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Short Communication

First molecular detection of a viral pathogen in Ugandan honey bees

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ABSTRACT

Ugandan honey bees (*Apis mellifera* L.) produce honey, and are key pollinators within commercial crops and natural ecosystems. Real-time RT-PCR was used to screen immature and adult bees collected from 63 beekeeping sites across Uganda for seven viral pathogens. No samples tested positive for *Chronic bee paralysis virus*, *Sacbrood virus*, *Deformed wing virus*, *Acute bee paralysis virus*, *Apis iridescent virus* or *Israeli acute paralysis virus*. However, *Black queen cell virus* (BQCV) was found in 35.6% of samples. It occurred in adults and larvae, and was most prevalent in the Western highlands, accounting for over 40% of positive results nationally.

Beekeeping is widespread throughout Uganda, where the most common hive species is *Apis mellifera* L. (Roberts, 1971). These bees are of immense economic significance as producers of honey and beeswax, and as pollinators of commercially-valuable coffee, cotton and sunflowers (Roberts, 1971; Ogaba, 2003; UEPB, 2005). Pollinating *A. mellifera* play an equally crucial role in conserving the biodiversity in many natural ecosystems (Crane, 1999; Commonwealth Secretariat, 2002). The new and expanding sector of Ugandan apiculture is integral to programmes for poverty alleviation and rural development (MAAIF, 2000). It is therefore important that Uganda maintains bee populations that are strong enough to sustain productivity and essential pollination services (MFPED, 2000; Ogaba, 2003; UEPB, 2005). Honey bees are prone to a range of viral diseases, but there is no literature pertaining to their prevalence or distribution in Uganda. Since Uganda is directly bordered by five other countries; Rwanda, Kenya, Tanzania, Sudan and the Democratic Republic of the Congo (DRC), where certain diseases are reported as being/suspected to be present, there is no safe assumption that the causative pathogens are absent in Uganda (Dubois and Collart, 1950; Rosario-Nunes and Tordo, 1960; Bauduin, 1966; Kigatiira, 1984, 1988; Kumar et al., 1993; Matheson, 1993; Mostafa and Williams, 2002; Ellis and Munn, 2005). The present study provides the first report of any virus in East African (Ugandan) honey bees.

Larval, pupal and adult *A. mellifera* were collected from 138 colonies at 63 sites in nine of Uganda's 10 agro-ecological zones (Table 1). With the exception of one site in the Western highlands, where dead worker-bees were found, samples came from colonies that were free from overt signs of disease. Sample size was fifteen individuals/site, to allow a high (95%) probability of disease detection, even in small colonies (<10,000 bees) and/or where disease prevalence was low (20% of the population affected) (Cannon and Roe, 1982). However, on three occasions sample sizes were unavoidably lower, where the destructive nature of traditional honey-harvesting left insufficient surviving brood. All samples were exported to the UK for molecular diagnoses in the Molecular Technology Unit of The Food and Environment Research Agency (Fera), York.

RNA was extracted on a Kingfisher mL magnetic particle processor (Thermo Electron Corporation) using the default RNA protocol and including the optional 5 min heating step at 65 °C prior to elution. Groups of five bees, were homogenised in 5 mL of Guanidine Lysis Buffer L6, and centrifuged (2 min, 6000*g*). Clarified extracts (1 mL) were mixed with 50 µl paramagnetic particles (Promega: MD1441) and processed on a Kingfisher mL following the manufacturer's protocol. Blank extraction controls were completed to monitor contamination.

Samples were screened for seven honey bee viruses using real-time RT-PCR: *Black queen cell virus* (BQCV), *Chronic bee paralysis virus* (CBPV), *Sacbrood virus* (SBV), *Deformed wing virus* (DWV), *Acute bee paralysis virus* (ABPV), *Apis iridescent virus* (AIV) and *Israeli acute paralysis virus* (IAPV). This panel of viruses was chosen because of their known associations with honey bees, the damaging effects of the diseases they cause, and because of their potentially high economic impact on beekeeping. Real-time RT-PCR testing used assays and cycling conditions as described by Chantawannakul et al. (2006). Primers for IAPV were modified from Pal-

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Table 1
Number of sites and bee samples from at least one district representing each agro-ecological zone.

Agro-ecological zone	Estimated density of beekeepers in district	Sites sampled (district/village or beekeeper)	No. of sites sampled
Eastern	Medium (1000–5000 beekeepers)	Mbale/Bufumbo	1
		Mbale/Municipality	1
		Mbale/Mutoto village	4
		Tororo/Kwappa	1
		Tororo/Rugbongi	2
Lake Albert crescent	Medium (1000–5000 beekeepers)	Hoima/Bulindi	2
		Masindi/Nyabyeya	3
Lake Victoria crescent	High (5000–10,000 beekeepers)	Kampala/Kawempe	2
		Kampala/Mengo	1
		Luwero/Bututumula	3
		Luwero/Katikamu	1
		Mubende/Mubende	1
		Nakasongola/Nakasongola	2
Mid northern	High (5000–10,000 beekeepers)	Kitgum/NAADS apiary	1
		Lira/Adingo	1
		Lira/Amone	1
		Lira/DickAmone	1
		Lira/Eswapu	1
		Lira/Ogur	1
South east	Low (<1000 beekeepers)	Busia/Busia	1
		Kamuli/Kisozi	3
Southern drylands	Low (<1000 beekeepers)	Mbarara/Rwampara	3
Southern highlands	Medium (1000–5000 beekeepers)	Rukungiri/Bujumbura	5
		Rukungiri/Bwambara	1
West nile	High (5000–10,000 beekeepers)	Arua/Katrini	3
		Arua/Ayivu county Pajulu	1
		Yumbe/Kechi	1
Western highlands	High (5000–10,000 beekeepers)	Kabarole/Bukuku	3
		Kabarole/Bwenderwe	1
		Kabarole/Fort Portal	5
		Kabarole/Karambi	1
		Kabarole/Katurru	1
		Kabarole/Mugusu	3
		Kyenjojo/Kasamba	1
Karamoja drylands	Low (<1000 beekeepers)	Not sampled	
Total			63

acios et al. (2008) to detect all known variants of IAPV (Jeffrey Hui, Centre for Infection and Immunity, Columbia University, USA, pers. comm.). All real-time RT-PCR protocols were validated using pure viral cultures and virus-infected bees. An internal control allowed interpretation of negative results (Ward et al., 2007). C_T values were assessed using Sequence Detection Software v2.2.2 (Applied Biosystems). Samples giving the lowest C_T values were selected for confirmatory testing using direct sequencing.

No samples tested positive for DWV, SBV, CBPV, ABPV, IAPV or AIV. However, screening with RT-PCR suggested that BQCV was present in a number of samples. To validate our findings, samples that tested positive for the presence of BQCV were subjected to confirmatory screening using conventional RT-PCR coupled with direct sequencing (Tentcheva et al., 2004a). Virus positive and no template controls were also performed for each virus. To ensure provenance of nucleotide data from Ugandan virus, RT-PCR products from Ugandan and BQCV-positive controls were sequenced directly. Initial sequence identity was confirmed by identifying the closest sequence matches from all the published sequences on the EMBL database using the BLASTN search algorithm. Alignments and phylogenetic analyses of closely related sequences were completed using the default setting of MEGA 4.0 (Tamura et al., 2007). The nucleotide and the translated protein sequences were analysed. Direct sequencing of the RT-PCR product from one positive sample confirmed the identity of BQCV (Accession number FJ495181). BQCV from Uganda clusters with other BQCV isolates from South Africa and the European Union and is distinct from isolates of other RNA viruses. Initially isolated in the 1970s, from the remains of decomposing queen larvae found within blackened cells, BQCV is widespread throughout the EU (Bailey and Woods, 1977). In Australia this virus is thought to be the most common cause of death in queen larvae (Anderson, 1993).

BQCV was found in 35.6% of Ugandan samples tested (Table 2). It occurred in 87.5% of adult samples and 12.5% of larvae, but was not found in any pupae that were screened, even those from BQCV-positive colonies. This pattern of infection is consistent with other studies, which show that although BQCV affects all life-stages of *A. mellifera*, it is usually detected in adults, rather than in brood or pupae (Siede and Büchler, 2003; Tentcheva et al., 2004a). Infected material came from seven of the nine zones that were sampled, the two exceptions being the South east and Southern highlands. BQCV was most prevalent in the Western highlands, where it was found in seven separate sites (accounting for over 40% of positive results for BQCV nationally). It was comparatively less widespread in the Eastern zone (found at three sites), and only present in single sites elsewhere. Although data collected in this study suggests that certain parts of Uganda may be virus free, given that infected zones directly border apparently healthy zones, this is highly unlikely to be the case. Absence of positive samples is more likely to reflect the comparatively small sample sizes, coupled with the possibility that virus is present at low (sub-clinical) levels. It is the view of these authors that BQCV should be assumed present throughout the country.

Regarding the implications of BQCV infection for Ugandan apiculture, with one exception, samples collected for the purposes of this study came from asymptomatic colonies, demonstrating

Table 2
The incidence of BQCV in different bee development stages from nine agro-ecological zones of Uganda.

Agro-ecological zone	District	Site no.	Bee life-stage	Mean C_T values (±SD)	
				BQCV + ve	Internal control 18S rRNA
Eastern	Mbale	1	Adult	38.48 (±2.143)	15.05 (±0.102)
	Mbale		Adult	30.91 (±0.044)	15.17 (±0.020)
	Mbale		Adult	29.97 (±0.125)	16.98 (±0.128)
	Mbale	2	Adult	34.77 (±0.071)	15.71 (±0.124)
	Mbale		Larva	32.16 (±0.320)	13.97 (±0.038)
	Mbale	3	Larva	21.56 (±0.312)	14.60 (±0.053)
	Mbale		Larva	28.12 (±0.007)	13.87 (±0.082)
Lake Albert crescent	Hoima	1	Adult	33.59 (±0.342)	16.05 (±0.007)
	Hoima		Adult	32.10 (±0.195)	15.32 (±0.227)
	Hoima		Adult	33.71 (±0.575)	15.56 (±0.034)
Lake Victoria crescent	Mubende	1	Adult	31.52 (±0.583)	22.70 (±0.367)
	Mubende		Adult	33.70 (±0.068)	28.77 (±0.115)
Mid northern	Lira	1	Adult	38.27 (±0.395)	18.45 (±0.072)
South east	No samples tested positive for BQCV				
Southern drylands	Mbarara	1	Adult	27.47 (±0.428)	16.96 (±0.060)
Southern highlands	No samples tested positive for BQCV				
West nile	Yumbe	1	Adult	30.22 (±0.468)	14.31 (±0.071)
	Yumbe		Adult	32.17 (±1.316)	14.37 (±0.062)
	Yumbe		Adult	30.54 (±0.171)	14.38 (±0.105)
	Yumbe		Adult	32.75 (±0.222)	15.20 (±0.132)
	Yumbe		Larva	36.97 (±4.279)	14.31 (±0.225)
Western highlands	Kabarole	1	Adult	35.84 (±0.122)	22.76 (±0.079)
	Kabarole	2	Adult	33.17 (±0.948)	17.45 (±0.364)
	Kabarole		Adult	30.34 (±0.027)	15.96 (±0.042)
	Kabarole		Adult	28.74 (±0.566)	16.75 (±0.020)
	Kabarole	3	Adult	35.72 (±2.277)	18.10 (±0.005)
	Kabarole	4	Adult	35.62 (±0.676)	16.90 (±0.365)
	Kabarole	5	Adult	32.83 (±0.500)	20.13 (±0.198)
	Kabarole		Adult	29.82 (±0.046)	16.04 (±0.030)
	Kabarole		Adult	30.00 (±0.075)	18.16 (±0.050)
	Kabarole		Adult	28.76 (±2.083)	17.02 (±0.027)
	Kabarole	6	Adult	38.36 (±2.326)	16.00 (±0.036)
	Kabarole		Adult	34.32 (±0.246)	16.87 (±0.167)
	Kyenjojo	7	Adult	32.03 (±0.199)	16.16 (±0.186)

that the presence of virus within a colony does not necessarily result in overt disease. It has been observed that certain viruses are found in apparently healthy adult bees and pupae (Dall, 1985; Anderson and Gibbs, 1988; Hung et al., 1996a), and only when they occur in colonies co-infested with the parasitic mite *Varroa destructor* will virus-induced mortality follow (Shimanuki et al., 1994; Hung et al., 1996b). This suggests that on some occasions, viruses multiply to lethal levels when activated by feeding mites. *Varroa* has a weakening effect on its hosts, rendering them more susceptible to infection (Yang and Cox-Foster, 2005); dissemination of pathogens is also facilitated when viruses enter through the lesions caused by feeding mites (Ball, 1989). For some viruses, transmission by *V. destructor* has been proven experimentally (Békési et al., 1999; Nordström et al., 1999; Chen et al., 2004; Tentcheva et al., 2004a), and *Varroa* may act as vectors for BQCV (Bailey, 1976). Although EU studies have been unable to confirm this assumption (Tentcheva et al., 2004b; Forgách et al., 2008), BQCV has been detected in mites from Thailand (Chantawannakul et al., 2006), and in South Africa, BQCV has been linked with notably increased honey bee mortality when associated with *Varroa* (Swart et al., 2001; Davison et al., 2003).

There is no available data for the incidence of *Varroa* in Uganda, but they are believed to be absent from this country, and neighbours Kenya, Tanzania, Sudan and DRC (Griffiths and Bowman, 1981; Kigatiira, 1984, 1988; Matheson, 1993; Ellis and Munn, 2005). The status of *Varroa* in Rwanda is unknown (Ellis and Munn, 2005). However, the mite is prevalent throughout much of the rest of the world, having spread from Asia to all continents except Australia (Webster and Delaplane, 2001); *Varroa* is confirmed as present and spreading in sub-Saharan Africa (Allsop, 1997a, b, 1999), including Nigeria (Ukattah, 2008). This means that while the current impact of BQCV in Uganda may be minimal, should *Varroa* reach districts where BQCV is endemic, then the combination of the mite and the virus could have a much more serious consequences. It is relevant to note that although this study sampled over 150 colonies, allowing detection of diseases with a low (10%) prevalence, no DWV was found. This virus is commonly associated with *Varroa* infestations elsewhere, and where mites are present prevalence of DWV typically exceeds 90% (de Miranda and Genersch, 2010).

A. mellifera is not the only type of bee utilised in Ugandan apiculture. At least six species of stingless bees are hunted and/or cultured for their honey and brood (Kajobe, 2007; Kajobe and Roubik, 2006). The incidence of viral pathogens in these additional bee stocks is yet to be investigated, and their susceptibility to honey bee viruses is entirely unknown.

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selection, nest architecture and colony characteristics of equatorial afro-tropical stingless bees), and the National Agricultural Research Organisation (NARO) of Uganda. All samples screened in the UK were imported under Defra Import Authorisation No. POAO/2008/373.

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