In partnership with

VIRUS TASKFORCE

Proceedings

Avignon, France, 6-7/04/2017

VIRUS TASKFORCE

Topic

The research on honey bee viruses is a quickly expanding frontier and there is an increasing need to address the appearance of new viruses, to establish standards to assess virus diversity and to gain better knowledge of the variability of viruses.

The Virus Task Force aims to fill the above gap, updating and compiling the new information on honey bee viruses for the bee research community and providing tools to allow honey bee-virus comparative analysis across the globe.

- Through our common task, we will bring together researchers from European, Asian, Africa, North and South-American countries where known and emerging viruses are reported.

- Promote innovative research (detection-identification and biological assays) on the
  Honey bee virus front for virus prevention and development of future means for control.
- -Facilitate exchange of information and ideas among interested researchers, beekeepers and other stakeholders;
- Use of easier and standardised methods to send/analyse data
- Provide updated tools for epidemiology studies
- Promote cooperation with other COLOSS working groups

When

- 6-7/04/2016

Where

- Avignon, France :
  INRA
  UR 406 Abeilles et environnement
  Centre de recherche Provence-Alpes-Côte d'Azur
  Site Agroparc - Domaine St Paul
  228, Route de l'aérodrome
  CS40509
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ORGANIZER CONTACTS	
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VIRUS TASKFORCE Schedule

Avignon, France, 6-7/04/2017

Thursday 06/04/2017 Registration: 13:30

Time	Session 1 – Kick-off meeting
14 :00- 15:20	Introductory talks (15 min talk + 5 min for questions per speaker) • Judy Chen- IAPV • Per Kryger- SBV, CBPV, DWV and BQCV screening relevance • Eric Dubois- Accuracy of RT-PCR method for quantifying the CBPV (inter-laboratory study) • Declan Schroeder- DWV quasispecies
15:20-15:40	Participants self-introduction to the group
15:40-16:00	**Coffee/snack break**
Time	**Session 2 – Virus variability**
16 :00-16 :30	Introduction to the topic "Virus variability" - Joachim de Miranda
16:30- 17:30	*Discussion* *Distribution of tasks on Virus variability*
Time	**Session 3 – New viruses and Biological assays**
17:30- 18:30	What viruses? *Group discussion*
20:00	**Dinner**

Friday 07/04/2017

8 :30-8 :50	What viruses (cont.)? *Group discussion*
8 :50- 9 :10	*Conclusion and distribution of tasks for session 3*
9 :10- 9 :30	Introductory talk : Pavel Plevka- Structure of honey bee viruses
9 :30- 10 :15	Introductory talk : Ewan Campbell- Varroa transmission of DWV
10:15-10:40	What assays? *Group discussion*
10:40-11:00	**Coffee/snack break**
Time	Small topic: Sample collection and transport
11:00-12:00	*Group discussion and distribution of tasks*
Time	General discussion
12:00- 12:45	*General discussion*
12:45-13:30	**Lunch**
13:30-14:30	*Summary and perspectives*

Virus TaskForce (VTF) Workshop Summary

Anne Dalmon, Marina Meixner, Per Kryger, Orlando Yañez, Nor Chejanovsky

Research on honey bee viruses is a quickly expanding frontier and there is an increasing need to address the appearance of new viruses, to establish standards to assess virus diversity and to gain better knowledge of the variability of viruses .These questions brought together Anne Dalmon (France), Marina Meixner (Germany), Per Kryger (Denmark), Orlando Yañez (Switzerland) and Nor Chejanovsky (Israel) to form the Virus Task Force that met last April in Avignon. The meeting was attended by 24 researchers from13 countries. Seven talks were the basis to discuss about virus variability, new viruses, biological assays, sample collection and transport. As a conclusion of the discussions several tasks were identified and defined:

- To write together 2 reviews, one regarding the routes of infection and the second about virus distribution and prevalence in different countries/regions, making a database of what viruses are present in different countries.
- To make a catalogue of old samples available in each lab (to be studied for the presence of DWV B).
- To design a sampling strategy to study virus variability and define an adequate sampling protocol including meaningful metadata. Then raised the need to list first the primers available for the different types of DWV, using different qPCR assays.

Finally it was agreed that the next meeting will take place in February 2018, in Warsaw, hosted by Anna Gajda.
The meeting will be reported to the General Assembly of COLOSS.

Abstract title:

Virus research topics in Denmark and beyond

Authors:

- Kryger Per, Department of Agroecology, Aarhus University, 4200 Slagelse, Denmark per.kryger@agro.au.dk

Abstract text:

It has been shown that sacbrood virus (SBV) is highly prevalent in Denmark. My lab frequently receive samples from beekeepers with severe outbreaks, causing major brood loss, impacting on honey production and even colony survival. Compared to our close neighbouring countries both prevalence and severeness of SBV is more pronounced. The can be caused by the presence of a virulent strain of SBV in Denmark, or Danish bees may have a poorer defence against SBV. Alternatively, the Danish nectar and pollen flow, based on 62 % arable land may result in poor nutrition.

Acute bee paralysis virus, Kashmir bee virus and Israeli acute paralysis virus all seem to occur. These viruses belong to the Acute bee paralysis virus complex, and we have developed a single primerset (AKI) that can detect all three, based on a conserved region found the available genomic sequences. It seems a financially advantageous solution, to use one PCR reaction to detect any of these three viruses. Some variation does exist within the targeted region, which may allow for determination of the more exact type, but this needs further exploration. Given the similarity in genomic sequence, it would seem likely that recombination can occur between the three viruses.

Deform wing virus (DWV) seems the agreed upon most problematic virus. In Denmark we have screened more that 500 samples and found that both type A and type B are widespread, whereas type C has eluded our detection thus far. The question of recombination has not been examined, but I would like to discuss the question and suggest strategies for research in this regard. The queen breeders in Denmark can be separated in those that have type A problems and those that have type B problems, while only a few have both. The reason for this is of interest.

Black Queen Cell Virus (BQCV) seems unproblematic in Denmark, so much so that I have decided to drop this virus from our regular surveys. Interviewing commercial queen breeders confirm that indeed BQCV is rarely noticed, if it was ever seen. The obvious thought is, the high level of resistance to Nosema of our honey bee population, has resulted reduced transmission of the virus. More virus have recently been found in Denmark, but diseases seem mainly concerned with those mentioned above.

I will be happy to share and exchange samples, primers and ideas on the above topics.

Abstract title:

Genetic diversity in a honey bee DWV pathogen

Authors:

- Schroeder; Declan; Marine Biological Association; Plymouth; UK; dsch@mba.ac.uk

Abstract text:

DWVs operate as a quaispecies and therefore its effects on the declining honey bee population must be seen in this context. The use of next-generation sequencing has revealed the possibility to the prospect of certain DWV quasispecies being responsible for colony losses. To date, we have identified at least three quasispecies in the DWV species complex.

Abstract title:

The role of viruses in honey bee colony losses

Authors:

- Yanping (Chen), USDA-ARS Bee Research Laboratory, Beltsille, MD 20705, USA

Abstract text:

Honey bees are inevitably subject to infection by a wide variety of pathogens. Among honey bee pathogens, viruses present one of the major threats to the health of honey bees and are responsible for significant colony losses. Lately, honey bee viruses have gotten a lot of international attention due to the significant disease status that they have caused in honey bees and the observation of tight correlation between Israeli acute paralysis virus (IAPV) and honey bee Colony Collapse Disorder (CCD) during the winter of 2006-2007 in the U.S. This presentation will summarize our recent research effects on improve our understanding of the morphology, genome organization, taxonomy, transmission, pathogenesis of honey bee viruses, and host defensive mechanisms to virus infections. The effect of RNA interference (RNAi), an new antiviral mechanism, in blocking replication of a honey bee virus will be also discussed in detail.

Abstract title:

Metabolic stress and viral infection in honeybee

Authors:

- Di Prisco; Gennaro; University of Napoli Federico II. Department of Agricultural Sciences; Portici; Italy; gennaro.diprisco@unina.it
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- Caprio; Emilio; University of Napoli Federico II. Department of Agricultural Sciences; Portici; Italy
- Pennacchio; Francesco; University of Napoli Federico II. Department of Agricultural Sciences; Portici; Italy

Abstract text:

Honeybees (Apis mellifera L.) play a key-role in the environment and are essential for pollination of many crop plants. Honeybee immunity can be adversely affected by a number of biotic and abiotic stress factors, which can synergistically interact. In the past few years, an increasing occurrence of colony decline and eventual collapse has been reported globally. These declines are often associated with colonies containing high loads of pathogens and parasites, with bees exhibiting clear signs of bee immunosuppression, but are also associated with increased exposure to pesticides and acaricides. One critical factor that could improve bee resistance to pesticides, parasites and pathogens is colony nutrition. Here we tested whether access to essential amino acids in diet influenced adult worker bee health. We also measured the transcriptomic profile in this population and pathogen load of deformed wing virus (DWV). Our results indicate that diets high in amino acids lead to a complex immunosuppressive syndrome impacting essential components of the social and innate immunity, reducing worker survival with proliferation of DWV. Our study shows that there are potentially severe and unpredictable impact of dietary components on immunocompetence and survival that are likely to account for honeybee colony collapse.

Abstract title:

Trueness and precision of the real-time RT-PCR method for quantifying the chronic bee paralysis virus genome in bee homogenates evaluated by a comparative inter-laboratory study

Authors:

- Dubois; Eric; Anses - European Union Reference Laboratory for Honeybee Health; Sophia Antipolis; France; eric.dubois@anses.fr
- Schurr; Frank; Anses; Sophia Antipolis; France
- European Network of National Reference Laboratories for Honey Bee Health
- Ribière-Chabert; Magali; Anses; Sophia Antipolis; France

Abstract text:

The ANSES Sophia Antipolis Laboratory has been appointed as the European Union Reference Laboratory (EURL) for bee health in 2011 and is also Reference Laboratory of the World Organisation for Animal Health (OIE) for bee diseases. The main scope of its reference activities is to harmonise the diagnosis of bee diseases by ensuring that the National Reference Laboratories (NRLs) use EURL-validated methods, and guarantee the proper use of these diagnostic methods.

One of such validated methods is the RT-qPCR method for quantifying Chronic bee paralysis virus (CBPV) genome copies per bee. This method quantifies the CBPV load between 4 to 10 log10 CBPV/bee with an accuracy of 0.5 log10 CBPV/bee. This method has been implemented in several European NRLs or official laboratories and is used notably to evaluate the prevalence of chronic bee paralysis in Europe.

In 2015, the accuracy (trueness and precision combined) of measurements of three CBPV loads (5, 8 and 9 log10 CBPV/bee) was assessed through an inter-laboratory comparison organised following the requirements of NF EN ISO/CEI 17043. Twenty-one participants, including 16 European National Reference Laboratories, received 13 homogenates of CBPV-infected bees adjusted to the three virus loads. Participants were requested to use the method usually employed for routine diagnosis. The

quantitative results (n=270) were analysed according to international standards NF ISO 13528 and NF ISO 5725-2.

The international network of NRLs responsible for diagnosing chronic bee paralysis by RT-qPCR provided accurate results. No significant systematic error (trueness) was found in the estimation of the number of genome copies in honeybee homogenates adjusted to the diagnostic threshold (8 log10 CBPV/bee). Only one laboratory had a lower precision (random error) than the others. The global standard deviations of measurement reproducibility (SR) were 0.83, 1.06 and 1.16 at viral loads 5, 8 and 9 log10 CBPV/bee, respectively. The inter-laboratory confidence of viral quantification (+/- 1.96 SR) at the diagnostic threshold (8 log10 CBPV/bee) was +/- 2.08 log10 CBPV/bee. These results highlight the need to take into account the confidence of measurements in epidemiological studies using results from different laboratories. Considering this confidence, viral loads over 6 log10 CBPV/bee may be considered to indicate probable cases of chronic paralysis.

Abstract title:

n/a

Authors:

- Campbell, Ewan; University of Aberdeen, Aberdeen, Scotland, e.m.campbell@abdn.ac.uk

Abstract text:

When adult female Varroa mites feed off the haemolymph of both larval and adult bees they transmit a number of pathogens to the bee hosts, most notably deformed-wing virus (DWV). We hypothesise that, similar to other Acari, Varroa mites suppress or alter the immune response of the bee host by secreting factors in the saliva.

This immune suppression may inadvertently facilitate DWV transmission from Varroa to bee and thus allow DWV to establish in the host.

In order to study the transmission of DWV and the suppression of immune response we have developed methodology for a novel micro-collection technique to stimulate and extract nanodrop amounts of saliva from individual reproductive-phase adult mites and micro-dissect pairs of salivary glands from feeding mites. DWV are detectable in saliva and in salivary glands and dominant strain types of DWV can be distinguished. This work and these techniques represent an important step forward toward a deeper understanding of the bee / Varroa / virus axis.

Abstract title:

n/a

Authors:

- Plevka; Pavel; CEITEC-Masaryk University; Brno; Czech Republic; pavel.plevka@ceitec.muni.cz
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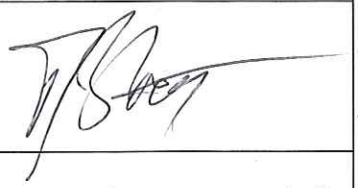
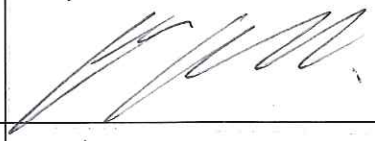
- Paxton; Robert; Martin Luther University Halle-Wittenberg; Halle; Germany; robert.paxton@zoologie.uni-halle.de

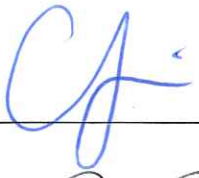
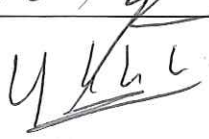
Abstract text:

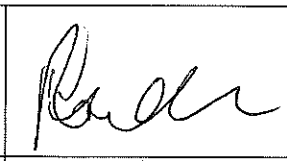
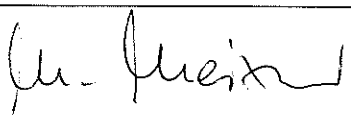
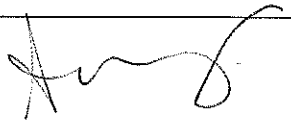
Viruses from the families Iflaviridae and Dicistroviridae are insect pathogens. Many of them cause lethal diseases in honeybees and bumblebees resulting in agricultural losses. These viruses have non-enveloped icosahedral virions containing single-stranded RNA genomes. However, their genome release mechanisms are unknown. The possibility of blocking virus genome delivery would provide a tool to prevent the spread of these pathogens. We describe the three-dimensional structures of virus particles in low-pH buffer, which imitates the conditions that the viruses encounter during cell entry. The low pH induces a reduction in the contacts between capsid proteins and formation of pores within the capsid that serve as channels for the genome release. We show that capsid proteins VP3 of iflaviruses deformed wing virus (DWV) and slow bee paralysis virus (SBPV) contain C-terminal globular domains that have not been observed in other viruses from the order Picornavirales. The protruding (P)-domains form “crowns” on the virion surface around each fivefold axis in native forms of the viruses. However, in buffers with low pH or high ionic strength the P-domains move and rotate at the capsid surface. Furthermore, the P-domains contain the ser-his-asp triad within a surface patch of eight conserved residues that constitute a putative catalytic or receptor-binding site. The movements of the domain might be required for efficient substrate cleavage or receptor binding during virus cell entry. Our work provides the first high-resolution structural characterization of iflavirus and dicistrovirus genome release.

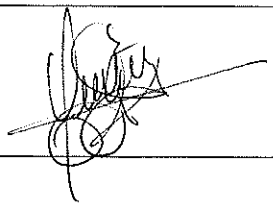
MEETING ATTENDANCE LIST

Meeting Title: Virus Taskforce	Start date: 6-04-2017	End date: 7-04-2017

	Participant	Country	Signature
1	Alexis Beaurepaire	France	
2	Tjeerd Blacquiere	The Netherlands	
3	Ewan Campbell	UK	
4	Yanping (Judy) Chen	USA	
5	Nor Chejanovsky	Israel	
6	Marianne Coulon	France	
7	Anne Dalmon	France	
8	Joachim de Miranda	Sweden	
9	Cecile Desbiez	France	

	Participant	Country	Signature
10	Eric Dubois	France	
11	Anna Gajda	Poland	
12	Christina Jenkins	Germany	
13	Per Kryger	Denmark	
14	Yves Le Conte	France	
15	Benoit Moury	France	
16	Peter Neumann	Switzerland	
17	Delfin Panziera	The Netherlands	
18	Niels Piot	Belgium	

19	Pavel Plevka	Czech Republic	
20	Marina Meixner	Germany	
21	Angela Minnameyer	Switzerland	

	Participant	Country	Signature
22	Melissa Oddie	Switzerland	
23	Declan Schroeder	UK	
24	Orlando Yañez	Switzerland	
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