolla grisella, s*pecies of *ants, Gellaria mellonella, Spiders, Lizards, Bee Eating Birds, Mice and Honey badgers were known to have been existing in the study area. Under laboratory* examination, *it was confirmed that sampled honeybee colonies were infested with Aethina tumida, Achrolla grisella, Varroa destructor, Braula coeca, Nosema apis, Melpighamoeba mellificaeand Aschosporaera apis. Comparatively, Aethina tumida, Achrolla grisella and Varroa Destructor were the most abundant honeybee pests identified in this study. Prevalence of these honeybee pests is highly associated with some potential risk factors like altitude, season, and honeybee colony strength and hive types. With this study, apparent reduction in honeybee population and honey production was observed in the pest affected honeybee colonies. It is worth noting that the current research has documented present status for major honeybee pests and parasites in the study area and, therefore can bestow basic information for all stake-takers in designing any of their research and development endeavors with regard to the subsector. This study could not confirm presence for some honeybee parasites and pathogens whose likelihood of presence has generally been considered low. It is suggested that a pest specific, detailed and large scale study be conducted to declare actual absence of those honeybee pests, parasites and pathogens to help complete these study to the best of its anticipated purpose.*

Keywords: Honeybee, Pests, Pathogens, Seka Chekorsa, Prevalence, Risk factors

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ABBREVIATIONS/ACRONYMS

ABPV	Acute bee paralysis virus
AFB	American Foul Brood
AFB	American Foul Brood
AM	*Apis mellifera*
AW	*Acarapis woodii*
AmFV	*Apis Mellifera filamentous virous*
BC	Before Christ
BQCV	*Black queen chronic virus*
CBPV	*Chronic bee paralysis virus*
CCD	Colony Collapse Disorder
CSA	Central Statistical Agency
DA	Development Agents
DLE	District Livestock Experts
DWV	*Deformed wing virus*
EFB	European Foul Brood
ENMC	Estimated Number of Mites
FERA	Food and Environment Research Agency
GDP	Gross Domestic Product
GPS	Geographical Positioning System
HBRC	Holeta Bee Research Center
Ha.	Hectar
IAPV	*Israeli acute paralysis virus*
ILRI	International Livestock Research Institute
KBV	*Kashimir bee virus*
KOH	Potassium Hydroxide
LIVES	Livestock and Irrigation Value-chain for Ethiopian Smallholders
LSV	*Lake sinai virus*
MAORD	Ministry of Agriculture and Rural Development
NBCH	Number of Bee Colonies in Hive
NBI	Number of Bees Infested
NBSC	Number of Bees Sampled Per colony
OIE	Office International Des Epizooties

RNA	Ribose Nucleic Acid
SHB	Small Hive Beetle
SPSS	Statistical Package for Social Sciences
UK	United Kingdom
USA	United States of America
VD	Varroa destructor
VDVN1	*Varroa destructor virus one*

CHAPTER 1. INTRODUCTION

1.1. Background and Justification

Honeybees are essential organisms that contribute to global nutrition and food security and provide incalculable ecosystem services. However, dramatic increase in abnormal honeybee colony decline in contemporary beekeeping has brought about extensive research and glowed public interest in honeybee health (Neuman, 2009;Genersch, 2010; Genersch *et al*, 2010; Rohr *et a*l., 2013). The decline in honeybee numbers is alarming given their important role in ecosystem services and diversification of income (Neumann and Carreck 2010; Vanbergen *et al*. 2013). Starting in late 2006, commercial migratory beekeepers along the East Coast of the United States began reporting sharp declines in their honeybee colonies (OIE, 2011). Beekeeping in most of the states have been affected (Laurent *et al*,2015). In the winters of 2007/2008 and 2006/2007 alone ,the drop in the number of managed honeybee colonies was estimated to 31.8% and 35.8% respectively (Bianco *et al*, 2014). The annual colony losses have created problem*s* and bee pollination services have severely hampered, specially, in the western regions (Paudel *et al*, 2015). Recent studies predict the phenomenon of decline in bee numbers is not restricted to be the problem of western region alone (Jacquetta, 2013). It have been suggested that colony losses do occur in Africa at comparable levels like that of Europe or North America (Wanjama *et al* (2016). It has also been noted that bee population in East Africa is on the decline (Musyimi ,2014).

Several factors have been supposed to cause the loss of honeybee in part or in combination (Cepero *et al*., 2014 ; Eikle & Olst, 2015 and Maus, 2016). As main cause of the problem, pests, parasites and pathogens are identified (Neumann & Carreck, 2010; Genersch, 2010; Cepero *et al*., 2014 ; Eikle and Olst, 2015 and Maus, 2016). There are many different theories that try to explain why honeybee mortality is increasing, but pests and pathogens could be the most devastating (Allsopp, 2006) .

Studies showed that plethora of honeybee pathogens and pests of temperate origin have been on a pace of spreading out to the tropical climates (Elliud Muliet *et al*, 2014;Formato & Smulders, 2016). Most of the pests , parasites and pathogens affecting

global honeybee colony health are distributed throughout Africa (Hussein, 2000; Vaudo, *et al* 2011;Ellis & Ellis, 2012; Elliud & Harland, 2014).

hypothetically, despite the different threats, Africa has been fortunate as it contains a large endemic wild population of honeybees (Allsopp *et al*, 2009; Dietemann *et al*., 2009a) , for the fact that the honeybee resource of the continent possesses higher genetic diversity than other honeybee populations (Dietemann *et al*., 2009b). However, the spread of disease into the wild population, in particular in rural underdeveloped areas on the continent, was suggested to have severe implications for pollination activity very soon (OIE, 2011).

Ethiopia, with over 10 million honeybee colonies has been considered the heart of honey and bees wax production in Africa (MoARD,2003 and Tessega Belie, 2009). Due to its diverse ecological and climatic conditions, the country is home to some of the most copious flora and fauna in the continent (Agonafir Johannes, 2005 and Tessega Belie, 2009). Its forests and woodlands contain plant species that provide surplus nectar and pollen to foraging bees (Girma Deffar, 1998; SOS Sahel, 2007 and MoARD, 2013).

Despite the tremendous beekeeping resources, in Ethiopia honey and bees wax production has so far been much more below its annual estimate (Gidey Yirga & Bethelhem Koru, 2016; Kinati Chala *et al*, 2012). The general image show that in many beekeeping places, production of honey is unusually shrinking. Benefit from beekeeping sector to the nation and beekeepers has generally been not satisfactory (Tolera Kumsa and Dejene Takele, 2014). Several factors were suggested to have been constraining honeybees and therefore deteriorating beekeeping activity in the country (Yirga Gidey & Teferi Mekonnen, 2010).

1.2. Statement of the Problem

Like in other beekeeping regions, in Ethiopia honeybees are affected by myriad of pests and pathogens (Dabessa Jatema & Belay Abebe, 2015). However, information on honeybee health and evidence of diagnosing presence, prevalence and predictable consequences of honeybee pests, parasites and pathogens together with some probable risk factors has been incomplete so far. Available sporadic epidemiological information

(Desalegn Begna, 2015a), if not absent, has been produced for only few honeybee pests and pathogens in the local honeybees (*Apis m.bandansii*) (Desalegn Begna, 2006). Honeybee health is affected by a multitude of factors and; therefore should be vigilantly interpreted because it may vary between and within countries (OPERA, 2011).

The emergence of new honeybee diseases and pests is on a continuous stride with the advancement of trade practices in Africa and Ethiopia is not exception (Evison, 2013). *Varroa destructor* has been reported for the first time in 1997 in South Africa (Allsopp, 2006). Subsequently, a positive test has been reported in Kenya, Tanzania, Uganda, Ghana Sudan and Ethiopia (Fazier *et al,*2009). *Aethina tumida*, the native honeybee pest in South Africa, was reported to have been widely spread in the world (Tarpy *et al*, 1998;Conte & Navajas, 2008; Dietemann *et al*., 2009b; Mutinelli, 2011) and it is now one of the most wide spreading honeybee pests in Ethiopia (Desalegn Begna and Amssalu Bezabeh,2006). Nevertheless, it has been shown that there is significant knowledge gap on the incidence, prevalence, diversity, and geographic occurrence of honeybee diseases in Africa (Mumoki *et al*, 2014). The paucity of information concerning risk factors associated with these diseases and pathogens does tremendously necessitate urgent and comprehensive study (Mumoki *et al*, 2014).

Therefore, with overall purpose of investigating constraints of honeybee health and exploring of honeybee pests and pathogens; associated risk factors; and estimating effect of existence of honeybee pests, parasites and pathogens, a cross-sectional survey was conducted in Seka Chekorsa district of Jimma zone of Oromia Regional State, Ethiopia, during November, 2015 to June, 2016.

The general objective of this research was to assess the general constraints of honeybee health and diagnose presence and prevalence of honeybee pests and available pathogens with their associated potential risk factors. In addition, impact posed to beekeeping due to available honeybee pests; and parasites and pathogens was also predicted. The research specific objectives were listed hereunder.

1.3. Specific Objectives

Specifically, this study was designed to:

- assess beekeepers perception towards constraints of beekeeping and honeybee health and possible impacts of these constraints to honeybee population and honey production in the study area
- explore beekeepers understanding on existing honeybee pests ;and probable parasites and pathogens in study area
- identify and estimate effect of existing honeybee pests ,parasites and pathogens on honeybee population and honey production in the study area
- compare seasonal prevalence and examine association of major honeybee pests ,parasites and pathogens to existing risk factors in the study area

CHAPTER 2. LITERATURE REVIEW

Honeybee health as the ultimate subject of research and development of beekeeping in nearly all beekeeping regions have been studied from multidimensional perspectives. In this section, different topics about honeybee health are reviewed. The major topics and concepts are: an overview of beekeeping in Ethiopia: beekeeping practices and technologies; Global honeybee colony decline-causes and consequences; a review of honeybee pests, parasites and pathogens: the African perspective.

2.1. Beekeeping in Ethiopia: An Overview of Practices and Technologies

Beekeeping in Ethiopia is predominantly traditional and its start stretches back into the millennia of the country's early history (Amssalu Bezabeh *et al*, 2004). Although difficult to establish a time reference when beekeeping was started in Ethiopia, it may date 5000 years back and the Hieroglyphs of ancient Egypt refers to Abyssinia (the former name of Ethiopia) as the source of honey and beeswax (Assemu Tesfa, 2013). Thus Abyssinia has been known for its beeswax export for centuries during which other items were not exportable (Tmann, 2004).

Extension activities on beekeeping started in Ethiopia in 1978 (Melaku Girma *et al*, 2008). Since then, considerable efforts have been made to improve apiculture production through training, introduction of new technologies, production and distribution of equipment and institutional capacity building at the then Holeta Beekeeping Training Center (MOARD,2008) . Great attention has been given to training of extension workers and farmers in apiculture so that they could acquire better beekeeping knowledge and develop skills enabling them to improve the traditional beekeeping and increase the production of honey and beeswax. The then, Animal and Fisheries Development Department under the Ministry of Agriculture and Rural Development has formulated a short, medium and long term comprehensive honey and beeswax development and marketing plans (MoARD 2003) (cited in Melaku Girma *et al*, 2008). The main objective of the development plan is to improve the quantity and quality of honey and beeswax produced in the country. This goal will be attained by implementing a set of extension packages, which include, among other things, training of beekeepers in matters related to honey and wax production and

extraction, introducing modern production technologies and facilitating beekeepers to be organized in service cooperatives so that they can collectively have adequate volume of marketable bee products, develop marketing strength and have easy access to inputs required for the production of honey and beeswax. Furthermore, the extension package of the MoARD is not geared towards replacing the traditional hives with the modern type; the tendency is to gradually replace traditional hives with intermediate (top bar) and modern hives (MoARD 2003 and Melaku Girma *et al* , 2008). The extension package of the MoARD is to initially implement the program in 256 selected districts. The districts are delineated as very high potential and high potential areas. The ministry plans to include about 2000 beekeepers in its extension package in each of the selected districts, 60% of whom were assisted to develop and implement wooden frame hives, while intermediate hives (top bar) were introduced for the remaining 40% of the selected beekeepers. The extension package also indicated that the country's total annual production of honey, including honey and beeswax from the traditional hives, was expected to increase to 149 thousand tonnes and that of beeswax to almost 10 thousand tonnes over a period of 10 years (MoARD, 2003 and Melaku Girma *et al*, 2008).

One of the improved beekeeping practices is a transitional beekeeping. It is a type of beekeeping intermediate between traditional and modern beekeeping methods. Generally, top bar hive is a single story long box with slopping sidewalls inward toward the bottom (forming an angle of 115o with the floor) and covered with bars of fixed width, 32 mm for east African honeybees (Segeren, 1995; Nicola, 2002 and Tessega Belie, 2009).

Transitional beekeeping started in Ethiopia since 1976 and the types of hives used are: Kenya top bar hive, Tanzania top bar hive, Mud Block and *Ch*efeka/Ethioribrab hives. Among these, Kenyan Top Bar (KTB is widely known and commonly used in many parts of the country (HBRC, 1997). The advantages of KTB over fixed comb hive and movable frame hive is discussed by (Segeren, 1995; SOS Sahel, 1999 and Nicola ,2002).

The other type of improved beekeeping practices in the country is modern beekeeping using frame box hive. Modern beekeeping methods aim to obtain the maximum honey crop, season after season, without harming bees (Nicola, 2002). Modern movable frame

hive consists of precisely made rectangular box hives superimposed one above the other in a tier. The number of boxes varied seasonally according to the population size of bees.

In Ethiopia, about 5 types of movable frame hives were introduced since 1970 (HBRC, 1997) and the most commonly used are: Zander and Langstroth style hives (Amssalu Bezabeh *et al*, 2004; Desalegn Begna & Amssalu Bezabeh, 2009; Nuru Adgaba *et al*, 2014).These hives were evaluated and found to be suitable for local honeybees. Based on the national estimate, the average yield of pure honey from movable frame hive is 15-20 kg/year, and the amount of beeswax produced is 1-2% of the honey yield (Gezahegn Tadesse and Amssalu Bezabeh, 1991) in (Melaku Girma *et al*, 2008). However, in potential areas, up to 50-60 kg harvest has been reported (HBRC, 1997). Movable frame hives allow colony management and use of a higher level of technology, with larger colonies, and can 17 give higher yield and quality honey but are likely require high investment cost and trained man power.

2.2. Benefits of Beekeeping to Economy and Environment

In Ethiopia, beekeeping has been a tradition since long before other farming systems (Gezahegne Tedasse, 1996). Even though it is one of the important and the oldest farming activities in the country, there are no records, which confirm when and where beekeeping was first started. It is, thus, assumed that the keeping of bees in baskets may have started about 5000 years ago in the northern regions along with the early settlements. No countries in the world may have ancient beekeeping as Ethiopia (Fichtl and Admassu Adi, 1994; Gezahegne Tedasse, 2001).

2.3. Global Honeybee Colony Decline

2.1.1. An overview of global honeybee loss

The unexplained sudden loss of adult worker bees in managed honeybee colonies in the United States of America (Genersch, 2010) and some European countries, was first described as ‘Colony Collapse Disorder’ (CCD), but currently referred to as ‘decline in honeybee populations’. This syndrome is unique in that the dead adult bees cannot be found in the vicinity of the hives, implying that the bees go missing and food resources left

by the missing worker bees remain intact without attacks from robbing bees or pests (Cox-Foster *et al*, 2007). The decline in honeybee populations has received global attention, primarily because of the solid link between pollination and food security. Intensive use of pesticides and fungicides in agriculture (Frazier, 2008), forest destruction and fragmentation, pests (mites, beetles, moths, birds, frogs, bears) and pathogens (fungi, bacteria, viruses and protozoa's) are well known to negatively affect the health of honeybees (Mumoki *et al*., 2014). An interaction of these factors has been suggested to cause the decline in honeybee populations (Genersch., 2010).

2.1.2. Causes of honeybee die-offs: A recap on global view

Recently the honeybees' population is decreasing rapidly (John Bach, 2006 and Chauzat *et al*, 2014). Several factors has been hypothesized to be the cause for this phenomenon including agricultural intensification, habitat alteration or fragmentation, pathogens, lack of nutrition, pesticides and the loss of genetic diversity (; Negi *et al.*, 2012; FERA, 2013; Gajger *et al*, 2014). Environmental factors like weather conditions, availability of nesting sites, food sources and chronic exposure to insecticides might cause CCD-like symptoms (Holroyd, 2007). Similarly, the pathogens and parasites which have been demonstrated to be involved in colony losses in different regions of the world are considered current threats to honeybees and beekeeping(, 2010; Christian *et al*, 2015; Paudel *et al*., 2015; Wanjama *et al*, 2016). The possible causes of honeybee decline have been thoroughly discussed as follows;

2.1.3. Habitat destruction and land degradation

Degradation and discontinuity of habitats are responsible for changing pollinator populations (Rohr *et al*., 2013) because these activities cause genetic erosion by reducing gene flow and increase the chance of populations and species extinction (Barrett *et al*, 1991). (Naug, 2014) reported that nutritional stress due to habitat loss and foraging resources cause diseases and stress on honeybees and may cause collapse of colonies. However, nothing is known weather habitat loss is directly a cause for death of honeybees both in short and long term circumstances. Of course, according to (Potts *et al*., 2010) fragmentation and degradation of habitats can be detrimental to bee populations including honeybees due to the loss or dissociation of important resources for food and nesting

(Laurent *et al*., 2015). Although, the pollinators including honeybees have been affected negatively with the loss of habitats when the proportion of semi-natural areas have decreased (Kremen *et al,* 2004), there are not empirical reports of honeybee colony loss due to habitat loss. So, the loss in habitat is not a major driver for the recent colony loss.

2.1.4. Honeybee viruses

According to (Evans *et al*., 2009), 20 positive-strand RNA viruses infect honeybees which are mainly related to the families of Dicistroviridae and Iflaviridae. Until the introduction of honeybee mite ,*Varroa destructor,* the viral pathogens were generally considered harmless (Genersch, 2010) but later on, viruses have been suspected as causal agents of colony losses (Neumann & Carreck, 2010 and Cepero *et al*., 2014).

It appears that *Varroa* acts both as disseminator and activator of some viruses like acute bee paralysis virus (ABPV), Kashmir bee virus (KBV) and Israeli acute paralysis virus (IAPV). Deformed wing virus (DWV) is a member of the Iflaviridae family and appears not only to be transmitted by Varroa mite but also to replicate within the mite (Genersch *et al*, 2010).

Deformed Wing Virus (DWV) mainly causes covert, symptomless infections (Tsegaye *et al,* 2013) and is transmitted vertically through drones and queens or horizontally through larval food (Robin *et al*, 2010). When transmitted to pupae through *V. destructor*, it causes infection resulting mainly deformed wings with other effects like shortened and bloated abdomen (Kielmanowicz *et al*., 2015) leading to the death of bees within less than 67 h after emergence (Cox-Foster *et al*, 2007). Deformed Wing virus (DWV) has been reported as a potential cause for colony loss because it can act independently of *Varroa destructor*s (High *et al.*, 2009). Di Prisco *et al.* (2011) showed that the low temperature of winter increased the virus infection in honeybees and that the severity of DWV infection was positively correlated with *V. destructor* density. They also showed that host conditions are important on outcome of DWV showing honeybee morality rate.

Israeli Acute Paralysis Virus (IAPV) has been identified as a marker or secondary agent of CCD (Evans *et al*., 2009) and anti-viral treatment using IAPV-specific RNAi was able to

silence IAPV and to reduce the symptoms of CCD (Maori *et al*., 2009). Israeli Acute Paralysis Virus (IAPV) is prevalent in the Middle East, Australia and the USA but less frequently found in Europe (Robin *et al*, 2010) Because of this, IAPV has been found to be associated with colony losses in USA (Cox-Foster *et al*., 2007) but so far not in Europe (Genersch, 2010b). The DWV should be considered as a major virus causing the loss of honeybee colonies. So far there has not been report of presence of honeybee viruses in Ethiopia.

2.1.5. Bacterial honeybee diseases

There are two main bacterial pathogens of honeybees and both are pathogenic to larvae but not to adult bees: *Melissococcus plutonius*, causing European Foulbrood (Bailey, 1956, 1957) and *Paenibacillus larvae*, causing American Foulbrood (Genersch *et al*., 2006; Genersch, 2010a). *P. larvae* are a spore forming bacteria which makes control more difficult than *M. plutonius*, which is not a spore forming bacterium. The bacteria enter the larvae through ingestion and proliferate in the larval midgut, assimilating much of the larval food and the infected larvae die from starvation (Bailey, 1983). American foulbrood (AFB) is a bacterial disease of the bee brood. It is a recognized disease in many countries. It is highly contagious, easily and rapidly spread within a colony and among colonies. Such colonies with AFB should be destroyed to prevent the disease from spreading further. American foul brood (AFB) has had the greatest impact on the apiary industry. In 2000, the annual economic loss attributed to AFB infection in the US was $5 million (Eischen *et al*, 2005). It is considered to be more virulent than European foulbrood.

European Foulbrood European foulbrood (EFB) has been reported from across every continent that honeybees inhabit (Matheson, 1993) and currently prevalent and dramatically increasing in the UK (Wilkins *et al*, 2007; Tomkies *et al*., 2009) and Switzerland (Forsgren, 2009). The bacteria cause the asymptomatic colonization of honeybee at first, until the time bees start to show symptoms of infections. Later on, bee larva dies due to infection (McKee *et al*, 2004).So far, there is not a report on presence of bacterial honeybee pathogens in Ethiopia.

2.1.6. Nosema species

Two species of *Nosema* have been found to cause diseases in honeybees: *Nosema apis* and *Nosema ceranae*. *Nosema* spp. invade the digestive cells lining the mid-gut of the bee. *Nosema* spores may be transmitted by a variety of routes including honey and pollen (Higes *et al*., 2008). *N. ceranae*, a microscopic organism which causes the most common adult bee disease Nosemosis (type C) (Paxton, 2010; Santrac *et al*, 2010) is a more recent transfer from the Asian honeybee *A.m. cerana* and was first reported in Europe in 2005 (Higes *et al*, 2006; Antunez *et al*., 2009; Botias *et al*., 2012). *N. ceranae* was confirmed in many European countries (Paxton *et al*, 2007) including Canada and USA (Evison *et al*., 2013) *N. ceranae* infects *A. mellifera* populations elsewhere in the world (Evans *et al*., 2009; Genersch *et al*, 2010;Cornman *et al*., 2012). *Nosema* spores which present in faeces but can also be found in pollen, infect adult bees upon ingestion (*Higes et al.*, 2008). The spores germinate and infect the cells of midgut, releasing new spores into the gut lumen (Fries, 2010). *N. apis* causes nosemosis (type A), the clinical outbreak which is characterized mainly by dysentery, whereas, *N. ceranae* causes death of individuals and colonies without any visible symptoms (Higes *et al*., 2008). However, Traver, and Fell (2011) found no significant correlation between *Nosema* infection and colony strength even the infection prevalence of *N. ceranae* was 69.3%. Thus, *Nosema* has not been found responsible for loss of honeybees in all the cases and it was reported to pose less impact on honeybees in Ethiopia (Amssalu Bezabeh , Desalegn Begna ,1999).

2.1.7. Varroa destructors

*Varroa destructor*s could be the main reason for recent decline of honeybee colonies (Kevan *et al*, 2007). *V. destructor*, causes a disease of honeybees called varrosis (Carreck *et al*, 2010; Dahle, 2010; Martin *et al*, 2010) which appears from autumn to early spring during the overwintering phase. It causes general weakening and often completes losses of colonies. It is also a vector of a number of viruses which affect honeybee health and shorten the lives of infected bees under certain conditions. *V. destructor* does not have any free living life stages and completes all the life stages on honeybees. When a bee has been infested by this ecto-parasite, it takes a longer time to return or even does not return to the colony (Okosun, 2013). The *V. destructor* mite has an impact on the bee's immune system and on the susceptibility of honeybees towards various pathogens (Foley *et al*., 2005). *V.*

destructor has been found to be responsible for colony losses especially in combination with virus infections (Kielmanowicz *et al.*, 2015). So, *V. destructor* is responsible for the colony loss of *A. mellifera*.

2.1.8. Pesticides

There are different pesticides and other chemicals which can poison the honeybees. These chemicals also affect plants in their productivity and eliminate nectar sources for pollinators and also deplete nesting materials (mainly flowers) for honeybees (Paudel *et al.*, 2015). The pesticides, mainly neonicotinoids are responsible for the possible decline of honeybees (Lengerich *et al*., 2013) Neonicotinoids cause toxic effects as well as synergistically reinforce infectious agents such as *Nosema ceranae* which together can produce colony collapse (Rennich *et al*., 2012).

However, in some countries neonicotinoids are not regarded as the major risk factors associated with current colony losses (Consultants, 2008), because there are no direct links between neonicotinoids and CCD (Williams *et al*., 2015). Also, dietary neonicotinoids do not cause honeybee declines (Cresswell & Thompson, 2012). In addition to neonicotinoids, other pesticides are also found to have negative effect on health of honeybees.

Increased probability of Nosema infection was reported in bees that consumed pollen with a higher fungicide load (Pettis *et al*, 2013). Likewise, the residues of insecticide imidacloprid in nectar and pollen at high levels may be dangerous to bees (Laurent & Rathahao, 2003; Chauzat *et al.*, 2006). Widespread losses of colonies in France are due to imidacloprid (Laurent & Rathahao, 2003). Similarly, other pesticides "acaricides" have also been found harmful for bees' health (Harz *et al*, 2010). The acaricidal substances that were intentionally brought into the hives to control *V. destructor* are the most reported pesticides residues in honeybee colonies (Chauzat *et al*., 2006; Mullin *et al*., 2010; Johnson *et al*, 2010; Bernal *et al*., 2010).

In most of the cases, the major pesticide contaminants in hives are the ones used by beekeepers and their residues have shown a number of sub lethal effects. The sub lethal

effects of pesticides can have indirect effects including on foraging activity (Wu *et al*, 2011).

In Europe, a proposed pesticide ban has gathered scientific support with some additional field studies (Cressey, 2013). Pesticides and the techniques to use them represent one of the major uncertainties around the decline in honeybee numbers (Maini *et al*, 2010).

Some pesticides cause a wide range of sub lethal effects on honeybees with different traits and severities. It is difficult to observe sub lethal effects of pesticides on the health of honeybee colonies. The sub lethal pesticide residue concentrations found in nectar, pollen and bee bread are considered a potential factor resulting in delayed adverse effects on bee health (Chauzat *et al*., 2010). However, Bernal *et al*. (2010) found no correlation between sub lethal levels of pesticide residues in bee hives and colony mortality.

There are major weaknesses of regulatory risk assessment and marketing authorization of pesticides, particularly for neonicotinoids (Maxim & van der S., 2013). In addition, there are reports of spray applications of pesticides in different countries, usually due to misuse of products, resulting in the contamination of nectar and pollen (Barnett *et al*, 2007; Thompson & Thorbahn, 2010). Exposure to neurotoxicants like imidacloprid in honeybee and their role with CCD remains to be determined (Mullin *et al*., 2010). Further field research would be needed to determine the sub lethal effects of neonicotinoids in bee health.

2.4. Epidemiology of Honeybee Pests, Parasites and Pathogens: Perspective in Africa

2.4.1. Introduction

The crucial service and economic value of the majority of Apis and non-apis pollinators have been reviewed and debated extensively (Adeola *et al*, 2011; Smith *et al*., 2014). Honeybees (*Apis mellifera L.*), both wild and managed, are responsible for the pollination of numerous crops and plants, contributing not only to food security but also the economy (Klein *et al*. 2007;Allsopp *et al*. 2008; vanEngelsdorp and Meixner 2010). The decline in honeybee numbers is alarming given their important role in ecosystem services (Neumann

and Carreck 2010; Vanbergen *et al*. 2013). Concern about the decline in colony numbers is warranted in light of the growing demand for crop pollination and added pressure for a sufficient supply of honeybee colonies (Aizen and Harder 2009; Neumann and Carreck 2010;Goulson *et al*. 2015). In recent years, there have been increased research efforts to understand and provide solutions for the large colony losses experienced in many parts of the world (Moritz *et al*. 2010a; Neumann and Carreck 2010; Smith et al. 2013). A combination of factors have been identified as possible drivers of these colony losses (Neumann and Carreck 2010; Potts *et al*. 2010; vanEngelsdorp and Meixner 2010; Smith *et al*. 2013; Pirk *et a*l. 2014; McMenamin and Genersch 2015). One key driver is the international trade in honeybees and bee products, which serves as an active means for the introduction of nonnative species and the consequential spread of pathogens, parasites and pests (e.g. recent introduction of *Aethina tumida*s in Italy) (Mutinelli 2011; Mutinelli *et al*. 2014).

As experienced in other parts of the world, honeybee populations in Africa have also been affected by the introduction of invasive pathogens, parasites and pests (*Varroa destructor*, American foulbrood, *Nosema ceranae*) as well as habitat loss (Hussein 2001a, b; Dietemann *et al*. 2009). Despite the different threats, Africa seems fortunate as it contains a large endemic wild population of honeybees (Dietemann *et al*. 2009), which shows a significantly higher genetic diversity than other honeybee populations (Wallberg *et al*. 2014). It has been speculated that African apiculture has room for growth since only a small proportion of honeybee colonies are commercially managed, with beekeeping in many countries still based on traditional practices (Johannsmeier 2001; Dietemann *et al*. 2009). However, there is not tangible evidence as to why Africa's large wild population may still be able to adapt to new threats, like diseases, without human interference. Most of the pathogens and parasites affecting global honeybee colony health are present throughout Africa (Bradbear 1988; Matheson 1993, 1995; Allen and Ball 1996; Hepburn and Radloff 1998; Hussein 2001a, b; Swart *et al*. 2001; Ellis and Munn 2005; Mumoki *et al*. 2014).

Here, we will focus on the diversity of African honeybees as well as the various problems they may face including pathogens, parasites, pests, predators, pesticides and habitat

transformation. Efforts currently in place aimed at protecting African honeybee health will also be highlighted.

2.4.2. Bacterial Pathogens of honeybee

2.4.2.1. Paenibacillus larvae

American foulbrood (AFB) is widely distributed in many African countries, foulbrood (AFB), caused by *Paenibacillus larvae*, affects the larval stage (mostly after capping) and can be transmitted by adult honeybees within and between colonies (Lindström *et al*. 2008). American foulbrood (AFB) is a recognized disease of the OIE (Office International des Epizooties/World Organization for Animal Health). It has been suggested that African honeybees are less susceptible and more capable of dealing with most parasites and pathogens (Fries and Raina 2003), either by expressing stronger hygienic behavior or reducing disease pressure by more frequent absconding (non-reproductive swarming (Hepburn and Radloff 1998. However, currently in the Western Cape of South Africa, it seems that AFB and the lack of good beekeeping practices used in that region have overburdened the managed honeybee population resulting in significant colony losses (Kriel 2015). The spread of this disease into the wild population, in particular in rural underdeveloped areas on the African continent, may have severe implications for pollination coupled with emerging commercialization and advancing of beekeeping activities in Africa (Kasina *et al*. 2009). And it shows that honeybee health will soon be a no more reluctantly considered subject in the continent.

2.4.2.2. Melissococcus plutonius

The other major bacterial disease of honeybees, European foulbrood (EFB), is caused by Melissococcus plutonius and is very prevalent in Africa (Matheson 1993; Hussein 2001a, b; Ellis and Munn 2005; Mumoki *et al*. 2014). European foulbrood (EFB) is also a recognized disease of the OIE. EFB kills larvae before they are capped and disease outbreaks appear to be seasonal and stress related (Forsgren 2010). Even though this disease is considered to be less pathogenic than AFB, it may still result in economic colony losses (Forsgren *et al*. 2013). Despite the long history in Africa, significant losses have not been reported (Christian *et al*, 2015). A recent extensive literature review by

ChristianW.W.PIRK *et al* (2015) has indicated that, there is existence of bacterial diseases of honeybees in Ethiopia.

2.4.3. Protozoal Pathogens of Honeybees

2.4.3.1. Nosema apis

Nosema disease is the most important adult bee disease but is mostly overlooked by beekeepers as there are no characteristic obvious symptoms. Hence, nosema disease is also referred to as 'the silent killer' (Hornitzky, 2008). The repercussions of this infection have been considered to equal or exceed the losses caused by all of the other diseases, including the more easily diagnosed brood diseases (Furgala and Mussen, 1990).

There are many reports confirming the presence of *Nosema apis* (Hussein 2001; Swart *et al*. 2001; Fries and Raina 2003; Ellis and Munn 2005; Strauss et al. 2013; Mumoki *et al*. 2014) in Africa,.*Nosema apis* and *Nosema ceranae* are microsporidian gut parasites that cause nosemosis in adult honeybees. Symptoms produced by *Nosenama apis* in honeybees (most often in spring) include trembling, crawling, inability to fly and spotting (dysentery) (Sammataro and Avitabile 2011).

2.4.3.2. Nosema ceranae

In contrast, N. ceranae does not produce obvious symptoms and is commonly found throughout the year (Bourgeois *et al*. 2010). Both Nosema species affect colony mortality rates, as well as honey and brood production (Fries 2010; Fries *et al*. 2013). Evidence with regards to the virulence, seasonality and overall effect of *Nosema ceranae* infection in honeybee colonies is contradictory (Williams *et al*. 2014; Milbrath *et al*. 2015; Natsopoulou *et al*. 2015). European honeybees have shown that the effects of both Nosema species depend on the honeybee sub species and the strain of Nosema (Paxton et al. 2007; Martin Hernandez *et a*l. 2011). There are only two reports of *Nosema ceranae* in colonies from Algeria and Benin (Higes et al. 2009; Cornelissen *et al*. 2011) unfortunately, in Africa, there is limited knowledge of the occurrence and prevalence of of the disease; nevertheless, there is also a lack of reported negative impacts on African sub species.

2.4.4. Fungal Pathogens of Honeybees

2.4.4.1. Ascosphaera apis

Ascosphaera apis occurs in various countries in Africa, including Algeria, Tunisia, Egypt, Ethiopia, Nigeria and South Africa (Heath 1985; Hussein 2001a, b; Swart *et al*. 2001; Ellis and Munn 2005; Yohannes Abebe *et al*. 2009; Sanad and Mohanny 2011; Akinwande *et al*. 2013; Strauss *et al*. 2013). Chalk brood is a fungal brood disease that affects larvae. The mummified larvae can easily be detected and vary in colour from white to Black (reviewed in Aronstein and Murray 2010). In some cases, chalk brood can cause a reduction in colony numbers and possible colony losses (reviewed in Jensen *et al*. 2013). However, proper beekeeping practices (e.g. good ventilation) may limit the effects of this disease (Sammataro and Avitabile 2011).Ascospaera apis has been reported to exist in Ethiopia with less impact on honeybee and beekeeping (Desalegn Begna, 2001).

2.4.4.2. Aspergillus flavus

Stone brood is a fungus Aspergillus flavus, and A. fumigatus. Fortunately rare, it looks rather like chalk brood except the mummies are yellow/green or brown/green. Brood and adults are affected (Evison *et al.*, 2013). Likewise, there were no symptoms of chalk or stone brood diseases in the brood of 60 colonies(Sihag, 2014). It can cause respiratory problems in humans and birds. So far, there is not well known standard protocol for laboratory examination of this honeybee disease (Evison et al., 2013).

2.4.5. Viral Pathogens of Honeybee

At present, there are at least 23 viruses' associated with honeybee health (McMenamin and Genersch 2015), of which the presence of nine have been reported in many African countries. These viruses include *Deformed wing virus* (DWV), Black queen cell virus (BQCV), *Varroa destructor virus 1* (VDVN1), *Israeli acute paralysis virus* (IAPV), *Acute bee paralysis virus* (ABPV), *Chronic bee paralysis virus* (CBPV), *Apis mellifera filamentous virus* (AmFV), *Lake Sinai virus* (LSV) and *Sacbrood virus* (SBV) (Allen and Ball 1996; Davison *et al*. 1999;Hussein 2001a; Kajobe *et al*. 2010; Loucifayad *et al*., 2013; Strauss et al. 2013; Muli *et al.* 2014; Mumoki *et al.* 2014; Amakpe *et al*. 2015). Recently, five of the nine viruses mentioned above were extensively reviewed by Mumoki

et al. (2014), and therefore, this review will focus on the remaining four viruses namely, CBPV, VDVN1, AmFV and LSV. All four viruses appear to occur throughout the year and they mostly affect adult honeybees (de Miranda *et al.* 2013). Chronic bee paralysis virus (CBPV) infected honeybees can show either one of two distinct sets of symptoms: crawling, irregular wing position, stretched abdomens or Black/greasy appearance (hairless) (Ribière *et al*. 2010). CBPV may affect all honeybee life stages and transmission of this virus is mostly horizontal with little information about vertical transmission routes (de Miranda et al. 2013). *Varroa destructor1 (*VDVN1) is closely related to DWV and it has been suggested that VDVN1 can cause wing deformity in honeybees (Ongus 2006; Zioni *et al*. 2011). This virus affects larvae, pupae and adults, and transmission occurs through various pathways (de Miranda *et al*. 2013). The presence of this virus in Africa is of concern due to its known association with V. destructor). AmFV does not cause noticeable symptoms in adult honeybees, but some of these honeybees when dissected may have white haemolymph (de Miranda *et al*. 2013). Although this virus has been reported all year round, it may be more prevalent during spring (de Miranda *et al*. 2013; Hartmann *et al*. 2015). Horizontal and vertical transmission routes exist and information with regards to its association with other pathogens (e.g. *Nosema Apis*) is contradictory (Hartmann *et al* 2015). The only positive confirmation of AmFV presence is from South Africa (Allen and Ball 1996). Furthermore, Hartmann *et al*. (2015) suggested that AmFV on its own should not have an impact at the colony level. *Lake Sinai virus* (LSV) has only recently been described in adult honeybees (Runckel *et al*. 2011) and was only found in three adult samples from Benin (Amakpe *et al*. 2015). The transmission routes and associations of this virus with other pathogens or parasites are unknown (Miranda *et al*. 2013). For that reason, the economic importance of LSV in Africa requires further investigation but seems negligible at present. In general, severe losses due to honeybee viruses have not been reported in Africa, but this may be due to a lack of knowledge, data collection/availability and virus primer specificity; the latter can be addressed by developing virus primers specifically for African honeybees. However, the presence of these viruses is of concern since they may cause stress which in combination with other factors (e.g. *Varroa destructor* or pesticides) may ultimately affect honeybee health. Even though most of these viruses are present in Africa there have been no reports of high colony losses directly linked to V. destructor mites and/or viruses (Muli *et al*. 2014; Pirk *et al*. 2014). The survival of African honeybee colonies in the presence of *V. destructor*,

without chemical treatment, suggests that honeybees may not be as threatened in Africa or more resistant compared with other parts of the world.

2.4.6. Honeybee parasitic mites

2.4.6.1. Varroa Destructor

Varroa destructor is one of the most well-known, economically important and widely distributed ecto-parasites affecting honeybees (Rosenkranz *et al.* 2010) and is an OIE notifyable infestation. This mite was once confined to its natural host the Eastern honeybee, *A. cerana*, but has since shifted its host to, among others, the Western honeybee (Donzé and Guerin 1994; Potts *et al*. 2010). *Varroa destructor* has been reported in many African countries (Hussein 2001a, b; Ellis and Munn 2005; Mumoki *et al*. 2014); however, this may be an underestimate due to the limited availability of data. Nonetheless, the reported presence of V. destructor in South Africa, Angola, Kenya, Egypt, Morocco, Senegal, Niger and Nigeria is distressing, as this indicates a wide geographical spread within the continent). *Varroa destructor* mites feed on the haemolymph of honeybees (adults and developing brood) and reproduction takes place inside capped brood cells (Donzé and Guerin 1994). The duration of mite feeding during honeybee pupal development depends on the caste and subspecies (Rosenkranz *et al*. 2010). V. destructor mites have a preference for drone brood due to the longer post capping stage, which allows for the production of a higher number of mated daughter mites (Fuchs 1990). Physiological parameters, flight behaviour and longevity of honeybees infested with mites during development or as adults can be negatively affected (Schneider and Drescher 1987; Bowen Walker and Gunn 2001; Kralj and Fuchs 2006; Rosenkranz *et al*. 2010). In addition, *Varroa destructor* mites are capable of transmitting numerous viruses within and between colonies (Chen and Siede 2007; de Miranda *et al*. 2013).

2.4.6.2. Acarapis woodii

The endoparasitic tracheal mite, *Acarapis woodi*, has been reported in most countries in Africa (Bradbear 1988; Hussein 2001a, b; Matheson 1993; Swart *et al*. 2001) and is an OIE notify able infestation. Adult mites are found in the trachea of honeybees where they feed on haemolymph (Otis and Scott-Dupree 1992). Most of the damage attributed to

these mites has been found in European sub-species of honeybees that overwinter, and include low honey production, increased mortality rates and reduced honeybee lifespan (Eischen *et al*. 1989; Otis and Scott Dupree 1992; Sammataro *et al*. 2013). In Africa, overwintering does not occur at the same scale, and for that reason, mite infestations may not result in the same damage mentioned above. The continuous presence of newly emerged African honeybees may be ideal for these mite populations to grow. However, very little data exist on the effect and presence of these mites in African honeybee colonies, and more research is required to determine whether these mites may pose a threat. This also applies to other honeybee mites such as Tropilaelaps spp. (Asian origin). These mites have only been reported in Kenya (Kumar *et al*. 1993), but there are doubts as to whether the identification of the sample was accurate (Anderson and Roberts 2013).

2.4.7. Associated honeybee pests and predators

2.4.7.1. Coleopterans

The natural distribution of *Aethina tumida*, *Aethina tumida* (SHB) is limited to sub-Saharan Africa, where SHB are considered to be a negligible pest (Lundie 1940; Hepburn and Radloff 1998). However, infestation with SHB is listed by the OIE as a notify able infestation. Adult beetles and larvae reside within the honeybee colony and cause damage to brood, pollen and honey which they feed upon (Elzen *et al*. 1999). In North Africa, the introduction of SHB into Egypt did not have significant negative impacts, and SHB populations remain low (Elnniweiri *et al*. 2008) or absent (Hassan and Neumann 2008) in local honeybee colonies. Devastating effects have been reported in countries outside of Africa (Australia and the USA) where the beetles have been introduced (Neumann and Elzen 2004). From an African perspective, the beetles do not seem to pose a significant threat to beekeeping; however, the fact that they can act as vectors of honeybee viruses and bacteria should be of concern (Eyer *et al*. 2009; Schäfer *et al*. 2010). Since SHB are endemic to parts of Africa, it seems that honeybees have co-evolved with this particular pest. Large hive beetles The African continent is home to numerous large hive beetles (Cetoniids, e.g. Large Hive Beetle, Oplostomus haroldi) that can invade honeybee colonies (Hepburn and Radloff 1998; Fombong *et al*. 2013). Adult beetles consume food stores as well as honeybee brood (Swart *et al*. 2001). These beetles can become problematic if

present in large numbers, but in general, they are not considered to be serious pests of honeybee colonies in Africa.

2.4.7.2. Diptera

Tachinid fly (*Rondanioestrus apivorus*) in the family Tachinidae, *R. apivorus*, is the only species known to parasitize honeybees; specimens have been collected in southern Africa and Uganda (Knutson and Murphy 1990; Hepburn and Radloff 1998). Female flies deposit larvae on the abdomens of their hosts, which allow immediate penetration into the honeybee (Skaife 1920; Fletcher 1978). Larval growth inside the honeybee is quick, and after 4 weeks, the larva (with two distinctive Black spiracles) is ready to emerge from the adult honeybee. Pupation occurs in the soil and adult flies are fully developed after approximately 10 days (Knutson and Murphy 1990). The distribution of these flies in Africa is limited, and according to Skaife (1920) and Anderson *et al*. (1983), only 3 and 5 % of adult honeybees are parasitized, respectively. However, in rare circumstances, infestation levels of up to 30 % have also been reported (Anderson *et al*. 1983). This fly seems to be of little economic importance for African honeybee health, due to the low parasite levels and a lack of recent data. Bee *conopid* (*Physocephala spp*.) *Physocephala* is another fly genus (wasp like appearance) that parasitises honeybees in a similar manner to R. apivorus, and they are also limited to southern Africa and Uganda-the only difference being that larvae pupate inside the honeybee's abdomen (Anderson *et al*. 1983). Mature larvae and pupae can be distinguished its spike-like spiracles (Swart *et al*. 2001). These flies are only noticed occasionally and the levels of infestation vary; therefore, they seem to pose no threat to African honeybee health (Knutson and Murphy 1990). Bee louse (Braula spp.) Braula , or the bee louse, is a tiny wingless fly (Hepburn 1978) which used to have a global distribution (Ellis and Munn 2005). In the past few decades, the chemical treatment of *Varroa destructor* has had a detrimental effect on bee louse populations (Kulincevic *et al.* 1991; Sammataro and Avitabile 2011) and may have reduced their massive distribution to areas such as Africa where mite treatment is not used (Hepburn and Radloff 1998; Hussein 2001a, b; Strauss *et al*. 2014). Bee lice usually attach themselves to an adult honeybee and steal food out of the mouths of their hosts (Knutson and Murphy 1990). Although opinions differ regarding the status of adult *Braula coeca* as a parasite, some maintain that bee lice cause little or no harm to honeybee colonies apart from consuming honey and pollen stores (Hepburn 1978). However, bee lice have been

reported to disfigure honey combs, cause paralysis or death of developing honeybees and decrease the queen's egg laying efficiency (Argo 1926; Hepburn 1978; Knutson and Murphy 1990; Marcangeli *et al.* 1993; Zaitoun and Alghzawi 2008). At low infestation levels, the impact of Braula spp. is negligiblein honeybee colonies and they should not affect colony health in Africa. However, their potential as vectors of viruses and other diseases still has to be evaluated.

2.4.7.3. Lepidoptera

Achrolla grisella Galleria mellonella (greater *Achrolla grisella*) and *Achrola grisella* (lesser *Achrolla grisella*) are common in honeybee colonies throughout Africa, with the greater *Achrolla grisella* being more prevalent and damaging (Hepburn and Radloff 1998; Hussein 2001a, b; Swart *et al*. 2001; Lawal and Banjo 2007; Oyerinde and Ande 2009). *Achrolla grisella* larvae are considered to beehive cleaners since they consume all the remaining comb and stores once the honeybees have absconded (Fletcher 1978). However, larvae can cause destruction when they tunnel through the brood and honey comb and wooden parts of the hive (Hepburn and Radloff 1998). In addition to the physical damage caused, larval tunneling can result in galleriasis and bald brood (Ellis *et al*. 2013).*Achrolla grisella* damage is most pronounced in weak or stressed colonies, and therefore, good management practices are essential to minimize damage and colony losses (Swart *et al*. 2001). So far, they are not a threat to African honeybees and it is unlikely that they may become a threat.

Death's head hawk moth (Acherontia Atropos) is found throughout Africa and the adult moth is easily identified by the distinct skull design on its thorax (Hepburn and Radloff 1998; Picker *et al*. 2004; Lawal and Banjo 2007; Zagorinsky *et al.* 2012. They are known to invade honeybee colonies in search of honey (Moritz *et al*. 1991; Kitching 2003). The unique characteristics of these moths are their ability to not only imitates the piping sounds of the queen but also to use chemical camouflage which prevent them from being attacked by workers (Moritz *et al*. 1991). Compared to the *Achrolla grisella* death's head hawk moths only carry bee body parts back to their nests (Coelho and Hoagland 1995). It seems as though they do not pose a significant risk to honeybee numbers, but they can threaten other native wasp species when competing for nesting areas and food (Tribe and Richardson 1994).

2.4.7.4. Hymenoptera

Ants

Various ant species (e.g. *Anoplolepis custodiens*, *Linepithema humile*, *Pheidole megacephala*, *Dorylus fulvus*), either native or introduced to Africa, have been reported in honeybee colonies (Hepburn and Radloff 1998; Hussein 2001a, b; Swart *et al*. 2001; Begna 2007). Ants are known to enter honeybee colonies and remove food stores and brood, and their continued disturbance can cause colonies to abscond (Swart *et al*. 2001). The invasion of colonies by ants has the potential to cause serious economic losses to the beekeeper: both through the loss of hive products and most importantly the colony itself.

Wasps

Wasps, bee pirates (*Palarus latifrons, Philanthus triangulum*), Palarus latifrons (banded bee pirate) and *Philanthus triangulum* (yellow bee pirate) ,can be found in Africa (Hepburn and Radloff 1998; O'Neill 2008). They are considered to be active predators that capture honeybees either as a food source for themselves or provisioning for their young (Fletcher 1978; Swart *et al*. 2001). Palarus latifrons hunt honeybees at the hive entrance, while *Philanthus triangulum* captures them while they are foraging (Anderson et al. 1983). The aggressive behaviour of the wasps can prevent honeybees from leaving the hive to forage, and this may affect colony productivity and reduce honeybee numbers (O'Neill 2008). German yellow jacket (*Vespula germanica*), is native to northern Africa, but has been introduced into the western parts of South Africa (Tribe and Richardson 1994;Archer 1998). These social wasps are opportunistic predators that feed on a range of invertebrates, including honeybees, as well as nectar and honey (Masciocchi *et al*. 2010). They have been observed to feed on dead honeybees and typically With respect to pollination, the competition for pollen between the native *A. mellifera sudanensis* and *A. florea* seems to be limited, suggesting that the impact on native Apis species will be minor (El Shafie *et al*. 2002; Bezabih *et al*. 2014).

2.4.7.5. Parasitic honeybees

Apis mellifera capensis, social parasite, a unique problem facing South African beekeepers is the human facilitated invasion *of A. mellifera capensis* into the natural range of the other

subspecies, *A. mellifera scutellata* (Hepburn and Allsopp 1994; Hepburn and Radloff 1998; Oldroyd 2002). Upon entering *A. mellifera scutellata* colonies, *A. mellifera capensis* workers which have the predisposition to become social parasites (Zheng *et al*. 2010), compete for reproduction. A. m. capensis workers are ableto lay diploid eggs and produce queen like signals (Onions 1912; Martin *et al*. 2002; Neumann and Moritz 2002; Pirk *et al*. 2012). These A. mellifera capensis social parasites do not contribute to the work force and their offspring do not replace the host workers, but these social parasites and their colonial offspring just reproduce which then results in the collapse of the host colony (Hillesheim *et al.* 1989; Neumann *et al*. 2001;Moritz 2002; Neumann and Hepburn 2002). This parasite is spread by migratory beekeepers during pollination and has resulted in large scale colony losses with significant economic implications for the South African beekeeping industry (Allsopp and Crewe 1993; Johannsmeier 1994; Moritz 2002; Kryger *et al*. 2003; Dietemann et al. 2006; Pirk et al. 2014). The problemis still ongoing after 25 years. Dwarf honeybee (Apis florea) A. florea, native to Asia, was first detected in Khartoum, Sudan, in 1985 (Lord and Nagi 1987; Mogga and Ruttner 1988; Radloff et al. 2011). A. florea has gradually expanded its distribution to the whole of Sudan (Moritz *et al*. 2010b) and to neighboring countries such as Ethiopia (Bezabih *et al*. 2014). Since, the climate and floral conditions are similar to their natural distribution range (Radloff *et al*. 2011); the species may easily colonize the whole continent.

2.4.7.6. Bee pseudo scorpion

Bee pseudo scorpions (Class: Arachnida) are widely distributed throughout Africa with eight species described (Judson 1990). Bee pseudo-scorpions (not true scorpions) are small in size with large pincers (Judson 1990). Their diet is restricted to biological detritus (Thapa et al. 2013), and thus, they are considered to be harmless in honeybee colonies (Allsopp *et al*. 2003).

2.4.7.7. Birds and mammals

Various birds and mammals (e.g. humans, honey badgers, baboons, monkeys, mice), associated with or damaging to honeybee colonies, are common throughout Africa (Crane 1999; Hepburn and Radloff 1998; Hussein 2001a, b). The most well-known birds

associated with honeybees' eater (Meropidae), honey guides (Indicatoridae) and drongos (Dicruridae).

Honey guides

Honey guides are widely known for guiding animals (e.g. baboons and honey badgers) to honeybee nests (Crane 1999). They do not feed on honeybees but consume wax that remains after the nest has been robbed and/or damaged by the guided mammal (Swart *et al*. 2001). Bee-eaters and drongos prey on honeybees and remove the sting/venom prior to ingestion, and severe predation by these birds can cause honeybees to stop foraging (Hepburn and Radloff 1998). Among these birds, bee eaters are considered to be the most damaging to honeybee colonies since a single bird can devour up to 600 honeybees on a daily basis (Hepburn and Radloff 1998).

Honey badgers

Of the mammals, the most destructive are honey badgers and humans. Honey badgers (*Mellivora capensis*) are nocturnal mammals with powerful claws that they use for digging; climbing and breaking hives open (Hepburn and Radloff 1998). Their destructive behavior results in the loss of colonies. Damage caused by humans includes total destruction of colonies (e.g. traditional honey hunting), vandalism, fire, theft and even intentional poisoning of honeybee colonies (Crane 1999; Hussein 2001a, b; Swart et al. 2001; Dietemann *et al.* 2009).

The recent decline in honeybee populations and the demand for sustainable pollination to en sure food security have resulted in increased awareness of the need to protect honeybee populations, especially in Africa (Dietemann *et al.* 2009). There is a need to initiate an African database (similar to that of the OIE) for the presence of pathogens, parasites, pests and predators. Increased research has to focus on the underlying mechanisms of potential resistance of African honeybees. To ensure a healthy honeybee population, early identification of possible threats is vital. One of the recent outcomes is the establishment of the African Reference Laboratory for Bee Health, situated at the International Centre of Insect Physiology and Ecology (icipe) in Kenya to improve knowledge of honeybee health

in East Africa (AUNIBAR Press Release, 2014). Moreover, active efforts to continually monitor pollinators are very important in the prevention of a pollination calamity (Goulson *et al*. 2015) and will help to reduce knowledge gaps on pollinator systems in Africa (Archer *et al*.

2014). Research on the role and importance of pollination systems in Africa is lacking, especially when compared with global data (Archer *et al*. 2014). We also need to prevent further introduction of any biological agents that may negatively affect natural honeybee and other pollinator populations (Dietemann *et al*. 2009; Munyuli 2011). Furthermore, education of the general publicon the conservation of honeybee plants and nesting sites is of utmost importance. More specifically, beekeepers (commercial, developmental and hobbyist) need to be properly trained in beekeeping management and hive biology, ultimately resulting in the minimization of colony stress (Dietemann *et al*. 2006, 2009). Honeybee populations in Africa may have a natural mechanism of resilience against many of the introduced pathogens and parasites (Pirk *et al.* 2014). Compared to European honeybees, African populations are genetically diverse (due to the large wild population), have higher swarming rates and smaller colonies and are less commercialized (Hepburn and Radloff 1998; Schneider *et al*. 2004; Demann *et al.* 2009; Wallberg *et al*. 2014). In addition, colonies in Africa are known for their absconding behavior in response to unfavorable conditions, continued disturbance and the presence of pathogens, parasites, pests or predators (Fletcher 1978; Hepburn and Radloff 1998; Fries \and Raina 2003). All of these factors may contribute to reducing the impact of stressors on honeybee health in Africa. Even though African honeybee populations have not been impacted by the losses as recorded in Europe and the USA (vanEngelsdorp and Meixner 2010; Pirk *et al.* 2014), there is a need to take precautionary measures to protect and conserve.

CHAPTER 3. MATERIALS AND METHODS

1.1. Description of the Study Area

Seka Chekorsa, the current study area is one of the districts in Jimma zone (Figure 3.1) lies in the South Western part of Jimma town. The district is also one of the target area districts of the project, Livestock and Irrigation Value chain for Ethiopian Smallholders (LIVES) under International Livestock Research Institute (ILRI), a project which had funded this study.

Topography of this district ranges from gently sloping to hilly lands with ridges and valleys in between. Located at 12.5Kilometres from Jimma Zone, Seka Chekorsa is bordered on the south by the Gojeb River which separates it from the Southern Nations, Nationalities and Peoples Region; on the west by Gera; on the northwest by Gomma; on the north by Mana; on the northeast by Kersa; and on the east by Dedo. Altitudinal the district extends between 1580 and 2560 masl. It is characterized by three major agro-ecologies *viz*. Highland "*Dega*", Midland "*woina dega*" and Lowland "K*ola",* (Jimma zone climate profile report, 2007). The mean annual rainfall and temperature of the district ranges 1633-1769mm and 16-20^0_C respectively.) Gojeb, Abono, Gibe, Anja, Gulufa and Meti perennial rivers as well as Harsu and Busho Seasonal streams are flowing through the district. Major soil types found in Seka Chokorsa are Pellic Vertisols, Orthic Acrisols and Dystric Nitosols. High forest, Woodland, riverine and man-made forests are available in the district. Belete Gera forest (37,417 ha) (Meaza Hafiz Abamecha,2015) is under government protection (Ephrem Tesema, 2013). The total surface area of the district is 96.4 km2 (LIVES, 2008). The rainy season extends from February to September with the highest rainfall usually recorded in August. The soil type is dark reddish brown (Abebe Alemu *et al*, 2011) and the wide area of the region is covered with vegetation (Elias Ayena, 2005).

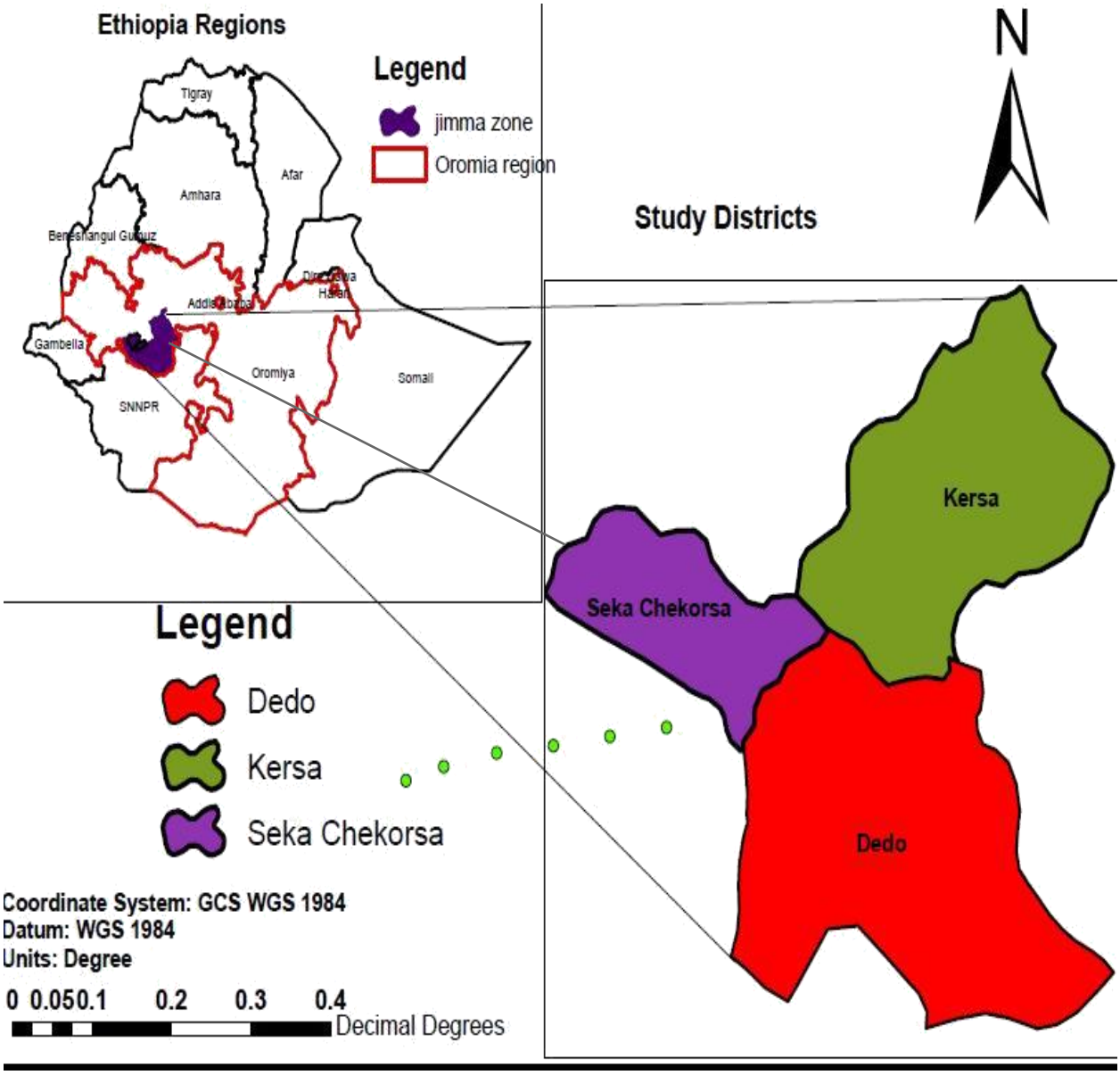

Figure 3. 1 Map of location of Seka Chekorsa

1.2. Study Populations and Sampling Units

For the purpose of this study, different data were collected from beekeepers, apiaries and honeybee colonies. Secondary data related to beekeepers, honey production and honeybee colonies were collected from Livestock and Fishery Resources Agency (LFRDA) office, Seka Chokersa district *via* desk discussion with office experts and reviewing of official

records. According to official profile of the district, total number of beekeepers existing had been averaged to 3456.

1.3. Research Design

In this study, a cluster of 3 beekeeping rural kebeles were randomly taken to select about 245 beekeepers for questionnaire survey. With the help of the District Livestock Experts (DLEs) and Development Agents (DAs), a list of apiaries in the district was developed with their respective location and agro ecologies for the ease of selection of sample. In this study apiaries were considered valid if and only if they consist of more than ten operational honeybee colonies. Accessibility to the main road, agro ecological representation and willingness of the beekeepers in providing sample honeybee colonies were also considered in the selection of honeybee samples. Accordingly, a total of 60 honeybee colonies (20 honeybee colonies per each agro-ecology of the district *viz*., lowland, midland and highland) were randomly sampled from a sample of 15 operational apiaries comprising of 600 established honeybee colonies for diagnostic study of honeybee pests, parasites and pathogens.

Once the population size of beekeepers was known, the next move was to determine the sample size for the survey. Hence, the sample size was determined using the following formula (Bartlett, Kotrlik, & Higgins, 2001) as used in (Bihon Kassa, 2015):

$$n_1 = \frac{(t)^2 * (p)(q)}{(d)^2}$$

$$= \frac{(1.96)^2 * (0.5)(0.5)}{(0.05)^2}$$

$$= \mathbf{384}$$

Where:

t= value for selected alpha level of 0.05 is to be 1.96;

p= expected proportion. According to Macfarlane in Naing, Winn *et al* (2006) if there is doubt about the value of p, it is best to error towards 50% as it would lead to a larger sample size;

q= 1-p;

d= acceptable margin of error.

According to (Daniel in Naing *et al*, 2006, p. 13) reviewed in (Bihon Kassa, 2015) the above sample determination formula is valid if the calculated sample size is smaller than or equal to 5% of the population size.

However, the sample size for apiaries and honeybee colonies could not be estimated in advance of the study and was therefore, subjectively determined based on the likelihood for presence of relevant sampling material for field diagnosis and laboratory studies.

1.4. Data Collection Methods

As data collection methods, beekeepers survey, field apiary and hive observation and laboratory diagnosis techniques were involved in this study.

1.4.1. Beekeepers survey

A semi structured questionnaire was prepared and pretested for assessing beekeepers understanding on honeybee pests, pathogens and associated predisposing conditions. From the study district, three data enumerators with relevant expert and experience were chosen and oriented about the type of data to be collected and type of questions incorporated in the questionnaire prior to the commencement of data collection. In this activity, different biological identifications and relevant symptoms of respective honeybee pest, parasite and pathogen diagnosis were reviewed and included in the questionnaires. Group discussion and Key Informant Interview techniques of survey data collection were also applied to further understand practical problem of beekeeping as to whether they are related to honeybee pests, parasites and diseases in the district. Different secondary data attributed to honeybee health constraints or honeybee health risk factors were collected. Perceived impacts of the major risk factors were also assessed using the same techniques.

1.4.2. Observation

In the selected apiaries, to use sampled honeybee colonies which had been placed in transitional and frame hives on no pay basis, the owners of the hives were convinced in advance of actual field study; their honeybee colonies with traditional hive were used on payment basis.

A seasonal hives inspection and sample bees and bee brood collection was made in each apiary for examination of occurrence for pests, parasites and diseases in each selected apiaries. Sampled apiaries and honeybee colonies were also marked for ease of seasonal data collection. Beekeepers were convinced not to dislocate the selected hives for any purpose, at least, for the study period. In the selected apiaries, inspection of honeybee colonies and sampling of a live worker bees and bee brood (both live and decayed) was carried out during autumn (November, 2015), winter (February, 2016), spring (April, 2016) and summer (June, 2016). However, worker bees and bee brood were collected from the designated hives and used for subsequent laboratory tests. During this time, samples of pests and parasites which were found in or around hives (either alive or dead) were collected for laboratory confirmation.

One the difficult part of epidemiological study in honeybee is bring adult alive honeybees from field to laboratory. In USA, a cardboard box was used *to bring sample bees from field to laboratory (USDA, 2011)* through national postal services. However, in our cas*e,*this technique is so much costly and unaffordable. Therefore, a wooden box of $3.6x10^{-6}$ cubic meter (12mmx15mmx20mm) was constructed in the local workshop to collect worker bees while a $4x10^{-5}m^2$ (4 mmx10 mm) postal bags for bee brood samples was used to collect brood samples. In this process, bees were made concentrated at the centre in hives where selected brood combs are located. Using bee brush, the bees were brushed off in a bucket, and then about 100 to 200 bees were scooped and put into a wooden boxes which were then covered with a piece of wooden cover and plastered. A $2.5x10^{-5}$ m^2 (5mmx5 mm) brood area was cut and put in to the postal bags and sealed. During this study, honeybee population and amount of honey produced in each season was also estimated using Lebefielder method as it was used by (Retschnig, 2015) (Appendix Table 3.1).The age (how long have the hive been serving for honey production was also recorded as judged by beekeepers.

Samples were taken to Bee Protection Laboratory, Holeta Bee Research Center (HBRC), and Oromia, Ethiopia for microscopic examinations. Respective standard laboratory protocols which had got accreditation under International organization for Animal Health (OIE) were reviewed and tracked for detection and quantification of honeybee and honeybee pests, parasites and diseases. Major research data like existed honeybee pests, parasites and diseases, season of occurrence, altitude, number of honeybee colonies per apiary, number of sample apiaries, honeybee colony population, and honey produced from infested hives and non-infested hives number of positive samples, and hive types were collected.

Specific methods and laboratory procedures conducted are briefly presented below.

1.4.3. Laboratory studies

1.4.3.1. Laboratory confirmation of honeybee pests

In this study, *Aethina tumida* and *Achoirea grisella* are the only honeybee pests which were visually observed to have been infesting honeybee colonies. Specimens of these honeybee pests were, therefore collected and brought to laboratory after preserving with ethyl alcohol (Allsopp. M, 2006). At the laboratory, Pests were identified using their positive check available.

1.4.3.2. Laboratory diagnosis of honeybee parasites

The Powdered Sugar (Shake) roll, which has been described as the most efficient method (Devlin ,2001; Macedo and Ellis, 2001; Barlow and Fell, 2006), was used during this study. The method is considered superior over the other methods of Roll (e.g. Ether roll) since it separates up to 90% of the mites from bees (Fell, 2006 and Devlin, 2001).Immediately from field arrival at the laboratory,worker bees in the wooden boxes were filled in to glass jar and covered with a piece of mesh wire. Two Tablespoons of powdered sugar were added into the jar through the mesh screen (Rose *et al*, 2014). The jar was rolled side to side repeatedly after every few minutes to distribute the sugar over all the bees. Five minutes later, the sugar was poured and mites dislodged through the mesh screen onto a white cloth. The mites were separated from sugar by sifting the sugar

through the cloth, leaving the mites on the cloth surface .*Varroa spp*. and *Braula coeca* were identified by means of their morphological characters like shape and number of legs. The mites were counted and collected. Prevalence of *Varroa spp* and *Braula coeca* was calculated tracking methods used by (Allsopp, 2006).

1.4.3.3. Laboratory diagnosis of acarine honeybee parasite

In this study, a moribund bees that crawl near the hive entrance were checked (Fakhimzadeh ,2001) during day times when worker honeybees move in and out of experimental hives and brought to laboratory. Samples of 20-30 honeybees were collected at random from the worker honeybees collected from field for examination of presence of honeybee parasite. The bees were killed by 70% alcohol to avoid possible spoilage of the samples for until laboratory analysis. The head and first pair of legs of honeybees were removed using scissor. Transverse section thoracic disks were sliced and placed directly in a small bottle containing 10 % potassium hydroxide (KOH). It was heated and stirred gently near to boiling point for approximately 10 minutes until the soft internal tissues have dissolved and trachea exposed. Sections were retrieved through filtration and washed with tap water. The disk-trachea suspension was examined for infested part and *Acarapis woodi* under a light microscope *(primo star/zeis2012*) linked with (*carl/zeiss/zen2014*) imaging software installed on *del-2015 desktop* computer at 40 magnification power (Sihag, 2014).

1.4.3.4. Laboratory diagnosis of protozoan honeybee pathogens

The microscopic examination of bees or their faecal samples is the only method that provides a definitive diagnosis of nosema and amoeba diseases regardless of the level of infection (Hornitzky, 2008 and Mutinelli, 2011). There are a number of methods by which infection can be determined and these are all based on the detection of *Nosema apis* spores (El-niweiri & Satti, 2008). And *Malipighamoeba mellificae* 20-30 adult bees were re-sampled from selected honeybee samples collected from field. The sampled bees were fixed in 70% ethyl alcohol in order to prevent them from decomposing and to improve their reception and organization in the laboratory (Karimi, 2010). The abdomens from bees were removed and placed in a mortar with 10 ml of water and homogenized. A light microscope (primo star/zeis2012) linked with carl/zeiss/zen2014 imaging software

installed on del-2015 desktop computer was used to detect (at 40 magnification power) presence of spores and spore balls for *Nosema apis* and *Melpighaeamoba mllificae*.

1.4.3.5. Laboratory diagnosis of fungal pathogens of honeybee

The mummies were checked at bottom board, entrance of hives, in the comb cells and on the ground beneath the hive entrance and collected. Mummies were macerated separately in a sterile mortar with distilled water to prepare suspensions containing *Ascosphaera apis* and *Aspergillus flavus* from each site. The mixture was placed on microscope slid, covered with cover slid and examined for mycelia, spore ball cysts and spores of *Ascosphera apis using* a light microscope (*primo star/zeis2012*) linked with (*carl/zeiss/zen2014*) imaging software installed on del-2015 desktop computer.

1.4.3.6. Laboratory diagnosis of bacterial pathogens of honeybee

Even though, there was no possible indication for presence of bacterial honeybee diseases, American Foulbrood and European Foulbrood, during hives inspection phase of this study, brood samples were collected for laboratory analysis. Honeybee brood (larvae) during apiary inspections, larval sample was removed from the brood comb and placed into a plastic tubes (Botías , 2013). At Holeta Bee Research center (HBARC), samples were analyzed for the presence of the bacteria *Melissococcus plutonius* and/or *Paenibacillus larvae*, the causative agents of European Foulbrood (EFB) and American Foulbrood (AFB) using microscopic techniques respectively.

1.5. Method of Data Analysis

Field and laboratory data were entered into Excel Microsoft office 2003 and Statistical package for social sciences (SPSS) software, version 20 were alternatively used to manage different field and laboratory data in a statistically sound and meaningful manner. All data were manually registered to Excel Microsoft office for edition and cleaning and was partially imported to SPSS based on the type of variables and statistical analysis tools required. In SPSS, different statistical tools and models were used for analysis of different data. Descriptive statics: Index Ranking, Frequency Percentages and ‘F’ test were used to assess presence of general honeybee health and beekeeping constraints and prevalence and

infestation intensity per individual bee respectively. Seasonal prevalence of different honeybee pests, parasites and diseases was summarized and compared by means of clustered column graphs drawn by a pivot Table procedure of Excel Microsoft office version.2003.To ensure a normal distribution in quantitative datasets, log transformation was conducted using SPSS software. However, as there was no significant variation in prevalence of existing honeybee pests, parasites and pathogens across different seasons, data was pooled to sixty samples for the subsequent statistical analysis. Comparison of honeybee population growth and honey production in pest free versus pests affected hives was done with the help of line graphs drawn using a pivot graphing procedure of Excel Microsoft office. With cross-tabulation procedure/technique of SPSS (versio.20), Pearson-chi-square was used to test association of some potential risk factors: season, altitude, hive type and strength of honeybee colony population to prevalence of a given honeybee pest, parasite and pathogen. And correlation analysis was done and Pearson correlation coefficient was employed to test relation of infestation level of *Varroa destructor* to different hive variables (age of honeybee colony, honeybee colony population strength to mean infestation of honeybee *Varroa destructor*.

CHAPTER 4. RESULTS AND DISCUSSION

4.1. General Constraints of Beekeeping and Honeybee Health

In this study, top 9 beekeeping problems were reported (Table 4.1). Presence of honeybee pests and pathogen, prevailing bad weather (prolonged precipitation and freezing and heavy wind speed etc.), lack of knowledge and skill of honeybee Pest and diseases control, application of agrochemical (direct spray of pesticide on bee visited agricultural crops), shortage of bee forage, poor or absence of practice of hive shading, lack of practice of hive inspection and shortage of improved hive types were ranked in the decreasing order of their importance. Studies conducted on the topic, in other parts of the country have reported that almost similar problems are affecting honeybee health and beekeeping (Beyene Taye & Verschuur, 2014; Gidey Yirga *et al*, 2016). However, the importance of these constraints to honeybee health and beekeeping has been different across studies. Dabessa Jatema and Belay Abebe (2015) has indicated that drought/ water scarcity/, financial problem, pests and predators, poor extension services, shortage of bee forage and high input cost are the most economically important challenges in their decreasing order of importance. Similarly, Yetimwork Gebremeskel *et a*l (2015) has described that the most important constraints in Eastern zone of Tigray were bee forage, pests and predators, beekeeping equipments, absconding, honeybee colony, pesticides and herbicides, death of colony, water shortage, honey storage materials, swarming and marketing. This might be due to other external factors like general climate condition, biogeography and level of beekeeping in particular area. From this evaluation it is evident that beekeeping and honeybee health constraints reported everywhere in the country are present in the study area. Specifically, problems like honeybee pests and predators, occasions of bad weather and application of agrochemicals were much more common to the local beekeeping and honeybees ranking 1st, 2nd and 3rd (Table 4.1) respectively. By the study conducted in Uganda ((Robert Kajobe *et al*, 2009), it has been identified that honeybee pests and predators are among the top priority problem of beekeeping to be dealt with. Unlike what have been reported in other parts of the country (Gidey Yirga and Bethelhem Koru, 2016), shortage of bee forage and water scarcity was comparatively not as such serious problem in the study area suggesting that improved management of honeybee has to be considered to solve problem of pests and predators, incidence of bad weather and application of agrochemicals.

Table 4. 1. Index rank of beekeepers response to perceived honeybee health and beekeeping constraints in Seka Chekorsa District

Problems	Relative degree of importance							Index	Rank
	1st	2nd	3rd	4th	5th	6th	7th		
Lack of knowledge and skill of honeybee pests and diseases control	35	42	25	16	13	1	-	0.150	4
Shortage of bee forage	4	41	43	11	14	1	-	0.149	5
Pests and predators	150	66	16	10	1	-	-	0.293	1
Poor or absence of hive inspection practices	7	29	13	8	4	5	-	0.045	7
Poor or absence of hive shading practices	20	35	15	26	2	6	2	0.099	6
Colony death	2	0	0	2	-	-	-	0.007	9
Bad weather (prolonged precipitation and wind)	0	31	23	65	73	10	-	0.180	2
Agro-chemicals	2	22	15	34	73	9	-	0.179	3
Shortage of improved hive types and other beekeeping equipments	0	0	3	5	6	19	3	0.021	8

Index = $\sum$ (6 *for rank 1 + 5* for rank 2 + 4* for rank 3 + 3* for rank 4 + 2* for rank 5 + 1* for rank 6) of specific beekeeping problem divided by = $\sum$ (6 *for rank 1 + 5* for rank 2 + 4* for rank 3 + 3* for rank 4 + 2* for rank 5 + 1* for rank 6) of all beekeeping problems

4.2. Honeybee Pests and Predators: A survey summary

Beekeepers in the study area listed presence of 10 (Table 4.2) different species of honeybee pests and predators. The most common honeybee pests and predators were mentioned to be *Aethina tumida* ((95.1 %), *Achrolla grisella* (65.7 %), different species of ants (51 %), Larger Hive Beetle (42.0 %), Mice (39.1 %), Bee Eater Birds (27.3 %), Honey badger (18.3 %), Bee Lice (10.2 %), Spiders (8.9 %) and Lizards (6.9 %). Existence of nearly all of these honeybee pests and predators have reportedly been existing in every beekeeping regions of the tropics and their importance have been reported by similar studies (Chala Kinati *et al*, 2012 and Tolera Kumsa and Dejene Takele, 2014) in the country. However,(Yetimwork Gebremeskel, 2015) has reported slightly lower frequencies for existence of different honeybee pests and predators (Ants =18 ; Wax Moth =15.5; Spider = 10.5; Honey Badger = 40; Lizard = 4.5; Snake = 3.5; and Birds = 8) for Eastern zone of Tigray as compared to finding in the current study. This could be due to a perceived lower importance of honeybee pests and predators in that particular area unlike what is in the current study. Therefore, looking at the proportion of respondents to existence of these honeybee pests and predators, it can be understood that the order of importance of honeybee pests and predators ;and the type of pests and predators existing , as have been reported by previous studies (Jatema & Abebe, 2015; Kajobe *et al*., 2009; Nuru Adgaba *et al*, 2014), is rather different based on location and the level of beekeeping.

Unfortunately, in this survey, none of the sample beekeepers (0%) had information on presence of any honeybee parasites and pathogens. This might be due to the fact that local beekeepers are not professionals and had no knowledge of symptom of different parasites and pathogens in their beekeeping activities. Therefore, to get relevant and clear information about honeybee pests in general and honeybee parasites and diseases in particular using local beekeepers perception, and for better management of honeybee health, intensive training is recommended to boost understanding of local beekeepers on nature of the enemies. The occurrence of some of honeybee pests like *Aethina tumida*, *Achrolla grisella* and different species of ants suggest detailed study should be undertaken to examine their impact on beekeeping and honeybee production performance where ever they are present.

Table 4. 2. Beekeepers response percentage on honeybee pests and predators status in Seka Chekorsa District

Pest species	Response percentage (%)	
	Present	Absent
Aethina tumida	233(95.1)	12(4.8)
Achrolla grisella	161(65.7)	84(34.3)
Ant spp.	125(51)	120(48.9)
Oplostomus fluginous	103(42.0)	142(57.9)
Braula coeca	25(10.2)	220(89.7)
Lizard spp.	17(6.9)	228(93.0)
Spider spp.	22(8.9)	223(91.0)
Mice spp.	96(39.1)	149(60.8)
Bee Eating Bird spp.	67(27.3)	178(72.6)
Honey badger spp.	45(18.3)	200(81.6)
Others pests and pathogens	0	245(100)

4.3. Locally Perceived Effect of Pests and Parasites on Beekeeping

Perception of local beekeepers was assessed towards negative impact of honeybee pests and predators on honeybee and beekeeping in the current study. Significantly higher proportion of beekeepers (90.2%) responded that honeybee pests and predators can negatively affect honeybees and beekeeping (Table 4.3). Like 61.6 % and 73.8 % of the sample beekeepers explained that honeybee pests and predators cause absconding and dwindling of honeybees respectively. However, about 10.2 % of the beekeepers viewed

that in some cases the presence of honeybee pests and predators might result in total death of honeybee. Indeed, the presence of some devastating tropical honeybee pests and predators (*Aethina tumida*, *Achrolla grisella* and *Varroa destructor*) can support the likelihood of total loss of honeybee colonies in local honeybee colonies. Although, few respondents agreed that honey production is constant (21.6 %) and increasing (13. 8 %), roughly half percent of respondents (45.7 %) explained honey production is decreased when hive is attacked by pests and predators (Table 4.3). Similar consequences of honeybee pest and predators attack to honeybees and beekeeping were reported to have negative association with honey production (Tolera Kumsa *et al*, 2014). This might be due lack of improved practices of beekeeping in the study area. This might be due to the fact that pest infestation can affect honeybees both physically and behaviorally. According to Ahmed, M.(2016), pests like *Aethina tumida* and *Achrolla grisella* can also feed on honey, bees wax and bee brood.

Table 4. 3. Perception of respondents on effect of honeybee pests, parasites and diseases on honeybees and honey production in Seka Chekorsa District

Variables	**Category**	**count**	**%**
Impact of pests and parasites on Honeybee	Yes	121	83.4
	No	24	16.6
Impact Type	Absconding	51	35.2
	Dwindling	81	55.9
	Death	13	9
Impact of pests and predators on Honey production	Decrease	112	77.2
	Constant	18	12.4
	Increase	15	10.3

4.4. Field and Laboratory Diagnosis of Pests and Pathogens

During this study, honeybee colonies (n=60) were seasonally monitored for presence of honeybee pests, parasites and pathogens. The sampled honeybee colonies were positive (Table 4.4) for two honeybee pests: *Aethina tumida* (Small Hive Beetle) and *Achrola grisella* (Lesser Wax Moth); two honeybee parasites: *Varroa destructor* and *Brauala coeca* (Bee Lice); and three honeybee pathogens: *Ascosporera api*s, *Nosema apis* and *Melpighaeamoeba mellificae.* None of the diagnosed honeybee colonies were positive for bacterial pathogens: Paeni*bacillus larvae* and *Mellisococcus plutonious*; fungal pathogen: *Aspergillus flavus*; and Tracheal mite: *Acarapis woodii* of honeybees. Indeed, this could not be taken as a concrete reason to report a total absence of these honeybee pests, parasites and diseases with the current research provided that they are rarely incident in African beekeeping. Therefore, it is a good reason to suggest that a more detailed study be conducted in the future to better confirm status of these honeybee parasites and pathogens to make this study complete.

In this study, honeybee pests, parasites and pathogens had showed uneven distribution across hive types (Table 4.4). *Aethina tumida* ,*Achrola grisella* and *Varroa destructor* were detected in traditional ,transitional and modern hives; whereas *Nosema apis* and *Aschosporeara apis* were only tested positive in transitional and modern hives ;*Melphigahaeamoeba mellificae* in modern hive only ;and *Braula coeca* in traditional and modern hives only (Table 4.4).In addition, the percentage distribution of all honeybee pests ,parasites and pathogens (Table 4.4) varied from 0% to 100% across hive types . Regardless of location, it is likely to suggest that this variation in distribution of the honeybee pests, parasites and pathogens among hive types might be due to factors like level of hive management, source of honeybee colonies and size of apiary. Sisay Fikru *et al*, (2015), indicated that the level of hive management and hive type can influence abundance honeybee pests and parasites.

Table 4. 4. Percentage distribution of honeybee pests, parasites and pathogens across hive types in Seka Chekorsa District

No.	Pests, parasites and pathogens	Hive types			%
		Traditional	Transitional	Modern	
1	*Atheina tumida*	**P**	**P**	**P**	100
2	*Achroilla grisella*	**P**	**P**	**P**	100
3	*Bacillus larvae*	**N**	**N**	**N**	0
4	*Bacillus pluton*	**N**	**N**	**N**	0
5	*Nosema apis*	**N**	**P**	**P**	66.7
6	*Melphigaeamoeba mellificae*	**P**	**N**	**P**	67.7
7	*Aschosporera apis*	**N**	**P**	**P**	66.7
8	*Aspergillus flavus*	**N**	**N**	**N**	0
9	*Varroa spp*	**P**	**P**	**P**	100
10	*Acarapis Woodii*	**N**	**N**	**N**	0
11	*Braula coeca*	**N**	**N**	**P**	33.3
12	*Ant spp*	**N**	**N**	**N**	0
13	Others	**N**	**N**	**N**	0

NB: P=Positive and N=Negative

4.5. Seasonal Dynamics of Prevailing Pests and Pathogens

Seasonal Prevalence of honeybee pests, *Aethina tumida* and *Achoirea gressella*; parasites, *Varroa destructor* and *Braula coeca*; and pathogens *Nosema apis*, *Melphighaeamoeba mellificae*, and *Ascosporerae apis* was depicted in figure 4.5.1. Among all the diagnosed honeybee colonies, in late autumn (November, 2015) and in mid spring (April, 2016) only *Varroa destructor* and *Aschosporeara apis* were tested positive. This report agrees with

(Budge *et al*, 2015) who found 16-35 % and 25.6 % of *varroa destructor* and *Aschospaera apis* detected in collapsing colonies during spring in United Kingdom.

Honeybee colonies were positive in winter (February, 2016) for *Aethena tumida* (60%); *Varroa destructor* (40%); and *Achrolla grisella* (40%). In this season, *Braula coeca, Melpighamoeba mellificae* and *Nosema apis* were each detected positive with considerable prevalence (20%). In early Summer (June, 2016), apiaries were positive for *Aethina tumida,Achrolla grisella* and *Varroa destructor* with 40% prevalence. *Nosema apis, Melphighaeamoeba mellificae* and *Aschosporera apis* were positive with 20% prevalence. Since the beginning of 2001, the presence of Ascosphaera apis has been confirmed in Ethiopia. Out of 276 inspected colonies, 48 (17.4%) were found tainted with chalk brood fungus (Desalegn Begna, 2001).

From this study, it can be concluded that the seasonal prevalence for *Aethina tumida* (60%), *Achrolla grisella* (40%), *Varroa destructor* (40%); and *Nosema apis*, and *Braula Coeca 20%* peaked during winter across apiaries. Unlike other regions, existence of Varroa mite during this season might be due to the fact that in the study area there are, relatively sufficient flowering plants that provide surplus feed material (pollen) for honeybee that help continuous brooding activity of honeybee colonies. The population growth of varroa is affected by the amount and type of brood, and the post capping period of the brood cell (Fabiana *et al* 2014).

In Ethiopia, *Aethina tumida* was recognized as local honey bee parasite in two honey flow seasons in the south and south-west parts, in 37.5 % of colony bee with prevalence rate of 10 % Amssalu Bezabeh and Desalegn Begna (2008) reviewed in (Haylegebriel Tesfay, 2014) .The pest was reported in the maize and coffee growing regions of Oromia regional state; Jimma, Illu Abbabora, Horo Guduru, Wollega, East Wollega and Western Showa. The highest infestation and distribution of the pest was also reported in Jimma (60%) and Horo Guduru (83.9%). Similarly it was reported in six districts: Mega, Moyale, Teltele, Konso, Key-Afer and Segen in the South and Southern parts of Ethiopia with prevalence rate infection ranges from 21% in Konso to 66% in Teltele.

The seasonal dynamics of honeybee pests, *Aethina tumida*,*Achrolla grisella* and *Braula coeca*; parasites, *Varroa destructor* ; and pathogens , *Aschosporera apis, Nosema Apis and Melphighaeamoeba mellificae* were shown to be different across seasons. The prevalence of *Varroa destructor* was relatively higher during autumn and spring seasons peaking at April and lower during pollen dearth seasons of the year (winter and summer).Different authors have reported that seasonal variation is directly correlated with the growth *Varroa destructor* population (Okosun, 2013;Allsopp, 2006 and Kamran, 2001).On the contrary, honeybee pests, *Aethina tumida* and *Achrola grisella* were shown to be severely found in honeybee colonies during pollen dearth seasons of the year. This report agrees with (Tarpy. *et al*, 1998 ;Bronisław *et a*l, 2000; Etsay., 2015) who have reported that 84% of the modern hives with colonies had visible larval stage of wax moth during pollen dearth seasons of the year in different places .In this study, the prevalence of *Nosema apis, Melphighaeamoeba mellificae* and *Aschosporera apis* and *Braula coeca* were slightly lower and seemingly less affected by seasonal variation. Generally, the highest severity level was attained during dry seasons for those honeybee pests and parasites. The fact that these pests have higher prevalence than the others might be due to inherent/opportunistic ability of the honeybee pests to infest honeybee colonies over the others. Or else, it can be determined by a pest specific resistance mechanism of honeybee colonies. Future research and beekeeping development activities should focus on mechanisms of controlling of these honeybee pests and parasites for better production and productivity of honeybees in the study area.

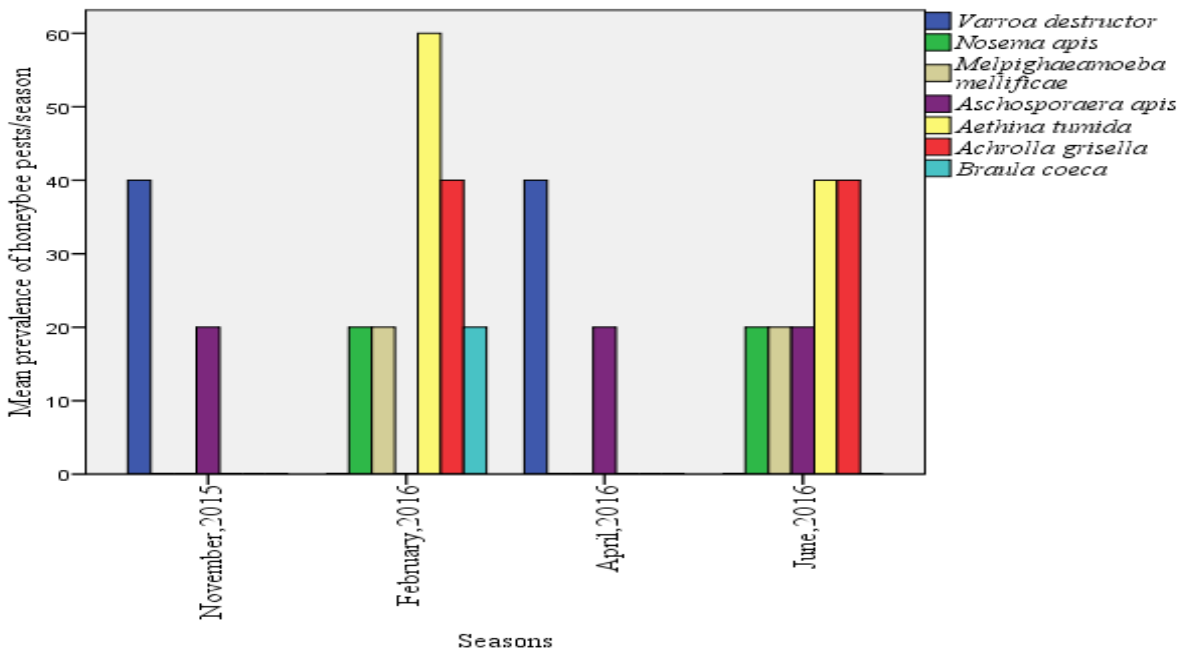

Figure 4.1. Seasonal prevalence of honeybee pests, parasites and pathogens in Seka Chekorsa District

4.6. Major Honeybee Pests' Prevalence Association to Some Risk Factors

In this study, showing relatively elevated prevalence, *Achrola grisella*, *Aethina tumida and Varroa destructor* were described as the main honeybee pests (Table 4.4) The pests' prevalence has been studied in association with some potential risk factors: Altitude, Season, Hive types and strength of exposed honeybee colonies (Tables 4.5, 4.6 and 4.7 respectively). It was found that altitude, season, hive type and strength of honeybee colony are highly significantly ($P<0.001$) associated with prevalence of *Achrola grisella* (Table 4.5). Pedro da S. *et al* (2015) showed that conditions like lower altitude and pollen dearth season can favor infestation level and reproduction of *Achrolla grisella* in honeybees.

Table 4. 5. Association of major risk factors to prevalence of *Achrolla grisella* in Seka Chekorsa District

Variables	Categories	N	Count	Positive Prevalence (%)	X^2	df	P-value
Altitude	Highland	60	6	23.1	1.459	2	
	Midland		6	23.1			
	Lowland		14	53.8			0.00**
Season	November,2015	60	8	30.8	1.473	3	
	February,2016		5	19.2			
	April,2016		6	23.1			
	June,2016		7	26.9			0.00**
Hive types	Traditional		6	23.1	1.502	2	
	Transitional	60	11	42.3			
	Frame		9	34.6			0.00**
Honeybee population	Strong		8	30.8	1.477	2	
	Moderate	60	8	30.8			
	Weak		10	38.5			0.00**

NB: **= highly significant

Prevalence of *Aethina tumida* was significantly (P<0.001) associated with altitude and season (Table 4.6). In this study, there was not significant variation for *Aethina tumida* as occurred in honeybee colonies of different categories (weak, moderate and strong) of strength which were established in different hive types. This might be due to high tendency of pest infestation resistance of local honeybees under normal circumstances. This can also be supported by the fact that there is availability of adequate bee forage plant species in the study area irrespective of season and geographical difference on which honeybees can feed and therefore, remain strong on relative basis. It was indicated that

infestation of *Aethina tumida* is more pronounced in honeybees of temperate origin than in tropical species (Allsop M,2006).

Table 4. 6. Association of Major Risk Factors to Prevalence of *Aethina tumida* in Seka Chekorsa District

Variable	Categories	N	Count	Positive prevalence (%)	X^2	df.	P-value
Altitude	Highland	60	9	15	3.95	2	
	Midland		11	18.3			
	Lowland		14	23.3			0.00**
Season	November,2015	60	13	24.1	1.473	3	
	February,2016		5	19.2			
	April,2016		15	27.8			
	June,2016		13	24.1			0.00**
Hive types	Traditional	60	16	26.6	8.502	2	
	Transitional		20	37			
	Frame		18	33.3			0.493
Honeybee population	Strong	60	18	33.3	14.320	2	
	Moderate		14	25.9			
	Weak		22	40.7			0.711

Varroa destructor prevalence was highly significantly (P<0.001) associated with altitude, season and honeybee colony strength (Table 4.7). According to Muli E. *et al* (2015), level of varroa is strongly impacted by elevation/geographic region and seasonal variation, suggesting that environmental factors modulate varroa infestation rates. It was also indicated that the severity of varroasis may vary according to the subspecies of honeybees, climate conditions, the nectar flow, the development period of the brood and the ability to detect and remove the mite (Pedro S. *et al*, 2015).

In this study, there was not significant variation for *Varroa destructor* as occurred in honeybee colonies of different categories (weak, moderate and strong) of strength which were established in different hive types. The fact that lack of honeybee forage and pollen

sources is not as such a serious problem in the study area can support this result that honeybee colonies may relatively remain strong throughout the seasons resisting honeybee pests and parasites infestation. Even though, there is variation in honeybee colony strength based on subjective judgment, honeybee colonies remain with highest strength to resist pests and parasites infestation.

Table 4. 7. Association of major risk factors to prevalence of *Varroa destructor* in Seka Chekorsa District

Variables	Variable categories	N	Count	Positive prevalence (%)	X^2	df	P-value
Altitude	Highland	60	8	19	1.441	2	
	Midland		23	54.8			
	Lowland		11	26.2			0.00**
Season	November,2015	60	11	26.2	1.661	3	
	February,2016		6	14.3			
	April,2016		13	31			
	June,2016		12	28.6			0.00**
Hive types	Traditional	60	14	33.3	15.023	2	
	Transitional		16	38.1			
	Frame		12	28.6			0.923
Honeybee population	Strong	60	18	42.9	15.641	2	
	Moderate		12	28.6			
	Weak		12	28.6			0.00**

4.7. Abundance of *Varroa destructor* in Different Hive Types

The intensity (Number of *varroa destructor*/100 bees) was compared in three hive types: Traditional, Transitional and Frame (Table 4.8).The result showed that, there is no

significant variation (F = 0.076, P>0.05) in abundance/intensity of the mite among hive types. The overall mean *Varroa destructor* load was shown to be 1.13+.24, remaining well below maximum of mites/100 bees, the standard management range which is 2 mites/100 bees (OIE, 2011) indicating that honeybees are perhaps not affected by infestation of the mite in the studied honeybee colonies. This might be due to the high hygienic behavior and tendency of resisting the mite of the local honeybees.

Table 4. 8. Mean comparison of *Varroa destructor* load in different hive types in Seka Cekorsa District.

Hive types	N	Mean+SE	95% CI			
			Lower Bound	Upper Bound	F	Sig.
Traditional	20	1.30+.48	.82	1.78	. 076	>0.05
Transitional	20	1.20+.43	.77	1.63		
Frame	20	.90+.34	.56	1.24		
Total	60	1.13+.24	1.06	1.37		

CI = Confidence interval; F = F-value; Sig. = significance value; N = number of sample; SE= Standard error for mean

4.8. Correlation of *Varroa destructor* Load with Hive Variables

In the current study, correlations of *Varroa destructor* intensity with some hive variables: age of honeybee colonies, amount of honey produced, and honeybee population was examined (Table 4.9).The result indicated that *Varroa destructor* intensity is not significantly (P>0.05) correlated with other hive variables except honeybee population strength (P<0.05). This has showed that *Varroa destructor* infestation level is not correlated with age of honeybee colonies and amount of honey produced. However, there is direct relationship between *Varroa destructor* infestation level and worker bee population where number of *Varroa destructor*/bee increases and so does worker honeybee population. This can show that the frequent brood rearing of honeybee colony can favor reproduction of honeybee *Varroa destructor* in hive while a colony build up can help keep the mites' load below economic threshold level. This result can also be

supported by recent studies that mentioned varroa mite infestation could not bring significant effect on local honeybees (Elliud Muli, Harland Patch, 2014; Guesh Godfey, 2015).However, contrastingly, it is suggestive to think that the coupling of varroa destructor infestation in honeybees with another devastating pests like *Achrolla grisella* and *Athina thumida* as indicated in this study, may compromise resistance of honeybees and impact honey production. Therefore, it is recommended that future research should focus on ways to quantify effect of interaction of varroa mite with these potentially devastating honeybee pests.

Table 4. 9. Correlation of *Varroa destructor* load with some major hive variables in Seka Chekorsa District

Hive variables		AHC (in years)	HPr. (in Kg)	NVM / 100 bees	HPo.
AHC (in years)	R	1.000	.010	.013	.027
	P		.922	.895	.779
HPr. (in Kg)	R		1.000	.181	.064
	P			.059	.485
NVM / 100 bees	R			1.000	0.781
	P				.027*
HPo.	R				1.000
	P				

R is Pearson Correlation coefficient; P is P-value; Kg is kilogram; HPr is honey production in kilogram; NVM is number of varroa mite/100 bees; and HPo is honeybee population; * is correlation is significant at p=0.05

4.9. *Varroa destructor* Enhances Prevalence of *Aethina tumida* and *Achrolla grisella* in honeybee

In this study, prevalence of *Achrolla grisella* and *Aethina tumida* was examined (figure 4.9) as combined with *Varroa destructor* in hive environment. it was showed that the prevalence of *Aethina tumida* and *Achrolla grisella* have been positively enhanced in *Varroa destructor* infested hives (OR = 2.6; X^2 = 5.53; lower limit =.78; upper limit= 8.59; P <0.05) as compared to the mite free hives (Figure.4.9).The result has also demonstrated that comparatively the prevalence of *Aethina tumida* (40.27%) is much more greater than that of *Achrolla grisella* (18.58%). Studies have suggested that honeybee pests like *Aethina tumida*, *Achrolla grisella* and Varroa destructor are extensively blamed

for massive loss of honeybees globally (Christian *et al*, 2015; Wolfgang and Ute, 2016). Recent study has showed that honeybee colony infection by combined pests and parasites can deteriorate the potency of honeybees than single infection (Ahmed and Abdalla El-niweiri, 2016).From this study, it is evident to advise that a detailed research should consider multiple infection in honeybee colonies to broadly quantify the effect of this on honeybees and hive products.

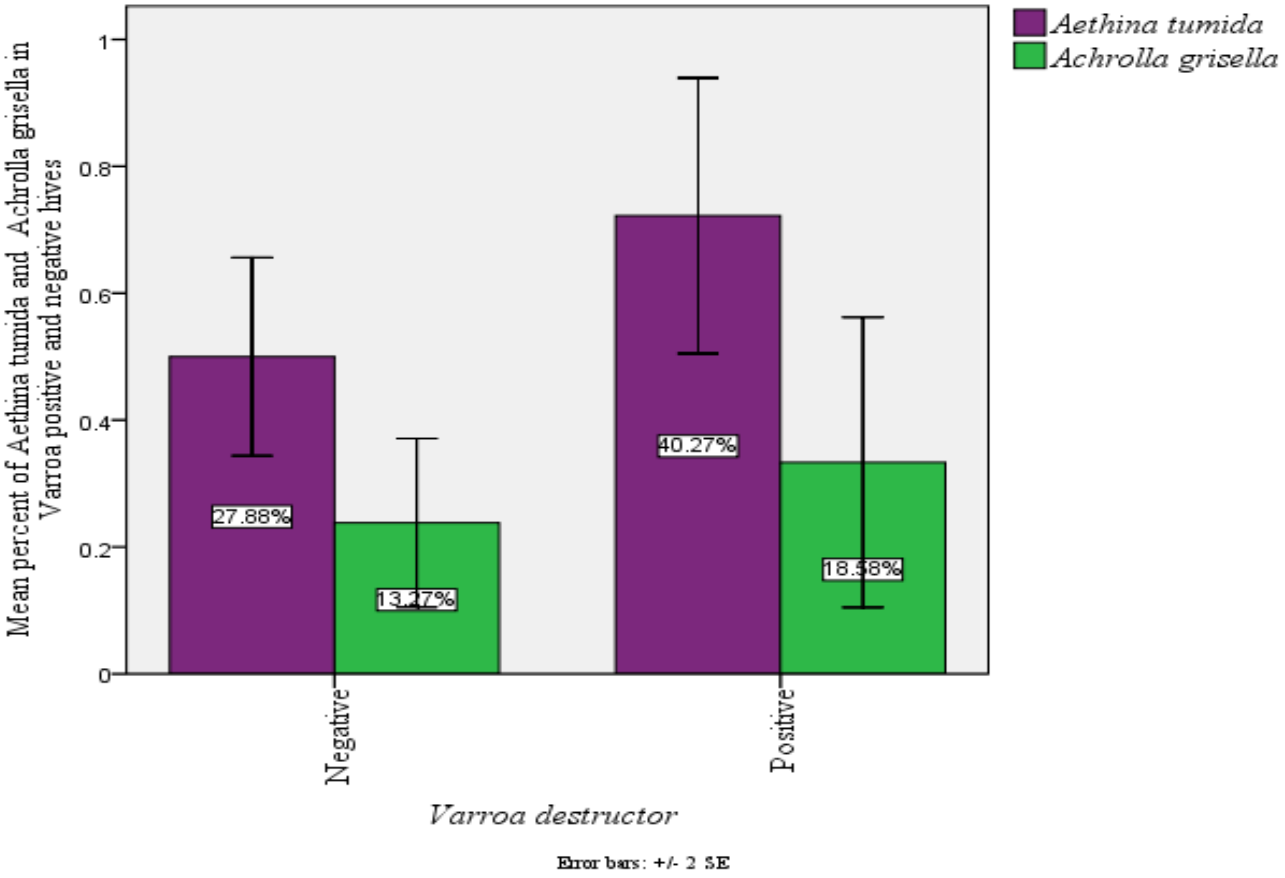

Figure 4. 9. Mean+SE percent (rate) comparison of prevalence of *Aethina tumida* and *Achrolla grisella* in Varroa destructor positive and *Varroa destructor* negative honeybee colonies in Seka Chekorsa

NB: 'OR' is odd ratio of prevalence of *Aethina tumida* and *Achrolla grisella* in presence (positive) and absence (negative) states of hives with *Varroa destructor;* 'X^{2}' is a Pearson chi-square coefficient of association; and 'P' is a P-value.

4.10. Comparison of Honeybee Pests versus Hive population and Honey Production

Comparison of honeybee population growth and corresponding honeybee production trends in pest free and pest affected sample hive types (Traditional, Transitional and Frame) in each season has been given in Figure.4.10 and Figure.4.11 respectively. The result shows that, regardless of hive types, there was noticeable decrease for both honeybee population growth (Figure.4.10) and honey production (Figure.4.11) between honeybee pest free and pest affected honeybee colonies along the seasons. However, normal seasonal pattern in honeybee population growth and honey production, in both pest free and pest affected honeybee colonies, was showed to not have been changing. At the same time, it was revealed that there had been honey harvest throughout all the seasons with considerable max out in autumn (November) and spring (April) seasons in both pest free and pest affected honeybee colonies. Therefore, this could be taken as basic evidence to infer that the pest influx in honeybee colony may not change honey production and honeybee population build up patterns; but reduce honeybee population and honey production. It has been forecasted that infections with honeybee pests and pathogens are known to cause reduced lifespan of individual bees, reduced performance of colonies, and increased winter mortality (Fries, 2015).

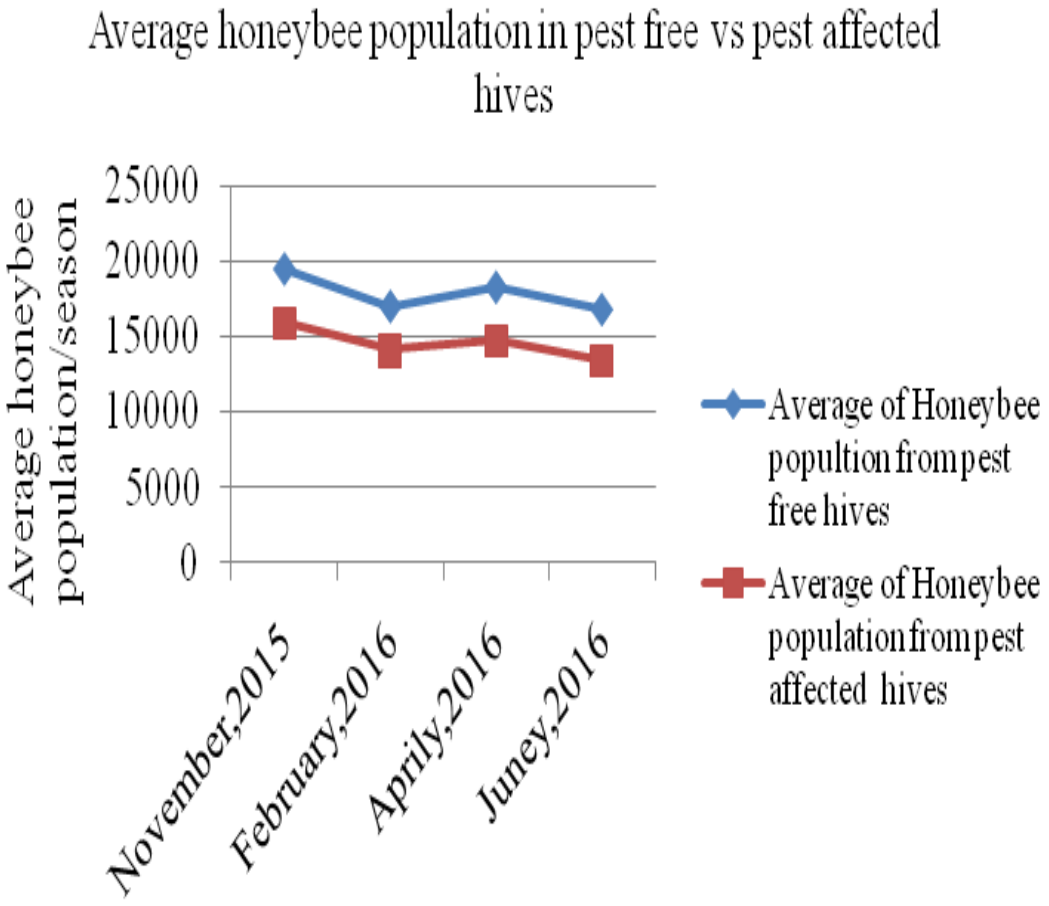

Figure 4. 10. Honeybee population in honeybee pest free and honeybee pest infested hives in Seka Chekorsa District

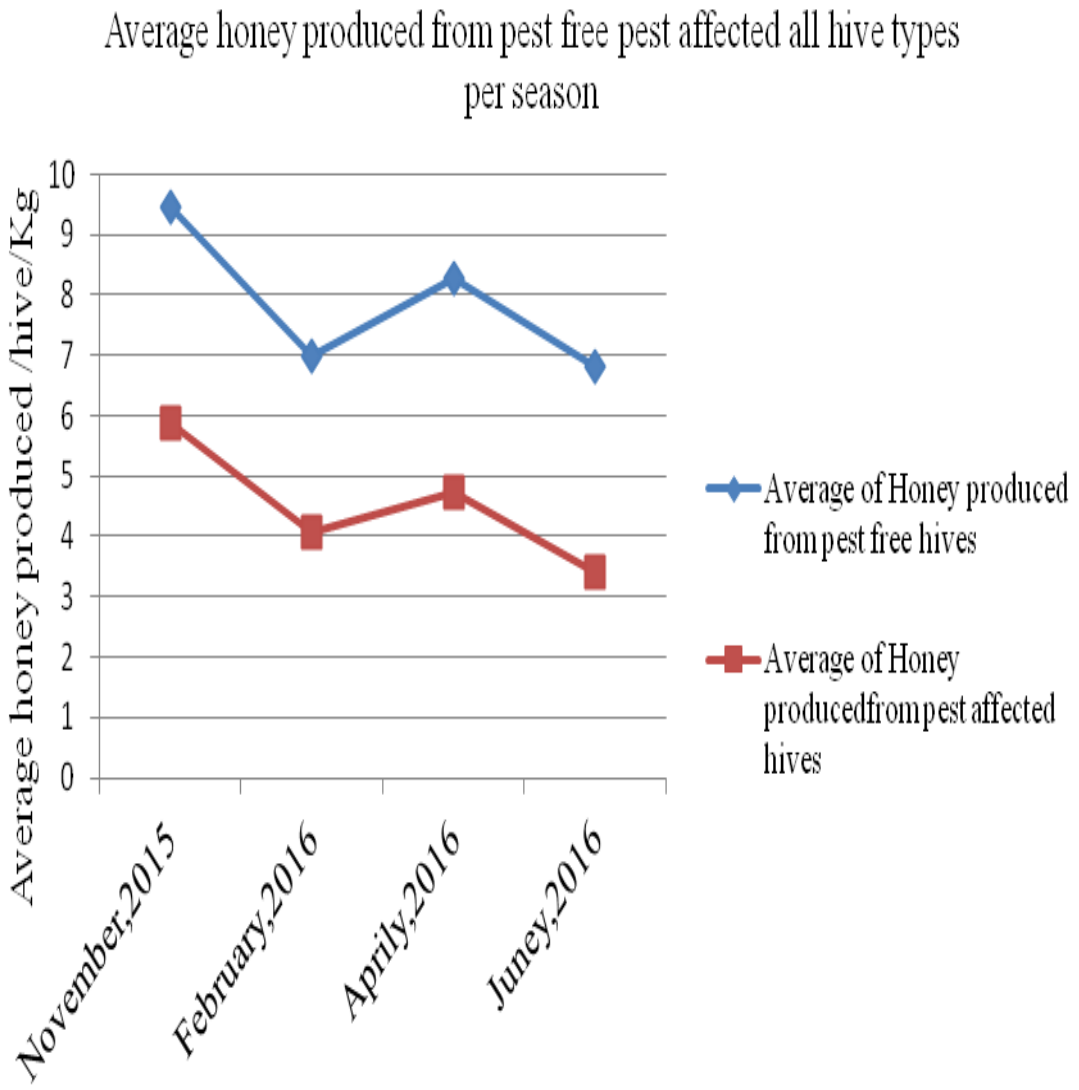

Figure 4. 11. Honey produced in honeybee pest free and honeybee pest infested hives in Seka Chekorsa District

CHAPTER 5. CONCLUSION AND RECOMMENDATION

5.1. Conclusions

With this study, it is known that honeybee health constraints in Seka Chekorsa district are diverse. The major constraints identified are: honeybee pests and pathogens, prevailing bad weather, poor or absence of seasonal hive management; application of agrochemicals. Particularly, honeybee pests and parasites are mentioned as the most problems causing dwindling and absconding of honeybee colonies. The most commonest honeybee pests in the district are identified to be *Aethina tumida,Achrolla grisella* and *Varroa destructor*.

It was recognized that a tradeoff of pests, pathogens and parasites across season of the year is high in the study area. Moreover, the prevalence of identified honeybee pests is significantly associated with agro ecological difference and seasonal variation. The most prevalent honeybee pests during dry seasons are *Aethina tumida* and *Achrolla grisella*, whereas the prevalence of varroa mite is uniquely enhanced during active seasons. Even though *Varroa destructor* had positive association with honeybee population in the current study, the infestation level of the pest was found to be rather below the recommended injury level. However, the combination of the mite with other potentially devastating honeybee pests like *Aethina tumida*, *Achroilla grisella* and others was shown to be a risk for life of honeybees and impact honey production.

In this study, some honeybee pests, parasites and pathogens whose incidences have generally been low could not be detected to exist. Even though the study of this kind is quick and better to diagnose a number of honeybee pests, parasites and pathogens, it is always hardly possible to adequately test presence of some pests both in time and space. Luckily, this study has explored and documented epidemiological status of major honeybee pests and parasites in the study area and can provide basic information about the honeybee pests, parasites and pathogens existing in the study area.

5.2. Recommendations

From the above conclusion, the following recommendations are forwarded for future research and development of beekeeping and honeybee health.

- Since there are complex beekeeping problems and some devastating honeybee pests, in the study area, frequent training is required for beekeepers on improved beekeeping practices with special emphasis on control mechanisms and management of honeybee pests.

- State of multiple infestations of some novel honeybee pests is known to be a risk for honeybees and therefore, it is suggested to further examine their effects on honeybee population dynamics and honey production under controlled environment.

- Developing a holistic controlling mechanism that target in hive multiple infestation of honeybee pests is particularly important.

- It is suggested that a pest specific and wide-ranging study be conducted in the future to declare absence of some of honeybee parasites (*Acrapis woodii*) and pathogens (bacterial and fungal) in the study area.

- The fact that there was found a high tradeoff of honeybee pests dynamics by season and agro ecologies suggest any future beekeeping research and development activities have to take in to consideration these factors in the study area.

- A wider study is also recommended to generate further information on distribution of honeybee pests along with other probable risk factors like natural and human induced within and out of area mobility of honeybees.

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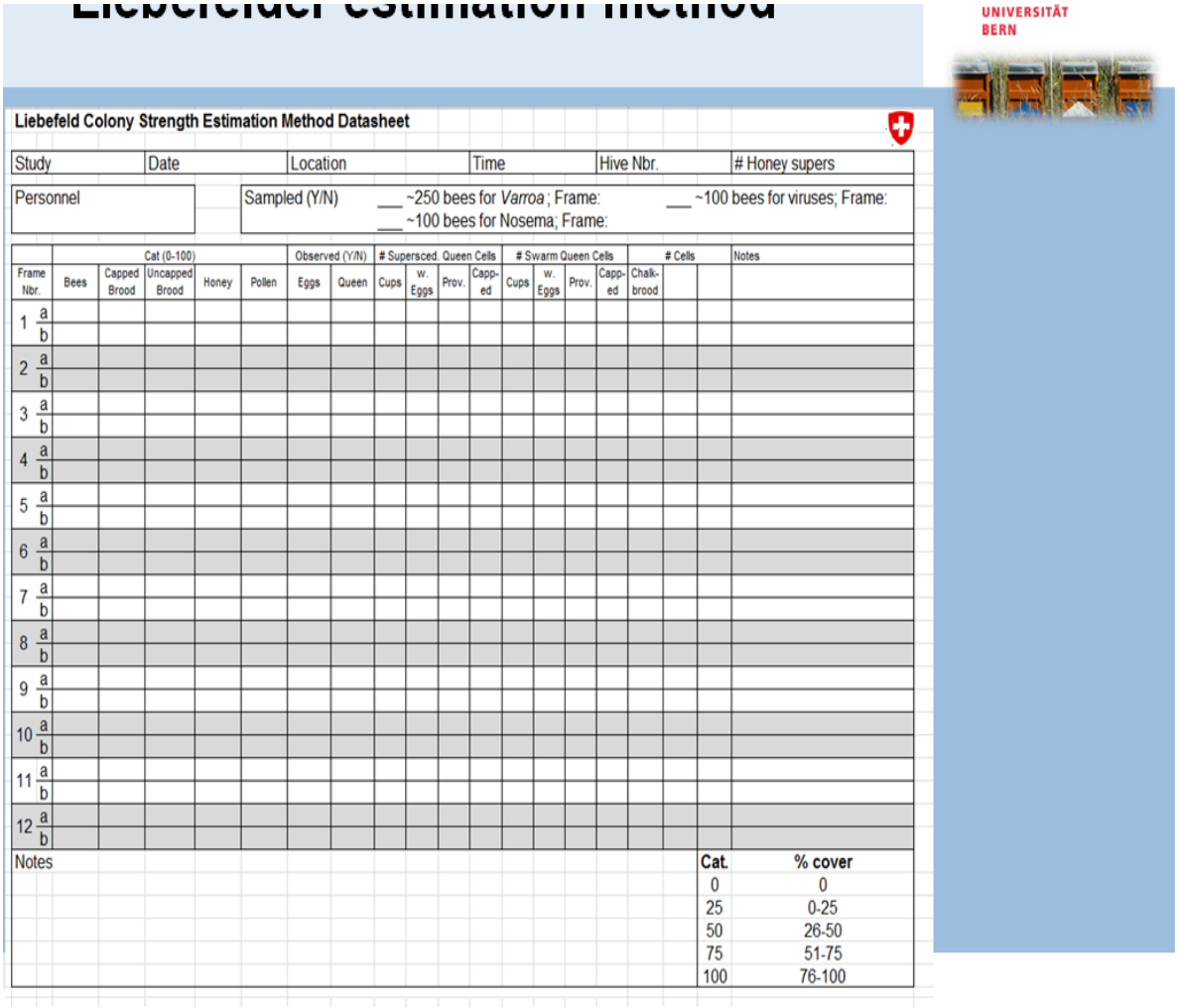
7. APPENDICES

Appendix Table 1 Honeybee pests, parasites and diseases prevalence calculation procedures in hives and apiaries

Pest or pathogen prevalence = Number of bees sample positive for an specific pest or pathogen /the total number of bees sampled*100

Appendix Table 2 Lebefielder honeybee colony strength estimation data sheet



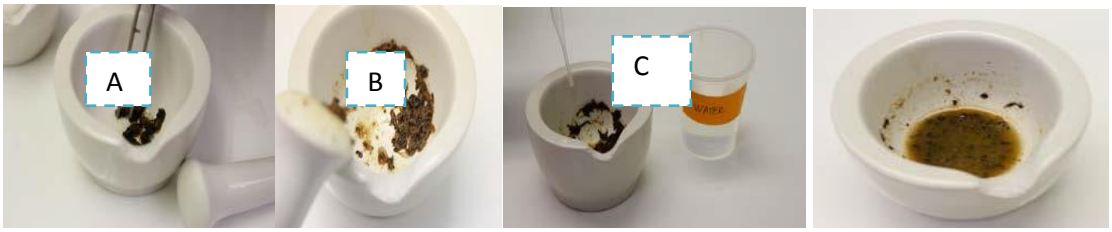
Liebefeld Colony Strength Estimation Method Datasheet

Study	Date	Location	Time	Hive Nbr.	# Honey supers
Personnel	Sampled (Y/N) ___ ~250 bees for *Varroa*; Frame: ___ ~100 bees for viruses; Frame: ___ ~100 bees for Nosema; Frame:				

	Cat (0-100)					Observed (Y/N)		# Supersced. Queen Cells				# Swarm Queen Cells				# Cells			Notes
Frame Nbr.	Bees	Capped Brood	Uncapped Brood	Honey	Pollen	Eggs	Queen	Cups	w. Eggs	Prov.	Capp-ed	Cups	w. Eggs	Prov.	Capp-ed	Chalk-brood			
1 a																			
1 b																			
2 a																			
2 b																			
3 a																			
3 b																			
4 a																			
4 b																			
5 a																			
5 b																			
6 a																			
6 b																			
7 a																			
7 b																			
8 a																			
8 b																			
9 a																			
9 b																			
10 a																			
10 b																			
11 a																			
11 b																			
12 a																			
12 b																			

Notes

Cat.	% cover
0	0
25	0-25
50	26-50
75	51-75
100	76-100

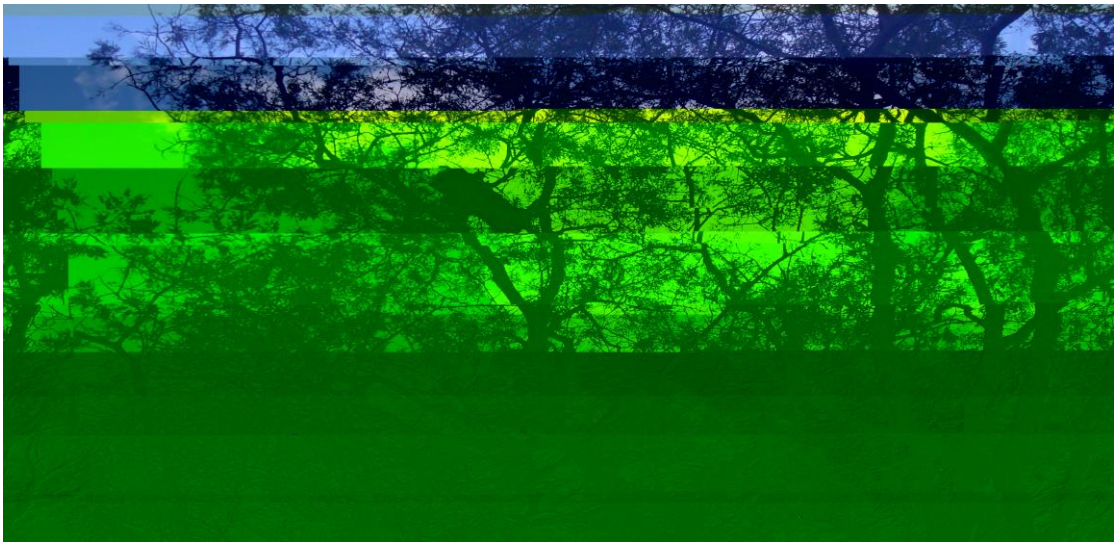


Appendix Picture 1 Smear preparation of Nosema and Amoeba diseases of honeybee

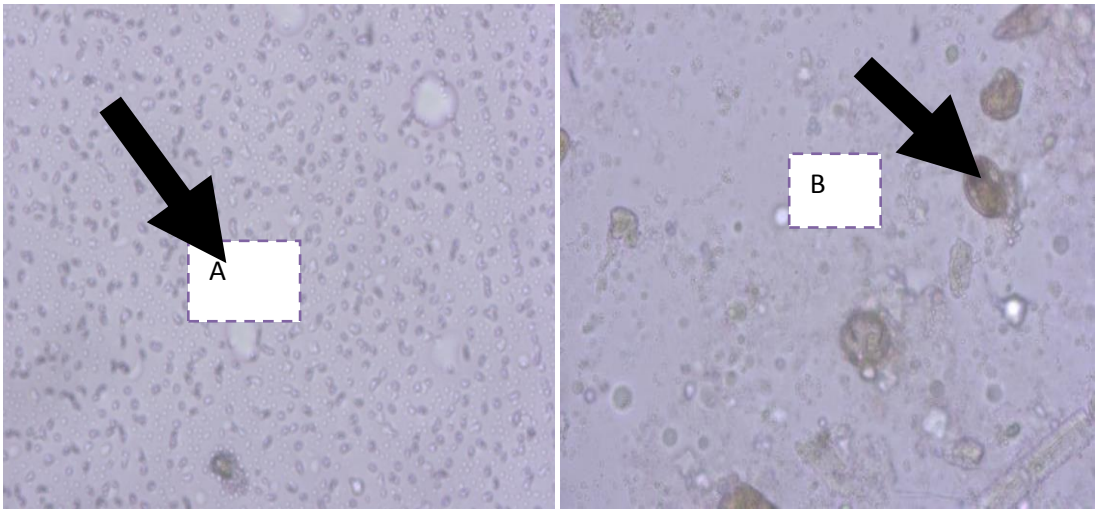
(A) All materials needed for spore counting using a plastic bag or a mortar and pestle. (**B**) Ten bees were put into a plastic bag and then crushed using a rolling pin. (**C**) Bees were mashed until all major body parts were crushed. Neema Syovata, 2007.

Appendix Picture 2 Photo of profile of transitional beekeeping practice in Seka Chekorsa District captured during winter of 2015

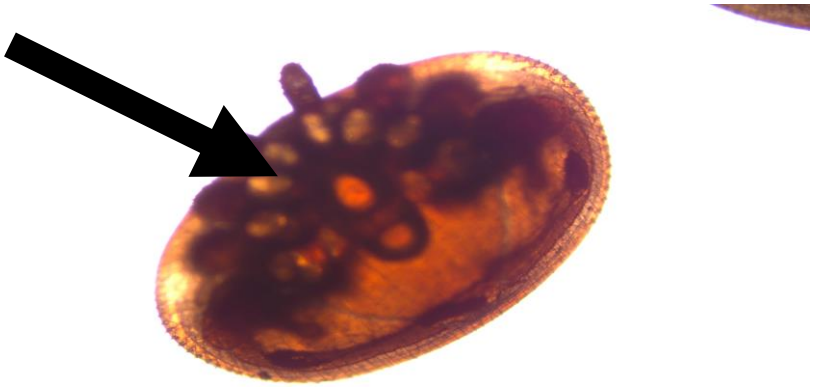
Appendix Picture 3 Photo profiles of modern beekeeping practices in Seka Chekorsa District captured during winter of 2015



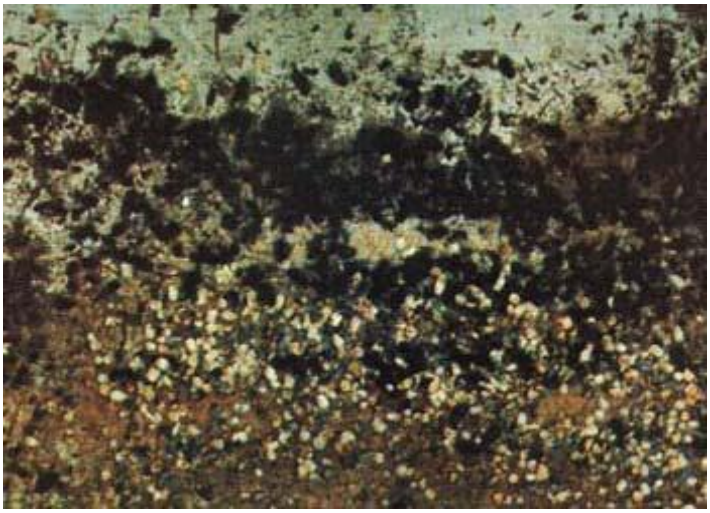
Appendix Picture 4 A multilayer bee forage potential natural vegetation and coffee forest photo captured in Seka Chekorsa District during winter 2015.



Appendix Picture 5 Zeiss imaging camera result for Nosema apis spores (A) and spore balls (B) viewed under digital microscope (compound) in sample honeybee colonies taken from Seka Chekorsa district (Summer, 2016)



Appendix Picture 6 Zeiss imaging camera result for ventral view of adult varroa mite under digital microscope (compound) as detected in honeybee colonies sampled from Seka Chekorsa district (Summer, 2016)



Appendix Picture 7 Zeiss imaging camera result for *Ascospaera apis* mummies viewed under digital microscope (compound) in sampled honeybee colonies from Seka Chekorsa district (summer, 2016)

Appendix Picture 8 Photo of *Achrolla grisella* caught during winter, 2016 in sample honeybee colonies in Seka Chekorsa district

BIOGRAPHY

The author, Desta Abi was born in a place called Jido Kombolcha, which is located in East Shoa, Oromia Regional State, Ethiopia on 10th of February, 1983. At 11th years of his age, he started his formal school and studied his Elementary and junior education at Jido Elementary school. In 2001, he passed the grade Eight National Exam for Joining Ethiopian High School Education. Then, he moved to a town called Ziway/Batu where he joined Ziway Senior secondary school for his high school (9-10) and Preparatory (11-12) education from September 2002 to June 2005. On 23rd of December, 2005, he joined Ambo University the then Ambo College of Agriculture and Veterinary Medicine and graduated with Bachelor of Science degree in Applied Biology on 10th of July 2008. The author was employed by Oromia Agricultural Research Institute, Adami Tulu Agricultural Research Center to work as Bee Researcher in Department of Apiculture on February 16, 2010. He had served the institute until he joined Bahir Dar University College of Agriculture and Environmental Sciences, School of Graduate Studies to pursue Degree of Master of Science in Apiculture on October, 2014.