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Short communication. First detection of Israeli Acute Paralysis Virus (IAPV) in Spanish honeybees

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Abstract

Spanish bee samples were analyzed for the presence of Israel Acute Paralysis Virus (IAPV). Some of these samples were collected from colonies presenting compatible symptoms with the colony collapse disorder (CCD, 240 out of 484) and the rest were asymptomatic. Only one of these samples was diagnosed as positive to IAPV by employing a one step RT-PCR that targets the ORF 2 of the IAPV genome. Specificity of the RT-PCR assay was evaluated by sequence analysis of size specific amplification products. IAPV nucleotide sequences already published in GeneBank were used to construct a phylogenetic tree that included the new Spanish IAPV sequence (FJ821506). They segregated in two main lineages and the Spanish isolate was mainly related with the American ones. As IAPV was detected in Spain in a very low frequency, no causal relation between IAPV and CCD in Spain was found.

Additional key words: colony collapse disorder, honeybee viruses, RT-PCR, phylogeny.

Resumen

Comunicación corta. Primera detección del virus de la parálisis aguda israelita (IAPV) en España

Se analizaron muestras de abejas españolas para detectar el virus de la parálisis aguda israelita (IAPV). Parte de las muestras (240 de 484) procedían de colmenas que mostraban síntomas compatibles con el síndrome de despoblamiento (SDC), el resto de las muestras eran asintomáticas. Solo una de las muestras fue diagnosticada como positiva frente a IAPV utilizando la técnica RT-PCR, que amplifica una parte del ORF 2 del genoma viral. La especificidad de la prueba fue confirmada por secuenciación del producto amplificado que presentaba el tamaño específico. Se emplearon secuencias de IAPV ya publicadas en GeneBank para construir un árbol filogenético que incluye la secuencia de IAPV detectada en muestras españolas (FJ821506). Se observaron dos grupos principales y la muestra española está agrupada con varias americanas. Debido a que en España IAPV fue detectado en muy baja frecuencia, no se encuentra relación causal entre el SDC y la presencia de IAPV.

Palabras clave adicionales: filogenia, RT-PCR, síndrome del despoblamiento de las colmenas, virus de abejas.

Honeybees (*Apis mellifera*), are key pollinators and are crucial for the maintenance of agriculture mostly in USA and Europe. Indeed, honeybees are the most economically valuable pollinators and it is estimated that ~35% of human food consumption depends directly or indirectly on insect mediated pollination (Klein *et al.,* 2006). In the past, periodic and significant losses of honeybee colonies have been reported. These days, such losses are increasing in frequency, magnitude and geographical distribution being described as a wide variety of symptoms gathered into the complex named colony collapse disorder (CCD).

However, the current state of knowledge is unable to explain the real causes of these honeybee losses. Recently, Cox-Foster *et al.* (2007), described a new pathogen associated with CCD by describing their high correlation rates. This new pathogen is the Israeli Acute Paralysis Virus (IAPV; Maori *et al.,* 2007), which was classified as a new member of the Dicistroviridae family (Christian *et al.,* 2005).

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Abbreviations used: BQCV (black queen cell virus), CCD (colony collapse disorder), DWV (deformed wing virus), IAPV (Israeli acute paralysis virus), KBV (Kashmir bee virus), SDC (síndrome despoblamiento de las colmenas).

To this moment, this virus has been already described in Israel, USA, Australia and France (Cox-Foster *et al.,* 2007; Maori *et al.,* 2007; Blanchard *et al.,* 2008; Palacios *et al.,* 2008) but discussion about the level of its implication in the CCD is still going on.

As Spain is the first European honeybee producer country and has also experienced colony losses in the past few years, it was considered important to assess the presence or absence of this virus in our colonies.

The presence of different honeybee viruses such as black queen cell virus (BQCV), deformed wing virus (DWV) or Kashmir bee virus (KBV) has already been reported (Higes *et al.,* 2005; Kukielka *et al.,* 2008) in Spain. Moreover, KBV is closely related to IAPV, which may lead to the idea of its more than possible presence in Spain. In this paper, the first detection of IAPV in bee samples from Spain is reported.

Four hundred and eighty four samples from almost all the Spanish territories were collected for a field study focused on honey bee viruses' prevalence between 2003 and 2008, and then, were retrospectively analyzed for the presence of IAPV, as this virus was newly described in 2007. They had already been analyzed for the presence of KBV and acute paralysis bee virus (ABPV). Some of these samples were taken from colonies presenting compatible symptoms with CCD (240 out of 484) and some others were asymptomatic (248).

Sample preparation, RNA extraction and cDNA synthesis were performed as described previously (Kukielka *et al.,* 2008). Molecular diagnosis (KBV, ABPV, andIAPV) were performed using primer pairs described previously (Stoltz *et al.,* 1995; Tentcheva *et al.,* 2004; Palacios *et al.,* 2008).

Only one of these samples was positive to IAPV by employing the diagnosis RT-PCR that targets the ORF 2 of the IAPV genome (Palacios *et al.,* 2008). It was collected in Valencia, May of 2006, and it belonged to the group of asymptomatic colonies. Specificity of the RT-PCR assay was evaluated by sequence analysis of size specific amplification products. The RT-PCR products were purified using QIAquick PCR purification Kit (Qiagen) and analyzed by the sequencing services of SECUGEN S.A. (Madrid, Spain). The sequence data of the amplification fragment was analyzed using the BLAST® server at the NCBI. Once the sample was confirmed as positive, another RT-PCR targeting the intergenomic region was performed to construct phylogenies. These were sequenced in both directions by the primers described by Cox-Foster *et al.* (2007) named IAPV_IGR_F and IAPV_IGR_R. Specificity of the amplification was confirmed by a BLAST search. A comparison with other IAPV, KBV and ABPV sequences already published in the GeneBank database was performed. This sample was also positive to KBV when analyzed with primers described by Stoltz *et al.* (1995).

IAPV nucleotide sequences already published in GeneBank (Table 1) were used to construct a phylogenetic tree that included the Spanish IAPV sequence (FJ821506). Sequence data were aligned with those of the other samples from GeneBank data base using Clustal X, version 1.83. Phylogenetic analysis was performed using the maximum likelihood method (MEGA software; Tamura *et al.,* 2007). The statistical significance of the tree topology was evaluated by bootstrap resampling of the sequences 500 times (Fig. 1).

It can be observed that IAPV isolates used to construct this phylogenetic tree segregated in two main lineages (group A and B). KBV and ABPV were used as outgroups and were clearly differentiated from the IAPV isolates. The group A includes mainly Australian and French isolates. The group B includes mainly the North American isolates, but the Spanish isolate is within this group with a Canadian and an Australian isolate. The pair wise comparison between the Spanish

Table 1. Identification of IAPV isolates used in phylogenetic analysis

Isolate (Acc. no.)	Country	Reference
EU604010	France	Blanchard *et al.* (2008)
EU604008	France	Blanchard *et al.* (2008)
EU604007	France	Blanchard *et al.* (2008)
EU604006	France	Blanchard *et al.* (2008)
EU436447	Australia	Palacios *et al.* (2008)
EU436448	Australia	Palacios *et al.* (2008)
EU436446	Australia	Palacios *et al.* (2008)
EU122348	Australia	Cox-Foster *et al.* (2007)
EU122347	Australia	Cox-Foster *et al.* (2007)
EU122349	Australia	Cox-Foster *et al.* (2007)
EU436444	USA	Palacios *et al.* (2008)
EU436432	USA	Palacios *et al.*. (2008)
EU436443	USA	Palacios *et al.* (2008)
EU122357	USA	Cox-Foster *et al.* (2007)
EU122358	USA	Cox-Foster *et al.* (2007)
EU122350	USA	Cox-Foster *et al.* (2007)
EU122361	USA	Cox-Foster *et al.* (2007)
EU122362	USA	Cox-Foster *et al.* (2007)
EU122356	USA	Cox-Foster *et al.* (2007)
EU436427	Canada	Palacios *et al.* (2008)
FJ821506	Spain	This report

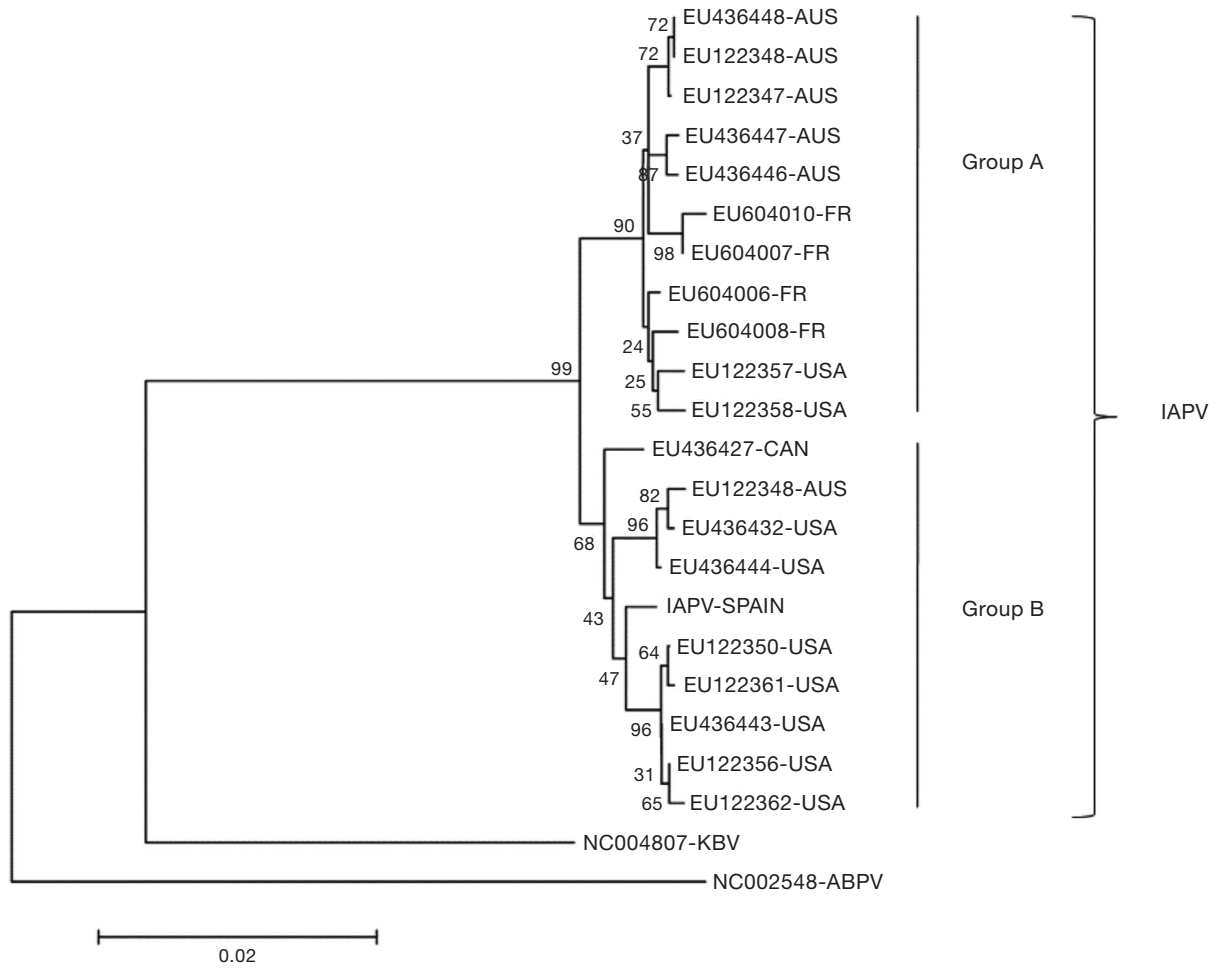

Figure 1. Phylogenetic analysis of IAPV sequences in the region between nucleotide 6193 to 6893. Sequence data were aligned with those of the other samples from GeneBank database using Clustal X, version 1.83. Programs from Mega package (Tamura *et al.,* 2007) were used to produce the phylogenetic tree, reconstructed by the maximum likelihood method. The statistical significance of the tree topology was evaluated by bootstrap resampling of the sequences 500 times. Sequences are named by their genebank accession number and country of origin.

isolate and the corresponding fragment from the complete IAPV genome (Maori *et al.,* 2007) showed a divergence of 1.4% (data not shown).

It is described in bibliography (Blanchard *et al.,* 2008; Palacios *et al.,* 2008) that KBV was found concomitantly with IAPV in all the positive samples for IAPV, raising the question of the specificity of the primers used to detect KBV (Stoltz *et al.,*1995). To address this issue, nine KBV positive samples from our study were analyzed with the IGR RT-PCR for detection of IAPV. These primers amplify a region of the RNA dependent RNA polymerase, which is highly conserved and therefore, useful only for establishing broad taxonomic relationships (Palacios *et al.,* 2008). After the gel electrophoresis, three of the samples yielded a band that when sequenced, the BLAST search gave KBV homologies. When these samples were subjected to the ORF2 RT-PCR for IAPV, one sample yielded a positive band that when sequenced, was IAPV. This result shows that samples already identified as KBV, could be IAPV and that specificity problems with IGR primers could arise if used in a diagnostic RT-PCR aimed to detect IAPV. Because the pathological relevance of viral infections in honeybees is unknown, wider studies should be conducted. As IAPV was detected in Spain in a very low frequency, more samples should be analyzed to know its influence in the colony losses that Spanish apiaries have been suffering in the past years. Assays to facilitate IAPV surveillance should be improved. Specificity of the primers should be assured before identifying samples as positive for any of these diseases, as multiple infec-

tions are quite common in honeybees (Evans, 2001; Chen *et al.,* 2004).

Inferred from our results, no causal correlation between IAPV and CCD in Spain was found although this study is still ongoing to improve the diagnostic assays for IAPV and study its prevalence in the Spanish honeybee population.

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References

BLANCHARD P., SCHURR F., CELLE O., COUGOULE N., DRAJNUDEL P., THIÉRY R., FAUCON J.P., RIBIÈRE M., 2008. First detection of Israeli acute paralysis virus (IAPV) in France, a dicistrovirus affecting honeybees (*Apis mellifera*). J Invertebr Pathol 99(3), 348-50.

CHEN Y.P., ZHAO Y., HAMMOND J., HSU H.T., EVANS J., FELDLAUFER M., 2004. Multiple virus infections in the honey bee and genome divergence of honey bee viruses. J Invertebr Pathol 87(2-3), 84-93.

CHRISTIAN P., CARSTENS E., DOMIER L., JOHNSON J., JOHNSON K., NAKASHIMA N., SCOTTI P., VAN DER WILK F., 2005. Family dicistroviridae. In: Virus Taxonomy (Fauquet C.M., Mayo M.A., Maniloff J., Desselberger U., Ball L.A., eds). Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier, San Diego. pp. 783-788.

COX-FOSTER D.L., CONLAN S., HOLMES E.C., PALACIOS G., EVANS J.D., MORAN N.A., QUAN P.L., BRIESE T., HORNIG M., GEISER D.M. *et al.,* 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. Science 318(5848), 283-287.

EVANS J.D., 2001. Genetic evidence for coinfection of honey bees by acute bee paralysis and Kashmir bee viruses. J Invertebr Pathol 78, 189-193.

HIGES M., ESPERÓN F., SÁNCHEZ-VIZCAÍNO J.M., 2005. First report of black queen-cell virus detection in honeybees (*Apis mellifera*) in Spain. Span J Agric Res 3, 322-325.

KLEIN A., VAISSIÈRE B., CANE J.H., STEFFAN-DEWENTER I., CUNNINGHAM S., KREMEN C., TSCHARNTKE T., 2006. Importance of pollinators in changing landscapes for world crops. Proc Biol Sci 274(1608), 303-313.

KUKIELKA D., ESPERÓN F., HIGES M., SÁNCHEZ-VIZCAÍNO J.M., 2008. A sensitive one-step real-time RT-PCR method for detection of deformed wing virus and black queen cell virus in honeybee *Apis mellifera.* J Virol Methods 147(2), 275-281.

MAORI E., LAVI S., MOZES-KOCH R., GANTMAN Y., PERETZ Y., EDELBAUM O., TANNE E., SELA I., 2007. Isolation and characterization of Israeli acute paralysis virus, a dicistrovirus affecting honeybees in Israel: evidence for diversity due to intra and inter-species recombination. J Gen Virol 88, 3428-3438.

PALACIOS G., HUI J., QUAN P.L., KALKSTEIN A., HONKAVUORI K.S., BUSSETTI A.V., CONLAN S., EVANS J., CHEN Y.P., VANENGELSDORP D. *et al.,* 2008. Genetic analysis of Israel Acute Paralysis Virus: distinct clusters are circulating in the United States. J Virol 82, 6209-6217.

STOLTZ D., SHEN X.R., BOGGIS C., SISSON G., 1995. Molecular diagnosis of Kashmir bee virus infection. J Apic Res 34, 153-165.

TAMURA K., DUDLEY J., NEI M., KUMAR S., 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24, 1596-1599.

TENTCHEVA D., GAUTHIER L., ZAPPULLA N., DAINAT B., COUSSERANS F., COLIN M.E., BERGOIN M., 2004. Prevalence and seasonal variations of six bee viruses in *Apis mellifera* L. and *Varroa destructor* mite populations in France. Appl Environ Microbiol 70(12), 7185-7191.