





Presentation

Since October 2018, the Laboratory for Animal Health has had two main sites: one in Maisons-Alfort on the campus of the National Veterinary School of Alfort (ENVA) and the other in Dozulé/Goustranville in Normandy, next to CIRALE (where ENVA's equine activities are based). The Laboratory also has a satellite site at the University of Reims that hosts two contracted units (USCs). The Laboratory has played a historically significant role in combating major infectious and contagious diseases of livestock (foot-andmouth disease, tuberculosis) and Equidae (glanders, dourine). It currently has 130 employees grouped into five main units that cover all spheres of infectiology in animal health. Both of its main sites have close ties with the ENVA campus, with which it shares premises and staff, and it also belongs to the Community of establishments of

eastern Paris (COMUE) and the Normandy University Community. It is primarily a reference laboratory (30 national, European or international reference mandates), but also carries out research activities supported by other bodies (INRAE, ENVA, universities) with accreditation from the French Ministry of Research. Its innovative work in equine autopsy is especially noteworthy, and it is the leading French centre in the field. The Laboratory's activities revolve

around the control of major animal epidemics (such as foot-and-mouth disease and bluetongue); bacterial, viral and parasitic zoonoses; fungal diseases; infectious equine diseases; and vector-borne diseases (carried by ticks, sandflies, *Culicoides*, lice, etc.). The dynamics of infectious agents within herds (epidemiology/ surveillance) is also a core study activity. "The Laboratory has 130 employees grouped into five main units."

First Annual Workshop of the NRLs for Foot-and-Mouth Disease organised by the <u>EURL ANSES/Sciensano</u>

VIROLOGY (UMR)

 \rightarrow Since 1 January 2019, the Laboratory for Animal Health has held the mandate of European Reference Laboratory for Foot-and-Mouth Disease (FMD) jointly with Sciensano.

On 7 and 8 October 2019, the Laboratory held the first annual workshop of the NRLs for FMD and the 11th workshop on vesicular stomatitis in Maisons-Alfort. About 60 participants from 35 countries, representing both EU NRLs and laboratories from neighbouring countries (Algeria, Georgia, Turkey, Montenegro, Serbia, Norway, Switzerland, etc.), attended this international meeting. It was the first workshop organised by ANSES under its new EURL FMD mandate, and provided an opportunity to present the results of the first interlaboratory trial organised by the EURL. This success would not have been possible without the full commitment of the staff of both the Laboratory for Animal Health and Sciensano.

Context

The Laboratory for Animal Health has been a pioneer in animal infectiology since its foundation in 1901. Its main focus being research, development and reference activities in animal infectiology, it studies several domestic animal species and major zoonotic agents. The Laboratory operates through contractual actions, the sharing of patents and reference reagents with research organisations from various countries, and partnerships with the Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE). Its reach extends across Europe owing to its three European Union Reference Laboratory (EURL) mandates and the coordination of its networks. Its activity in the Near/Middle/Far East, West Africa and the Mediterranean Basin is growing considerably, particularly the monitoring of foot-and-mouth disease (training, participation in the European Foot-and-Mouth Disease network) and work on emerging or re-emerging zoonoses. The Laboratory has a long history of cooperation with several research centres in Asia - particularly in China, South Korea and Thailand - but also in Brazil. Students trained here have moved on to various university and research centres, giving the Laboratory access to an extensive network of institutions in China and other countries.

The Laboratory plays a leading role in many areas:

→ European Union Reference Laboratory for infectious equine diseases, brucellosis and foot-and-mouth disease (coordination of 26 national laboratories).

→ OIE Reference Laboratory for foot-and-mouth disease, epizootic haemorrhagic disease, bovine tuberculosis, glanders and chlamydiosis.

→ OIE Collaborating Centre for foodborne parasites, Europe region (appointed in 2014). FAO Collaborating Centre for foot-and-mouth disease.

→ Participation in numerous European or national research contracts (H2020, COST, ANR, EJP One Health/ DIM1Health) and bilateral contracts with various third countries (China, Brazil, several French-speaking African countries).

Its reference/surveillance activity, which is subject to ISO 17025 accreditation assessments (the highest level available), is balanced with original, dynamic research supported by numerous contracts. Research in equine pathology (autopsy, epidemiology and microbiology) in the Pathophysiology and Epidemiology of Equine Diseases (PhEED) Unit has been reorganised and contractual opportunities with various partners are currently being explored (USC project with the University of Caen). The Unit runs the equine mortality surveillance network (RESUMEQ) and is involved in another network (RESPE) as an equine clinic.

The Laboratory also includes a new USC, set up in partnership with Paris-Est-Créteil University (UPEC) and ENVA, which is developing research on fungi and lice (resistance).

The new iCUBE platform became operational in late 2018. This enabled the EURL for foot-and-mouth disease to launch its reference activity in January 2019 and the EURL for equine diseases to transfer its activities.

"The Laboratory's reach extends across Europe owing to its three European Union Reference Laboratory (EURL) mandates and the coordination of its networks."

KEY FIGURES



national or international reference mandates

145

5 international category A+/A publications



presentations, invitations, posters at national/international congresses



joint research units (UMRs) assessed by the High Council for Evaluation of Research and Higher Education (HCERES) in 2019

High prevalence of dengue and Zika virus infections in Equidae in New Caledonia and French Polynesia

VIROLOGY UMR

→ Many emerging arboviruses, such as dengue, chikungunya and more recently Zika, are circulating in the South Pacific. In 2015-2016, a serological survey was conducted in New Caledonia and French Polynesia to determine the seroprevalence of flavivirus infections in the equine population. This showed that 16.6% (27/163) of Equidae from New Caledonia and 30.8% (40/130) from French Polynesia tested positive by ELISA. Confirmatory serological tests with the main flaviviruses circulating in the South Pacific showed that 6 to 8% of Equidae had antibodies neutralising dengue virus (DENV) serotype 1, while 4.3% of Caledonian sera and 15.4% of Polynesian sera neutralised Zika virus (ZIKV). The seroprevalence study in the equine population showed that horses can be infected with ZIKV and DENV, leading to their seroconversion. This study implicitly indicates that the main vector of these viruses in New Caledonia and French Polynesia, *Aedes aegypti*, takes its blood meals mainly on primate hosts but also on other mammals.

The consequences of this infection on the equine population, as well as the role of horses in the epidemiological cycle of ZIKV and DENV, are two questions requiring further investigation.

Work undertaken and key events

The Laboratory contributes to the early detection of major emerging animal pathogens affecting multiple species including horses, characterises their pathogenicity and implements preventive measures (vaccines or therapy). It follows the "One World, One Health" approach and has intensified its work on various subjects relating to emerging zoonotic diseases, in particular by developing a research topic on tickborne pathogens and Culicoides. In 2019, the Laboratory obtained new contracts from the French National Research Agency (ANR) and from Europe, particularly via the EJP One Health call for tenders. Its publication output grew to more than 145 papers in international high-impact peer-reviewed journals (categories A and A+). This remarkable progress over the past year underlines the increased efforts of the teams to enhance the value of their research.

The Laboratory continued to coordinate the field of major interest (DIM) for the Île-de-France Region on the "One Health" theme for the third year (www.dim1health.com). The mid-term assessment of this project was very positive, with the budget being fully renewed despite the addition of four more DIMs.

Acquisition of a confocal microscope

The DIM1Health project financed the acquisition of a confocal microscope for the Maisons-Alfort site, an instrument accessible to the entire campus and the ANSES units.

The VIRO and BIPAR UMRs and the Dynamic USC were assessed very favourably by HCERES, with their mandates being renewed for a further five years. This collective audit confirmed each unit's main scientific orientations and highlighted the strong synergies between reference activities and research for the UMRs.

The Laboratory for Animal Health has had to tackle the re-emergence of various equine viruses (equine infectious anaemia, West Nile, equine viral arteritis), ruminant viruses (monitoring of bluetongue, especially development of the serology reference activity) and Usutu virus. It has participated in the isolation of *Bacillus anthracis*, mycobacteria, *Chlamydia* and *Brucella*, in different livestock rearing conditions. To carry out its missions, the Laboratory strengthened its relations with various partners, mainly through four contracted units (USCs). The sequencing and analysis of many viral and bacterial genomes (*Francisella, Burkholderia, Brucella, Anaplasma*) continued in 2019, leading to the publication in high-impact journals of new knowledge on taxonomy and genetic diversity. The Laboratory reinforced its phylodynamics capability with two application models: foot-and-mouth disease virus and mycobacteria.

The PhEED Unit at the Normandy site specialises in infectious and parasitic diseases of reproduction, persistent and/or emerging infections of major importance for the equine sector, and epidemiological surveillance of these diseases and equine mortality.

In 2019, the Laboratory also obtained a new OIE reference mandate for *Burkholderia mallei* and *pseudomallei*, as well as a favourable assessment as the future OIE Reference Laboratory for dourine.

"The sequencing and analysis of many viral and bacterial genomes continued in 2019, leading to the publication in high-impact journals of new knowledge on taxonomy and genetic diversity."

Outlook and projects initiated

A number of Laboratory modernisation projects are currently in the preliminary study stage and will be actively pursued in 2020 (in particular for the bacterial zoonoses building and the Bressou building). The same applies to the Normandy site, which has obtained regional funding for its overall modernisation (the "equine campus" project, with different phases starting in 2021).

Pathogen genome analysis using high-throughput approaches is experiencing rapid growth and benefits from synergies between site units. These approaches are optimising ties with the IdentyPath platform and fostering the emergence of a new platform on host-pathogen interactions. The Normandy region intends to develop higher education and research activities in the field of equine health. This objective will take shape through the creation of an equine campus on the Laboratory's Normandy site, with the activities of ENVA's equine clinic in Maisons-Alfort being transferred there by 2023. Both the Laboratory for Animal Health's Normandy site and its key partner, ENVA, are involved in these discussions with the regional authorities, which also include future investments to be allocated by the region.

"Development of genomic and phylodynamic analysis of pathogens in collaboration with ANSES entities and other institutes"

First French-Czech meeting on ticks

BIPAR UMR

 \rightarrow Tick-borne diseases are a growing problem for human and animal health in Europe and around the world. Europe needs to develop its research leadership on this topic, so it is essential for European institutions to promote integration and complementarity.

Integration is the key to European science. The first French-Czech meeting on ticks (FCTM 1) brought together around 50 participants at Maisons-Alfort. Over two days, 30 French and Czech researchers presented scientific results related to tick research. Several presentations on methodologies for studying parasites or pathogens were also included in the programme. The French researchers were from ANSES, INRAE, and ENVA, while the Czech researchers came from the Institute of Parasitology at the Biology Centre of the Czech Academy of Sciences in Ceske Budejovice. Synergies and future collaborations were discussed during the round table, leading to the creation of a French-Czech cooperation group in tick research.

The BIPAR UMR (French partner) and the Institute of Parasitology (Czech partner) are working on the joint agenda and the creation of an international laboratory. A framework agreement between the two institutions will provide the basis for the formation of a group to strengthen scientific leadership in tick research at European and international level.

<u>Research on</u> <u>the vaccination</u> <u>of ibexes against</u> <u>brucellosis</u>

BACTERIAL ZOONOSES UNIT

→ Brucellosis is a disease of animal origin caused by bacteria of the genus *Brucella*. It is widespread throughout the world and can affect humans and most other mammalian species, including both domestic and wild ruminants. *Brucella* comprises 12 species, including *Brucella melitensis*, which mainly affects sheep and goats. In 2012, via the Brucellosis team of the Laboratory for Animal Health, ANSES spearheaded the identification of an outbreak of brucellosis in ibexes from the Massif du Bargy, related to cases of bovine and human contamination.

In 2015, vaccination of ibexes was suggested as a means of controlling *Brucella* infection in this area, in addition to the slaughter of infected individuals. The Ministries of Agriculture and the Environment then asked ANSES, INRA, the ONCFS and the MNHN to conduct experimental work to assess and compare the safety of a Rev.1 conjunctival vaccine between Alpine ibexes and domestic goats. The article's authors showed differences between ibexes and goats in immune responses, distribution in the tissues analysed and shedding of the vaccine strain used. In particular, the authors observed that, unlike goats, ibexes could produce urogenital excretions that could contaminate their congeners through contact, as well as the environment.

This work paves the way for further research, particularly on the effectiveness of vaccinating ibexes with the *B. melitensis* Rev.1 vaccine strain. It is also an example of collaboration, sharing and synergy of scientific efforts between four scientific institutions: ANSES, INRAE, the ONCFS and the MNHN, in order to assess the risks and study the best control strategies for this disease in the ibexes of the Massif du Bargy. Through its Brucellosis Reference Laboratory (national, European and international mandates), ANSES will continue to contribute its bacteriological, molecular and serological expertise to these large-scale studies.

<u>Presentation during</u> <u>the Fourth OIE International</u> <u>Conference on non-tsetse-</u> <u>transmitted animal</u> <u>trypanosomose [NTTAT]</u>

PHEED UNIT

 \rightarrow The PhEED Unit of the Laboratory for Animal Health (Normandy site) is currently in charge of national and European reference activities for dourine, and aims to obtain the OIE Reference Laboratory mandate for this disease. A major strategic challenge is active participation in international meetings organised by the OIE-NTTAT network, and establishment of collaborations and exchanges of biological material with countries in which dourine is endemic.

The Unit took part in the Fourth OIE International Conference on non-tsetse-transmitted animal trypanosomose, organised in Mongolia by the Institute of Veterinary Medicine of Mongolia and the National Research Center for Protozoan Diseases (Obihiro, Japan). This conference, which brought together 34 participants from Africa, Asia and Europe, provided an opportunity for the PhEED Unit to present the results of a sero-epidemiological study set up in partnership with the Argentinian laboratory Clinica Equina SRL, which revealed circulation of surra, equine infectious anaemia, West Nile fever and equine piroplasmosis in a herd of semi-wild horses in northern Argentina.

Prospects in the short term include technical organisation of exchanges of biological materials between the PhEED Unit and its Mongolian and Japanese partners (respectively the Institute of Veterinary Medicine of Mongolia and the National Research Center for Protozoan Diseases), and organisation of a Japanese scientist's collaborative secondment. In the medium term, the involvement of the PhEED Unit in the OIE-NTTAT network could be stepped up through a proposal to take over the network's secretariat.

"Vaccination enabled the country to recover its disease-free status in late 2012, but the epizootic flared up again in 2015."

<u>Bluetongue in France: from emergence to re-emergence</u>

EPIDEMIOLOGY UNIT

<u>Assessment of antimicrobial</u> <u>susceptibility of polymicrobial</u> <u>biofilms (filamentous fungi</u> <u>and bacteria)</u>

DYNAMIC USC

→ The fungus Aspergillus fumigatus and the bacterium Stenotrophomonas maltophilia are two opportunistic pathogens that can coexist as a biofilm, particularly in the respiratory tract of immunocompromised patients or those suffering from cystic fibrosis. In the hospital setting, *A. fumigatus* is the second most common fungal agent causing mortality in patients with impaired respiratory function, e.g. due to COVID-19. Micro-organisms in biofilm form have reduced susceptibility to antimicrobial treatments.

Lolita Roisin's thesis work focused on the *in vitro* antimicrobial susceptibility of *A. fumigatus* and *S. maltophilia*, alone or in combination, in biofilm form. The antimicrobials amphotericin B, itraconazole, levofloxacin and rifampicin were selected for their known action on these microorganisms. The results of this work showed that amphotericin B was effective against the *A. fumigatus* biofilm, unlike itraconazole. The presence of *S. maltophilia* seemed to increase the susceptibility of *A. fumigatus* to amphotericin B, whereas both antibiotics were less effective against *S. maltophilia* in the presence of *A. fumigatus*. The presence of the fungus may therefore confer protection to the bacterium, possibly due to the fungal extracellular matrix acting as a barrier to diffusion of the antimicrobial agents. This hypothesis is supported by the fact that in the presence of an enzyme (proteinase K) capable of cleaving proteins from the fungal matrix, the results show an increase in the viability of *S. maltophilia* when exposed to levofloxacin.

With the appearance of COVID-19, which affects the respiratory tract, assessing the susceptibility of polymicrobial biofilms (filamentous fungi and bacteria) in contact with bronchial cells will be a priority in 2020.

→ Bluetongue virus serotype 8 (BTV-8) spread widely in France in 2007/09 and had a major economic impact. Vaccination, which was first mandatory and then optional, enabled the country to recover its disease-free status in late 2012, but the epizootic flared up again in 2015. A series of studies focusing on BTV-8 helped clarify its epidemiology from emergence to re-emergence and were the subject of Noémie Courtejoie's thesis, defended in 2019. This research was used to conduct a critical analysis of control procedures using various modelling methods.

Although developed for BTV-8, it should eventually be possible to use these modelling tools to investigate situations where several BTV serotypes are circulating simultaneously (BTV-1 and BTV-4, in particular). Although several epidemiological studies suggest the absence of a wild reservoir, these tools should also enable the question of wildlife's role in the increase in BTV persistence to be addressed.

JANUARY

→ Transmission of the summary document on the DIM1Health activity (2 years). Mid-term evaluation of the DIM1Health project by the Îlede-France Region's Scientific Council.

FEBRUARY

→ Collective audits by HCERES of the VIRO UMR, Dynamic USC and BIPAR UMR. Renewals validated by the three supervisory ministries

APRIL

→ Participation with stand and presentations at the Normandy Equine Festival (FENO, 12-14 April). DIM Scientific Council on 15 and 16 April to assess all the dossiers submitted to the platform (60 dossiers submitted for a budget of €2.8M)

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→ DEPAQUIT J, AKHOUNDI M, HAOUCHINE D, MANTELET S, IZRI A. No limit in interspecific hybridization in schistosomes: observation from a case report. Parasite. 2019;26:10.

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17-21 JUNE

7-19 SEPTEMBER

→ Korean delegation in Maisons-Alfort for a presentation of the European reference activity for foot-andmouth disease → Workshop of the EURL for Brucellosis and visit by the IVDC (China)

7-10 OCTOBER

→ Workshop of the EURL for Equine Diseases and the EURL for Foot-and-Mouth Disease, Maisons-Alfort **24-25 OCTOBER**

→ First French-Czech meeting on ticks with the Institute of Parasitology, Academy of Sciences (Czech Republic)

4-7 DECEMBER

→ Presentations at the Paris Horse Show

→ JAY M, FREDDI L, MICK V, DURAND B, GIRAULT G, PERROT L, et al. Brucella microti-like prevalence in French farms producing frogs. Transboundary and Emerging Diseases. 2019.

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→ VANCOVA M, BILY T, NEBESAROVA J, GRUBHOFFER L, BONNET S, PARK Y, et al. Ultrastructural mapping of salivary gland innervation in the tick Ixodes ricinus. Scientific Reports. 2019;9(1):6860.

→ YOUNG JJ, COULOMBIER D, DOMANOVIC D, ZELLER H, GOSSNER CM. One Health approach for West Nile virus surveillance in the European Union: relevance of equine data for blood safety. Euro Surveillance. 2019;24(16).

→ GAJADHAR AA, NOECKLER, K., BOIREAU, P., ROSSI, P., SCANDRETT, B., GAMBLE, H.R., . International commission on Trichinellosis: Recommendations for quality assurance in digestion testing programs for Trichinella. Food and Waterborne Parasitology [Internet]. 2019.; https://doi. org/10.1016/j.fawpar.2019.e00059.

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