First detection of Israeli acute paralysis virus (IAPV) in France, a dicistrovirus affecting honeybees (Apis mellifera).

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FIRST DETECTION OF ISRAELI ACUTE PARALYSIS VIRUS (IAPV) IN FRANCE, A DICISTROVIRUS AFFECTING HONEYBEES (*Apis mellifera*).

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Abstract

Bee samples were collected in French apiaries that displayed severe losses and mortality during the winter (from November 2007 to March 2008). They were screened for the presence of Israeli acute paralysis virus (IAPV) by using RT-PCR. Five out of 35 surveyed apiaries, located in two different geographical areas, were found positive. This represents the first reported detection of IAPV in France. The specificity of the PCR products was checked by sequencing. The phylogenetic analysis showed that French isolates of IAPV were closely related to a cluster including American and Australian isolates. Nevertheless, most of American isolates previously reported to be associated to Colony Collapse Disorder (CCD) and an Israeli isolate first isolated in 2004 from dead bees were included in another cluster. Since IAPV was detected in only 14 % of the affected apiaries, it was not possible to establish a causal link between IAPV and the severe winter losses that occurred.

Keywords: Israeli acute paralysis virus (IAPV), RT-PCR detection, winter losses, *Apis mellifera.*

Short communication

Israeli acute paralysis virus (IAPV) was first described in 2004 in Israel, where severe bee mortality has inflicted heavy losses on Israeli apiculture (Maori et al., 2007). Based on homology and genomic structure, IAPV was characterized as a new member of the *Dicistroviridae* family (Christian et al., 2005), closely related to Kashmir bee virus (KBV) and Acute bee paralysis virus (ABPV), but genetically and serologically distinct (Maori et al., 2007). Recently, the presence of IAPV has been strongly correlated with a new syndrome of honey bee losses observed in the United States, called the Colony Collapse Disorder (CCD) (Cox-Foster et al., 2007). These authors reported that IAPV could be a statistically significant marker for CCD. However, this hypothesis still remains under discussion (Stokstad, 2007; Chen and Evans, 2007; Anderson and East, 2008; Cox-Foster et al., 2008). IAPV has been isolated in Israel, Australia and different states of USA such as Florida, California, Maryland

47 and Pennsylvania (Chen and Evans, 2007; Cox-Foster et al., 2007; Maori et al., 2007). In this
48 paper, we report the first detection of IAPV in bee samples from France, collected in 2008.
49 During the last winter, honey bee colony losses and mortalities have occurred in French
50 apiaries. In some cases, beekeepers have reported up to 90% mortality rate of the colonies.
51 A preliminary survey was conducted on 35 apiaries showing severe winter losses in various
52 parts of France (16 departments) to assess the pathological context. Given that last winter
53 losses can suggest those observed in case of CCD such as a rapid loss from a colony of its
54 adult bee population (Cox-Foster et al., 2007), it appeared interesting to assess the presence
55 of IAPV. To date, IAPV has never been investigated in France. Furthermore, Acute bee
56 paralysis virus (ABPV) and Kashmir bee virus (KBV) have been looked for, since (i) they are
57 genetically closely related to IAPV and (ii) all positive samples for IAPV also contained KBV in
58 a recent report on CCD (Cox-Foster et al., 2007). Moreover, ABPV was detected in bees from
59 colonies infested with *Varroa destructor*, and presenting high winter mortality (Bakonyi et al.,
60 2002; Siede et al., 2006).
61 Thirty-five apiaries distributed on all the French territory were sampled (one hive per apiary).
62 Sample preparation, RNA extraction and cDNA synthesis were performed as described
63 previously (Blanchard et al., 2007; Ribière et al., 2002). Molecular diagnosis (ABPV, IAPV
64 and KBV) were performed using primer pairs described previously (Bakonyi et al., 2002; Cox-
65 Foster et al., 2007; Maori et al., 2007, Stoltz et al., 1995) (Table 1). Unexpectedly, among the
66 35 apiaries, IAPV was detected in five apiaries located in two distinct regions, including three
67 in the department of Lozère and 2 in the department of Rhône in France. PCR products
68 (768bp) obtained from IAPV-positive samples were sequenced in both orientations by using
69 primers IAPV_IGR_F and IAPV_IGR_R described by Cox-Foster et al. (2007) and compared
70 to IAPV, ABPV and KBV sequences available on GenBank (Cox-Foster et al., 2007; Maori et
71 al., 2007; de Miranda et al., 2004; Govan et al., 2000). After exclusion of the primer
72 sequences, the nucleotide sequences reported in Table 2 were aligned by using the
73 CLUSTAL_X program (Thompson et al., 1997). The phylogenetic tree was constructed by
74 using the maximum likelihood method as implemented in the PHYLOWIN program (Galtier et
75 al., 1996) and 500 bootstrap replicates. The phylogenetic tree was visualized using TreeView
76 (Page, 1996) (Figure 1). The IAPV sequences from French isolates described in this paper

77 were submitted to the GenBank database under Accession Nos. EU604006, EU604007,
78 EU604008, EU604009 and EU604010.
79 IAPV isolates segregated in two main lineages supported by strong bootstrap values and
80 clearly separated from KBV and ABPV as already shown by Cox-Foster et al. (2007). Lineage
81 A contained two isolates from the United States, two from Australia and all French isolates
82 that grouped together. Lineage B contained most of American isolates (15/17), including the
83 isolate first described in Israel and two Australian isolates. Overall IAPV isolates tended to
84 segregate according to their geographical origin.
85 During this study, 85% of apiaries were diagnosed with one or several diseases and/or
86 pathogens, such as varroasis (50%), ABPV (40%) and nosemosis (30%) (unpublished
87 results), in agreement with previous studies demonstrating the crucial role of diseases in
88 winter losses (Bakonyi et al., 2002; Faucon et al., 2002, Siede et al., 2006). All IAPV-positive
89 apiaries were also positive for varroasis, three were positive for nosemosis (*N. ceranae*) and
90 three for ABPV. Furthermore, KBV was also detected, but only in the samples where IAPV
91 was found. Therefore, although IAPV was detected in a significant number of the surveyed
92 apiaries (14%), it was not possible to establish a causal relationship between IAPV and the
93 severe winter losses which occurred in France, unlike the CCD-related cases described by
94 Cox-Foster et al. (2007). Future work will seek to investigate the prevalence of IAPV in
95 France. In this purpose sensitivity of PCR test will be assessed.
96 Since KBV and IAPV were always concomitantly detected in the analysed samples, the
97 relationship between these viruses was investigated further. The RT-PCR products obtained
98 from KBV positive samples were checked by sequencing. Pair-wise comparison with IAPV
99 sequences (Maori et al., 2007) and KBV sequences (de Miranda et al., 2004) showed that the
100 sequences obtained in our study were more closely related to IAPV (8% of divergence) than
101 to KBV (13% of divergence). French KBV-like sequences obtained in this study were also
102 closely related (2-3% mean distance) to a putative KBV sequence from Australia (AUSbee
103 AF034541), previously shown to be genetically distant to other KBV isolates obtained from
104 USA (Hung et al., 2000). The same primers were used to identify KBV in French bee samples
105 (Tentcheva et al., 2004). However, KBV-like sequences reported by the same authors
106 (AY669845 - AY669846) are more closely related to the IAPV sequence described by Maori

et al. (2007) (2% divergence), than to the KBV sequence (14% divergence) described by de Miranda et al. (2004) (not shown). Altogether, these observations raise the question of the specificity of the primers used (Stoltz et al., 1995) and suggest that they could also amplify IAPV. If so, it could be hypothesized that IAPV was already present in France in 2002, but identified as KBV by Tentcheva et al. (2004). Further studies are necessary to ascertain this hypothesis, such as full length sequencing of various IAPV and KBV isolates, and retrospective analysis of available honey bee samples.

The exact role of IAPV in winter mortalities of the bee colonies in France and the conditions of its importation are not known at present. French isolates are clustering in sub-lineage A with two Australian isolates coming of apparently healthy bees and two isolates from USA, whereas other IAPV isolates recovered from cases associated with mortalities (Cox-Foster et al., 2007; Maori et al., 2007) are included in sub-lineage B (Figure 1). This suggests that different IAPV isolates may possess different pathogenic properties, as already pointed out by Chen and Evans (2007). Alternatively, other factors such as the influence of the environment or concurrent pathologies may affect the health status of the apiaries. This survey is currently ongoing to further investigate the involvement of other pathologies such as varroasis, nosemosis, or due to other viruses in the severe winter losses that occurred in France in 2007-2008.

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Captions to figures

Table 1.

137 List of primers used for the detection of ABPV, IAPV and KBV.

138

139 Table 2.

140 IAPV isolates used in phylogenetic analysis

141 [a,b,c,d] 100 percent identity between each sequence

142

143 Figure 1.

144 Maximum likelihood phylogenetic tree based on 727 nt sequence, including the intergenic
145 region (IGR) of 20 IAPV isolates from Israel (Maori et al., 2007), the United States, Australia
146 (Cox-Foster et al., 2007) and France (this study). ABPV and KBV were used as an outgroup.
147 The number of each node represents the bootstrap values as the result of 500 replicates.
148 Bootstraps values <50% were omitted. The scale corresponds to the number of substitutions
149 per site.

150 Table 1
151

Primer	Sequence (5'-3')	Length (bp)	Amplification target	Position (GenBank accession no.)	Reference
ABPV 1	CATATTGGCGAGCCACTATG	398	Viral RNA capsid gene	8115 - 8512 (AF126050)	Bakonyi et al., (2002)
ABPV 2	CCACTTCCACACAACTATCG				
IAPV_IGR_F	CGATGAACAACGGAAGGTTT	767	Viral RNA Intergenic Region	6128 – 6894 (NC009025)	Cox-Foster et al., (2007)
IAPV_IGR_R	ATCGGCTAAGGGGTTTGTTT				
IAPV F	AGACACCAATCACGGACCTCAC	475	Viral RNA capsid gene	8860 – 9334 (NC009025)	Maori et al., (2007)
IAPV R	AGATTTGTCTGTCTCCCAGTGCACAT				
KBV 1	GATGAACGTCGACCTATTGA	414	Viral RNA polymerase gene	5406 – 5819 (AY275710)	Stoltz et al., (1995)
KBV 2	TGTGGGTTGGCTATGAGTCA				

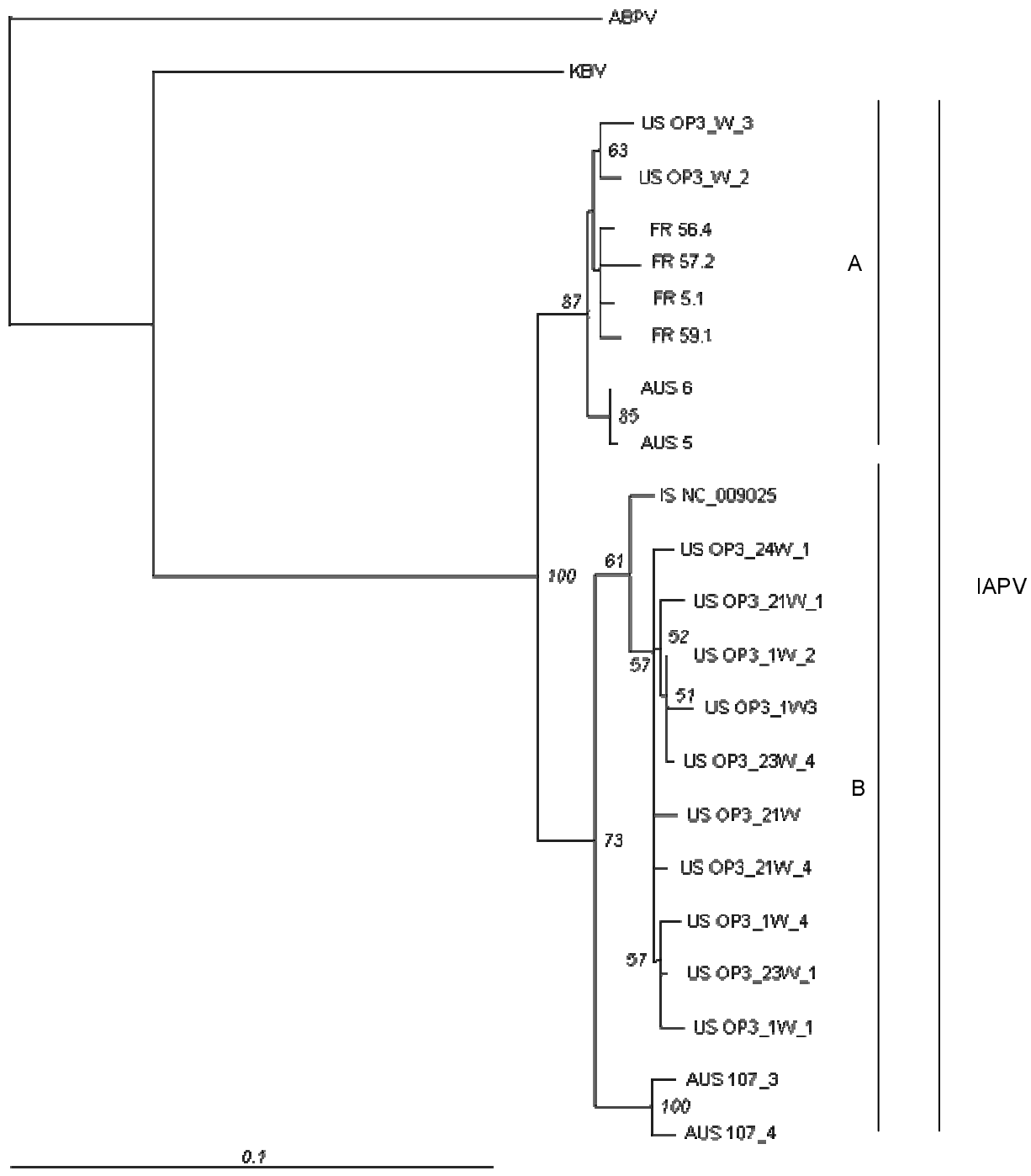
152

153 Table 2
154

Isolate	Country	Reference	GenBank accession No.
	Israel	Maori et al., (2007)	NC_009025
107_4	Australia	Cox-Foster et al. (2007)	EU122346
5	Australia	Cox-Foster et al. (2007)	EU122347
6	Australia	Cox-Foster et al. (2007)	EU122348
107_3	Australia	Cox-Foster et al. (2007)	EU122349
OP3_1W_1	United States	Cox-Foster et al. (2007)	EU122350
OP3_1W_2	United States	Cox-Foster et al. (2007)	EU122351[a]
OP3_1W_3	United States	Cox-Foster et al. (2007)	EU122352
OP3_1W_4	United States	Cox-Foster et al. (2007)	EU122353
OP3_20W	United States	Cox-Foster et al. (2007)	EU122354[a]
OP3_21W	United States	Cox-Foster et al. (2007)	EU122355[b]
OP3_21W_1	United States	Cox-Foster et al. (2007)	EU122356
OP3_W_2	United States	Cox-Foster et al. (2007)	EU122357
OP3_W_3	United States	Cox-Foster et al. (2007)	EU122358
OP3_21W_4	United States	Cox-Foster et al. (2007)	EU122359
OP3_23W	United States	Cox-Foster et al. (2007)	EU122360[a]
OP3_23W_1	United States	Cox-Foster et al. (2007)	EU122361
OP3_23W_4	United States	Cox-Foster et al. (2007)	EU122362
OP3_24W_1	United States	Cox-Foster et al. (2007)	EU122363[c]
OP3_24W_2	United States	Cox-Foster et al. (2007)	EU122364[c]
OP3_24W_4	United States	Cox-Foster et al. (2007)	EU122365[c]
OP2	United States	Cox-Foster et al. (2007)	EU122366[b]
5.1	France	This report	EU604006 [d]
5.2	France	This report	EU604009 [d]
57.2	France	This report	EU604007
59.1	France	This report	EU604008
56.4	France	This report	EU604010
ABPV	South Africa	Govan et al., (2000)	AF150629
KBV	United States	de Miranda et al., (2004)	AY275710

155

Figure 1


ABPV
KBV
US OP3_1W_3
63
US OP3_1W_2
FR 56.4
FR 57.2
87
FR 5.1
FR 59.1
AUS 6
85
AUS 5
A
IS NC_009025
US OP3_24W_1
61
100
US OP3_21W_1
52
US OP3_1W_2
57
51
US OP3_1W3
US OP3_23W_4
B
US OP3_21W
73
US OP3_21W_4
US OP3_1W_4
57
US OP3_23W_1
US OP3_1W_1
AUS 107_3
100
AUS 107_4
IAPV
0.1

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167

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