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SOPHIA ANTIPOLIS LABORATORY

2020 Annual Report

About 30 people work at the Sophia Antipolis Laboratory, which comprises two research and reference units, respectively on bee diseases and animal Q fever. It holds several national and international reference mandates in these two areas. It is also the European Union Reference Laboratory (EURL) for bee health and the National Reference Laboratory (NRL) for research into pesticide residues, in association with ANSES's Laboratory for Food Safety. Its teams carry out applied research projects, based on observations in the field (development of means of prevention, studies on the modes of transmission of diseases, etc.), and provide scientific and technical support to veterinary services (supervision of networks of official laboratories, development of diagnostic methods, provision of reagents, etc.). Lastly, the Laboratory is involved in the epidemiological surveillance of Q fever and bee colony mortality phenomena.

KEY FIGURES

5 scientific articles in international journals

108 analyses

938 samples processed

1 inter-laboratory test organised

In 2020, the Laboratory's work was heavily marked by the COVID-19 pandemic. The Laboratory was able to maintain its public service activities (diagnosis of regulated diseases, provision of reference materials, etc.) thanks to a business continuity plan. However, its research projects and reference activities were impacted. The year was also marked by a decrease in the number of requests for analyses received by the Laboratory, probably due to a drop in the number of health visits in the field. Some activities had to be delayed or postponed, such as the organisation of inter-laboratory tests, while workshops and meetings were held remotely as part of the Laboratory's missions for the coordination of French and European laboratory networks. In spite of the above, significant results were achieved in the Laboratory's two areas of work.

BEE HEALTH

SUPPORT FOR IMPLEMENTATION OF THE EUROPEAN ANIMAL HEALTH LAW AND MONITORING OF BEE MORTALITY

The new European Animal Health Law, which entered into force in April 2021, lists four bee diseases that are regulated in the European Union: infestation with *Aethina tumida* (small hive beetle), infestation with *Tropilaelaps spp.*, infestation with *Varroa spp*. (varroosis), and American foulbrood. In 2020, scientific and technical support was provided to the Directorate General for Food and to the beekeeping sector, as part of the national implementation of this regulation.

Furthermore, the Laboratory renewed its involvement in the national survey on winter mortality in bee colonies, in collaboration with several beekeeping organisations. The third edition of this survey was launched online on 15 June 2020 and was aimed at the 56,844 beekeepers who had declared their hives at the end of 2019. A section addressing the management of varroa mites was added to the questionnaire, as requested by health organisations. There was also a question on the impact of the COVID-19 crisis on the health monitoring of colonies. The results will soon be available on the website of the epidemiological surveillance platform for animal health, on which the findings of the 2018-2019 survey have been published.

REFERENCE ACTIVITIES INVOLVING PATHOGENS

The continuity of the Laboratory's reference activities was maintained despite the various lockdowns and restrictions relating to the health crisis. A new real-time PCR method for the identification of the *N. apis* and *N. ceranae* parasitic fungi was assessed and accredited by the French Accreditation Committee (COFRAC). It will replace the conventional PCR method.

The methods used for the morphological and molecular identification of the small hive beetle were also revised, to better meet needs for the diagnosis of this exotic health hazard that was detected in 2014 in Southern Italy and is so far still absent from the rest of Europe. In particular, a method for the molecular identification of the larvae was validated and accredited by COFRAC. Lastly, a real-time PCR method for the detection and quantification of several bee viruses was made available and then adopted by three laboratories in the bee network.

REFERENCE ACTIVITIES INVOLVING PESTICIDE RESIDUES

The Laboratory also continued its analytical development and validation work aimed at broadening the range of plant protection compounds tested in various bee matrices. For example, two methods for multi-residue analyses in nectar were developed and applied to samples collected in the field as part of the European PoshBee project. The LC-MS/MS[1] method for measuring neonicotinoids in pollen was extended to a new compound, sulfoxaflor. Lastly, analyses of neonicotinoid residues in pollen and nectar were undertaken as part of several studies on the monitoring of environmental contamination by plant protection products.

Impact on bees of co-exposure to a pesticide and virus in natural conditions

Exposure to multiple stress factors contributes to honeybee colony decline. However, little is known about how co-exposure to stress factors can alter the survival and behaviour of free-living honeybees. The results of a thesis, co-funded by ANSES and INRAE, on potential interactions between a neonicotinoid pesticide, thiamethoxam, and a highly prevalent honeybee pathogen, deformed wing virus (DWV), were published in 2020. Tagged bees were exposed to DWV by feeding or injection, and/or to field-relevant doses of thiamethoxam, and were then left in colonies equipped with optical bee counters to monitor flight activity. DWV loads and the expression of immune genes were quantified. A reduction in vitellogenin expression levels was observed in DWV-injected bees and was associated with precocious onset of foraging. Combined exposure to DWV and thiamethoxam did not result in higher DWV loads compared to bees only exposed to DWV. However, it induced precocious foraging, increased the risk of not returning to the hive after the first flight, and decreased survival when compared to single stress exposure. We therefore provided the first evidence for deleterious interactions between DWV and thiamethoxam in natural conditions.

[1] Liquid chromatography-mass spectrometry.

CO-EXPOSURE AND STRESS FACTORS

In terms of research, the results of a study dealing with the impact on bees of pesticide-virus co-exposure in natural conditions were published (see box on page 5).

As part of the European PoshBee project, one of whose work packages is coordinated by the Laboratory, new methods were developed for the detection and quantification of several bee pathogens, as were methods for the quantification of pesticides in nectar. Moreover, a database was created to collect the results of the project's partners.

Organisation of the first circulating test for an exotic parasite in Europe

Every year, as part of its reference missions, the Laboratory organises one or more inter-laboratory tests for the laboratory networks it coordinates. The aim of these tests is to assess these laboratories' ability to implement diagnostic methods. This year, for the first time, the Laboratory organised a test on the *Tropilaelaps pathogenic* bee mite, whose infestation is regulated at European level. This mite is native to Asia and absent from Europe. The European Union laboratories approved for its detection have to implement reliable and robust diagnostic methods to be able to rapidly respond in the event that the mite is introduced into Europe. The EURL for bee health was faced with the extreme difficulty of obtaining mites of the target species to carry out tests. It therefore used an innovative format: a circulating test. A single panel of specimens was prepared, unlike with conventional inter-laboratory tests where a panel is produced for each participating laboratory. This panel was successively sent to the participating European Union laboratories. Between each consignment, the EURL verified the integrity of the samples. This test, which included six laboratories, ran over a period of 10 months. Its successful implementation demonstrated the ability to carry out circulating tests on a larger scale and thus make up for the low number of reference specimens. Moreover, the test established the reproducibility of the morphological identification method developed by the EURL and used by the participants.

ANIMAL Q FEVER

EPIDEMIOLOGICAL SURVEILLANCE: "ONE HEALTH" APPROACH TO Q FEVER

The Laboratory continued to be involved in the public health investigations coordinated by the Directorate General for Health and Directorate General for Food, concerning situations of clustered human cases of Q fever. An investigative group was created within the epidemiological surveillance platform for animal health, to take into account debates relating both to animal health and to human health as far upstream as possible. In particular, this group was asked to put preventive measures against Q fever into place in an agricultural secondary school where the disease was found to be circulating. Human cases had been reported the year before in another agricultural secondary school, which continued to be monitored by the Laboratory in 2020 (see box on page 10). These situations show the difficulty of defining actions to be taken in the specific case of facilities open to the public (educational farms, agricultural secondary schools, direct sales outlets, farm campsites, occasional fairs and cultural events, zoos, etc.) and demonstrate the importance of collecting case descriptions and feedback.

With the aim of pooling the knowledge of the National Reference Laboratory for animal Q fever and the National Reference Centre for human Q fever – the *Méditerranée Infection* University Hospital Institute – a scientific partnership agreement was signed in 2020. This collaboration will enable progress to be made in terms of the comparison of *C. burnetii* genomes isolated from animals, or from the environment and patients. Genotyping methods may also be improved, to better understand the conditions in which human cases emerge.

Lastly, an epidemiological study undertaken in collaboration with the University of São Paulo in Brazil detected a risk of contamination for laboratory workers (see box on page 8).

IMPROVEMENT OF Q FEVER DIAGNOSTIC TOOLS ON RUMINANT FARMS

As part of a doctoral thesis carried out at ANSES in partnership with VetAgro Sup and INRAE, a study was finalised comparing the performance of the three tests marketed to detect this infection in animals. This work, undertaken in collaboration with the French Federation of Health Protection Groups, the French National Centre for Scientific Research (CNRS) and the French Livestock Institute (IDELE), will enable the test results obtained to be interpreted more accurately, to better detect the disease.

The three serological tests marketed to diagnose the infection in ruminants were compared in terms of their performance. This showed that all three ELISA tests had high specificity, meaning that they correctly identified healthy animals. However, their sensitivity, which is the ability to reliably detect animals carrying antibodies against the bacterium, was lower and varied among the tests and for different species (between 39.3% and 93.8%). The optimum number of animals to be tested to detect the infection at herd level was also determined. This varied from three to more than 20 depending on the test and the species. Following this study, an application will be developed to help testing laboratories interpret the reliability of results and therefore the probability that the positive or negative results obtained for the animals are accurate. All of these findings have been submitted for publication in a scientific journal.

Q fever: a risk in slaughterhouses in Brazil

As part of a scientific partnership with the University of São Paulo in Brazil, a study was carried out to estimate the risk of slaughterhouse workers in this Brazilian state being infected with C. burnetii, responsible for Q fever. The prevalence of *C. burnetii* in cattle, the main reservoir for the pathogen, was therefore evaluated. An immunofluorescence assay (IFA) was undertaken with 1515 cattle serum samples collected from nine slaughterhouses. Of these, 23.8% were positive, indicating past or recent exposure to *C. burnetii* infection. Of the 54 cities sampled during the study, 83.3% had at least one seropositive animal. All of the positive samples were tested by qPCR, and *C. burnetii* DNA was detected in 12.2% of cases, suggesting active or recent infection. These significant findings highlighted the risk for slaughterhouse workers that results from exposure to contaminated aerosols produced during slaughter procedures. This study's results were published in PLOS One in 2020.

OUTLOOK AND PROJECTS INITIATED

THE EXPAIRCOX PROJECT

In 2017, some clustered human cases of Q fever were reported in the vicinity of Niort, France. After Q fever was found to be circulating in nearly half of the herds tested in the area, a project involving several stakeholders (INRAE, ANSES Sophia Antipolis and Niort, Qualyse laboratory) was carried out in 2018 and 2019. Dust samples were collected in 300 ruminant (cattle, sheep, goats) farm buildings and in public places. The results showed that *C. burnetii* DNA was frequently present in the farms' environments, sometimes in very high quantities. In 2020, seven strongly positive goat farms were selected to monitor airborne exposure during manure handling and spreading operations. These two studies have provided data to better protect farm workers and the population. For all of the positive samples, the different strains will be genotyped. To that end, the Laboratory has prepared a panel of representative *C. burnetii* strains, which will also be used to produce an antibody against *C. burnetii* spores. The aim is to develop a rapid viability test that can be applied to environmental samples to assess the infectious risk associated with the bacteria found in the environment. In parallel, a human and social sciences component will study how risks are determined and handled by experts, managers and farmers.

THE JOINT TECHNOLOGICAL UNIT FOR THE PROTECTION OF BEES IN THE ENVIRONMENT (PRADE)

In 2020, the Laboratory joined the Joint Technological Unit (UMT) for the Protection of Bees in the Environment (PRADE), a group of researchers and innovation stakeholders accredited by the Ministry of Agriculture, Food and Fisheries. Its members include the Network of Animal and Plant Sector Institutes, the Beekeeping Institute, the Association for the Development of Beekeeping in Provence, INRAE and now, for the first time, the bee diseases unit of ANSES Sophia Antipolis. Partnerships have already been initiated as part of this UMT. For example, the Laboratory is developing a project to model the impact of the small hive beetle's introduction into the French beekeeping sector, together with several UMT partners. Moreover, it has committed to the joint supervision of a thesis with a UMT partner; this thesis, which will begin in 2021, will focus on viral interactions in honeybees and their consequences for colony dynamics.

Epidemiological investigation following reports of human cases of Q fever

In late January 2019, the first human cases of Q fever were reported to the Regional Health Agency of Nouvelle-Aquitaine. They involved students from an agricultural secondary school with a dairy sheep farm. Q fever had previously caused abortions in some of the farm's sheep. Of the 12 suspected human cases, five were confirmed in May 2019; no new cases occurred. However, almost 300 individuals could be considered as exposed (students, staff). This led public health and veterinary stakeholders to conduct investigations and recommend management measures.

The first measure was the discontinuation of activities open to the public. All of the animals were vaccinated. The sheepfold was cleaned and the manure was piled away from the wind and composted. Two cleaning and disinfection operations were carried out in 2019. Changes in bacterial levels were monitored, showing a decrease in and then absence of excretion by the animals in 2020; however, the cleaning and disinfection operations were found to be almost completely ineffective, with a large amount of bacteria persisting in the environment. Building contamination only decreased in the dairy production area. The effect of the cleaning and disinfection operations was negligible and their feasibility was complex. Manure cleaning led to a slight increase in levels, probably due to the re-suspension of dust. The wool was clearly positive in the year following the abortion outbreak, including upon the animals' return from summer grazing. A significant decrease was measured only upon their return from summer grazing in the second year of the study.

Overall, transmission to the human population was low given the high levels of excretion and environmental contamination. The quantitative tests used to assess environmental contamination are essential for this airborne zoonosis. However, both dead and live bacteria can be detected by real-time PCR. Technical upgrades are therefore necessary to be able to define risk levels and criteria for lifting measures such as the bans on activities open to the public.

KEY DATES

14 – 19 JANUARY *General meeting of the European PoshBee project*

21 – 22 OCTOBER *Annual workshop of the European Union Reference Laboratory for bee health*

26 – 27 NOVEMBER *Évaluation des activités d'analyses par le COFRAC*

MAIN PUBLICATIONS

Benkirane, Y., C. Dupuy, M. Laurent, C. Ruger, C. Sourdeau, J. Vallon, and S. Wendling. 2020. "Enquête nationale sur les mortalités hivernales des colonies d'abeilles. Mortalité des colonies d'abeilles durant l'hiver 2018-2019. Résultats descriptifs pour la France métropolitaine". *La santé de l'abeille* 300: 508-529.

Dubois, E. and A. Dalmon. 2020. "Relations entre le virus des ailes déformées, l'acarien Varroa destructor, l'abeille mellifère et leurs conséquences sur la santé des colonies". *La santé de l'abeille,* 181-194.

Bicudo de Almeida-Muradian L., D.Barth, O. Dietemann, M. Eyer, A. Da Silva de Freitas, A.-C. Martel, G. Marcazzan, C. Marchese, C. Mucignat-Caretta, A. Pascual-Maté, W. Reybroeck, M. Sancho, and J. Gasparotto Sattler. 2020. "Standard methods for Apis mellifera honey research". *Journal of Apicultural Research* 59 (3): 1-62.

Coulon, M., A. Dalmon, G. Di Prisco, A. Prado, F. Arban, E. Dubois, M. Ribiere-Chabert, C. Alaux, R. Thiéry, and Y. Le Conte. 2020. "Interactions Between Thiamethoxam and Deformed Wing Virus Can Drastically Impair Flight Behavior of Honey Bees". *Front Microbiol* 11: 766.

Medkour, H., B. Davoust, M. Angelakis, R. Thiéry, D. Raoult, E. Rousset, P. Parola, and C. Eldin. 2020. "A sporadic case of acute Q fever and identification of the animal source of the infection". *Folia Microbiologica*. https://doi.org/ doi: 10.1007/s12223-020-00788-3.

Mioni, M. S. R., F. B. Costa, B. L. D. Ribeiro, W. S. R. Teixeira, V. C. Pelicia, M. B. Labruna, E. Rousset, K. Sidi-Boumedine, R. Thiéry, and J. Megid. 2020. "Coxiella burnetii in slaughterhouses in Brazil: A public health concern". *PLoS One* 15 (10): e0241246.

Wendling, S., F. Meziani, P. Hendrikx, and S. Franco. 2020. "Bilan de la surveillance des maladies règlementées et troubles de l'abeille mellifère Apis mellifera pour l'année 2016". *Bulletin épidémiologique, santé animale et alimentation.* Special issue on regulated and emerging animal diseases: 20.

30 NOVEMBER – 4 DECEMBER

Internal audit according to the ISO 17043 standard, concerning proficiency testing schemes

15 DECEMBER

Meeting of the network of accredited laboratories for bee diseases

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