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Original article

Detection of chronic honey bee (*Apis mellifera* L.) paralysis virus infection: application to a field survey

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Abstract – Chronic paralysis of honey bees is a viral disease caused by chronic paralysis virus (CPV). As its clinical signs are similar to those observed in intoxications or during intense work, it requires sensitive and specific diagnostic tests to identify the infection in colonies. In this report, a rabbit polyclonal antiserum against CPV was obtained after purification of the virus in a Renografin gradient. The viral polypeptide composition was analysed by SDS-PAGE and Western blotting. Four viral-associated polypeptides of molecular weights 75 kDa, 50 kDa, 30 kDa and 20 kDa were identified. Western blotting and an agar gel immunodiffusion test were used for the diagnosis of CPV during a field survey of the prevalence of CPV infection. The two tests demonstrated the presence of the virus in several colonies in the south-east of France.

chronic paralysis virus (CPV) / polypeptide composition / diagnosis / epidemiology

1. INTRODUCTION

The honey bee, *Apis mellifera* L., is host to a large variety of viruses, but only two (*Apis* iridescent virus and sacbrood bee virus), have been characterised and assigned to a recognised virus family [5, 13]. Chronic paralysis virus (CPV) was one of the first viruses isolated from honey bees [3] and has been detected in adult bees from all continents, except South America [2]. Symptoms of trembling, flightless clusters of crawling bees have long been recognised, but these signs are very similar to those occurring during acute or chronic intoxications with pesticides, or following intense work of colonies particularly during the summer period.

In France, many colonies present these signs, but the diagnosis based on the experimental reproduction of the infection [16] is not routinely feasible. For this reason,

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there is a need to improve our knowledge of CPV in order to develop simple and reliable methods of diagnosis, and to determine the importance of chronic paralysis in the present bee pathology.

CPV consists of ellipsoidal particles which fall into four size classes with modal lengths of about 30, 40, 55 and 65 nm, each approximately 20 nm wide. The components have sedimentation coefficients of 80S to 130S. In caesium chloride gradients, all size classes have a buoyant density of 1.33 $g \cdot ml^{-1}$ [4]. The virus particles have one capsid protein of 23.5 kDa [4] and contain five single-stranded RNAs [20].

In this report, the virus was purified in a Renografin gradient, thereby enabling production of a rabbit polyclonal antiserum. The viral polypeptide composition was analysed by SDS-PAGE and by Western blotting. Four viral associated polypeptides of molecular weights 75 kDa, 50 kDa, 30 kDa and 20 kDa were identified. This approach was used to develop diagnostic protocols for the detection of CPV based on simpler tests, such as agar gel immunodiffusion test (AGID), in comparison to the Western blot as reference test. In experimental infections, the two techniques detected CPV in infected honeybees from day 1 post-injection, before the appearance of symptoms, to the sixth day corresponding to the death of infected bees.

A first field survey was performed by using these two diagnostic tests, which demonstrated the presence of CPV in 6 apiaries out of 11 showing typical symptoms.

2. MATERIALS AND METHODS

2.1. Virus analysis

2.1.1. Identification of CPV infection

For the first identification of CPV in honey bees, samples of bees showing typical symptoms of chronic paralysis were tested positive as described below: (i) by experimental infection to reproduce symptoms and mortality, and (ii) with an agar gel immunodiffusion test (AGID) using an anti-CPV serum kindly provided by Dr. B. Ball (Rothamsted Experimental Station, UK). These two tests permitted the selection of naturally-paralysed bees from infected colonies for the production of large amounts of virus. For this purpose, heads of infected bees were selected because CPV has a particular tropism for nervous tissues and mandibular and hypopharyngeal glands [14, 15, 18]. As another report described the presence of CPV-associated particles (CPVA) in the abdomens rather than in the heads of bees [6], only heads of paralysed bees were chosen for this study in order to eliminate sources of other bee viruses, such as Kashmir bee virus (KBV), which replicate in most tissues excepting nervous tissues [11].

Moreover, the preparations used for CPV propagation were checked for the absence of acute paralysis virus (APV) [7] with a specific antiserum by agar gel immunodiffusion (AGID) as previously described [12].

2.1.2. Virus propagation

Heads of 10 paralysed bees were removed and crushed in a mortar at 4 °C in 5 ml physiological saline. After one cycle of centrifugation at $3\,000 \times g$ for 30 min, the supernatant was used for inoculation of healthy bees at the greatest dilution causing 100% mortality one week after infection, as described by Bailey and Woods [8, 9]. CPV was propagated by intra-thoracic injection. Controls were inoculated with physiological saline.

Inoculated bees were obtained from our laboratory apiary without any evidence of viral or bacterial infections. A control injection of extracts from heads of these healthy bees into other healthy bees induced no mortality, demonstrating that the intra-thoracic injection of head extracts did not activate unapparent viruses in the healthy bees of our laboratory.

2.1.3. Virus purification

After incubation for 6 days at 35 °C, a viral suspension was made from 10 heads crushed in a mortar in 1 ml 0.2 M potassium phosphate buffer at pH 7.5 containing 0.02% sodium diethyldithiocarbamate, 1/5 vol. diethylether [3, 10] and a protease inhibitor cocktail (Sigma, Saint Quentin Fallavier, France). The mixture was then emulsified with carbon tetrachloride [10], coarsely filtered, and cleared at 3 000 × *g* for 30 min.

The crude viral suspension was layered onto a Renografin (Radioselectane, Schering Laboratories, Lys lez Lannoy, France) preformed density equilibrium gradient (20 to 75% w/v) and centrifuged at 30 000 × *g* for 15 h [19]. After one cycle of centrifugation, a visible band at 43% of Renografin was removed, diluted in 0.2 M potassium phosphate buffer at pH 7.5 and centrifuged at 180 000 × *g* for 3 h. The pellet was resuspended in 100 µl of this buffer and kept at 4 °C. Similar preparations were made with 10 heads of healthy bees as negative samples by using the same steps of purification (Fig. 1).

Five microliters of each preparation were examined by electron microscopy to check for the presence of virus. Briefly, preparations were loaded on formvar-coated grids and were negatively stained with 1% phosphotungstic acid, pH 7, and examined with a Hitachi H600 (75 kV) transmission electron microscope [1].

Protein concentration was measured using the Bio-Rad Protein Assay (Bio-Rad, Ivry sur Seine, France), with bovine serum albumin (BSA) as standard.

2.1.4. Production of antiserum

A large preparation of virus from 300 contaminated bee heads was purified

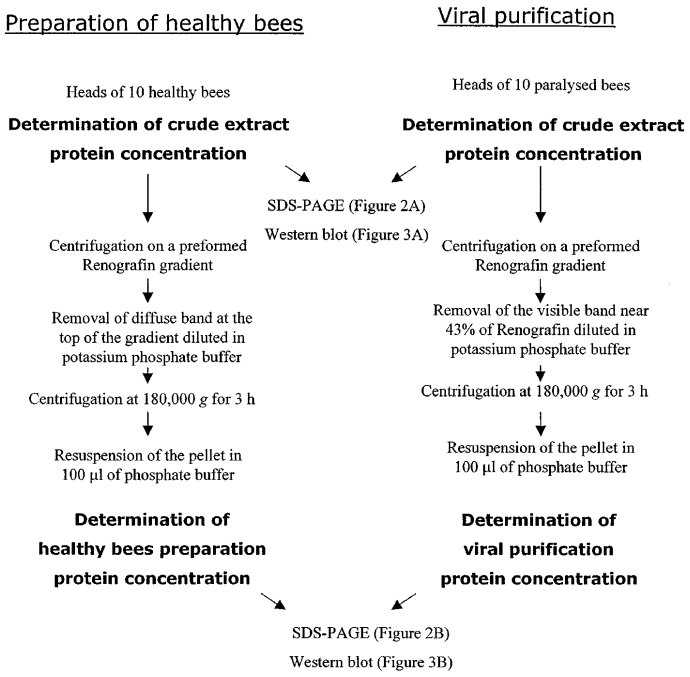

Figure 1. Flowchart of the purification protocol.

as described above in order to produce an antiserum against CPV.

Polyclonal antibodies were raised in female New Zealand white rabbits using 500 μg of purified CPV injected intramuscularly. The initial injection using virus preparation mixed with Freund's complete adjuvant was followed by 3 booster injections, using Freund's incomplete adjuvant, at week 3, week 5 and week 7 post-injection. Exsanguination by cardiac puncture was carried out 3 weeks following the final injection.

2.1.5. Absorption of antiserum

To check the specificity of the antiserum, blocking tests were performed using extracts from healthy bees similar to those used as negative samples.

Following several tests with different volumes, and different protocols, the following protocol was adopted: one volume of antiserum was put in contact with 0.5 volume of denatured bee extract and 0.5 volume of bee extract for 1 h at 37 °C. This absorbed antiserum was centrifuged for 1 h at 12 000 × *g* and used in Western blotting and AGID in order to compare results with a non-absorbed antiserum.

2.1.6. Electrophoresis and Western blotting

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli [17]. Twelve percent gels or gradient gels (10–15%) were used for the separation of proteins, and bands were stained with SYPRO™ Orange (Bio-Rad, Ivry sur Seine, France).

Samples were denatured by boiling with loading buffer (0.25 M Tris-HCl pH 6.8, 25% β-mercaptoethanol, 10% SDS, 50% glycerol, 0.5% bromophenol blue) for 5 min at 100 °C.

Polypeptides were transferred, for the blotting following SDS-PAGE, to nitrocellulose membranes (Hybond-ECL, Amersham, Slough, UK), using a BioRad Mini Trans-Blot apparatus. Prestained molecular weight markers (BioRad) were used routinely. After the transfer, membranes were exposed for 1 h at 37 °C to blocking buffer (0.2 M potassium phosphate buffer, 5% skimmed milk). The CPV antiserum was used diluted in 0.2 M potassium phosphate buffer: the optimal dilution was previously determined by titration, in Western blotting and AGID, against a panel of positive and negative purified extracts of experimental infections in serial dilutions. It was used at a 1/250 dilution for one hour at 37 °C, and then carefully rinsed off with potassium phosphate buffer. Absorbed antiserum was used at 1/100 dilution. Another CPV antiserum was kindly provided by Dr. B. Ball (Rothamsted Experimental Station, UK) and was used at 1/250 dilution. Membrane filters were then exposed to mouse anti-rabbit IgG (H + L) antibodies conjugated with horseradish peroxidase (Biosys, Compiègne, France). Enzyme activity was detected by enhanced chemiluminescence systems according to the manufacturer's recommendations (Amersham, Buckinghamshire, UK). The protein concentration of samples was measured using the Bio-Rad Protein Assay.

2.1.6.1. Virus analysis

SDS-PAGE analysis was performed at all steps of preparation for all the samples, i.e. negative controls and viral preparations. Samples were loaded at the same protein concentration.

After production of the rabbit antiserum, Western blotting was routinely used at each step of purification both for the viral suspension and the extract of healthy bees.

2.1.6.2. Western blotting for diagnosis

Bee samples were obtained from colonies with symptoms of chronic paralysis. Heads of 10 bees were removed and crushed in a mortar at 4 °C in 0.2 M potassium phosphate

buffer, pH 7.5. This preparation was cleared at 3 000 × *g* for 30 min and used as antigen for Western blotting.

2.1.7. Agar gel immunodiffusion test

Heads of 10 paralysed bees, obtained following an experimental infection or from colonies with symptoms of chronic paralysis, were removed and crushed in a mortar at 4 °C in 0.5 ml of 0.2 M potassium phosphate buffer, pH 7.5. These crude antigenic preparations were used directly in AGID tests. Glass slides were precoated with 5 ml agar (Institut Pourquier, Montpellier). The central well (5 mm in diameter) in each case contained the rabbit antiserum raised against CPV and the outer wells contained 20 µl of samples to be tested. After incubation at room temperature for 48 h, gels were washed in phosphate buffered saline, pH 7.5 and distilled water. The positive lines were visualised with Coomassie Brilliant Blue R (Sigma).

2.2. Field survey

This survey was carried out during the summer in the south-east of France, where lots of colonies presented signs similar to those of chronic paralysis. Bees from 17 colonies presenting signs from 11 different apiaries were tested serologically. Three types of samples were taken: dead bees, bees trembling and crawling outside the hive, and apparently healthy bees collected inside the hive.

As a few samples were not taken, 42 samples in all were analysed by AGID and Western blotting as described before.

3. RESULTS

3.1. Virus purification

Following purification of CPV in a Renografin gradient, one band was visible at 43% of Renografin and was removed for further concentration by centrifugation. Observation in electron microscopy showed mostly ellipsoidal particles corresponding to the previous description of CPV particles [3]. No band was visible at the same level in control gradients and when this zone was sampled no protein was detected. Only a diffuse band at the top of the gradient was visible and removed: this band was assumed to contain the extract of healthy bees (supported by this polypeptide composition in SDS-PAGE, Fig. 2B, lane 1).

3.2. SDS-PAGE and Western blotting for analysis of CPV following purification

SDS-PAGE of the viral preparation before Renografin gradient (Fig. 2A) revealed the presence of one intense band at 30 kDa. Following purification, this was revealed as a major band (Fig. 2B). In the extract of healthy bees, there was a band at a similar molecular weight but much less intense (Figs. 2A and 2B). In Figure 2B (lane 2), high molecular weight bands corresponding to bee proteins were still present after viral purification.

In Figure 3, Western blotting with the non-absorbed antiserum and with the absorbed antiserum revealed the presence of four polypeptides in the viral suspension, with molecular weights close to 75 kDa, 50 kDa, 30 kDa and 20 kDa. The absorbed antiserum did not reveal the presence of bands in the healthy bee extract at the high molecular weights (Fig. 3B, lane 1).

Using the antiserum raised against CPV provided by Dr. B. Ball, we checked the specificity of these previous bands, demonstrating that they were associated with the virus: this antiserum revealed the presence of three polypeptides in the viral preparation, with molecular weights close to 50 kDa, 30 kDa and 20 kDa (data not shown). At high molecular weights, the background was too high to be able to distinguish other viral polypeptides.

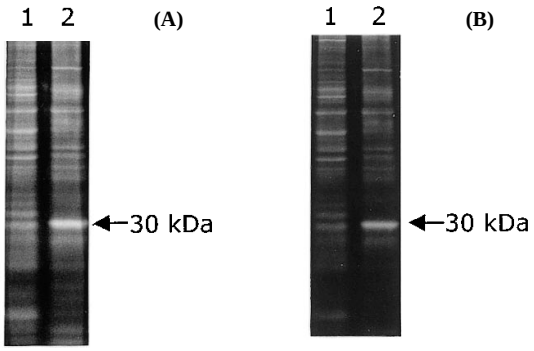

Figure 2. Visualisation, following SDS-PAGE, of polypeptides from extracts before separation on a Renografin gradient (2A) and of polypeptides after purification on a Renografin gradient (2B). Gels (12%) was stained with SYPRO Orange. (**A)** Each lane was loaded with 10 µl (10 µg of protein) of bee extract. Lane 1: extract of healthy bees. Lane 2: extract of bees inoculated with CPV. **(B)** Each lane was loaded with 5 µg of protein. Lane 1: purified extract of healthy bees (5 $\mu g \cdot \mu l^{-1}$). Lane 2: purified extract of bees inoculated with CPV (1 $\mu g \cdot \mu l^{-1}$).

3.3. Western blotting and AGID applied to diagnosis of experimental and natural infections

In crude extracts from naturally-infected bees, Western blotting with the non-absorbed antiserum revealed a larger number of polypeptides (Fig. 4). This antiserum produced a high background, but viral-associated polypeptides were still visible. In the negative controls *(*Fig. 4, lanes 4 and 5), three polypeptides were recognised at high molecular weights with a weak background as in Figure 3A. An additional experiment showed that the absorbed antiserum did not recognise these three polypeptides.

In the case of naturally-infected bees, by using the antiserum at a dilution of 1/250, CPV-associated polypeptides were still detectable at the 10^{-3} dilution of an extract containing ten heads of infected bees.

With the AGID test, viral purified extract produced one precipitin line against the rabbit antiserum against CPV (absorbed or non-absorbed). Similar precipitin lines were obtained with crude extracts of experimentally-infected bees (Fig. 5).

Before being applied to field samples, the AGID test was standardised with samples from experimentally-infected bees in order to determine its sensitivity. The sensitivity was appreciated first by the capacity to detect the virus in experimentally-infected bees before the appearance of signs, and

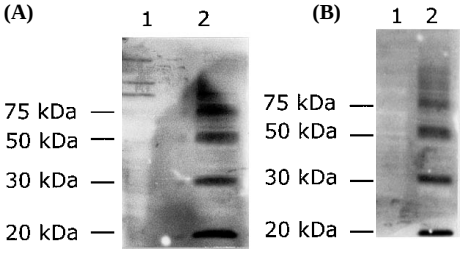

Figure 3. Western blots of purified extracts after purification on a Renografin gradient, using the non-absorbed antiserum (**A**) or absorbed antiserum (**B**) (5 µg per lane). Lane 1: purified extract of healthy bees. Lane 2: purified extract of bees inoculated with CPV.

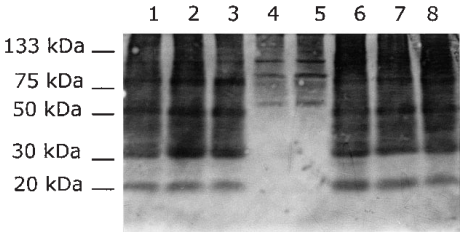

Figure 4. Diagnosis of CPV infection by Western blot. The rabbit antiserum against CPV was used at dilution 1/250 and antigen/antibody complexes were detected by chemiluminescence. Each lane was loaded with 15 µg of protein. Lanes 1, 2, 6, 7 and 8: crude extracts from bees with symptoms. Lane 3: positive control (crude extracts of experimentally-infected bees). Lanes 4 and 5: negative controls (crude extracts of healthy bees).

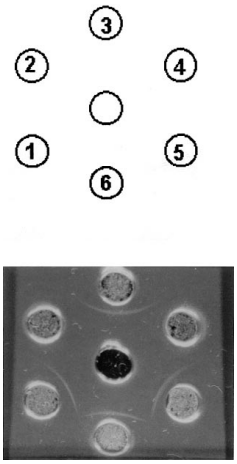

Figure 5. Agar gel immunodiffusion test for diagnosis of CPV. The inner well contained the rabbit anti-CPV antiserum; the outer wells contained 20 µl of crude extracts. Wells 3 and 6: positive controls (crude extracts of experimentally-infected bees). Wells 2 and 4: negative controls (crude extracts of healthy bees). Wells 1 and 5: positive field samples.

second by the proportion of infected heads in a sample necessary to give a positive response. In the first set of tests, AGID was able to detect the virus at day 1 post-infection before the appearance of symptoms, till days 6 or 7 post-infection corresponding to the death of all infected bees. In the second set of tests, AGID was able to give a positive response when only one head from an infected bee was present among the ten heads of a sample. Moreover, when testing 10-fold dilutions, only the 10^{-1} dilution gave positive results.

3.4. Field survey

Results of AGID and Western blotting were concordant for the three types of samples: dead bees, trembling and crawling bees, and apparently healthy bees. Eight colonies out of the 17 with signs gave a positive result and came from 6 apiaries out of 11. A total of 15 samples out of 42 were

Table I. Results of the field survey of CPV infection in 17 colonies of honey bees.

		Type of sample			
Apiaries	Colonies number	Dead bees	Bees with signs	Apparently healthy bees	Final result
A	1	+[a]	+	+	+
	2	–[b]	–	...	–
B	1	–	–	–	–
	2	–	–	...	–
C	1	–	+	+	+
	2	–	+	...	+
D	1	–	+	+	+
	2	–	+	...	+
E	1	–	–	...	–
F	1	–	+	+	+
	2	–	–	...	–
	3	–	–	...	–
G	1	–	–	–	–
H	1	–	+	+	+
I	1	...[c]	+	+	+
J	1	...	–	–	–
K	1	–	–	–	–

[a]+: positive samples for presence of CPV in Western blotting and gel diffusion test; [b]–: negative samples for presence of CPV in Western blotting and gel diffusion test; [c]...: no sample.

tested positive (Tab. I). The first use of these specific diagnostic tests for natural CPV infection demonstrated the presence of CPV in the south-east of France.

4. DISCUSSION

The aim of this study was to develop specific tools for diagnosis of CPV infection in honey bees. To achieve this goal, we first used a new protocol for purifying the virus. In our laboratory conditions, a Renografin gradient was simpler and more resolvent than sucrose gradient [3, 19].

In this study, a CPV antiserum obtained in rabbit and raised against CPV revealed four viral-associated polypeptides, with molecular weights close to 75 kDa, 50 kDa, 30 kDa and 20 kDa. The serum provided by Dr. B. Ball clearly recognised three of these four viral bands under our laboratory conditions. These results differ from those of a previous study [4] where SDS-PAGE revealed only one protein at 23.5 kDa corresponding to the CPV capsid. The detection of three additional polypeptides in comparison to the previous study underlines the great sensitivity of Western blot: these three polypeptides are likely of viral origin but we cannot exclude the possibility that they may be the result of an overexpression of host proteins as a consequence of the CPV infection.

Western blotting with the chemiluminescent detection of antigen/antibody complexes proved to be effective in the diagnosis of CPV infection. Simultaneously with the development of a Western blotting technique, a simpler technique such as AGID was used. Although less sensitive than Western blotting, AGID is simple and rapid and does not require large amounts of reagents. The comparison of results from the two techniques showed a good concordance.

The first field survey presented in this report demonstrated the presence of CPV infection in the south-east of France, where many colonies presented symptoms similar to those of chronic paralysis. All the samples came from hives with signs but only 8 colonies out of 17 gave a positive result. The positive results came from samples of live bees with symptoms or without evidence of symptoms (Tab. I). In contrast, samples of dead bees in positive colonies were negative except for one colony. These negative results may be the consequence of the quality of the sample: in particular, the desiccation of dead bees in summer may hamper detection of viral infection. It would be advisable to sample symptomatic bees while they are still alive, in front of the colony. As the diagnostic tests developed in this study allowed detection of CPV before the appearance of symptoms, the absence of positive response in the 9 colonies with symptoms may be due to other bee diseases: the signs presented by the colonies, similar to those induced by a CPV infection, may be due to other causes such as acute or chronic intoxications by pesticides, intense work of colonies or other infections.

The continuation of this project will turn to (i) detailed investigations of the epidemiology of this honey bee virus which may yield options for reducing the incidence of the virus by identifying the factors that initiate its multiplication and its spread in colonies; (ii) the study of the viral RNA in order to improve knowledge of this virus and to develop diagnostic techniques based on the detection of the viral genome during experimental and natural infections by CPV.

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Résumé – Détection des infections par le virus de la paralysie chronique de l'abeille domestique (*Apis mellifera* L.) : application lors d'une enquête de terrain. Le virus de la paralysie chronique (CPV) a été l'un des premiers virus isolés chez l'abeille domestique [3]. Le CPV est responsable d'une maladie infectieuse et contagieuse des abeilles adultes, la paralysie chronique de l'abeille connue par les apiculteurs français sous le nom de « Maladie noire » [16]. À l'origine de mortalités, cette pathologie affaiblit les colonies et provoque des pertes de production. Elle se manifeste au niveau de la colonie par la présence d'abeilles traînantes, tremblantes, incapables de voler et dans certains cas d'abeilles mortes, noires et aux ailes écartées. La recrudescence de symptômes imputables à cette virose dans le sud-est de la France a motivé la reprise des recherches sur ce sujet.

Cependant, l'ensemble de ces symptômes peut être confondu avec ceux d'intoxications aiguës ou chroniques par des pesticides ou d'un surmenage de la ruche particulièrement durant les périodes estivales.

Le diagnostic de la paralysie chronique par reproduction expérimentale de la maladie [16], n'est pas envisageable en routine. Dans le but de déterminer l'importance des infections par le CPV dans les pertes observées à l'heure actuelle, il est apparu nécessaire de mettre au point des techniques d'étude et de diagnostic plus sensibles.

Afin de sélectionner des abeilles naturellement infectées, des prélèvements d'abeilles présentant les symptômes de la paralysie chronique ont été testés (i) par infection expérimentale, afin de reproduire les symptômes et les mortalités caractéristiques de la maladie, (ii) par diagnostic en immunodiffusion en gélose (IDG) utilisant un sérum anti-CPV. Le CPV a ensuite été multiplié par inoculation à des abeilles saines par voie intra-thoracique de préparations issues de ces abeilles contaminées, à la dilution causant 100 % de mortalité une semaine après injection, comme décrit par Bailey et Woods [8, 9].

Le virus a été purifié sur gradient de Rénografine (Fig. 1) et ceci a permis la production d'un antisérum polyclonal de lapin anti-CPV. La composition polypeptidique de cette purification virale a été analysée en SDS-PAGE (Fig. 2B) et en Western blot. Quatre polypeptides associés à l'infection virale et de poids moléculaires 75 kDa, 50 kDa, 30 kDa et 20 kDa ont été identifiés (Fig. 3). Un diagnostic simple de détection du CPV par immunodiffusion en gélose (IDG) a ensuite été mis au point (Fig. 5) et comparé avec la technique de Western blot comme référence (Fig. 4). Lors de tests sur des abeilles infectées expérimentalement ces deux techniques ont permis de détecter la présence du CPV depuis le premier jour post inoculation avant même l'apparition des symptômes, jusqu'au sixième jour correspondant à la mort des abeilles. Une enquête de terrain utilisant ces deux tests diagnostics (Tab. I), a été réalisée afin de déterminer la prévalence du CPV, et a démontré la présence du virus dans six ruchers sur les onze compris dans l'étude qui présentaient tous des symptômes attribuables à la paralysie chronique.

Virus Paralysie Chronique / CPV / polypeptide / diagnose / épidémiologie

Zusammenfassung – Nachweisverfahren von Infektionen mit dem chronischen Honigbienen – Paralyse Virus in der Freilandüberprüfung. Der Chronische Paralyse Virus (CPV) war einer der ersten Viren, der bei Honigbienen (*Apis mellifera* L.) isoliert wurde [3]. CPV führt zu einer ansteckenden, direkt übertragbaren Krankheit von adulten Bienen, bei französischen Imkern als "Maladie Noire" [16] bekannt. Diese Krankheit schwächt die Völker, führt zu Bienensterben und verursacht ökonomische Verluste.

Als Symptome treten Zittern und Ansammlungen von flugunfähigen Bienen, seltener schwarze Bienen mit abgespreizten Flügeln auf. Die Wiederaufnahme der Forschung

über diese Krankheit wurde durch kürzliches Auftreten dieser Krankheitssymptome in Südfrankreich veranlasst. Ähnliche klinische Symptome treten jedoch auch nach akuten oder chronischen Vergiftungen mit Pestiziden oder sogar nach einer intensiven Bearbeitung der Völker besonders im Sommer auf. Die Diagnose einer CPV Infektion, die auf der künstlichen Vermehrung der Krankheitserreger beruht [16] ist jedoch für Routineprüfungen nicht geeignet. Aus diesem Grund ist es wichtig, unsere Kenntnis über CPV zu erweitern, um eine einfache und verlässliche Methode zur Diagnose und die Rolle von CPV im jetzigen Krankheitsstand der Bienen zu bestimmen.

Für die erste Identifizierung von CPV in Honigbienen wurden die Bienenproben mit typischen Symptomen folgendermaßen getestet: (i) durch künstliche Infektion zur Verstärkung der Symptome und des Totenfalls und (ii) mit einem Agar – Gel – Immunodiffusions – Test (AGID) unter Verwendung eines Anti-CPV Serums. Diese beiden Tests erlaubten eine Selektion von natürlich erkrankten Bienen aus infizierten Völkern zur Erzeugung von groβen Virusmengen. CPV wurde durch Injektion in den Thorax von gesunden Bienen verbreitet. Hierbei wurde die höchste Verdünnung verwendet, die nach Bailey und Woods [8, 9] eine Woche nach der Infektion eine 100 % Sterblichkeit zur Folge hat

In dieser Untersuchung wurde das Virus für die Erzeugung von polyclonalem Antiserum in Kaninchen in einem Renografin Gradienten (Abb. 1) gereinigt. Die Zusammensetzung der viralen Polypeptide wurde mit SDS-PAGE (Abb. 2B) und mit Western Blotting analysiert. Vier lose miteinander verbundene virale Polypeptide mit Molekulargewichten von 75 kDa, 50 kDa, 30 kDa und 20 kDa wurden identifiziert (Abb.3). Dieser Ansatz wurde benutzt, um ein diagnostisches Protokoll für die Erkennung von CPV zu entwickeln, das auf dem im Vergleich zum Western Blot Test (Abb. 4) einfachen AGID Test (Abb. 5) beruht. Bei der künstlichen Infektion erkannten beide Tests CPV bereits am Tag 1 nach der Injektion, bevor Symptome auftraten, bis zum 6. Tag, dem Todeszeitpunkt der infizierten Bienen. Ein Feldtest über die Verbreitung von CPV Infektionen wurde mit Hilfe dieser beiden diagnostischen Tests durchgeführt (Tab. I). Von 11 Bienenständen, bei denen die typischen Symptome auftraten, waren 6 Bienenständen von CPV befallen.

Chronische Paralyse Virus (CPV) / Polypeptidzusammensetzung / Diagnose / Epidemiologie

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