Archives of Razi Institute, Vol. 68, No. 2, December (2013) 101-104

Full Article

Molecular detection of Acute Bee Paralysis Virus in Iran

Moharrami*, M., Modirrousta, H.

1. Department of Honey Bee, Silk Worm & Wildlife, Razi Vaccine and Serum Research Institute, Karaj, Iran

Received 17 Sep 2012; accepted 20 Jan 2013

ABSTRACT

Acute bee paralysis virus (ABPV) is a small single stranded RNA virus recently classified within the family *Dicistroviridae*, genus *Cripavirus.* Here, we describe the first study of ABPV in unhealthy bee colonies, which has been an unusual loss in adult bee population and significant honey bee mortality during the year. The aim of this study was evaluation of ABPV infection in honey bee colonies in apiaries with loss of population in different geographical provinces in Iran. Adult bee samples were collected between July - September 2011 and 2012 and were originated from 23 provinces with different geographic areas of Iran. Following the RT-PCR reaction with the specific primers on the isolated RNA, an approximately 618 bp product was detected. We demonstrated the presence of ABPV RNA in 9 (5.62 %) out of 160 samples collected from Iranian apiaries.

Keywords: Acute Bee Paralysis, Virus, RT-PCR, Honey bee, Iran

INTRODUCTION

Honeybee, *Apis mellifera* L., through its various products and by contribution to the pollination of agricultural plants is one of the most important economical insects. However, many pathogens including viruses threaten honeybee hives and cause severe loss to apiculture **(**Fievet *et al* 2006, Chen & Reinhold 2007). They usually cause unapparent infections and may not be perceived by beekeepers for many years. Although honeybee viruses are reported from many countries (Berenyi *et al* 2007, Forgách et al 2008), there are very limited number of reports about viral honeybee diseases and their causative agents in Iran. Acute bee paralysis virus (ABPV) is one of the important viruses found in *Apis mellifera.* The virus persists as inapparent infections in nature and is probably transmitted in the absence of *Varroa destructor*, via the salivary gland secretions of adult bees and the food to which these secretions are added (Bailey 1976). Mites can also act as a vector in the spread of the virus from bee to bee (Ball 1989). Honey bee larvae can also become infected with the virus by ingesting food contaminated with viral particles secreted by infected nurse bees (Ball & Allen 1988). ABPV is a small (particle diameter 30 nm) single stranded RNA virus morphologically and physiochemically resembling picornaviruses. It is now considered to be a cricket paralysis-like virus and is classified as a *Cripavirus* in the family *Dicistroviridae*

*Author for correspondence.Email: m.moharrami@rvsri.ir

(Mayo 2002). ABPV was diagnosed first by Bailey et al.1963 and is considered to be a common infective agent of bees which is frequently detected in apparently healthy colonies. However, it has been presumed that this virus plays a role in cases of sudden collapse of honeybee colonies (Nordstrom *et al* 1999). Reverse transcription-PCR (RT-PCR) assays have been developed for the detection of ABPV (Benjeddou *et al* 2001 & Evans 2001).

MATERIALS AND METHODS

Sampling of bees. Infected honeybees from colonies suffering with symptoms of depopulation, sudden collapse, paralysis, and varroa infestation, used in this study, originated from 23 provinces of Iran (various geographic regions): Alborz, Ardebil, Chaharmahal and Bakhtiari, West Azarbayejan, East Azarbayejan, Fars, Ilam, Isfahan, Ghazvin, Gilan, Golestan, Hamedan, Kerman, Kermanshah, South Khorasan, Khorasan Razavi, North Khorasan, Kohkilouyeh and Boierahmad, Mazandaran, Markazi, Semnan, Yazd, Zanjan)(Table). Bee samples were collected between July - September 2011 and 2012 and sent to the Honey Bee Department of Razi Vaccine and Serum Research Institute, by collaborating colleagues in Veterinary Organization. Altogether, samples from 160 apiaries were collected and submitted for virus screening. From each apiary, 100-500 adult worker bees were sampled. All samples were transported by airplane or express mail in carefully wrapped paper sacks or boxes using cold chain and stored at -20 ºC.

Isolation of RNA. The bees were homogenized in ceramic mortars with sterile diethylpyrocarbonate treated water. The homogenates were centrifuged at 20,000 *g* for 1 min, and 140 µl of supernatant was used for RNA extraction (Berenyi *et al* 2006). RNA was extracted employing the QIAmp Viral RNA Mini kit (QIAGEN, Germany) according to the manufacturer's instructions.

Primer used. For detecting ABPV infection in the honey bee, ABPV- specificprimers (GTGCTATCTTG GAATACTAC) and (AAGGYTTAGGTTCTACTAC T) that lead to a fragment of 618 bp were used according to a previous work by Bere´nyi et al. 2006.

RT-PCR. Positive control was prepared from Feredrich-Loeffler-Institute in Germany**.** RT and amplifications were performed with a RT-PCR method by employing a One Step RT-PCR kit (QIAGEN, Germany), following the manufacturer's recommendations. Amplifications were performed in a Master Cycler Gradient Eppendorf. The reverse transcription at 50 °C for 30 min was followed by a denaturation and polymerase activation step at 95 °C for 15 min and by 40 cycles of PCR, each consisting of 30 s at 94 °C, 50 s at 55 °C, and 1 min at 72 °C. Reactions were completed by a final elongation step for 7 min at 72 °C (Bere´nyi *et al* 2006). The PCR products were electrophoresed in a 1.2 % agarose gel and stained with ethidium bromide. Bands were photographed under UV light with Panasonic Digital Camera.

RESULTS

A total of 160 honeybee samples originated from 23 Iranian provinces, were investigated by RT-PCR for the presence of the ABPV one of the important honeybee viruses. Following the RT-PCR reaction with the specific primers on the isolated RNA, an approximately 618 bp product was detected (Figure). Out of the 160 apiaries examined, 9 (5.62%) were infected with ABPV (Table).

DISCUSSION

Many reports on the presence of honey bee viruses in *A. mellifera* L. populations have been published in various countries, some of which were before the invasion of *V. destructor* in Europe. Most of these reports were based on symptomatic or dead bees collected at the hive entrance, sometimes after colony collapse; i.e., most of the time the data were obtained from a few samples that were not representative of the natural occurrence of virus infections in bee colonies. The diagnosis of viral infections in honeybee has been rather complicated compared to other fields of veterinary virology. The lack of characteristic clinical

symptoms and pathological alterations makes the recognition of most diseases difficult (Bakonyi *et al* 2002).

Table. Collected sample from apiaries during summer 2011- 2012. The geographical localization of each apiary in Iran is indicated. Detection of ABPV in samples of adult bee.

No	Province	Code of province	Number of apiary	Positive Spiary	Negative apiary
1	Alborz	Al	1	1	0
2	Ardebil	Ar	6	0	6
3	Chaharmahal & Bakhtiari	C.B	6	2	4
4	East Azarbayijan	E.A	24	0	24
5	Esfahan	Is	3	0	3
6	Fars	Fa	12	0	12
7	Ghazvin	Gz	2	2	0
8	Gilan	Gi	6	1	5
9	Golestan	Go	2	0	2
10	Hamedan	Ha	6	0	6
11	Ilam	Il	7	0	7
12	Kerman	Ke	6	0	6
13	Kermanshah	Kr	5	0	5
14	Khorasan Razavi	Kh	35	0	35
15	Kohkilouyeh & Buyer Ahmad	K.B	4	1	3
16	Markazi	Mr	4	0	4
17	Mazandaran	Ma	1	1	0
18	North Khorasan	N.K	10	1	9
19	Semnan	Se	4	0	4
20	South Khorasan	S.K	4	0	4
21	West Azarbayijan	W.A	2	0	2
22	Yazd	Ya	8	0	8
23	Zanjan	Za	2	0	2
	Total		160	9 (5.62%)	151

Since cell cultures of bee origin are not available, the only way of isolation and artificial propagation of viruses is the experimental infection of pupae. Furthermore, as bees do not produce antibodies against pathogens, the indirect determination of viral infections (widely used in other fields of veterinary praxis) is not possible. Therefore the RT-PCR method worked out to amplify unique regions of the viral nucleic acid present in the samples, seems to be very promising in the diagnosis of bee virus infections (Bakonyi *et al* 2002). This is the first study of ABPV detection in Iranian apiaries. The samples were collected from unhealthy bee colonies chosen from 160 apiaries. Bee samples were sent to Razi Institute for causal diagnosis of health problems in influenced colonies from different provinces of Iran. Our study is part of honey bee viral infections assessment program in Iran, where there has been an unusual loss in adult bee population and significant honey bee mortality during the year. Viral infections could be directly or indirectly involved in such loss. The results are shown in table. Out of the 160 apiaries examined, (9 apiaries) 5.62 % were infected with ABPV. Bakonyi et al., 2002 demonstrated the presence of ABPV RNA in 14 from 114 seemingly healthy colony samples collected from Hungarian honey bees. Their investigation revealed that two third of the apiaries were infected with ABPV at a 12.2% infection rate. Haddad et al. 2008 (Jordan) reported that from 13 colonies examined, 92% were infected with DWV, 8% with SBV and 16% with ABPV. In 90 Austrian honeybee colonies the most prevalent virus was DWV, present in 91% of samples, followed by ABPV, SBV, and BQCV (68%, 49%, and 30%, respectively) (Berenyi *et al* 2006). Samples were collected from 10 Brazilian apiaries, 20 colonies in each apiary, ABPV was detected in 27.1% of colony samples (Teixeira *et al* 2008). Samples of adult bees and pupae were collected from 36 apiaries in the spring, summer, and autumn during 2002 in France. Acute bee paralysis virus (ABPV) was found in 58% of the apiaries. (Tentcheva 2004 b).

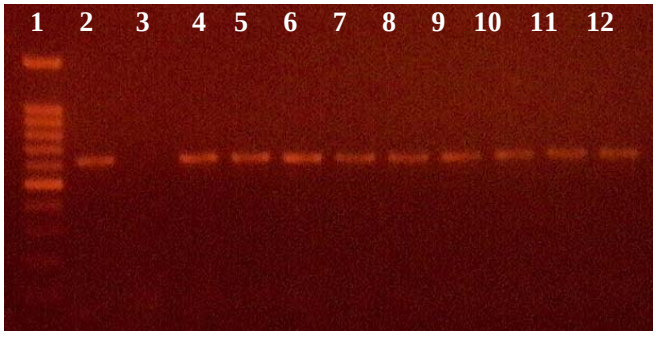

Figure. Visualization of the RT-PCR products obtained with amplifying RT-PCR fragments of 618 bp.1: Ladder 100bp - 2: Positive Control - 3: Negative Control - 4: Al - 5, 6: C.B - 7, 8: Gz - 9: Gi – 10: K.B - 11: Ma -12:N.K.

The reasons for differences in prevalence of bee viruses worldwide are not fully known and may be related to bee management and propagation practices or possibly the presence of alternative hosts or vectors for these viruses (Welsh *et al* 2009). Also, some variation in

prevalence is undoubtedly due to different methods of honeybee sampling and the analysis of results. ABPV is known to persist as inapparent infection in seemingly healthy bee colonies, but its presence was found to be correlated with the mortality of colonies infested with *V. destructor* (Tentcheva *et al* 2004 b). This is very important in the future, that in order to maintain the health of honeybee colonies (local and migratory colonies), bee viruses shoule be controlled and monitored.

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