Journal of Environment and Earth Science
ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Vol.7, No.5, 2017 

# Correlation between Pest Abundance and Prevalence of Honeybee Pathogens at Selected Apiaries in Kenya, 2013/2014

Juliette Rose Ongus[*1,2] Janet Irungu [2] Suresh Raina [2]
1.Pan African University Institute for Basic Sciences, Technology and Innovation (PAUSTI). P.O. Box 62000, 00200 Nairobi
2.International Centre of Insect Physiology and Ecology (icipe), Duduville Campus, Kasarani, off Thika Road. P.O. Box 30772-00100 Nairobi, Kenya

**Abstract**
Routine screening for pathogens plays an invaluable role in the detection of diseases in pre-clinical stages and prevention of losses. The present study was part of a larger surveillance effort to identify the determinants of African honeybee health, and particularly, to quantify honeybee pest abundance and to determine the prevalence of pathogens across Kenya, where 161 colonies from 32 apiaries were examined. From each colony, 20 individuals of foragers, nurse bees, worker pupae and drone pupae were sampled separately. These were organized as 30 foragers, 32 nurse bees, 28 worker pupae and 10 drone pupae pools. The pest abundance was determined by counting the number per colony and their occurrence in each apiary was computed by calculating the arithmetic mean values. Honeybee diseases were detected by PCR and the prevalence per apiary was computed. *Varroa destructor* mites and *Aethina tumida* were the most abundant pests. There was near universal presence of *Varroa* and to a smaller extent *A. tumida* in majority of the locations visited. Overall, *Varroa destructor* virus 1, deformed wing virus and black queen cell virus were detected with the highest prevalence in the apiaries at 66%, 69% and 69% respectively. Other pathogens detected were Sacbrood virus, Israeli acute paralysis virus, Acute bee paralysis virus and *Nosema ceranae* at 28%, 22%, 19% and 13% respectively. Spearman Ranked Correlation between the mean pest count and the specific pathogen prevalence revealed an overall positive though non-significant correlation between the pests and most pathogens. The exceptional finding of this study was the identification of a key association between the abundance of *A. tumida* and *Nosema* infection, with a statistically significant positive correlation (R = 0.89803; P = 0.01507). Further studies will be required to understand the nature of this association with the aim of unravelling if the *A. tumida* has a biological role in *Nosema* transmission.
**Keywords:** Aethina tumida, Nosema, DWV, VDV 1, BQCV

## 1. Introduction

Honeybees, *Apis mellifera*, are essential pollinators of wild and cultivated plants and are vital to food production and biodiversity. Pollination by honeybees accounts for approximately US$3.2 million added crop value in Kakamega, western Kenya, which is almost 40% of the annual market value (Kasina *et al.* 2009). These crops include fruits (such as mangos, guava, avocados, passion fruit papaya and tomatoes), vegetables (such as onions, beans, carrots coriander and cabbage), nuts (such as macadamia nuts) and seeds (like sunflower and sesame). The added crop value is expressed as increased yield and quality achieved. The value of honeybee pollination worldwide exceeds $215 billion (Gallai *et al.* 2009). It is estimated that around one third of the food consumed by humans is produced from bee pollinated plant life (Klein *et al.* 2007). Honeybees are important emerging livestock in many African countries, and an increasing number of small-scale farmers are adopting apiculture to supplement their livelihoods.

The diversity of wild honeybees has decreased in many regions of Western Europe, mostly due to habitat destruction (Biesmeijer *et al.*; Fitzpatrick *et al.* 2007) and many managed honeybee populations are infected by diseases (Graystock *et al.* 2016). Additionally, populations of wild pollinators are declining in several regions in the world (Kluser & Peduzzi 2007; Potts *et al.* 2010), raising concern of a potential global pollination crisis that could threaten our food supply (Withgott 1999; Kremen & Ricketts 2000; Richards 2001; Westerkamp & Gottsberger 2002; Steffan-Dewenter *et al.* 2005). Epidemiological data indicate that honeybee pathogens have spread worldwide (OIE 2012) and infected colonies may experience reduced size and sudden losses. In the USA, honeybees have been reported to experience colony collapse due to a combination of mostly pathogens and parasites and other harmful environmental factors (vanEngelsdorp *et al.* 2007). Among the parasites accountable for colony losses is the invasive ectoparasitic mite *Varroa destructor* which poses a threat to honeybees' health directly by sucking bee haemolymph and indirectly by injecting the bee with pathogens resulting in diseases such as deformed wing virus eventually leading to loss or weakened colonies.

In contrast to many countries in different regions of the world, so far there is no evidence to show that African honeybees have experienced the devastating effects of *V. destructor* and honeybee pathogens (Muli *et al.*; Strauss et al., 2013). There is also very limited continental data (Mumoki *et al.* 2014) on the epidemiological status of diseases among populations of African honeybees due to the near absence of routine screening (Pirk *et*

*al.* 2016). Routine screening for pathogens plays an invaluable role in the detection of diseases in pre-clinical stages and prevention of losses (Ryba *et al.* 2012). Standards set by the World Organisation for Animal Health (OIE) are recognised internationally as a basis for declaring a country or region free from disease and maintaining disease free status therefore, any successful surveillance program should meet OIE criteria (OIE 2012).

Honeybees being social insects live in densely crowded populations with a high contact rate between colony members related to feeding and chemical communication. This presents many opportunities for disease transmission (Chen *et al.* 2006a). Generally, disease transmission can occur either or both horizontally and vertically through multiple routes in social organisms. In horizontal transmission, pathogens can either be transmitted directly, for example, via air-borne infection, food-borne infection, and venereal (sexual) infection, or indirectly by an intermediate biological host, like a vector, which acquires and transmits the pathogen from one individual to another (Chen *et al.* 2006a). Horizontal transmission of honeybee viruses by the parasitic mite *V. destructor* has been demonstrated experimentally by Bowen-Walker *et al.* (1999). The mite acted as a vector to transmit the virus to uninfected bees (Chen *et al.* 2006b).

*Varroa* mites are the most serious problem for honeybees, and are the leading cause of colony death in the Western honeybee. The mites infest and feed on the blood of both adult and immature stages of bees (Frazier 2011). It is through this feeding that the mite is able to acquire and transmit viral infections among the bees (de Figueiró Santos *et al.* 2016). *Varroa* acts as a vector of viruses, propelling an increased need to monitor virus prevalence (Traynor *et al.* 2016). When infestation levels are high, the mites cause extreme damage and death to honeybee colonies.

The small hive beetle (*Aethina tumida*) is a native honeybee pest in sub-Saharan Africa (Cuthbertson *et al.* 2013; Neumann *et al.* 2016). In this region this species does not inflict severe damage on strong colonies since the bees have developed strategies to combat them. Adult female beetles lay eggs near or on combs within colonies, and the larvae tunnel through wax combs eating mostly pollen, but also honey, bee eggs and larvae (Neumann *et al.* 2016). They can cause considerable damage to new combs. The beetles defecate in the honey, causing it to ferment, froth and stink like rotten oranges (Frazier 2011). To complete their development, the larvae must crawl out of the colony and enter the soil to pupate (Cuthbertson *et al.* 2013).

Of all the identified honeybee pests, *Varroa* has been demonstrated to actively vector diseases (Traynor *et al.* 2016) by having the unique ability to multiply viruses in its body (Ongus *et al.* 2004; Traynor *et al.* 2016), thus exacerbating poor colony health. Other pests of the African honeybee such as *A. tumida,* the larvae of the greater wax moth (*Galleria mellonella*) and the lesser wax moth (*Achroia grisella*) (Frazier, 2011), which co-exist intimately with the bees, need to be investigated for any potential role in disease transmission. *A. tumida* was first reported as an exotic pest in the United States in 1996 and has since spread to many countries in the world (Neumann *et al.* 2016). In these naïve bee populations the beetle has been demonstrated to vector endemic pathogens such as *Paenibacillus larvae* (Schafer *et al.* 2010) and potentially deformed wing virus (Eyer *et al.* 2009). The present study was part of a larger surveillance effort to identify the determinants of African honeybee health, particularly, to quantify honeybee pest abundance and disease prevalence across Kenya with the intention of obtaining empirical evidence supporting any linkages between native pests and disease transmission.

## 2. Materials and Methods

### 2.1 Selection of study sites

The selection of study sites was guided by agro-ecological zones. Kenya has 7 agro-ecological zones as per the index developed by Sombroek (Sombroek *et al.* 1982). These are: Humid, Sub-humid, Semi-humid), Semi-humid to Semi-arid, Semi-arid, Arid, and very arid (Figure 1). The six selected study sites represent 5 of the 7 agro-ecological zones of Kenya. These are: Kakamega 0°16'60.00" N 34°44'59.99" E (humid), Mt. Kenya 0°07'15.60" N 37°20'7.20" E (humid), Isiolo 0°52'60.00" N 38°40'0.12" E (arid), Mwingi 0°55'59.99" N 38°03'60.00" E (semi-arid), Taita Hills -3°22'59.99" S 38°33'59.99" E (semi-humid to semi-arid) and Coastal Kenya -4°02'20.40" S 39°40'12.59" E (semi-humid)

### 2.2 Scope of survey and selection of apiaries to sample

The epidemiological unit considered in this study was the apiary. A stratified sampling design was adopted. In order to qualify to be included in the study, only apiaries with more than 10 hives were considered. The study sites were conveniently selected from locations with established beekeeping activities (Table 1; Figure 1). For this cross-sectional study, sampling was done during the hot dry months from September 2013 up to the end of February 2014.

### 2.3 Sampling of honeybees and their pests

In total, 32 apiaries were visited (Table 1). On average, 5 colonies were randomly selected from each apiary, giving a total of 161 colonies. The sampling process was previously described by Ongus *et al.* 2017 and is

briefly mentioned here. From each colony, the bees that were sampled included 20 individuals of foragers, nurse bees, worker pupae and drone pupae were sampled separately. The foraging bees were collected by vacuum aspiration into 50mL sterile Falcon tubes as they returned to the hive entrance. Nurse bees were scooped directly from the brood comb into 50mL sterile Falcon tubes. The pink eye pupal stage of worker and drone pupae were picked individually from the brood comb using toothpicks with care not to poke their delicate bodies and placed into separate 50mL sterile Falcon tubes. All *A. tumida* observed in a colony were counted as they were aspirated into a 50mL falcon tube. *Varroa* mites were collected using the sugar shake method. A 250ml plastic cup was used to scoop a group of bees (approximately 300) into a 500ml Bee Mason glass jar with a mesh lid whose openings were too small to allow the bees to escape but large enough for *Varroa* mites to fall through. Two heaped tablespoons of icing sugar was poured on the trapped bees in the Mason jar via the perforations of the lid and the bees were vigorously shaken for about 30s. The jar was left undisturbed for about one minute after which it was turned upside down and shaken onto a white sheet of A4 paper to dislodge the mites. This was repeated twice with a fresh scoop of bees to make a total of three sugar shakes per colony. The dropped mites were then counted. The individual colonies were inspected for any manifestation of honeybee disease signs or symptoms. The sampled bees were preserved directly on dry ice at the field site and transported to the laboratory, where they were stored at –80$^{o}$C until processing. Repeated sampling was not carried out since the study was not designed to look at changes in the same colonies over time.

### 2.4 Molecular diagnosis of honeybee pathogens

The methods applied were fully described by Ongus *et al.* 2017. Briefly, in the laboratory, the bees from each apiary (hives 1-5) were pooled in groups as foragers, nurse bees, worker pupae and drone pupae. Twenty bees were randomly selected from each pool and homogenised in 1 x phosphate buffered saline (PBS) pH 7.4. Viral RNA/DNA and microbial DNA were extracted from 200µL of the bee homogenate as described by Ongus *et al.* 2017. Molecular assays were designed to detect the presence of honeybee disease agents (viral, bacterial and fungal) that have been previously identified to have the gravest consequences on honeybee health (OIE 2012; Dietemann *et al.* 2013). Each sample in this study was screened using 18 different PCR reactions with a panel of primers as described by Ongus *et al.* 2017. All the amplified PCR bands were excised from the agarose gel, purified and sequenced from both the forward and reverse directions at Macrogen (Korea) using universal SP6 and T7 primers.

### 2.5 Mean pest counts as a measure of pest abundance

The mean number of pests detected was determined to infer pest abundance in an apiary by calculating the arithmetic mean of the pest counts from all the colonies sampled in the apiary.

### 2.6 Data analysis

Disease prevalence was measured as the proportion of units that tested positive for a honeybee pathogen. The relationship between pest abundance and pathogen presence was investigated. The Spearman Correlation Coefficient was used to measure the strength of association of co-occurrence between two variables (mean pest count and pathogen prevalence per site). The correlation of each pathogen detected was weighed separately against a specific pest. A positive correlation coefficient indicates a positive relationship between the two variables (the larger the pest count, the larger the pathogen prevalence) while negative correlation coefficients expresses a negative relationship (the larger the pest count, the smaller the pathogen prevalence). A correlation coefficient of 0 indicates that no relationship between the variables exists at all.

## 3. Results

A total of 161 colonies from 32 apiaries (Table 1**)** were surveyed from September 2013 up to the end of February 2014 across 5 agro ecological zones in Kenya. The arthropod pests, *V. destructor* (ectoparasitic mite) and *A. tumida* were the most abundant pests, which were present in majority of the locations visited. The Varroa mite was present in all the locations visited except Isiolo with is an arid area. *A. tumida* was only located in Mt. Kenya region, Mwingi and the Coast (Table 2). Mt. Kenya recorded the highest mean *Varroa* count (at 53.51) followed by Mwingi then Kakamega and Taita Hills in that order. Coastal Kenya recorded the highest mean *A. tumida* count (at 36.97) followed by Mwingi and Mt. Kenya (Table 3).

Overall, *Varroa destructor* virus 1 (VDV 1), deformed wing virus (DWV) and black queen cell virus (BQCV) were detected with the highest prevalence in the apiaries at 66%, 69% and 69% respectively (Table 2). The other honeybee disease agents detected were Sacbrood virus (SBV), Israeli acute paralysis virus (IAPV), Acute bee paralysis virus (ABPV) and *Nosema ceranae* at 28%, 22%, 19% and 13% respectively (Table 2). Kakamega and coastal Kenya were the most burdened locations by honeybee diseases followed by Mt. Kenya region (Figure 1; Table 2). The semi-arid region of Mwingi had reduced disease burden and the arid region of Isiolo did not register any honeybee pathogens. A summary of honeybee disease agents detected in each apiary

(Table 2) indicated a co-existence of multiple infections of both pathogens and pests within apiaries across the study sites. Co-infections within colonies were however not investigated in this study. All infected bees in the present study were asymptomatic.

The Spearman Correlation Coefficient (Rho) was used to measure the strength of association of co-occurrence between two variables (mean pest count and pathogen prevalence per site) using the values indicated in Table 3. VDV 1 had a negative correlation with the *V. destructor* mite. All the other pathogens showed from low to moderate positive correlations to the mean *V. destructor* count, though none was statistically significant (Figure 2). The Spearman Rank correlations between mean *A. tumida* counts and honeybee pathogens prevalence (%) at the study sites (Figure 3) indicated general positive correlation between the *A. tumida* counts and honeybee pathogens prevalence, though not statistically significant, except the association between *Nosema ceranae* and *A. tumida* counts, which was statistically significant with a strong R value of 0.89803 and a supporting P value of 0.01507.

## 4. Discussion

This survey provided the opportunity to obtain data that would support the establishment of an epidemiological baseline of pests and diseases in honeybee colonies in Kenya. *V. destructor* and *A. tumida* were the most abundant pests. There was near universal presence of *V. destructor* and to a smaller extent *A. tumida* in majority of the locations visited. These two pests are very intimately associated with the colonies they infested. This makes them pests of interest especially where they may have a role as potential agents of disease transmission.

No clinical symptoms of any known honeybee disease (either viral, bacterial, fungal or microsporidian) was observed. However, molecular diagnosis identified the presence of 7 pathogens causing the greatest disease burden to honeybees in Kenya. These are DWV, BQCV, ABPV, VDV 1, SBV, IAPV and *N. ceranae*. In this study, no bacterial pathogens that cause American foulbrood (*Paenibacillus larvae*) or European foulbrood (*Melissococcus plutonius*) or fungal pathogens that cause chalkbrood (*Ascosphaera apis*) and stonebrood (*Aspergillus fumigatus, Aspergillus flavus, and Aspergillus niger*) were found. A previous nationwide study to evaluate the status of honeybee populations conducted in 2010 identified only four honeybee disease agents: DWV, BQCV, ABPV and *N. apis* (Muli *et al.* 2014). In the present study, the identified disease agents were almost evenly spread across the study sites. VDV 1, DWV and BQCV were equally spread across apiaries in different agro-ecological zones. The apiaries in Kakamega (humid), presented the highest positivity rate for majority of the disease agents detected and would form an ideal location for future focused honeybee disease studies. An investigation of the presence of the agents in apiaries revealed the common occurrence of more than one pathogen per apiary. The identified honeybee pests were also present in apiaries with mixed viral infections. Other studies investigating the prevalence of honeybee viruses in and around the Gauteng region of South Africa (Strauss *et al.* 2013) and in the Biobío Region of Chile (Rodríguez *et al.* 2014) reported similar mixed infections in apiaries. There appears to be a heavy disease burden on the Kenyan bees and it would be important to investigate the mixed infections further to see if they manifest as co-infections within the colonies. Since the samples were analysed as apiary samples, incidences of co-infection within colonies could not be elucidated. It would also be important to measure pathogen loads to fully understand the extent of the disease burden and lack of clinical symptoms as observed in this study. This study further describes *N. ceranae* infections in both foragers and nurse bees at the coast (Semi-humid) and at another inland location in the semi-arid region of Mwingi. Muli *et al.* described *N. apis* infections in foragers at three sites along the coast and one interior site on the western border of Kenya and Uganda (Muli *et al.* 2014).

Spearman Ranked Correlation between *Varroa* and pathogen prevalence revealed an overall weak and non-significant positive correlation with most of the pathogens. As *Varroa* has been demonstrated to transmit honeybee viral pathogens, the observed positive correlation with the viral infections, though weak, agrees with previous findings (Ongus *et al.* 2004; Traynor *et al.* 2016).

(ectoparasitic mite)

Spearman Ranked Correlation between *A. tumida* and pathogen prevalence however revealed an exceptional finding. The analysis identified a key association between the abundance of *A. tumida* and *N. Ceranae* infection, with a statistically significant positive correlation (R = 0.89803; P = 0.01507). This is the first time that *A. tumida* is implicated in a possible role of being part of the transmission pathway of *Nosema* infection. Followup studies will be required to understand the nature of this association with the aim of unravelling if *A. tumida* has a biological role in *Nosema* transmission. *A. tumida* has previously been reported to be able to transmit the spores of *Paenibacillus larvae*, the bacterium that causes American Foulbrood (Schafer *et al.* 2010). In that study, they demonstrated that the larval and adult SHB became contaminated with *P. larvae* spores when exposed to honeybee brood combs with clinical American foulbrood and spread it around the colony as they tunnelled through wax combs. *Nosema* is a fungal disease affecting adult bees. This single-celled microsporidian lives in the gut of bees (Frazier 2011). The spore stage of this disease is excreted in the bees' faeces. Infected bees may defecate within the hive, and the nest cleaning behaviour of the bees spreads the disease (Forsgren &

Fries 2010). There is a likelihood of the beetle being able to vector the pathogen through contact with infected faecal matter excreted by infected bees. However, this relationship has not been thoroughly studied and is worthy of further investigation given the close interaction between the beetle and honeybees (Ellis *et al.* 2003; Neumann *et al.* 2001; Suazo *et al.* 2003). The Spearman Ranked Correlation between *A. tumida* and the other pathogens however revealed general weak and non-significant positive correlation with most of the pathogens.

A laboratory-based study by Eyer *et al.* (2009) demonstrated evidence of DWV replication in the beetle making it a potential vector of honeybee viruses. Thus, the observations in this study supports previous findings, which demonstrate the beetle's potential as a vector of bee pathogens and expands on the knowledge of the specific pathogens that could be transmitted by the beetle. However, it remains unknown whether the beetle can transmit other viruses and pathogens, support their replication within its body and be affected health-wise. Thus, further investigations are required to address these questions.

Based on the present study, the Coastal lowlands and High altitude cool and humid forests (Kakamega and Mt. Kenya) can be considered high-risk zones for beekeeping given the high prevalence of pests and pathogens. The coastal highlands (Taita hills) and semi-arid zones (Mwingi) present moderate risks while the arid zone (Isiolo) presents the lowest risk to beekeeping as far as honeybee pests and diseases are concerned. These findings suggest that arid and semi-arid zones are the most appropriate for beekeeping in Kenya from a health perspective. Of particular interest was the absence or complete absence of pathogens in the arid zone.

## 5. Conclusion

The most important finding of this study was the identification of a key association between the abundance of *A. tumida* and *Nosema* infection, with a statistically significant positive correlation ($R = 0.89803$; $P = 0.01507$). Further studies will be required to understand the nature of this association with the aim of unravelling if *A. tumida* has a biological role in *Nosema* transmission. This study has also demonstrated that the semi-arid and arid regions may be the most appropriate for beekeeping since they experience fewer incidents of pest infestation and in some cases there is total absence of honeybee diseases.

## 6. Acknowledgements

This study was funded by the European Union Grant, Contract Number DCI-FOOD/2013/313-659 for the project entitled ICIPE: African reference laboratory (with satellite stations) for the management of pollinator bee diseases and pests for food security. We thank *icipe* for providing the facilities to conduct the study. We also thank the bee health team for assisting in collecting samples in the field. We acknowledge all beekeepers who allowed us to access their apiaries to obtain the samples that were used in this study. The European Union Reference Laboratory (EURL) for Bee Health provided plasmid controls of 7 viruses (DVW, BQCV, KBV, CBPV, SBV, IAPV, ABPV), *M. plutonius* (EFB), *P. larvae* (AFB), *Nosema* (*N. apis* and *N. ceranae*). Biological material (cDNA and gDNA) of honeybee pathogens was provided by Joachim de Miranda of the Swedish University of Agricultural Sciences (SLU) Honeybee Research Group.

## References

Biesmeijer, J.C., Roberts, S.P., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J. & Kunin, W.E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*. 313(5785): 351-4.

Bowen-Walker, P.L., Martin, S.J. & Gunn, A. (1999). The transmission of deformed wing virus between honey bees (*Apis mellifera L.*) by the ectoparasitic mite *Varroa jacobsoni* Oud. *Journal of Invertebrate Pathology*. 73:101–106.

Chen, Y., Evans, J. & Feldlaufer, M. (2006a). Horizontal and vertical transmission of viruses in the honey bee, Apis mellifera. *Journal of Invertebrate Pathology*. 92: 152–159.

Chen, Y.P., Pettis, J.S., Collins, A. & Feldlaufer M.F. (2006b). Prevalence and Transmission of Honeybee Viruses. *Applied And Environmental Microbiology*. 72(1): 606–611. doi:10.1128/AEM.72.1.606–611.2006

Cuthbertson, A.G.S., Wakefield, M.E., Powell, M.E., Marris, G., Anderson, H., Budge, G.E., Mathers, J.J., Blackburn, L.F. & Brown, M.A. (2013). The small hive beetle Aethina tumida: A review of its biology and control measures. *Current Zoology*. 59(5): 644–653.

de Figueiró Santos, J., Coelho, F.C. & Bliman, P.-A. (2016). Behavioral Modulation of Infestation by Varroa destructor in Bee Colonies. Implications for Colony Stability. *PLoS ONE*. 11(9): e0160465. https://doi.org/10.1371/journal.pone.0160465

Dietemann, V., Ellis, J.D. & Neumann, P. (Eds). (2013). *The COLOSS BEEBOOK, Volume II: standard methods for Apis mellifera pest and pathogen research*. International Bee Research Association; Cardiff, UK. 356 pp. ISBN 978-0-86098-275-3

Ellis, J.D., Hepburn, H.R., Ellis, A.M., & Elzen, P.J. (2003). Social encapsulation of the small hive beetle

(Aethina tumida Murray) by European honeybees (Apis mellifera L.). *Insectes Sociaux*. 50(3): 286–291.

Eyer, M., Chen, Y.P., Schäfer, M.O., Pettis, J. & Neumann, P. (2009). Small hive beetle, Aethina tumida, as a potential biological vector of honeybee viruses. *Apidologie*. 40(4): 419 – 428. DOI https://doi.org/10.1051/apido:2008051

Fitzpatrick, Ú., Murray, T.E., Paxton, R.J., Breen, J., Cotton, D., Santorum, V., et al. (2007). Rarity and decline in bumblebees – A test of causes and correlates in the Irish fauna. *Biological Conservation*.136: 185–194.

Forsgren, E. & Fries, I. (2010). Comparative virulence of *Nosema ceranae* and *Nosema apis* in individual European honeybees. *Veterinary Parasitology*. 170(3-4): 212-217.

Frazier, M. (2011). Department of entomology. The Pennsylvania State University. A Field Guide to Honey Bees And their Maladies. Code # AGRS-116.

Gallai, N., Salles, J-M., Settele, J., & Vaissière, B.E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*. 68(3): Pages 810–821.

Graystock, P., Blane, E.J., McFrederick, Q.S., Goulson, D. & Hughes, W.O.H. (2016). Do managed bees drive parasite spread and emergence in wild bees? *International Journal for Parasitology: Parasites and Wildlife*. 5(1): 64–75. http://dx.doi.org/10.1016/j.ijppaw.2015.10.001

Kasina, J.M., Mburu, J., Kraemer, M. & Holm-Mueller, K. (2009) Economic Benefit of Crop Pollination by Bees: A Case of Kakamega Small-Holder Farming in Western Kenya. *Journal of Economic Entomology*. 102(2): 467-473.

Kenya Soil Survey, 2004. Ministry of Agriculture of the Government of Kenya.

Klein, A-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. and Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B*. 274: 303–313.

Kluser, S. & Peduzzi, P. (2007). Global pollinator decline: a literature review. Geneva: UNEP/GRID.

Kremen, C. & Ricketts, T. (2000). Global perspectives on pollination disruptions. *Conservation Biology*. 14: 1226–1228.

Muli, E., Patch, H., Frazier, M., Frazier, J., Torto, B., Baumgarten, T., Kilonzo, J., Kimani, J.N., Mumoki, F., Masiga, D., Tumlinson, J., & Grozinger, C. (2014). Evaluation of the Distribution and Impacts of Parasites, Pathogens, and Pesticides on Honey Bee (Apis mellifera) Populations in East Africa. *PLoS One*. 9(4): e94459.

Mumoki, F.N., Fombong, A., Muli, E., Muigai, A.W.T. & Masiga, D. (2014). An inventory of documented diseases of African honeybees. *African Entomology*. 22(3): 473-487.

Neumann, P., Pirk C.W.W., Hepburn, R., Elzen, P.J. & Baxter, J.R. (2001). Laboratory rearing of small hive beetles Aethina tumida (Coleoptera, Nitidulidae). Journal of Apicultural Research. 40: 111–112.

Neumann, P., Pettis, J. & Schäfer, M.O. (2016). Quo vadis Aethina tumida ? Biology and control of small hive beetles. *Apidologie*. 47: 427–466.

OIE (World Organisation for Animal Health). (2012). Terrestrial Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees). 7th Edition, Volumes 1 and 2, 1404 pages. ISBN 978-92-9044-878-5.

Ongus, J.R., Peters, D., Bonmatin, J.-M., Bengsch, E., Vlak, J.M., Van Oers, M.M. (2004). Complete sequence of a picorna-like virus of the genus *Iflavirus* replicating in the mite *Varroa destructor*. *Journal of General Virology*. 85: 3747–3755.

Ongus, J.R., Fombong, A.T., Irungu, J., Masiga, D. & Raina, S. (2017). Prevalence of common honeybee pathogens at selected apiaries in Kenya, 2013/2014. *International Journal of Tropical Insect Science*. 37(3).

Pirk, C.W.W., Strauss, U., Yusuf, A.A. Démares, F. & Human, H. (2016). Honeybee health in Africa—a review. *Apidologie*. 47(3): 276–300.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O. & Kunin, W.E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology and Evolution*. 25: 345–353. http://dx.doi.org/10.1016/j.tree.2010.01.007

Rodríguez, M., Vargas, M., Antúnez, K., Gerding, M., Castro, F.O. & Zapata, N. (2014). Prevalence and phylogenetic analysis of honey bee viruses in the Biobío Region of Chile and their association with other honey bee pathogens. *Chilean Journal of Agricultural Research.* 74(2): 170-177.

Richards, A.J. (2001). Does low biodiversity resulting from modern agricultural practice affect crop pollination and yield? *Annals of Botany*. 88: 165–172.

Ryba, S., Titera, D., Schodelbauerova-Traxmandlova, I. & Kindlmann, P. (2012). Prevalence of honeybee viruses in the Czech Republic and coinfections with other honeybee disease. *Biologia.* 67(3): 590—595.

Schäfer, M.O., Ritter, W., Pettis, J. & Neumann, P. (2010). Small hive beetles, Aethina tumida, are vectors of

Paenibacillus larvae. *Apidologie*. 41(1): 14–20.
Sombroek, W.C., Braun, H.M.H. & van der Pour, B.J.A. (1982). Explanatory Soil Map and Agro-climatic Zone Map of Kenya. Report E1. National Agricultural Laboratories, Soil Survey Unit, Nairobi, Kenya, 56 pp.
Steffan-Dewenter, I., Potts, S. G., & Packer, L. (2005). Pollinator diversity and crop pollination services are at risk. *Trends in Ecology and Evolution*. 20(12): 651-653.
Strauss, U., Humana, H., Gauthiera, L., Crewea, R.M., Dietemanna, V. & Pirka, C.W.W. (2013). Seasonal prevalence of pathogens and parasites in the savannah honeybee (*Apis mellifera scutellata*). *Journal of Invertebrate Pathology*. 114(1): 45–52.
Suazo, A., Torto, B., Teal, P.E. & Tumlinson, J.H. (2003). Response of the small hive beetle *Aethina tumida* to honey bee *Apis mellifera* and beehive-produced volatiles. *Apidologie*. 34: 525–533.
Traynor, K.S., Rennich, K., Forsgren, E., Rose, R., Pettis, J., Kunkel, G., Madella, S., Evans, J., Lopez, D., VanEngelsdorp, D. (2016). Multiyear survey targeting disease incidence in US honey bees. *Apidologie*. 47: 325–347. DOI: 10.1007/s13592-016-0431-0
vanEngelsdorp, D., Underwood, R., Caron, D. & Hayes, J. Jr (2007). An estimate of managed colony losses in the winter of 2006–2007: a report commissioned by the Apiary Inspectors of America. *American Bee Journal*. 147: 599–603.
Westerkamp, C. & Gottsberger, G. (2002). *The costly crop pollination crisis*. In: Kevan P, Imperatriz Fonseca V, editors. Pollinating bees – The conservation link between agriculture and nature. Brasilia: Ministry of Environment; pp. 51–56.
Withgott, J. (1999). Pollination migrates to top of conservation agenda. *Bioscience*. 49: 857–862.

**Table 1:** Surveillance sites visited across 5 agroecological zones in Kenya.

| Site | Agroecological zone | No. of Apiaries | No. of Colonies |
|---|---|---|---|
| 1. Kakamega | Humid | 8 | 40 |
| 2. Mt. Kenya | Humid | 8 | 38 |
| 3. Isiolo | Arid | 1 | 4 |
| 4. Mwingi | Semi-arid | 7 | 33 |
| 5. Taita Hills | Semi-humid to semi-arid | 2 | 10 |
| 6. Coastal Kenya | Semi-humid | 6 | 36 |
| **TOTAL** | | **32** | **161** |

**Table 2:** Co-existence of honeybee pests and pathogens at the study apiaries.

| | | Honeybee Pathogens Detected * | | | | | | | Mean Honeybee Pest Counts per Apiary § | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Apiary code** | **VDV 1** | **DWV** | **BQCV** | **SBV** | **IAPV** | **ABPV** | ***Nosema ceranae*** | ***Varroa destructor*** | ***Aethina tumida*** |
| **Kakamega** | AKA | | | | | | | | 19 | 0 |
| | ISKT | | | | | | | | 6.4 | 0 |
| | BKH | | | | | | | | 13.8 | 0 |
| | AMY | | | | | | | | 23 | 0 |
| | FUR | | | | | | | | 35.2 | 0 |
| | BAN | | | | | | | | 25 | 0 |
| | MAK | | | | | | | | 18.6 | 0 |
| | CHR | | | | | | | | 20 | 0 |
| **Mt. Kenya** | TIM | | | | | | | | 0 | 0 |
| | MEG1 | | | | | | | | 21.6 | 0.2 |
| | MEG2 | | | | | | | | 7.4 | 1 |
| | NKB | | | | | | | | 23.6 | 0 |
| | MEF | | | | | | | | 65.7 | 5.3 |
| | NYK1 | | | | | | | | 192.6 | 0 |
| | NYK2 | | | | | | | | 96 | 0 |
| | EMBW | | | | | | | | 21.2 | 17.8 |
| **Isiolo** | OLD | | | | | | | | 0 | 0 |
| **Mwingi** | KAS1 | | | | | | | | 54.2 | 1.6 |
| | KAS2 | | | | | | | | 14.8 | 2 |
| | KAS3 | | | | | | | | 32 | 0.4 |
| | NGN | | | | | | | | 11.6 | 0.4 |
| | ITZ | | | | | | | | 70.3 | 1.3 |
| | WNG | | | | | | | | 30.4 | 6.6 |
| | KITH | | | | | | | | 43 | 59.25 |
| **Taita Hills** | THUC | | | | | | | | 9 | 0 |
| | THC | | | | | | | | 0.8 | 0 |
| **Coastal Kenya** | KTC | | | | | | | | 24.1 | 128.4 |
| | BKM | | | | | | | | 24.4 | 65.8 |
| | TBT | | | | | | | | 6.6 | 0 |
| | HAB | | | | | | | | 9.2 | 23.4 |
| | MALL | | | | | | | | 23.2 | 1.8 |
| | BAH | | | | | | | | 5.6 | 2.4 |
| **Percent apiaries infected with each pathogen** | | **66%** | **69%** | **69%** | **28%** | **22%** | **19%** | **13%** | | |

* The presence of a pathogen at an apiary is indicated using the symbol. Pathogens not listed were not detected in any sample.

§ The mean pest count represents the average number of a particular pest counted from all sampled colonies in an apiary.

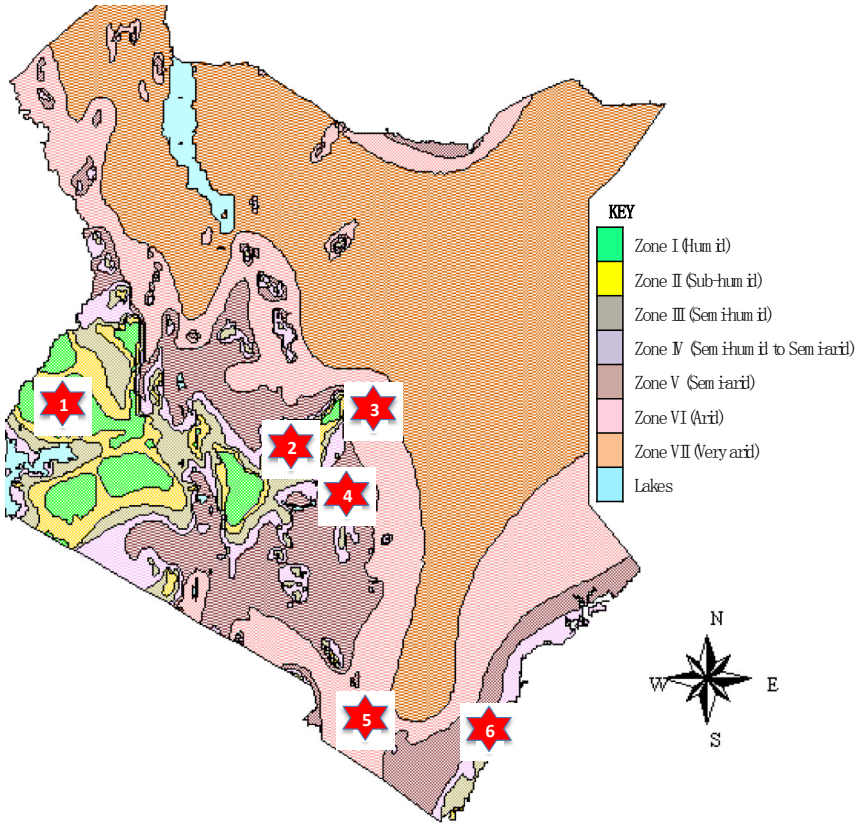
Abbreviations used: *Varroa destructor* virus 1 (VDV 1), Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV), Sacbrood virus (SBV), Israeli Acute Paralysis Virus (IAPV), Acute Bee Paralysis Virus (ABPV).

**Table 3:** Prevalence of individual honeybee pathogens and pest abundance per agroecological location.

| | Honeybee Pathogens Detected | | | | | | | Mean Honeybee Pest Count per site | |
|---|---|---|---|---|---|---|---|---|---|
| **Site** | **VDV 1** | **DWV** | **BQCV** | **SBV** | **IAPV** | **ABPV** | ***Nosema ceranae*** | ***Varroa destructor*** | ***Aethina tumida*** |
| **Kakamega** | 100% | 100% | 100% | 50% | 62.50% | 12.50% | 0% | 20.13 | 0 |
| **Mt. Kenya** | 62.50% | 62.50% | 75% | 25% | 12.50% | 62.50% | 0% | 53.51 | 3.038 |
| **Isiolo** | 0% | 0% | 0% | 0% | 0% | 0% | 0% | 0 | 0 |
| **Mwingi** | 0% | 71.43% | 57.14% | 0% | 0% | 0% | 28.57% | 36.61 | 10.22 |
| **Taita Hills** | 100% | 0% | 100% | 0% | 0% | 0% | 0% | 4.9 | 0 |
| **Coastal Kenya** | 100% | 66.67% | 33.33% | 66.67% | 16.67% | 0% | 33.33% | 15.52 | 36.97 |

Abbreviations used: *Varroa destructor* virus 1 (VDV 1), Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV), Sacbrood virus (SBV), Israeli Acute Paralysis Virus (IAPV), Acute Bee Paralysis Virus (ABPV).




| Location | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| | **Kakamega** | **Mt. Kenya** | **Isiolo** | **Mwingi** | **Taita Hills** | **Coastal Kenya** |
| **Pathogens present** | VDV-1<br>DWV<br>BQCV<br>SBV<br>IAPV<br>ABPV | VDV-1<br>DWV<br>BQCV<br>SBV<br>IAPV<br>ABPV | —— | DWV<br>BQCV<br>*N. Ceranae* | VDV-1<br>DWV<br>BQCV<br>IAPV | VDV-1<br>DWV<br>BQCV<br>SBV<br>IAPV<br>*N. Ceranae* |
| **Pests present** | *Varroa* | *Varroa*<br>SHB | —— | *Varroa*<br>SHB | *Varroa* | *Varroa*<br>SHB |

**Figure 1:** A map of Kenya highlighting the presence of honeybee pathogens and pests at the study sites superimposed on the agro-ecological map adapted from the Kenya Soil Survey, 2004. The study locations are highlighted with a numbered star shape and the pathogens detected at those sites are listed in the panel beneath the map. Abbreviations used: Varroa destructor virus 1 (VDV 1), Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV), Sacbrood virus (SBV), Israeli Acute Paralysis Virus (IAPV), Acute Bee Paralysis Virus (ABPV) and small hive beetle (SHB).

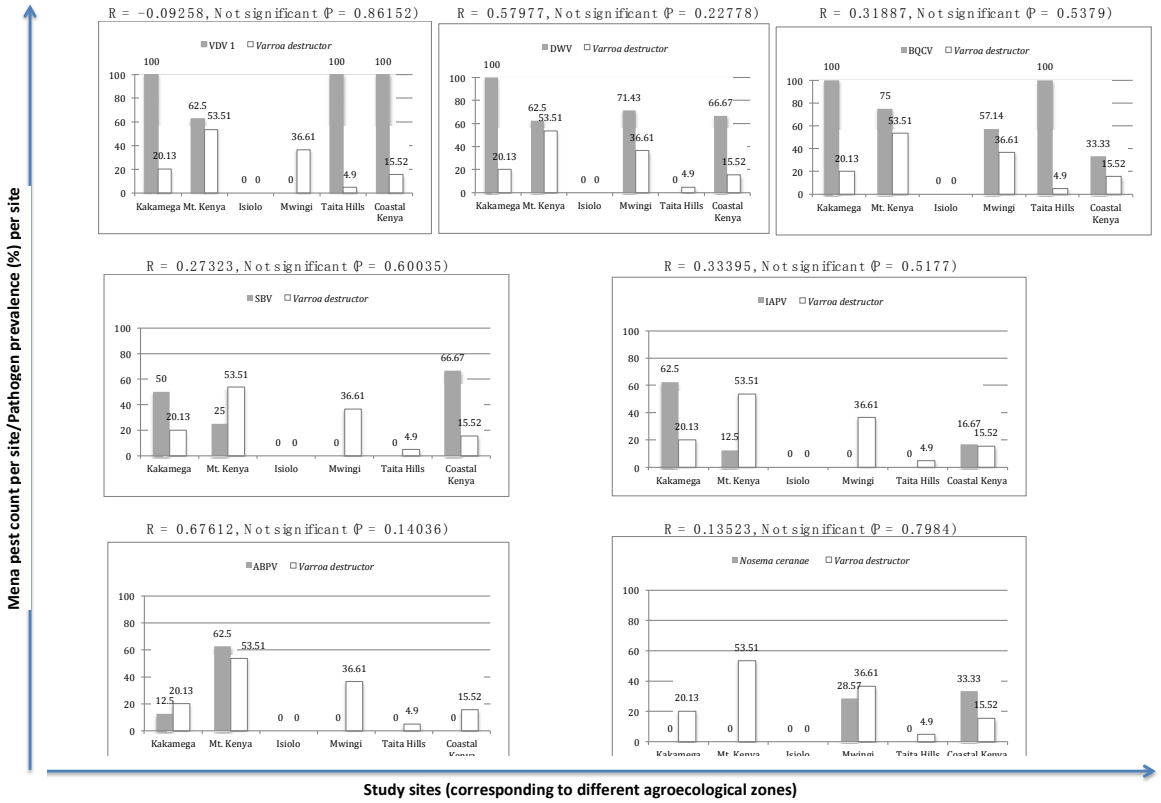



**Figure 2**: Spearman Rank correlations (Rho) between mean *Varroa destructor* counts and honeybee pathogens prevalence (%) at the study sites.

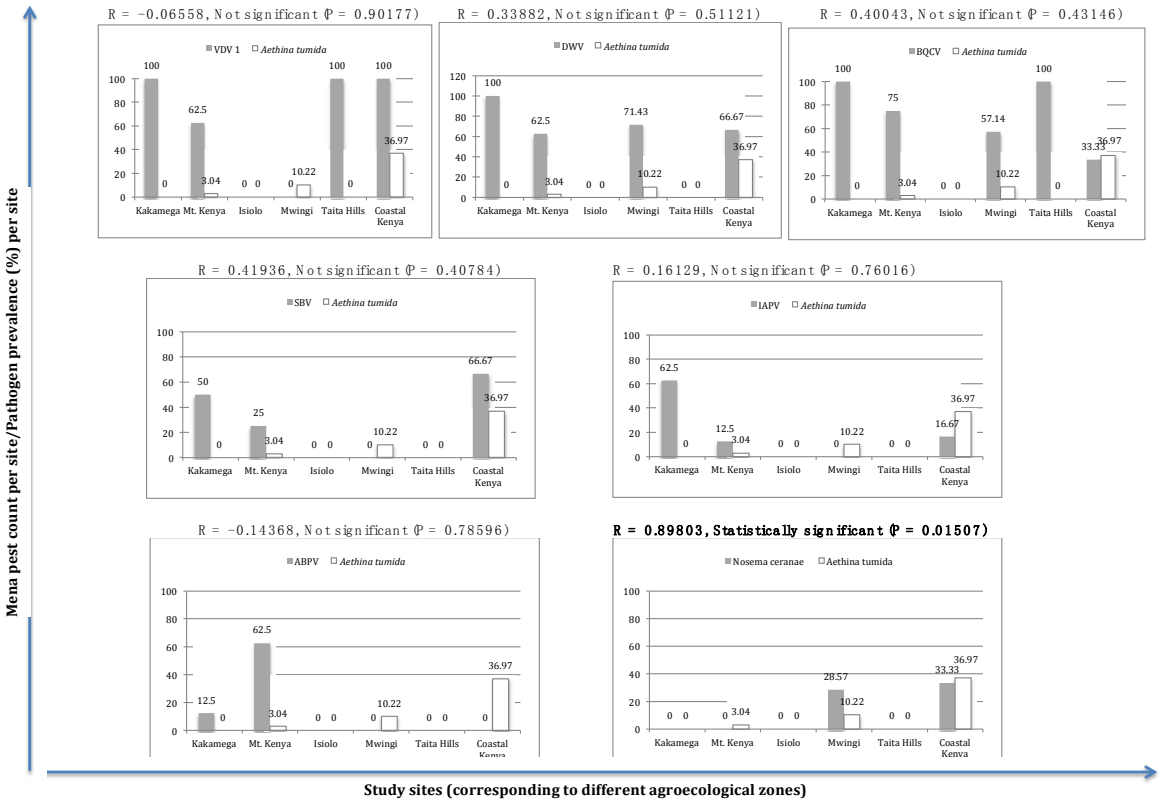



**Figure 3**: Spearman Rank correlations (Rho) between mean *Aethina tumida* counts and honeybee pathogens prevalence (%) at the study sites.