Centre de Recerca en Sanitat Animal (CReSA)

### Services commissioned by the Generalitat de Catalunya

**2012 Executive Summary** 









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### Centre de Recerca en Sanitat Animal

### Working for the animal health sector

### Origin and mission

The Centre de Recerca en Sanitat Animal (CReSA) is a foundation created in 1999 to conduct research into animal health. It was founded by initiative of the Universitat Autònoma de Barcelona (UAB) and Institut de Recerca i Tecnologia Agroalimentàries (IRTA). CReSA joins the human potential for research into animal health of both founding institutions, and takes advantage of a technologically advanced building, with level-3 biocontainment (BSL3), grouping efforts and channeling new resources in this field.

The CReSA researchers search for innovative and effective vaccines, study epidemiology, immunological responses and pathogenic mechanisms, while assessing risks for human health and developing standardised infection models and diagnosis techniques.

### Research subprograms of CReSA

- · Veterinary epidemiology and risk analysis
- Bacterial and endoparasitic infections, and antimicrobial resistance
- Transboundary viral infections
- · Endemic viral infections

### **Objectives**

The CReSA objectives are the research and technological development (R&D), and all aspects of studies and education in the field of animal health. The projects are carried out in collaboration with the UAB, IRTA, other institutions, and the private sector.

To achieve our objectives we:

- i) develop R&D programs within the field of animal health
- ii) transfer the scientific advances that we achieve to the agrifood sector
- iii) offer services by means of arranged R&D programs
- iv) advise agrifood companies and public administration and offer technological support; and v) organize scientific and technical training programs.



### Services for the public administration

In parallel to scientific interest, CReSA researchers perform studies that have important implications for consumers, producers and regulatory institutions. For this reason, CReSA carries out different initiatives for the government departments of the *Generalitat de Catalunya* with competencies in animal and public health. From 2001 until the present, CReSA has been working closely with different public institutions to improve animal and public health on a regional and national level. This collaboration takes the form of annual services or research activities contracts, or occasional contracts for specific activities.

At the regional level, CReSA has an annual contract with the Department of Agriculture, Livestock, Fisheries, Food and Natural Environment (Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural, DAAM) and the Department of Health (Departament de Salut, DS) of the Catalonian Government. There are also some occasional collaborations with the Catalan Food Safety Agency (ACSA), ascribed to the DS, for some specific activities.

On the national level, the CReSA has started a collaboration with the Ministry of Environmental, Rural and Marine Affairs (Ministerio de Agricultura, Alimentación y Medio Ambiente, MAGRAMA) and has conducted some studies for other regional governments, such as those of Andalusia and Galicia.

In the framework of these contracts, CReSA has worked in epidemiology, diagnostics, and general studies of diseases, including bovine tuberculosis (bTB), bluetongue (BT), avian influenza (AI), West Nile Disease (WND), Newcastle disease (ND), bovine spongiform encephalopathy (BSE), Aujeszky's disease (AD), classical swine fever (CSF), Maedi-Visna, paratuberculosis, border disease, rabies, and brucellosis in cattle and small ruminants.

In 2012, CReSA received 546,000€ from DAAM and 372,107€ from DS to carry out the services commissioned.



### **Advanced facilities**

The CReSA building, which opened in 2003, consists of highly-specialised equipment and technologically advanced facilities that enable studies to be performed in the fields of microbiology, immunology, molecular biology, entomology and prions. The CReSA has technologically advanced facilities for such studies, with two clearly differentiated areas:

### The level 2 biosafety laboratories

The laboratory zone, of biosafety level 2, occupies 717 m<sup>2</sup>. The area consists of eleven laboratories and equipment rooms in which specific activities are carried out: bacteriology, virology, immunology, molecular biology, pathological anatomy, cell culture, termocyclers, PCR sample extraction, electrophoresis, entomology, ultrafreezing, equipment, preparation of reagents, etc.

#### The level 3 biocontainment unit

The centre has a Biocontainment Unit with biosecurity level 3, which has several laboratories and stables that mainly house food supply animals (pigs, poultry, cattle, sheep, goats and rabbits), as well as wild animals (chamois, deer, quails, partridges, falcons, ferrets) and laboratory animals (rats, mice, guinea pigs). This Biocontainment Unit, of a total surface area of 4500 m2 distributed over three floors, is equipped with strict access control measures and biocontainment barriers that prevent the pathogens from getting outside, and which are studied using hermetic isolation systems.

This unit enables the research team to carry out research into pathogenic agents listed as diseases notifiable to the World Organization for Animal Health (OIE).

#### Biocontaintment systems, barriers and protocols

- Safe management of high-risk infectious agents
- Hermetic isolation systems
- Negative pressure gradients
- Absolute air filtration
- Treatment of liquid and solid wastes
- · Mandatory showers on leaving the biocontainment unit
- 6 high security laboratories: virology, bacteriology, cell culture, equipment, molecular biology and prions
- 12 high security boxes for experimental inoculations to house pigs, poultry, cattle, sheep, goats and rabbits, among others.

All laboratories have independent ventilation systems, with negative pressure gradient with regard to the corridors and HEPA filters for air on entry and exit. The boxes have strict control and containment measures, in addition to having negative pressure with respect to the corridors. All air entering and exiting the boxes is filtered through absolute HEPA filters. Waste coming from this zone, such as stools and waste water, is subjected to a chemical decontamination process before it leaves the building.

Personnel entering the boxes must change clothes and take a shower before leaving. Animals are on conventional livestock slats, with standard feeding and drinking troughs, living in a controlled atmosphere. All pathological clinical variables are thoroughly supervised. A videosurveillance system records images 24 hours a day, allowing control of the animals inside the boxes at all times. In order to maintain these strict conditions of biocontainment and biosecurity, there is a complex centralised management system that permits direct and quick control of all elements and parameters that directly influence the running of the facilities.



# Services commissioned by the Department of Agriculture, Livestock, Fisheries, Food and Natural Environment



### Program of surveillance for avian influenza and Newcastle disease in wild birds

The monitoring of the avian influenza (AI) in wild birds in Catalonia in 2012 belongs to the vigilance of AI being undertaken by the European Union. This program is developed in coordination with the rest of Autonomous Communities as part of the AI surveillance program in Spain, 2012.

The main objective in wild birds during 2012 has been the detection of highly pathogenic avian influenza viruses such as A/H5N1. The implementation of these programs includes the participation of the DAAM, CReSA, the Algete Central Veterinary Laboratory (LNR) and MAGRAMA. In addition, taking advantage of the operative net-

work to collect samples from avian wildlife for AI, a monitoring of the Newcastle disease viruses (NDV) has been carried out mainly in columbiform dead birds as differential diagnostic of AI. To collect information on the H5N1 AI virus and NDV circulating in wild birds, different activities based on passive surveillance have been implemented.

None of the total of 59 sick or dead bird samples have tested positive for AI. Whereas, four in the total of 37 bird samples analysed for NDV 3 *Streptopelia decao*cto and 1 *Passer domesticus*, have resulted positive for highly pathogenic NDV.



### **Program of surveillance for West Nile virus**

The monitoring of the West Nile virus in wild birds and equines in Catalonia in 2012 belongs to the vigilance of West Nile disease (WND) being undertaken since 2006. The aim of the surveillance program is the early detection of the West Nile virus (WNV) in Catalonia in the main reservoirs (birds) and domestic animal hosts (equines), basically in the main risk areas. The program involves the participation of the DAAM, CReSA, the Mosquito Control Services,

the Wildlife Recovery Centres, equine veterinary clinics, the Algete Central Veterinary Laboratory (LNR) and MAGRAMA. The program is based on different components: active and passive surveillance of equines (291 samples), wild birds (144 samples d and entomologic monitoring.

In 2012, seropositivity against WNV-like has been detected in both horses and migratory and resident wild birds. Nine equine sera have tested positive by ELISA, being 3 of them positive by seroneutralitzation test (SNT) with a low titers (<1/20)) and 11 avian sera have resulted positive by ELISA, although uniquely one sample has been positive by SNT with a low titer (1/20)). These results indicate that the enzootic cycle of WNV-like not only has remained in Catalonia in wild birds near highly populated urban areas, but also that the incursion of this virus is probable in other areas of the region.

### Virological analyses

The service of virological analysis has as main objective to provide diagnosis of the main viral diseases of domestic animals subjected to official control programs by the Servei de Sanitat Animal (animal health service) of the DAAM.

The diseases subjected to diagnosis are: classical swine fever (CSF) and other pestiviruses, swine vesicular disease (SVD), bluetongue (BTV) and Schmallenberg (SBV):

- CSF is a contagious viral disease of pigs, the causative virus is a member of the genus Pestivirus of the family Flaviviridae, and is closely related both antigenically and structurally to the viruses of bovine viral diarrhea (BVD) and Border disease (BD).
- SVD is a contagious swine disease, caused by an enterovirus; the main importance of SVD is that it is clinically indistinguishable from foot and mouth disease (FMD), and any outbreaks

- of vesicular disease in pigs must be assumed to be FMD until investigated by laboratory tests and proven otherwise.
- BTV infection involves domestic as a sheep, goats, cattle and wild ruminants, BTV is a member of the Orbivirus genus of the family Reoviridae. The BTV species, or serogroup, contains 24 recognized serotypes.
- SBV belongs to the Bunyaviridae family, within the Orthobunyavirus genus. The SBV is related to the Simbu serogroup viruses. SBV affects domestic ruminants as sheep, goats, cattle and wild ruminants.

In the 2012 period, 11.238 samples were analysed. These samples came from Laboratoris de Sanitat Ramadera, Seccions Territorial de Ramaderia i Sanitat Animal and Serveis Veterinaris Oficials de les Oficines Comarcals of DAAM.

### Assessment and diagnostic for the control and eradication program of bovine tuberculosis

Tuberculosis (TB) is a zoonotic disease mainly caused by *Mycobacterium bovis* and *M. caprae* affecting a range of domestic and wild animal hosts. TB in cattle is subjected to an eradication programme in Catalonia. By commission of the DAAM, CReSA conducts the diagnosis, the epidemiological follow-up of positive herds and provides expert guidance.

In 2012, 24 cattle herds were affected by TB, which supposed a 0.6% of annual prevalence, a reduction of 0.3% in comparison to 2011, whereas the incidence of new TB infected herds was reduced from 0.5% in 2011 to 0.2% in 2012. Furthermore, 17 out of the 24 positive herds recovered the Official TB-Free qualification during 2012.

The Mycobacteria Diagnostic Unit of CReSA performed a total of 4144 analyses by the Interferony assay, 380 by the antibody detection ELISA test, 422 by anatomopathological evaluation, 309 by mycobacterial culture and 280 by PCR. The laboratory techniques were carried out under international quality standards (UNE-EN ISO/IEC 17025).

The data obtained from epidemiological surveys and the integral diagnostic results were assessed and discussed in a monthly workshop formed by CReSA's researchers and veterinarians of DAAM, which recommended specific measures for decision making.



### Entomological surveillance program for bluetongue disease

Bluetongue (BT) is a viral infectious, noncontagious disease affecting ruminants. The transmission of the virus among susceptible hosts is through the bite of hematophagous midges of the genus *Culicoides*. Worldwide there are over 1400 species of *Culicoides* and only few of them can transmit arboviruses such as Bluetongue virus (BTV), Schmallenberg virus (SBV) or African horse sickness virus (AHSV) among others as well as many other parasites.

Since the year 2003, CReSA has designed and implemented the Entomological Surveillance Program for BT, as a service for the administration.

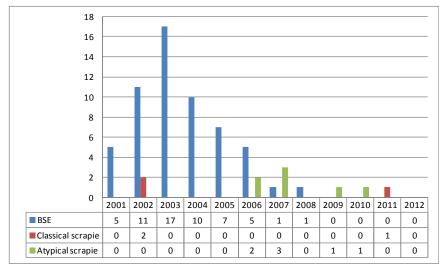
The objectives of the Entomological Surveillance Program are: i) to monitor the recent introduction and expansion *Culicoides imicola* in Catalonia (the main Afroasiatic vector for BTV), ii) perform the monitoring of other autochthonous species that are either confirmed or suspected BTV vectors, some of them being even more abundant than *C. imicola*, and iii) to improve the knowledge of the seasonal activity and the ecological requirements determining the presence of specific *Culicoides* throughout the year. These data is expected to be of importance in order to predict transmission risk periods according to the seasonal distribution and abundance of vectors.

During the year 2012, a total of 14.820 Ceratopogonid dipterans were diagnosed, being 12.295 species to be reported to the Government for being potential vectors of BTV. According to the results obtained, data analyzed suggested the presence of two periods of high risk for BTV transmission, late spring (May-June) and early autumn (September-October).

### Diagnostic for the surveillance plan of the animal transmissible sponfigorm encephalopaties

Central nervous system samples from fallen stock population (both cattle and small ruminants) are analysed as part of the active Transmissible Spongiform Encephalopathies (TSE) surveillance programme. Apart from routine tests, in cases with an initially positive result from rapid tests, confirmation tests are conducted. In 2012, 6.265 samples were analysed and no cases of TSE were diagnosed.





TSE cases in Catalonia (2001-2012).

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# Services commissioned by the Department of Health



## Diagnostic for the control and surveillance plan of the animal transmissible spongiform encephalopaties of animals destined for human consumption

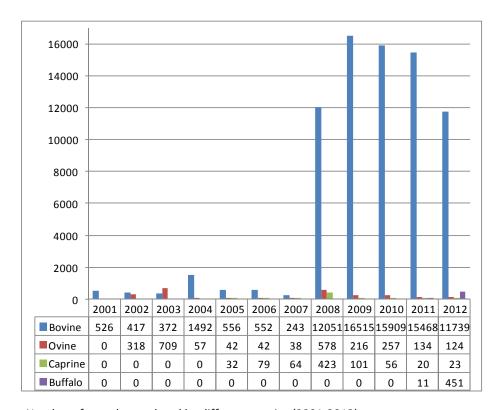
The PRIOCAT laboratory performs, by commission of the Catalan Public Health Agency (Agència de Salut Pública de Catalunya, ASPCAT) belonging to the Health Department, an active Transmissible Spongiform Encephalopathies (TSE) surveillance programme, whereby it specifically analyses samples from all of Catalonia of the central nervous system of a sample of bovines and small ruminants destined for human consumption in order to determine the presence of prion diseases.

Apart from routine tests, in cases with an initially positive result from rapid tests, confirmation tests are conducted. In 2012, 12.337 samples were analysed and no cases of TSE were diagnosed. The laboratory has also developed a research line funded by national and European research projects regarding the study of different aspects of TSE such as the transmission barriers determinant factors of animal prions (BSE and Scrapie) and the characterisation of the transmissibility of atypical

variants of Scrapie to other livestock species and humans.

In October 2012 the VIII scientific-technical meeting on Transmissible Spongiform Encephalopathies was organised in which research and surveillance results were reported.





Number of samples analysed by different species (2001-2012).

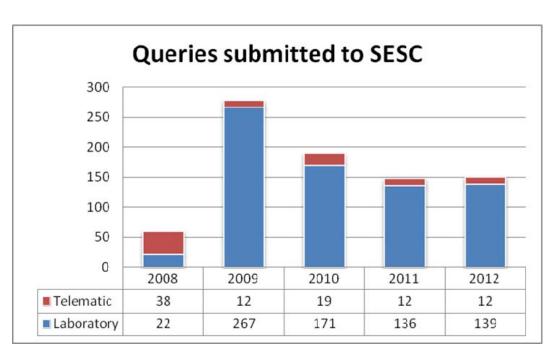
### **Slaughterhouse Veterinary Support Service**

In 2012, the Slaughterhouse Veterinary Support Service (Servei de Suport a Escorxadors, SESC) managed a total of 151 queries by official vets conducting inspections of slaughterhouses in Catalonia. Of these, 12 were telematic queries and the other 139 corresponded to requests for sample laboratorial analysis. Among the queries received there was a prominence of lesions of suspected bovine tuberculosis, followed by cattle muscle lesions to rule out bovine cysticercosis and Marek's disease in poultry.

A total of 33 posts were published in the SESC Case Archive website: a specialised blog on slaughterhouse veterinary pathology (www.cresa.cat/blogs/sesc) which provides continuing education to meat inspectors and other related animal health professionals. The blog was migrated during 2012 to a Wordpress platform

and protected under a creative commons license. It was also translated to Spanish and English languages; this quadrupled the number of visitors to the blog (over 19K since the new platform kicked off). Diffusion through social media (Twitter, Facebook and Linkedin) has also been implemented.





Queries submitted to SESC in 2012.

### **Transfer of knowledge**

### **Publications**

Alba A, Bicout DJ, Vidal F, Curcó A, Allepuz A, Napp S, García-Bocanegra I, Costa T, Casal J. Model to track wild birds for avian influenza by means of population dynamics and surveillance information. PLoS One. 2012;7(8):e44354.

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### Other pulications related with these topics

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Fernández-Sirera L, Cabezón O, Dematteis A, Rossi L, Meneguz PG, Gennero MS, Allepuz A, Rosell R, Lavín S, Marco I. Survey of Pestivirus infection in wild and domestic ungulates from South-Western Italian Alps. Eur J Wildl Res (2012) 58:425–431.

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García-Bocanegra I, Arenas-Montes A, Napp S, Jaén-Téllez JA, Fernández-Morente M, Fernández-Molera V, Arenas A. Seroprevalence and risk factors associated to West Nile virus in horses from Andalusia, Southern Spain. Vet Microbiol. 2012 Dec 7;160 (3-4):341-6.

García-Bocanegra I, Cabezón O, Pabón M, Gómez-Guillamón F, Arenas A, Alcaide E, Salas-Vega R, Dubey JP, Almería S. Prevalence of *Toxoplasma gondii* and *Neospora caninum* antibodies in *Spanish ibex* (*Capra pyrenaica hispanica*). Vet J. 2012 Feb;191 (2):257-60.

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Rodríguez-Prieto V, Martínez-López B, Barasona JA, Acevedo P, Romero B, Rodriguez-Campos S, Gortázar C, Sánchez-Vizcaíno JM, Vicente J. A Bayesian approach to study the risk variables for tuberculosis occurrence in domestic and wild ungulates in South Central Spain. BMC Vet Res. 2012 Aug 30;8:148.

Rodriguez-Campos S, González S, de Juan L, Romero B, Bezos J, Casal C, Álvarez J, Fernández-de-Mera IG, Castellanos E, Mateos A, Sáez-Llorente JL, Domínguez L, Aranaz A; Spanish Network on Surveillance Monitoring of Animal Tuberculosis. A database for animal tuberculosis (mycoDB.es) within the context of the Spanish national programme for eradication of bovine tuberculosis. Infect Genet Evol. 2012 Jun;12(4):877-82.

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### Doctoral thesis related with these topics

Immune response to influenza infection and vaccination

Júlia Vergara Alert Director: Ayub Darji

Epidemiology, vaccination and infection in wild ruminants with bluetongue virus

Cristina Lorca Oró

Director: Mariano Domingo, Ignacio García Boca-

negra, Jorge R. López Olvera

### Research master works related with these topics

La vigilancia de triquina en España: Situación actual y valoración de un sistema alternativo

L Cárdenas Contreras

Director: Sebastián Napp, Jordi Casal

Aedes albopictus en Cataluña: estudio de la estructura genética poblacional y análisis filogeográfico

Marco Brustolin

Director: Nonito Pagès, Núria Busquets

Ecogeografía del corzo en Aragón y las relaciones entre distribución, abundancia y dinámica poblacional

J Ferreres

Director: Pelayo Acevedo

Uso del espacio por el jabalí en montes de Toledo centrales: implicaciones como reservorio de enfermedades

Javier Gutiérrez

Director: Pelayo Acevedo, J Vicente

Tendencias poblacionales de especies de interés cinegético: datos de 20 años de monitorización

M Boadella

Director: Pelayo Acevedo

### **Technical seminars**

(Pla anual de transferencia tecnológica DAAM)

**Tuberculosi: situació actual i avenços científics** 05/06/2012 75 attendees



VIII jornada sobre EET's 01/10/2012 40 attendees



**Bioseguretat a les granges** 12/11/2012

69 attendees



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