Bee World

ISSN: 0005-772X (Print) 2376-7618 (Online) Journal homepage: https://www.tandfonline.com/loi/tbee20

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To cite this article: Agnes S. Otim, Robert Kajobe, Patrick P'Odyek Abila, Patrice Kasangaki & Richard Echodu (2019): Viruses Circulating in African Honey Bees in Uganda, Bee World, DOI: 10.1080/0005772X.2019.1698103

To link to this article: https://doi.org/10.1080/0005772X.2019.1698103

ARTICLE

Viruses Circulating in African Honey Bees in Uganda

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Introduction

Worldwide, honey bees are hosts of more than 20 identified bee viruses (de Miranda, Gauthier, Ribiere, & Chen, 2011); most of them are single stranded RNA viruses belonging to order Picornavirales which has five families, two of which (Iflaviridae and Dicistroviridae) contain the common bee viruses (De Smet et al., 2012). Nine of these bee viruses on the African continent are reviewed by Pirk, Strauss, Yusuf, Démares, and Human (2016). The Acute Bee Paralysis Virus (ABPV), Israeli Acute Paralysis Virus (IAPV) and Black Queen Cell Virus (BQCV) were reported in South Africa (Strauss et al., 2013). In Kenya, Muli et al. (2014) detected ABPV, BQCV and Deformed Wing Virus (DWV). In Benin, Amakpe, De Smet, Brunain, and Ravoet (2015) reported Lake Sinai Virus (LSV), while Onyango et al. (2016) added to Kenya’s bee viruses the Varroa Destructor Virus-1 (VDV-1) and Kakugo Virus. Apis melifera Filamentous Virus (AmFV) in South Africa is reviewed by Allen and Ball (1996). In Uganda, Kajobe et al. (2010a) detected the BQCV and none of the samples screened in their study tested positive for Chronic Bee Paralysis Virus (CBPV), Sac Brood Virus (SBV), ABPV, DWV, IABP, nor Apis iridescent virus, AIV. Studies of Chemurot (2017) show presence of ABPV, SBV, DWV, LSV and BQCV in bee colonies in eastern and western agro-ecological zones (AEZs) of Uganda.

The viruses persist as covert asymptomatic infections but their levels increase in stressful conditions causing reduced colony growth, foraging, resource availability, worker longevity and brood survival (Degrandi-Hoffman & Chen, 2015; Mingxiao, Jinhua, Yingjin, Li, & Yongfei, 2013). Most of these viruses have been detected in varroa mites and are transmitted through parasitism (de Miranda et al., 2013; Degrandi-Hoffman & Chen, 2015; Genersch & Aubert, 2010; Gisder, Aumeier, & Genersch, 2009; Ongus et al., 2004). By feeding on the haemolymph of bee brood and adult bees, varroa mites suppress the bees’ immune system (Degrandi-Hoffman & Chen, 2015; Rosenkranz, Aumeier, & Ziegelmann, 2010). These viruses infect all the developmental stages and castes of honey bees (Degrandi-Hoffman & Chen, 2015). Single and multiple virus infections in honey bees were detected by Chen et al. (2004), in USA and in Kenya by Muli et al. (2014).

The presence of varroa mites was reported on the African continent. Whereas Fazier et al. (2010) reported varroa mite presence in Kenya, Kasangaki, Otim, Abila, Chemurot, and Kajobe (2016) found mites in Uganda’s bee colonies. When BQCV was detected by Kajobe et al. (2010), varroa mites had not been reported in Uganda’s bee colonies and serious consequences were expected with BQCV disease after the detection of varroa mites in the districts endemic with BQCV (Kajobe et al., 2010). With the presence of varroa mites in Uganda’s bee colonies, there was an assumption of existing colonies harboring other bee viruses that could be impacting on the country’s honey production through reduced colony growth, foraging and resource availability. This study was designed with the objective of identifying the bee viruses circulating in Uganda’s bee colonies and their effect on colony strength.

Materials and Methods

Study Location

Honey bee colonies were sampled from seven apiaries in two districts representing two agro ecological zones of Uganda; Kabarole (00^{0}36’N, 30^{0}18’E) and Lira (2^{0}20’N, 33^{0}06’). Kabarole represents the western highlands, while Lira represents the Mid-North. These districts were selected purposefully for intensive beekeeping in their AEZ.

Sample Collection and Storage

Samples of adult worker honey bees were collected in plastic jars from the brood nest of honey bee colonies that were weak and those that apparently looked healthy (Table 1). The collected samples were then transferred into cryo-vials filled with RNA-Later while still in the field to stabilize the RNA and stored at -20 °C on arrival at the laboratory until analysis. A total of 98 samples of adult bees were collected from seven apiaries. Ninety-seven of these samples were taken from 10% of honey bee colonies randomly sampled from six apiaries, while one sample was a collective sample pooled from 10 honey bee colonies of one apiary ‘nd’ (Table 1). A sample consisted of ten adult worker bees. All the sampled colonies had varroa mites and did not show virus symptoms. The samples were collected from October to November 2017.

Pathogen Diagnosis

Each sample was screened for six bee viruses using RT-PCR methods: Acute Bee Paralysis Virus (ABPV), Black Queen Cell Virus (BQCV), Deformed Wing Virus (DWV), Sacbrood Virus, (SBV), Chronic Bee Paralysis Virus (CBPV) and Kashmir Bee Virus (KBV). The laboratory analyses described were performed at the Gulu University Bioscience Laboratory.

RNA Extraction

Total RNA from the pooled samples[1] was extracted using TRIzol® LS Reagent following the manufacturer’s protocol. 10 worker bees pooled per honey bee colony and 45 varroa mites (phoretic on adult bees and in brood) pooled per apiary were crushed in liquid nitrogen using a sterilized glass-Teflon and homogenized in TRIzol® LS Reagent. Total RNA was extracted from the samples using TRIzol® LS Reagent following manufacturer’s protocol. After homogenizing the sample with TRIzol® LS Reagent, Chloroform was added and the homogenate allowed to

DOI: 10.1080/0005772X.2019.1698103

separate into a clear upper aqueous layer containing RNA and inter-phase and red lower organic layer containing DNA and proteins. The total RNA was then precipitated from the aqueous layer using 100% Isopropanol. Thereafter the precipitated RNA was washed in 75% ethanol to remove impurities and re-suspended in ribonuclease free water/distilled water. The sample (RNA) was then used for the downstream molecular reactions.

Complementary DNA/cDNA Synthesis

Complementary DNA (cDNA) synthesis was performed using M-MuLV reverse transcriptase. All reaction steps were performed in a thermo cycler with a heated lid (100^{0}C) using PCR tubes. 2 µl of the total extracted RNA and 1 µl of reverse primer was added to a tube containing a 17 µl master mix (5 µl of M-MuLV reverse transcriptase buffer, 4 µl of 200 µM dNTP mix, 1 µl of 10 mg/µl BSA, 1 µl of 1 U R-Nase Inhibitor, 5.5 µl of PCR water and 0.5 µl of 100 U M-MuLV reverse transcriptase) in a final reaction volume of 20 µl for each sample. The samples were then incubated at 22° for 10 minutes, 42° C for 40 minutes, then 95° C for 4 minutes to inactivate the enzyme.

PCR Amplification

For PCR amplification reaction, 2 µl cDNA template and 1 µl of 10 µM of each forward and reverse primer was added to 21 µl of a master mix containing 5 µl of 1X Taq reaction buffer, 4 µl of 200 µM of dNTP mix, 7 µl of 2 mM of MgCl$_{2,}$ 4.875 µl PCR water and 0.125 µl of 1.25 U Taq DNA polymerase in a reaction volume of 25 µl. The PCR was run at 94° C for 5 minutes, (94° C for 30 seconds, 50° C for 30 seconds, 72° C for 1 minute) for 35 cycles with a final extension step of 1 cycle at 72° C for 10 minutes.

PCR analyses

The PCR reaction amplification products were analysed by electrophoresis on 1.5% high resolution agarose gel stained with 3 µl ethidium bromide and visualized under UV light.

Primer design

The reverse and forward PCR primers used for screening BQCV, ABPV, KBV, DWV, SBV and CBPV (Table 2) were according to Antúnez, Alessandro, Corbella, Ramallo, and Zunino (2006). All the primers were supplied by Inqaba biotec East Africa Limited.

Data analysis

Each detected virus was analysed individually using Fisher's exact test of independence of the fife Package (Fife, 2014) in R. The analysis was conducted using the program R (R Core Team, 2014) to test whether the proportions of number of viruses detected are heterogeneous with the honey bee colony strength. A pairwise Fisher's test followed by Bonferroni pairwise correction was implemented to identify the significant pairwise comparisons.

Results

Five honey bee viruses (BQCV, ABPV, SBV, CBPV, and DWV) (Table 3) were detected in 35.7% of the samples tested. KBV was not detected while samples taken from two apiaries (Ngetta 1 and Kyenjojo) did not test positive for any of the screened six bee viruses. Two viruses, CBPV and BQCV were detected from samples from Western-highlands agro-ecological zone, while all the five viruses detected in this study came from samples of Mid-North agro-ecological zone.

CBPV was the most frequently detected in the adult bee samples (20.4%), followed by BQCV (15.3%), then SBV (14.3%), ABPV (11.2%) and DWV (1.0%) (Table 4). Occurrence of more than one virus in a single apiary or in a bee colony (Table 5) was detected in samples from four apiaries. However, none of the six bee viruses (ABPV, BQCV, SBV, KBV, CBPV, ABPV) screened was detected in the varroa mite samples collected from the colonies.

Table 1. Apiary sites and number of bee samples taken from Mid North and Western highlands agro-ecological zones.

Agro-ecological zone	Apiary	Colony strength	
		Weak (<2000 bees)	Strong (>10,000 bees)
Mid North	Amach 1 (n = 15)	2	13
	Amach 2 (n = 21)	1	20
	Ngetta 1 (n = 13)	0	13
	Ngetta 2 (n = 18)	1	17
Western Highlands	Kyenjojo (n = 10)	4	6
	Rwebitaba (n = 20)	6	14
	nd (n = 10)	1	9

Numbers represent-number of samples from weak/strong colonies drawn from the apiary; <2000 bees and >10,000 bees are parameters used to rate weak and strong colonies respectively.

Table 2. Primers used for screening BQCV, ABPV, KBV, DWV, SBV and CBPV.

Virus name	Primer name	Amplification target	Sequence (5'-3')	Length bp	Reference
BQCV	BQCV-F	Structural poly-protein gene	TGGTCAGCTCCCACTACCTTAAAC	700	Benjeddou, Leat, Allsopp, and Davison (2001)
	BQCV-R		GCAACAAGAAGAAACGTAAACCAC		Benjeddou et al. (2001)
ABPV	ABPV-F	RNA polymerase gene	TTATGTGTCCAGAGACTGTATCCA	150	Benjeddou et al. (2001)
	ABPV-R		GCTCCTATTGCTCGGTTTTTCGGT		Benjeddou et al. (2001)
KBV	KBV-F	RNA polymerase gene	GATGAACGTCGACCTATTGAA	393	Stoltz, Shen, Boggis, and Sisson (1995)
	KBV-R		TGTGGGTTGGCTATGAGTTCA		Stoltz et al. (1995)
DWV	DWV-F	Gene for poly-protein	TTTGCAAGATGCTGTATGTGG	395	Tentcheva et al. (2006)
	DWV-R		GTCGTGCAGCTCGATAGGAT		Tentcheva et al. (2006)
SBV	SBV-F	Poly-protein gene	GGATGAAAGGAAATTACCAG	426	Tentcheva et al. (2004)
	SBV-R		CCACTAGGTGATCCACACT		Tentcheva et al. (2004)
CBPV	CBPV-F	Viral capsid gene	AGTTGTCATGGTAACAGGATACGAG	900	Ribière et al. (2002)
	CBPV-R		TCTAATCTTAGCACGAAAGCCGAG		Ribière et al. (2002)

Another aspect that was investigated was virus presence and colony strength. CBPV presence was significantly ($p = 0.0074$, 95% confidence interval: 1.4 to 21.4) correlated with colony strength, the proportion of infected colonies with CBPV scored as strong was less than that of weak infected colonies.

Presence of the other viruses detected in the honey bee colonies, ABPV ($p = 0.655$, at 95% CI: 0.1303 to 7.9770), SBV ($p = 0.2095$, 95%CI: 0.0 to 1.7) and DWV ($p = 1.0000$, 95%CI: 0.0 to 233.1) were all not correlated with colony strength. The total number of viruses detected was not significantly ($p = 0.1839$) correlated with the colony strength.

Discussion

In this study, five different bee viruses (BQCV, ABPV, SBV, CBPV and DWV) were detected, with CBPV as a new virus in Uganda's bee colonies. This report on the presence of bee viruses in Uganda follows reports by Chemurot (2017) and Kajobe et al. (2010). In their study, Kajobe et al. (2010) detected only BQCV from Uganda's bee colonies and none of their samples tested positive for DWV, SBV, ABPV, CBPV. The findings of Kajobe et al. (2010) show that BQCV was more prevalent (40%) in Western-highlands agro-ecological zone with a single positive sample from the Mid-North agro-ecological zone.

Another study by Chemurot (2017) in Eastern and Western Highlands agro-ecological zones reported the presence of DWV, ABPV, BQCV and SBV in Uganda's honey bee colonies. He did not detect CBPV but found BQCV, DWV, ABPV and SBV in samples from the two zones with DWV as the most prevalent (20%). In the present study, all the five different bee viruses (BQCV, ABPV, SBV, CBPV and DWV) were present in the Mid-North AEZ whereas only two (CBPV and BQCV) were detected in samples from Western Highlands AEZ.

The presence of CBPV, which was not detected in the studies of Chemurot (2017) and Kajobe et al. (2010), suggests recent invasion in Western Highlands and Mid-North AEZs. The possible explanation for CBPV not being detected before in Uganda and its neighbors is that CBPV onset of symptoms can be sudden and the outbreak location unpredictable (Budge, 2017). Its occurrence could not have been influenced by season of sample collection in the present study. The study of Chemurot (2017) in Western-highlands was also undertaken in the second wet season.

The high prevalence of CBPV (20.4%) in this study shows that its prevalence could be increasing as described by Traynor et al. (2016) in their study from USA, where CBPV presence doubled annually from 2010 when it was first detected in 0.7% of the colonies. According to Budge (2017), the disease is not commonly detected when random apiaries are tested, the prevalence is higher when apiaries with poor health are targeted. The implication of this is that the prevalence would have been even higher in the present study, if apiaries with poor health would have been targeted.

In this study, CBPV presence significantly correlated with colony strength; occurring more in weak colonies than in strong ones. This finding is in agreement with that of Ribière, Olivier, and Blanchard (2010) who state that the disease affects strong colonies and thousands are seen dead in front of hives making it a risk factor for colony weakness. In our study, symptoms of the disease as described by Ribière et al. (2010) were not observed in apiaries. Similar findings were reported in Uruguay, where infected colonies that did not show any symptoms eventually died after the study (Antúnez et al., 2006). The implication of this for our study is that colony strength may continue to decline due to CBPV.

A possible explanation for the absence of DWV, ABPV, and SBV in samples obtained from Western highlands, as compared to the findings of Chemurot (2017), is that some apiaries might be still free of viruses. The present study and that of Chemurot (2017) in Western highlands agro-ecological zone sample collection was done in different apiaries. The implication of this is that spread of these viruses should be controlled.

Table 3. Bee viruses in apiaries in Mid North and Western Highlands agro ecological zones of Uganda. Samples collected in October and November 2017.

Location		Honey bee viruses					
AEZ	Apiary	CBPV	BQCV	KBV	DWV	SBV	ABPV
Mid North	Amach 1	+	+	–	–	+	+
	Amach 2	+	+	–	+	+	+
	Ngetta 1	–	–	–	–	–	–
	Ngetta 2	+	+	–	–	+	+
Western Highlands	Kyenjojo	–	–	–	–	–	–
	Rwebitaba	+	+	–	–	–	–
	nd	+	–	–	–	–	–

+: presence; –: absence.

Table 4. Relative abundance of honey bee viruses in apiaries of Mid North and Western Highlands agro ecological zones of Uganda.

	Location/origin						
	Mid North AEZ				Western highlands AEZ		
Honey bee virus	Amach 1(n = 15)	Amach 2 (n = 21)	Ngetta 1 (n = 13)	Ngetta 2 (n = 18)	Kyenjojo (n = 10)	Rwebitaba (n = 20)	Total (%) (n = 98)
CBPV	5	4	0	1	0	10	20 (20.4%)
ABPV	6	4	0	1	0	0	11 (11.2%)
SBV	4	9	0	1	0	0	14 (14.3%)
DWV	0	1	0	0	0	0	1 (1.0%)
BQCV	5	6	0	1	0	3	15 (15.3%)
KBV	0	0	0	0	0	0	0 (0.0%)

Numbers represent number of infested colonies. Numbers in bracket in total the column represent the percentage of infected colonies.The samples were collected from October to November 2017.

Table 5. Co-occurrence of viruses in honey bee colonies in Mid North and Western Highlands agro ecological zones.

		Location/origin					
		Mid North AEZ				Western highlands AEZ	
No of viruses	Type of infection	Amach 1 n = 15	Amach 2 n = 21	Ngetta 1 n = 13	Ngetta 2 n = 18	Kyenjojo n = 10	Rwebitaba n = 20
1	CBPV	–	–	–	–	–	8
	ABPV	1	–	–	–	–	–
	SBV	1	4	–	–	–	–
	DWV	–	–	–	1	–	–
	BQCV	2	1	–	–	–	1
2	CBPV- ABPV	1	–	–	–	–	–
	ABPV- SBV	–	1	–	–	–	–
	SBV- BQCV	–	1	–	–	–	–
	CBPV- BQCV	–	–	–	–	–	3
3	CBPV-ABPV-SBV	1	–	–	–	–	–
	CBPV-ABPV-BQCV	1	1	–	–	–	–
	CBPV-SBV-BQCV	–	1	–	–	–	–
4	CBPV-ABPV-SBV- BQCV	2	2	–	1	–	–

nd all samples from this apiary were pooled to make one sample. Numbers represent number of infested colonies.

The finding that shows the presence of the other viruses (BQCV, ABPV, SBV, DWV) detected in the honey bee colonies not significantly affected by the colony strength is related to findings of Onyango et al. (2016). In their study, the detected viruses were not associated with colony strength. The implications for this are that the invasion of Uganda's honey bee colonies by different bee viruses requires time to monitor infected colonies to ascertain the impact of the disease.

Conclusion

Here we report one new honey bee virus (CBPV) that probably only recently invaded Uganda's bee colonies in addition to DWV, SBV, ABPV, and BQCV. We also report multiple bee virus infections in some colonies. CBPV is impacting colony strength and thus affecting honey production. Its high prevalence shows infected colonies may increase. Thus the colony strength of more honey bee colonies may continue to decline due to CBPV, suggesting monitoring, early detection of CBPV in honey bee colonies and formulation of control methods. The presence of other viruses did not affect colony strength.

Note

1. A pooled sample consisted of 10 bees taken from a single honey bee colony.

Acknowledgement

The authors acknowledge the support of; Diana Namayanja, Angiro Christopher, Mugume Ronald and Akullo Immaculate in data collection.

Disclosure statement

No potential conflicts of interest are reported by the authors.

Funding

This work was supported by the Government of Uganda and World Bank through the National Agricultural Research Organisation (NARO) under a project title Agricultural Technology and Agribusiness Advisory Services (ATAAS).

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