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French agency for food, environmental and occupational health & safety

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The Sophia-Antipolis Laboratory: 40 years of research and reference activities

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Press release

The Sophia-Antipolis Laboratory: 40 years of research and reference activities

Since its creation, ANSES's Sophia Antipolis Laboratory has established itself as a national and international reference in bee and ruminant health. Indeed, since 1976, it has been awarded five national, European and international reference mandates for bee health and bee matrices, as well as the national reference mandate and World Organisation for Animal Health (OIE) reference mandate for Q fever. This year, 2016, is thus an opportunity to take stock of 40 years of reference activities for the benefit of animal health. Moreover, its location at the heart of the Sophia Antipolis international technology centre, in an area boasting a dynamic beekeeping sector, helps raise the profile of the scientific community at the regional level, through research agreements and projects conducted in partnership with regional scientific centres of excellence.

The Sophia Antipolis Laboratory specialises in the study of the main diseases and poisoning phenomena of bees, and certain ruminant diseases, especially Q fever. As such, it implements research projects based on observations in the field and provides scientific and technical support to veterinary services (supervision of networks of accredited laboratories, development of detection, identification and quantification methods, provision of reference reagents, expert appraisal of diagnostic tools, etc.).

Reference laboratory for bee health

For some regulated or emerging pathogens (viruses, bacteria, parasites) or chemical contaminants of major importance, the health authorities need an effective surveillance system based on a network of reliable laboratories to conduct the official analyses.
For each regulated pathogen or contaminant requiring surveillance, the health authorities designate accredited laboratories for conducting analyses, as well as a "reference" laboratory. This reference laboratory ensures the reliability of the analyses carried out by all the accredited laboratories.

Since 1976, the Sophia Antipolis Laboratory has established itself as the reference in its field of expertise. Thus, in the field of bee health, it holds three reference mandates and is involved in two national mandates on screening for pesticides in bee matrices. It coordinates a network of eight accredited laboratories at the French level, and 27 national reference laboratories at the European level.

In particular, in 2011, ANSES's Sophia Antipolis Laboratory was appointed as the European Union Reference Laboratory for bee health, a key step that acknowledges the efforts put into the research and reference activities in this area over the past few years. This mission and the resources it provides, through the strengthening of skills in microbiology and epidemiology, has led to significant advances in understanding the multiple factors responsible for the disorders affecting bee colonies, thanks in particular to the European Epilobee survey, an extensive epidemiological surveillance programme in Europe that is seeking to better characterise the phenomenon of excess bee mortality. The data obtained in the framework of this programme are currently still being analysed, with the aim of identifying the different factors that can influence colony mortality.

In this context, the Laboratory is continuing its research to develop and validate ever more powerful analytical methods for the diagnosis of bee diseases and their pathogens, and for the detection and identification of the pesticide residues that are most hazardous to bee health.

Furthermore, the Laboratory took part in the expert appraisal led by ANSES on the issue of co-exposure of bees to various stress factors and their respective roles in bee colony weakening, collapse and mortality, with an emphasis on the interactions between these factors. This expert appraisal highlighted the multifactorial nature of the causes of bee colony mortality, and emphasised the role of co-exposure to pesticides and infectious agents in determining their collapse.

Reference laboratory for Q fever

The Sophia Antipolis Laboratory holds the national reference mandate and World Organisation for Animal Health reference mandate for Q fever, a zoonotic disease that can have a major impact on public health and farming.

To limit the clinical impact and spread of Q fever in ruminant herds and towards the human population, the Laboratory develops, improves or assesses tools for detecting the bacterium responsible for the disease, conducts epidemiological research in order to contribute knowledge on the survival of this bacterium in the environment and on the virulence characteristics of strains, takes part in studies on the assessment of control and prevention measures, participates in expert appraisal work at national and European levels in the framework of the drafting of recommendations and opinions, takes part in the national platform for epidemiology in animal health on abortions in ruminants, and contributes to veterinary field investigations during outbreaks of clustered human cases.

What next for the Laboratory?

In the coming years, the Laboratory will aim to continue its reference and research work on:

- understanding the causes of weakening and mortality of bees and colonies, by addressing both the main microbiological and parasitic hazards of bees, and the chemical contaminants that can affect bee health in isolation or simultaneously. It will continue its main actions to characterise these hazards while focusing on assessing their effects alone or in co-exposure. It will continue to invest in documenting and understanding weakening through epidemiological surveillance of colony losses and bee diseases, while seeking to take the impact of pesticides into account.

- bee diseases, in particular by participating in the programmes launched by the European Commission and EFSA on the risks incurred by bees, taking into account the pesticide risk and the impact of co-exposure (biological agents and nutrition).

- biological hazards, by pursuing work on the agent responsible for Q fever: development of tools for detecting bacterial reservoirs of the agent of Q fever, characterisation and molecular dynamics of strains of *C. burnetii,* studies on the viability and virulence of strains.

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A brief history

The Sophia Antipolis Laboratory sprang out of the regional veterinary research laboratory, founded in Nice in 1950, in premises made available by the Alpes-Maritimes *département*. This brand new laboratory's mission was to examine the region's primary health concerns. Given the importance of the sheep production sector in the Provence-Alpes-Côte d'Azur region, it took on the role of departmental testing laboratory for the diagnosis of diseases in sheep, and also in small pets, as well as for questions of food hygiene.

After incorporating the beekeeping section of the Alpes-de-Haute-Provence departmental veterinary laboratory, it extended its research to bee diseases and hygiene of hive products. On the one hand, it specialised in the study of infectious bee diseases due to viruses, bacteria and parasites, and on the other, it worked on methods for detecting unwanted chemical contaminants in honey: pesticides, antibiotics, antifungal agents and heavy metals. It also participated in the standardisation of these methods.

In 1974, the Laboratory was integrated with all the National Laboratories for Veterinary Research, and became part of the external services of the Ministry of Agriculture.

In 1976, the Laboratory became the National Laboratory for Diseases of Small Ruminants and Bees. This period saw the creation of national veterinary laboratories all reporting to the Ministry of Agriculture, bringing together under this name all the laboratories specialising in animal health and food hygiene distributed across the country. The mission of these laboratories was to provide scientific and technical support for the Ministry of Agriculture, which itself was responsible for shaping health policy in these areas. Although not yet designated "reference laboratories", in practice this was their role in their respective fields.

Over time, the Laboratory started to specialise. As early as 1977, it began developing virological and biochemical research, both for bees and small ruminants, in order to develop, improve and push forward diagnostic methods, by taking on board the latest scientific and technical advances. The end of the 1970s and the beginning of the 1980s saw the identification and isolation of several viruses affecting sheep or bees, some of which were detected on French territory for the first time: the virus of *Maedi-visna* disease in sheep, caprine arthritis-encephalitis virus, sacbrood virus in bees and chronic bee paralysis virus, which is still the subject of research today.

At the beginning of the 1980s, the arrival in France of *Varroa*, a parasitic mite of the honeybee, marked a turning point for beekeepers. Very early on, it was identified as having a major impact and surveys were conducted to assess its role in bee mortality. At the same time, different ways of treating the parasite were developed. Similarly, given the growing use of plant protection products in agriculture, it was necessary to conduct eco-toxicological investigations to measure the quantities of toxin residues present in bee matrices. In 1984, epidemiology and ecotoxicology research therefore began to be developed within the Laboratory.

In 1989, the Laboratory joined forces with the other National Laboratories for Veterinary Research to form the National Centre for Veterinary and Food Studies (under the name CNEVA Sophia Antipolis, Laboratory for Diseases of Small Ruminants and Bees), and then moved to the Sophia Antipolis technology park in 1990. Its establishment on the Sophia Antipolis technology park has had several advantages, in particular the proximity to a number of research organisations such as INRA, CNRS and the University of Nice Sophia Antipolis.
The Laboratory then became part of the French Food Safety Agency (AFSSA) in 1998.

In 1999, its facilities were supplemented by an L3 high-containment level laboratory. This facility was necessary for developing research on the agent responsible for Q fever, on which the Laboratory had chosen to concentrate in 1998, because at the time few studies had been undertaken on this disease, despite its high prevalence and resulting losses in herds of small ruminants, but also because of the regular occurrence in France of human cases. Significant progress has been made in diagnosis in ruminants, and in the management of alerts in humans, in close consultation with the health authorities.

In November 2008, the Laboratory became known as the AFSSA Sophia Antipolis site, Laboratory for study and research on small ruminants and bees.

Following the creation of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) through the merger of AFSSA with the French Agency for Environmental and Occupational Health Safety (AFSSET), the Laboratory became the Sophia Antipolis Laboratory.

The Sophia-Antipolis Laboratory: 40 years of research to benefit reference and health surveillance activities

For some regulated or emerging pathogens (viruses, bacteria, parasites) or chemical contaminants of major importance, the health authorities need an effective surveillance system based on a network of reliable laboratories to conduct the official analyses.
For each regulated pathogen or contaminant requiring surveillance, the health authorities designate accredited laboratories for conducting analyses, as well as a "reference" laboratory. This reference laboratory ensures the reliability of the analyses carried out by all the accredited laboratories. The Sophia Antipolis Laboratory has seven reference mandates.

About thirty people work at the Sophia Antipolis Laboratory, which contains two research and reference units: the bee diseases unit and the animal Q fever unit. The teams carry out applied research projects, based on observations in the field (development of means of prevention, studies of the modes of transmission of diseases, development of diagnostic tools, etc.) and provide scientific and technical support to veterinary services (supervision of networks of accredited laboratories, development of detection, identification and quantification methods, provision of reference reagents, expert appraisal of diagnostic tools, etc.).

For **bee health**, the Sophia Antipolis Laboratory holds **five** mandates:

- Three at the national level for unifying a network of field laboratories: bee health, pesticide residues in honey, and single-residue methods (shared with the Agency's Laboratory for Food Safety).
- **European Union** Reference Laboratory for bee health: it heads a network of national reference laboratories.
- At **international** level, it is also Reference Laboratory of the World Organisation for Animal Health (OIE).

As the reference laboratory for the European Union, the Laboratory is currently involved in several international calls for research projects and programmes, such as Must B, led by the European Food Safety Authority. These seek to extend knowledge on the effects of pesticides and co-exposures on bee health, and improve the surveillance tools.

The Laboratory pursues this work in collaboration with its national partners, INRA and the Technical and Scientific Institute of Beekeeping and Pollination (ITSAP - Bee Institute), as well as its network of European reference laboratories and various European and international research teams.

For Q-fever, a disease of ruminants caused by the bacterium *Coxiella burnetii* and transmissible to humans, it is the national reference laboratory and OIE Reference Laboratory, thus holding two reference mandates for this zoonosis.

A laboratory that is a point of reference for bee diseases

For several years now, bee colony mortality and weakening have been reported in numerous countries around the world. Multiple factors are potentially involved: bee diseases and parasites, poisoning by pesticides, stress related to changes in food resources, climatic conditions, etc. This situation requires monitoring systems to be harmonised, and research to be strengthened and coordinated, at national and European levels.

The Laboratory studies the major bee diseases and the pathogens involved:

- bacterial diseases: American and European foulbrood;
- parasitic diseases: varroasis, nosemosis, tracheal mites, infestation by the *Tropilaelaps* mite;
- viral diseases: deformed wing virus, chronic bee paralysis virus, sacbrood virus, acute bee paralysis virus, Kashmir bee virus, Israeli acute paralysis virus, black queen cell virus.

The Laboratory also investigates bee predators and develops diagnostic methods to identify and detect them: the Asian hornet and the small hive beetle.

It conducts research on pesticide and veterinary drug residues:

- in honey, in order to improve the safety of hive products,
- in the different bee matrices, in the framework of bee health.

It develops diagnostic tools and analytical methods for residues, and provides scientific and technical support: expert appraisals, sample analysis, provision of reference reagents, field investigations, *etc*.

Lastly, the Laboratory harmonises the diagnostic methods used by the official national and international laboratories in the framework of its reference mandates. To do this, it validates and disseminates methods, organises inter-laboratory tests and trains the employees of the official national (NRL mandate) and international (EURL mandate) laboratories.

Main research subjects and programmes

- Studies of diseases of viral, bacterial or parasitic origin and the diversity of pathogens
- Detection and identification of the main predators
- Study of the factors of bee colony weakening and loss
- Development of diagnostic methods and residue analysis methods
- Epidemiology
- Effect of pesticides and phenomena of co-exposure
- Fate of xenobiotic residues in honey and hive products

A reference laboratory for Q fever

Q fever is caused by the bacterium *Coxiella burnetii*. Responsible for abortions in goats, sheep and cows, it can have a significant public health impact: regular occurrence of outbreaks, clinical consequences over several years (morbidity, chronicity). Its prevalence and incidence are still insufficiently assessed, mainly because it is difficult to diagnose.

To limit the clinical impact and spread of Q fever in ruminant herds and towards the human population, the Sophia Antipolis Laboratory:

- develops, improves or assesses tools for detecting *Coxiella burnetii*;
- conducts epidemiological research in order to contribute knowledge on the survival of the bacterium in the environment and on its shedding and dissemination methods, as well as on the virulence characteristics of strains;
- takes part in studies on the assessment of control and prevention measures;
- participates in expert appraisal work at national and European levels in the framework of the drafting of recommendations and opinions;
- takes part in the national platform for epidemiology in animal health on abortions in ruminants;
- contributes to veterinary field investigations in conjunction with the health authorities and the national reference centre on *Coxiella* during outbreaks of human cases.

In terms of surveillance, in 2015, the Laboratory was closely involved in a programme conducted in ten pilot *départements* on the epidemiological situation of Q fever in France. This scheme had two components: monitoring of serial abortions due to Q fever, and a survey of seroprevalence in farms selected at random. A harmonised protocol for differential diagnosis of abortions was set up.

This pilot programme helped strengthen the Laboratory's reference activities: assessment of the performance of the quantitative PCR methods used in the network's laboratories, proposal for a more economical relative PCR method, and creation of a serum bank containing 4319 sera from goats, sheep and cattle with a view to characterising the performance of commercial diagnostic kits based on the ELISA method.

The European Epilobee programme

The Sophia Antipolis Laboratory has been mandated by the European Commission to coordinate Epilobee, a programme for the active surveillance of bee colony mortality, implemented in 17 Member States. Over two consecutive years, more than 176,745 bee colonies were visited and the data collected were used to estimate colony mortality rates according to the geographical areas in Europe, figures which had never previously been recorded in an active and harmonised manner.

Winter mortality rates varied according to the countries from 3.2% to 32.4% for winter 2012-2013 and from 2.4% to 15.4% for the following winter. These figures underline the annual and interregional variability, but also reveal colony mortalities that can exceed the generally accepted evidence-based rate of 10%. A milder winter in the second year of the study could, at least in part, explain the lower mortality observed. Colony mortality rates during the 2014 beekeeping season were between 0.04% and 11.1%. Lower than the winter mortality rates, they were overall identical to those of the previous season.

The prevalence of diseases affecting bee health fell overall in the second year of the programme, although this decline was not statistically significant for several countries. American foulbrood and European foulbrood (two brood diseases caused by bacteria) had prevalences below 12% and 8% respectively in all Member States for the two years.
No outbreaks of *Aethina tumida* (small hive beetle) or *Tropilaelaps* spp. (mites), both pests exotic to Europe, were detected during the course of the project. It should be noted that *A. tumida* was discovered in Italy in September 2014 after the end of the apiary visits carried out in the framework of Epilobee. Cases of varroasis were recorded in almost all the Member States. Cases of nosemosis were observed in 10 countries during the second year of the programme, with its prevalence exceeding 10% in only three of them. Lastly, only a few cases of chronic paralysis were detected during the two years of the programme.

The European Epilobee programme helped obtain a large quantity of data on bee colony mortality but also on the health situation of the colonies, the use of veterinary treatments, and the environment of the apiaries visited, within the 17 Member States participating in the project. The analysis of all of these data has enabled an exploration of the statistical links between colony losses and the data collected, and identified some of the risk factors affecting bee health.

Impact of co-exposure of bee colonies to pesticides and infectious agents

ANSES issued a formal internal request in 2012 on the co-exposure of bees to various stress factors and their respective roles in bee colony weakening, collapse and mortality, with an emphasis on the interactions between these factors.

The world has many species of insect pollinators that contribute to the survival and evolution of over 80% of plant species. These pollinators include some 20,000 species of bees, of which around 850 are found in France, in particular the honeybee *Apis mellifera*.

Bee colony mortality and weakening have been observed in numerous countries for several years now, and are partly responsible for the fall in the production of honey. These phenomena of bee colony weakening, collapse and mortality, observed in most of the countries where intensive agriculture is practised (Europe, the Americas), have in recent years been the focus of many studies seeking to understand the mechanisms involved in these disorders.

Better knowledge of concomitant or successive exposure to stress factors

In this context, the Agency issued an internal request in 2012 on the question of co-exposure of bees to stress factors and the interactions between these factors. Bees are exposed to many stress factors liable to interact with each other: infectious, chemical, physical, or related to food, beekeeping practices, weather conditions, *etc*. A wide variety of infectious and parasitic agents can affect bee colonies, and many xenobiotics (primarily insecticides, fungicides and acaricides) are found in bee matrices.

Apiaries are co-exposed to multiple combinations of factors. The presence of infectious agents within colonies, and the exposure of the bees to pesticides with a variety of origins and mechanisms of action, in all likelihood cause a transition from a "normal" state of health to the expression of pathologies that can lead to their collapse, through a decrease in immunity or a reduction in the bees' mechanisms of detoxification.

The Agency’s recommendations

Even though in some cases bee mortalities result from the action of a single factor, the Agency's work emphasises the often multifactorial nature of the causes of colony mortality and insists in particular on the importance of the co-exposure of bees to pesticides and infectious agents.

These phenomena are even more marked when the bees are exposed to multiple compounds. Certain interactions have already been demonstrated in the scientific literature and the Agency recommends further research to explore other potential interactions.

In general, ANSES notes that in spite of the urgency of the phenomena of bee colony weakening and the fact that this situation has existed for some time, the deployment of multiple studies, supported by various stakeholders over the past few years, has been unable to provide a consolidated diagnosis of the state of health of colonies nationwide, nor of their co-exposure to infectious and chemical hazards.

In this context, and although it is not possible to act on stress factors such as climate in the short term, ANSES stresses the need to intervene on all of the factors identified as contributing to colony weakening.

To do this, in order to avoid compounding the situation with other stress factors, in particular zootechnical or nutritional, it reiterates the importance of maintaining biodiversity and complying with good beekeeping practices to keep the bees and colonies healthy. It also recommends decreasing the use of inputs in agricultural practices in order to reduce the overall exposure of bees to plant protection products.

In the framework of discussions to be held at European level, the Agency recommends including, in the procedure for assessing the toxicity of plant protection products (insecticides in particular) before marketing authorisation is granted, tests to measure the effect of chronic chemical co-exposure to other compounds:

- an anti-*Varroa* acaricide;
- a fungicide also known to inhibit detoxification mechanisms in bees;
- an insecticide with the same mode of action as the test product and also known to be present in bee matrices, if the plant protection product to be tested is an insecticide.

The effective implementation of this proposal is subject to an adaptation of the EU regulations, following development of the necessary tests and procedures.

Lastly, the Agency recommends the use of quantitative methods for qualifying the status of the hive with regard to infectious agents, as well as the creation of reference apiaries, brought together in a network to cover the country as fully as possible, in order to define regional references for the different actors.

This recommendation should eventually lead to a unified and well-structured observation network, coordinated at national level and able to produce updates on the health status of colonies, their co-exposure to infectious and chemical agents, and how they change over time.

Prospects for the Laboratory

In the coming years, the work of the Sophia Antipolis Laboratory will focus on the following.
Concerning bees:

- the effects of exposure and co-exposure on bee health,
- characterisation and eco-dynamics of the hazards: identification and characterisation of pathogens at the molecular level and/or the carriers of their pathogenicity,
- epidemiological surveillance of colony losses and bee diseases with account taken of the role of pesticides.

Concerning ruminants and other animal species:

- development of tools for detecting bacterial reservoirs of the agent of Q fever (*Coxiella burnetii),*
- characterisation and molecular dynamics of strains of *C. burnetii*, as well as studies on the viability and virulence of strains.

Concerning bees, the research topics will be:

- development of tools for detecting and quantifying pesticides in bees and hive products,
- development of tools for diagnosing and characterising the main bee pathogens (bacterial, viral, parasitic),
- genomics of the main bee pathogens,
- epidemiological surveillance of bee colony losses.

Concerning Q fever:

- genomic plasticity of strains of *C. burnetii,*
- virulence and resistance of *C. burnetii* in the environment,
- epidemiology of Q fever and the detection of animal reservoirs.

Some of the work started in 2015

- **Study of the interaction between the chronic bee paralysis virus (CBPV) and thiamethoxam in bees**

This thesis project is part of a collaboration with INRA Avignon and is examining the issue of co-exposure of bees to viruses and toxic agents. The work led to the Laboratory studying the interaction between the chronic bee paralysis virus and thiamethoxam, an insecticide belonging to the neonicotinoids class. During the first year of the project, a method of transmitting the virus from a sick bee to a "healthy" bee was used, as well as chronic oral exposure to thiamethoxam, at different concentrations. A synergistic effect of the co-exposure on mortality was observed at the highest concentrations of the pesticide.

- **The European SmartBees project**

The SmartBees (Sustainable Management of Resilient Bee populations) project will run until 2018. It is seeking to improve understanding of the natural resistance of bee populations to infectious and parasitic diseases, with an emphasis on the parasite *Varroa destructor*. The Laboratory is a member of the consortium of around fifteen European partners (bee geneticists, pathologists, etc.), and is particularly involved in studying the diversity and virulence of the deformed wing virus (DWV), a pathogen transmitted by *V. destructor*.

- **A multi-species serological test for Q fever**

In order to obtain a diagnostic tool applicable to various situations, and therefore to respond to epidemiological questions, the unit began work to develop and optimise a multi-species ELISA method.

The Laboratory's partners

Ministry of Agriculture, Food and Forestry

European Commission

Provence-Alpes-Côte d'Azur Regional Council

Alpes-Maritimes Departmental Council

CNRS, INRA, the InVS, National Laboratory for Control of Breeder Stock, French Association for Directors and Executives of Public Veterinary Analysis Laboratories, Institut Mérial, AES Laboratoire Group, Universities, Veterinary schools, the Méditerranée-Infection University Hospital Institute (IHU), etc.

Inter-professional organisations: Regional Federation of Health Protection Associations, National Inter-professional Association for Goats, ITSAP (Bee Institute)

Association for Animal Health Certification, National Federation of Departmental Bee Health Organisations, French Association of Honey Producers, etc.

Epidemiological Surveillance Platform for Animal Health.

French Agency for Food, Environmental and Occupational Health & Safety - ANSES

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) is a scientific body working in the areas of food, the environment, work, animal health and welfare, and plant health.

Collective and independent expert appraisal

Through its monitoring, expert appraisal, research and reference activities, ANSES assesses all the risks (microbiological, physical or chemical) to which a person may be exposed, intentionally or otherwise, at all ages and times of their life, including at work, while travelling, while engaging in leisure activities or via their food.

This is based on the deployment of independent, pluralistic scientific expertise by expert groups, also taking into account the economic and social dimensions of a risk.

To carry out its various missions, the Agency relies on a network of eleven reference and research laboratories, spread out across the country, which contribute to health monitoring. It also works in partnership with many external, national and international bodies.

ANSES also assesses the effectiveness and risks of veterinary drugs, plant protection products, fertilisers, growing media and their adjuvants, as well as biocides, in order to issue marketing authorisations. It also carries out the assessment of chemical products in the framework of the REACh Regulation.

An agency open to society

The Agency is committed to openness to society and works closely with its stakeholders (public authorities, professional bodies, trade unions, consumer associations, environmental associations, associations representing occupational accident victims, elected officials, qualified individuals).

The Board of Administrators, which consists of the five colleges of the *Grenelle* environmental round table, has set up thematic steering committees which help determine ANSES's policy orientations and work programme priorities, by making it aware of the main concerns of civil society.

Lastly, on subjects that are key issues for society, the Agency is also able to create specific dialogue committees with stakeholders, whose mission is to inform the Agency about society's expectations in terms of risk assessment and research.

ANSES systematically publishes its work on its website www.anses.fr and organises or participates in some twenty scientific events each year.

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