



PROFILING OF HONEY BEE VIRUSES IN KENYAN HONEY BEE COLONIES

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INTRODUCTION

- ❑ Honey bee is the mainstay of honey production and crop pollination
- ❑ Most managed and efficient pollinator of most crops worldwide

(Potts et al., 2010).

- ❑ Global value of insect pollination is estimated at US dollars 212 billion worldwide (Gallai *et al.*, 2009).
- ❑ Recent declines in bee populations in most parts of the world (Granberg et al., 2013) due to interaction of multiple factors (Anderson and East, 2008); environmental and human induced (Moritz et al., 2010).
- ❑ Of interest in this study were bee viruses



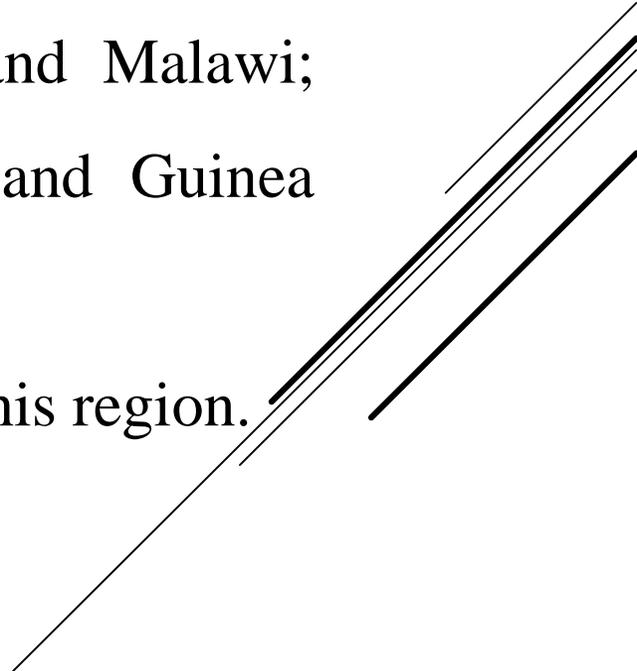
Bee viruses

- Honey bees have been reported to host about 18 viruses
- Bee viruses affect morphology, physiology, and behavior of bees
- Behavioral aberrations in bees include shivering, paralysis, disorientation, aggression or altered foraging preferences or changes in brood care
- Viral infections in honeybees are low-medium titre and thus asymptomatic, hence healthy looking bee colonies may harbor a number of potentially harmful virus infections (Di Prisco et al., 2011)

- Bee viruses are grouped into Iflaviridae and Dicitroviridae
- *Iflaviridae* include deformed wing virus (DWV), Sac brood virus (SBV), Varroa destructor virus-1 (VDV-1) and Kakugo virus (Chen et al., 2012)
- *Dicitroviridae* include black queen cell virus (BQCV), acute bee paralysis virus (ABPV), Kashmir bee virus (KBV), Israel acute paralysis virus (IAPV)
- Symptoms associated with specific viruses include wing deformities (DWV), hairless, dark, shiny bees (CBPV), swollen yellow larvae and/or dark-brown larva carcasses in the cells of worker-bees (SBV) or in queen cells (BQCV).

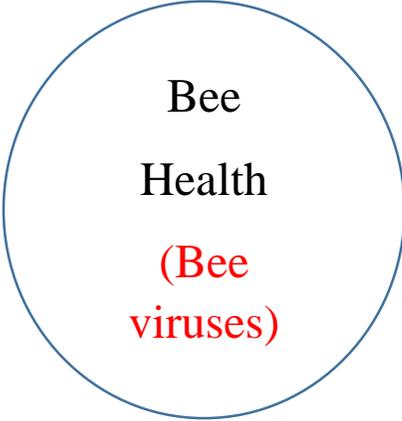


Bee viruses in Africa

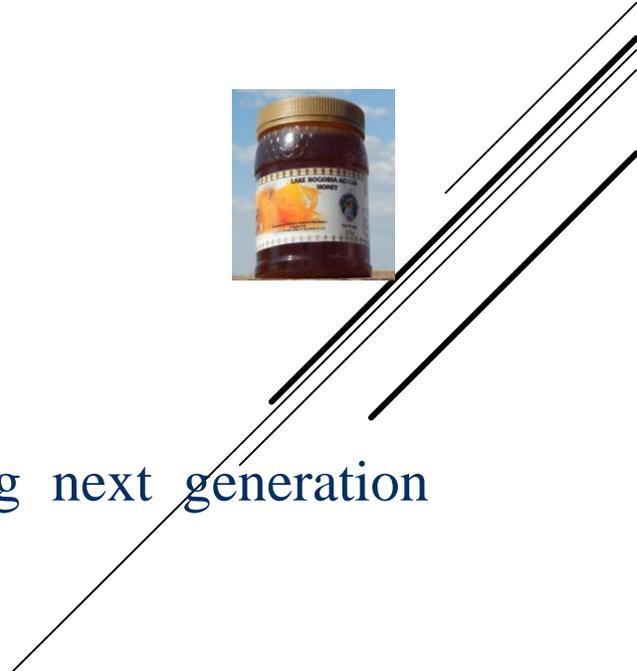
- + ABPV, IAPV, SBV, BQCV and DWV have been reported in Africa (Mumoki et al., 2014).
 - + In Kenya, deformed wing virus, black queen cell virus and acute bee paralysis virus have been reported.
 - + Bacteria *Melisococcus plutonius*, been reported in Algeria and Malawi; South Africa, Libya, Morocco, Tanzania, Tunisia, Senegal and Guinea Bissau (Mumoki et al., 2014).
 - + Lee et al., (2010) suggests presence of more benign diseases in this region.
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Pollination and Crop
production



Production of Honey and
other hive products



What is the current status of bee viruses across the study sites using next generation sequencing on 454 GS FLX platform.



MATERIALS AND METHODS

i) Study sites

Ijara, Magarini, Busia, Siaya, Narok, Marigat, Kwale and Voi in Kenya duration of sample collection was between November 2012 and June 2013.



ii) Sample collection

- ❖ Three apiaries totalling ten hives in each site
- ❖ Langstroth and Kenya top bar hives
- ❖ Samples of adult bees and immature bees (brood) were collected
- ❖ The samples were preserved singly in barcoded cryovials; and information entered in excel spread sheets
- ❖ Samples were transported in dry ice and immediately transferred to liquid nitrogen gas on arrival at the ILRI laboratory.



iii) Library preparation and amplification



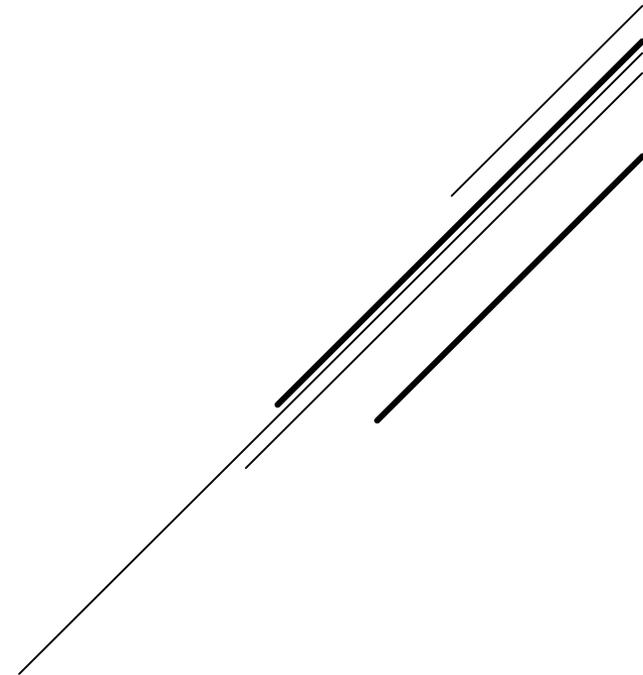
- RNA extraction using RNeasy extraction kit following the manufacturer's protocol.
- Good quality RNA from brood and adult bees was pooled for each site.
- Sixteen libraries were produced and used in downstream analysis.
- Complementary DNA (cDNA) for each of the libraries was synthesized using superscript II
- cDNA converted to double stranded DNA using Klenow reaction.
- PCR was performed at 94⁰C for 1minute, 30 cycles of 94⁰C for 30 seconds, 50⁰C for 30 minutes, 72⁰C for 1minute, and final extension of 72⁰C for 7 minutes and 4⁰C for infinite.
- PCR products sequenced using 454 GS FLX (Roche Diagnostics Corporation) at BecA hub ILRI

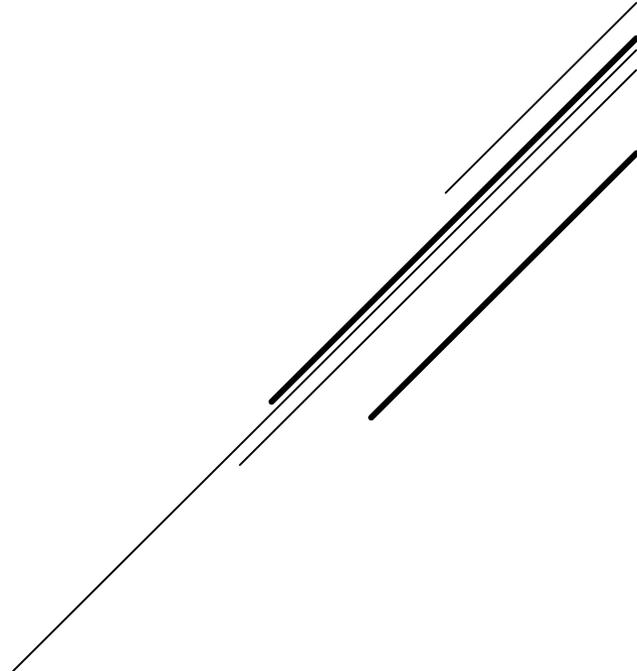
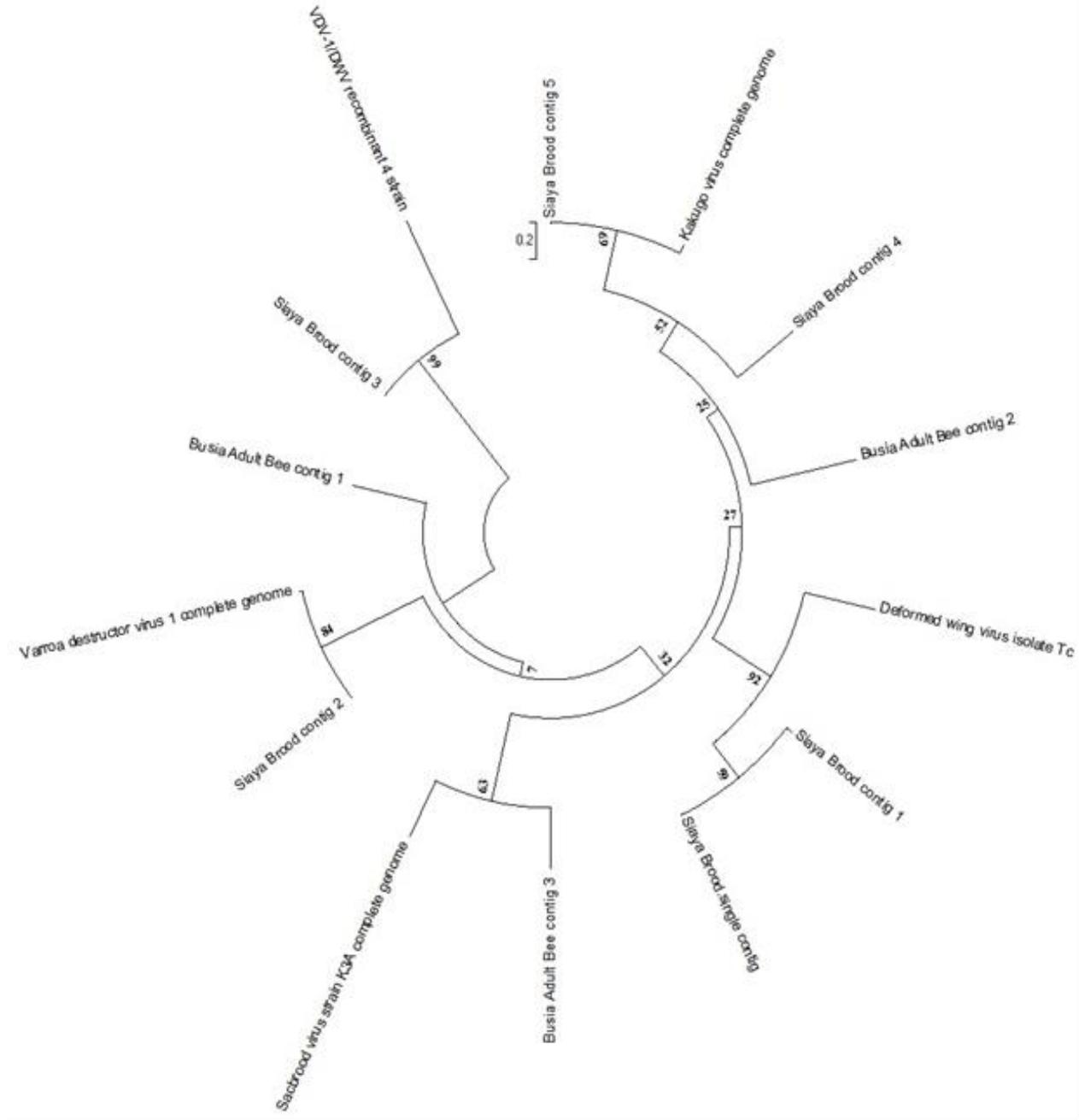
Virus sequences

- Five viruses associated with honey bees' health were detected in the general blast; **Kakugo virus, deformed wing virus, sac brood virus, varroa destructor virus-1 and Tomato Ring Spot virus.**
- Mapping on reference genomes and de novo assembly, generated contigs for Kakugo virus (8), deformed wing virus (1) and varroa destructor-1 (6).
- Further interrogation through BLAST on NCBI database gave matches for the above mentioned viruses plus a recombination of VDV-1 and DWV.
- Phylogenetic analysis revealed that contigs from Siaya brood library grouped with deformed wing virus, Kakugo virus, Varroa destructor-1 virus and recombinant VDV-1/DWV.
- Contigs from Busia library grouped with SBV, KV and VDV-1 (Fig 1).

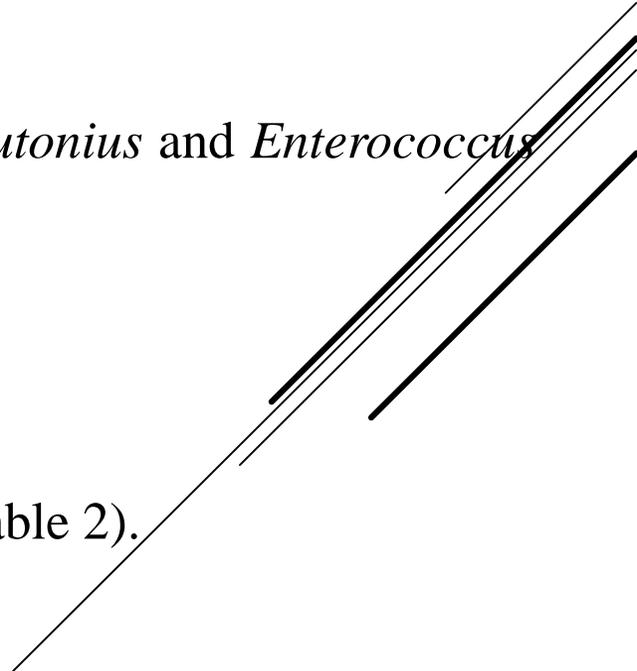
| Virus | Virus family | Reads | Contigs |
|---------------------------|-----------------------|--------------|----------------|
| Kakugo virus | Iflaviridae/Iflavirus | 59 | 8 |
| Deformed wing virus | Iflaviridae/Iflavirus | 2 | 1 |
| Sac brood virus | Iflaviridae/Iflavirus | 2 | 0 |
| Varroa destructor virus-1 | Iflaviridae/Iflavirus | 76 | 6 |
| Tomato ring spot virus | Secoviridae/Nepovirus | 6 | 0 |

Table 1: Shows viruses identified in raw reads; family classification; number of reads and contigs formed in each virus; the most prevalent viruses were *Varroa destructor* virus-1 and Kakugo virus





Bacteria sequences

- *Paenibacillus larvae* was detected through general blast of the study libraries in raw reads.
 - All the sixteen study libraries were mapped against the full genome of *Paenibacillus larvae* as the reference.
 - The libraries which mapped with the reference were de novo assembled using CLC genomic work bench and the resulting contigs were interrogated on local blast on NCBI database.
 - Contigs for five libraries out of the sixteen libraries matched with *Melisococcus plutonius* and *Enterococcus faecalis*.
 - These included Voi_adult, Ijara_adult, Busia_brood, Busia_adult and Narok_adult.
 - Busia_adult and Narok_adult libraries had the most number of matching contigs (Table 2).
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| Library | Reads | Number of Contigs formed | Number of contigs that matched with <i>M. plutonius</i> and <i>E. faecalis</i> |
|----------------|-------|--------------------------|--|
| Voi_adult | 239 | 8 | 1 |
| Voi_brood | 132 | 2 | 0 |
| Ijara_adult | 124 | 5 | 1 |
| Ijara_brood | 143 | 0 | 0 |
| Magarini_brood | 267 | 7 | 0 |
| Marigat_adult | 6 | 0 | 0 |
| Marigat_brood | 78 | 0 | 0 |
| Narok_adult | 157 | 9 | 5 |
| Narok_brood | 102 | 5 | 0 |
| Busia_adult | 1556 | 24 | 7 |
| Busia_brood | 777 | 7 | 2 |

Table 2: Libraries whose raw reads mapped with *Paenibacillus larvae* but matched with *M.plutonius* and *E.faecalis*

Discussions

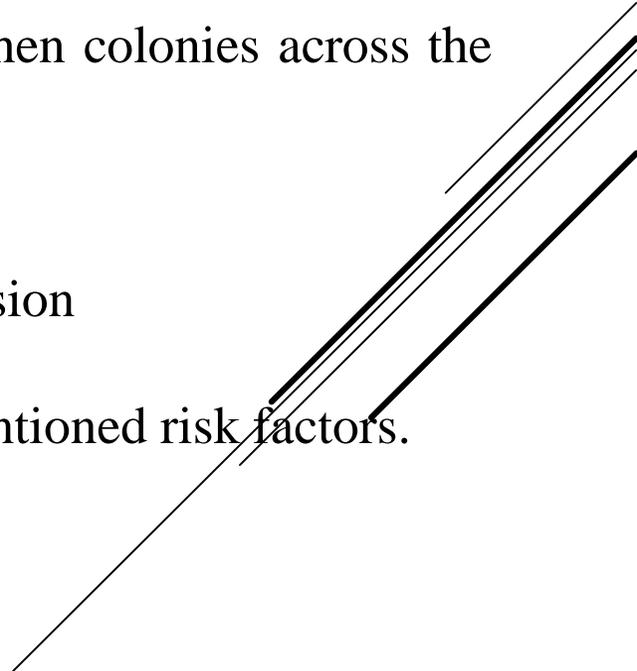
- ❖ The viruses revealed in this study belonged to family *iflaviridae*, **deformed wing virus**, *Varroa destructor virus-1*, and **Kakugo virus**.
- ❖ Prevalence was higher in Siaya_brood and Busia_adult libraries which had more contigs matching with the said viruses.
- ❖ Phylogenetic analysis of the study showed that contigs from Siaya_brood library matched with KV, VDV-1, DWV and VDV-1/DWV recombinant strain; while contigs from Busia_adult bee library matched with KV, VDV-1 and SBV.
- ❖ Siaya_brood library had the highest number of contigs (six) matching with Iflaviruses compared to Busia_adult bee library which had three contigs.

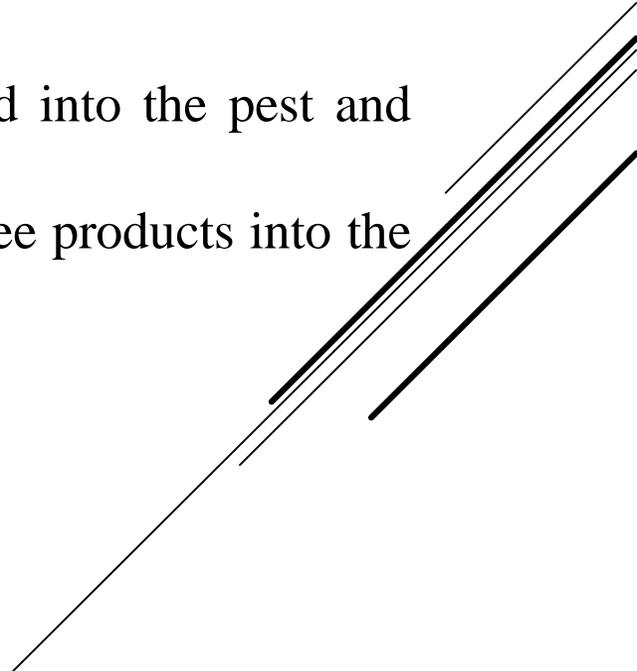
- ❖ Bacteria *M. plutonius* and *E. faecalis* were observed in the study contigs of five libraries Voi_adult, Ijara_adult, Busia_brood, Busia_adult and Narok_adult.
- ❖ Phylogenetic analysis using neighbor –joining method, revealed some contigs from Busia_adult, Busia_brood and Narok_adult libraries grouped with the reference *M. plutonius* strain DAT561e from the NCBI database.
- ❖ Two contigs from Narok_adult library grouped together, a contig from Busia_adult library grouped with *E. faecalis* strain 14 obtained from the NCBI database.
- ❖ Another contig from Busia_adult grouped with a contig from Narok_adult library. Other contigs from Busia_adult, Voi_adult, Ijara_adult and Busia_brood libraries grouped together.

Summary

- ❖ The virus incidences were high in Busia_adult library and Siaya_brood library.
- ❖ The bacteria detected was *Melisococcus plutonius* which causes European foul brood (EFB) and *Enterococcus faecalis* which is a secondary agent of EFB.
- ❖ Bacteria incidences was higher in Busia_adult library and Narok_brood library.
- ❖ *Paenibacillus larvae* which is a causative agent of AFB was observed in raw reads, however, after interrogations, the contigs matched with European foul brood, *M. plutonius* and the secondary causative organism of EFB, *E. faecalis*.
- ❖ The libraries from Busia indicated presence of both bacteria and viruses.
- ❖ Libraries, from Siaya did not reveal presence of bacteria but contigs from Siaya libraries also grouped with VDV-1/DWV recombinant strain, DWV, KV and Varroa destructor-1 virus

Conclusions and Recommendations

- ✓ The main risk factors for the spread of pests and diseases of honey bees is lack of knowledge on presence and identification of bee pests and pathogens.
 - ✓ Beekeepers and traders are not aware that bee pests and pathogens are transmitted through trade, sharing of bee equipment and transfer of live bees and queen bees to strengthen colonies across the country
 - ✓ There is need for capacity building of stakeholders to reduce possible transmission
 - ✓ There is also need to have regulatory measure in place to control the above mentioned risk factors.
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- ✓ It is important to carry out regular monitoring and intense honeybee pests and disease surveillance to detect changes in bee health status and to ensure early detection of disease threats.
 - ✓ Determination of disease free status of certain pests and diseases are critical in the trade of bees and other hive products. The data obtained in this study would feed into the pest and disease monitoring plan that the country lacks towards export of bee and bee products into the European Union market.
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