

PUBLICATIONS

SCI Publications

Abad, F.X., Busquets, N., Rivas, R., and Majó, N. **Persistence of H7N1 highly pathogenic avian influenza virus in fomites.** Submitted to: *Applied and environmental Microbiology*. MS AEM02682-08.

Aramouni, M., Busquets, N., Majó, N., and Abad, F.X. **Decay of H7N1 avian influenza virus infectivity by saline content.** Submitted to: *Applied and Environmental Microbiology*. MS AEM02930-08.

Busquets, N., Dolz, R., Abad, F.X., Rivas, R., Ramis, A., and Majó, N. **Quantification of H7N1 avian influenza virus shedding from experimentally infected chicks: feather pulp as an useful simple for HPAI virus detection.** Submitted to: *Virus Research*. VIRUS-D-09-00034.

Busquets, N., Serrano, E., Alba, A., Núñez, J.I., and Majó, N. 2006. **False-positive results obtained by following a commonly used reverse transcription-PCR protocol for detection of influenza A virus.** *Journal of Clinical Microbiology*, 44: 3845.

Other Publications

Alba, A., Busquets, N., Abad, F.X., Serrano, E., Allepuz, A., Napp, S., and Majó, N. 2008. **Seguimiento de la vigilancia de influenza aviar en aves silvestres en Cataluña durante el 2007.** In: *Selecciones Avícolas*.

Alba A., Majó, N., Busquets N., Serrano E., Cordon R., Lozano C., Pujols J., Napp S., Allepuz A., Abad F., Casal J., and Domingo M. 2008. **Avian Influenza Surveillance in wild birds in Catalonia, Spain (2007).** Annual Report for the Competent Authorities, February 2008.

Alba, A., Majo, N., Busquets, N., Casal, J. 2007 **Reseau de surveillance de la grippe aviaire chez les oiseaux sauvages en Catalogne, Espagne.** *Epidémiol. et santé anim.* 52, 49-57.

Alba A., Majó, N., Busquets N., Serrano E., Cordon R., Lozano C., Pujols J., Napp S., Allepuz A., Abad F., Casal J., and Domingo M. 2007. **Avian Influenza Surveillance in wild birds in Catalonia, Spain (2005-2006).** Annual Report for the Competent Authorities February 2007.

Alba, A., Majó, N., Busquets, N., Núñez, J.I., and Serrano, E. 2006. **Vigilancia de influenza aviar en aves salvajes en Cataluña (Período de marzo del 2005 a marzo del 2006).** Web de AECA (Asociación Española de Ciencia Avícola-WAPSA.). Sección Sanidad. <http://www.wpsa-aeca.com/>.

Alba, A., Majó, N., Busquets, N., Serrano, E., Pujols, J. and Domingo, M. 2006. **Vigilància enfront de la influència aviària en aus salvatges.** Memòria de l'any 2005.

Busquets, N., Abad, F.X., Ramis, A., Rivas, R., Serrano, E. and Majó, N. 2008. **Persistencia del virus influenza aviar altamente patógeno H7N1 en aves muertas.** In: *Selecciones avícolas*, August.

Busquets, N., Alba, A., Serrano E., and Majó, N. 2008. **Detección de virus de influenza aviar en aves silvestres en Cataluña durante el 2007.** In: *Selecciones avícolas*.

Congress Contributions

Abad, F.X. *Environmental persistence of highly pathogenic H7N1 avian influenza virus.* In: **6th Management Committee of COST B28 Array Technologies for BSL3 and BSL4 pathogens.** Bucharest. Oral contribution. September 2008.

Alba, A., Busquets, N., Abad, F.X., Serrano, E., Allepuz, A., Napp S and Majó, N. *Seguimiento de la vigilancia de influenza aviar en aves silvestres en Cataluña (España), 2007.* In: **WPSA, Expoaviga.** Poster, March 2008.

Abad, F.X., Busquets, N., and Majó, N. *Persistence of highly pathogenic H7N1 avian influenza virus in different environments.* In: **Current Developments in food and environmental virology Symposium, Pisa.** Poster, October 2008.

Abad, F.X., Busquets, N. and Majó, N. *Influenza virus persistence in several environmental conditions.* In: **The Bangkok International Conference on Avian Influenza 2008.** Poster, January 2008.

Abad, F.X., Busquets, N., Rivas, R. and Majó, N. *Persistencia de la infectividad del virus influenza aviar altamente patógeno H7N1 en superficies.* In: **WPSA, Expoaviga.** Poster, March 2008.

Alba, A., Majó, N., Busquets, N., Casal, *Réseau de surveillance de la grippe aviaire chez les oiseaux sauvages en Catalogne, Espagne.* J. In : **AEEMA.** Poster, June 2007.

Alba, A., Casal, J., Majó, N., Busquets, N., Serrano, E., Pujols, J., Domingo, M. *Vigilancia de Influenza aviar en aves salvajes en Cataluña (marzo 2005-marzo 2006).* In: **V Jornadas de Epidemiología y Medicina Preventiva Veterinaria.** Poster, April 2006.

Busquets, N., Alba, A., Napp, S., Serrano, E., Rivas, R., Núñez, J.I. and Majó, N. *Detection of Avian Influenza Viruses in Wild Birds in Catalonia (North-Eastern Spain) from 2006 to 2008.* In: **International Meeting on Emerging Diseases and Surveillance. IMED 2009, Viena** Poster, February 2009.

Busquets, N., Abad, F.X., Ramis, A., Rivas, R., Serrano, E., y Majó, N. *Persistencia del virus Influenza aviar altamente patógeno H7N1 en aves muertas.* In: **WPSA, Expoaviga 2008.** Oral contribution, March 2008.

Busquets, N., Serrano, E., Abad, F. X., Alba, A., Núñez, J. I. y Majó N. *Detección de virus de influenza de baja patogenicidad en aves salvajes en Cataluña.* In: **WPSA-AECA.** Poster, October 2007.

Busquets, N., Serrano, E., Abad, F. X., Alba, A., Núñez, J. I. and Majó, N. *Detection of Low Pathogenic Avian Influenza Virus in Wild Birds in Catalonia.* In: **Paris Anti-Avian Influenza 2007 Conference.** Oral contribution, June 2007.

Majó, N., Busquets, N., Abad, F.X., Campos, N., Ramis, A., Valle, R., Chaves, A. and Darji, A. *New insights on Highly Pathogenic Avian influenza pathogenesis in birds.* In: **The Bangkok International Conference on Avian Influenza 2008.** Poster, January 2008.

Ramis, A., Busquets, N., Abad, F.X., Dolz, R., Valle, R., Chaves, A., Darji, A., and Majó, N. *Pathogenesis of an Highly pathogenic avian influenza virus (H7N1) in chickens.* In: **26th Annual Meeting of the European Society of Veterinary Pathology.** Dubrovnik, Croatia. Oral contribution. September 2008.

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THE EXPERTISE OF CReSA IN AVIAN INFLUENZA

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RELEVANT FACT SHEETS

The **CReSA Avian Influenza Research Group** is a multidisciplinary group made up of researchers, graduate students and technicians. This information sheet reflects the expertise of **CReSA** in Avian Influenza since this group was created in 2005.

Tools developed:

- ➔ Influenza virus propagation and titration in embryonated chicken eggs and MDCK cells.
- ➔ Influenza virus detection by real time RT-PCR from different sources (swabs, alantoic fluid and tissues).
- ➔ Viral sequencing for selected genes.
- ➔ Immunohistochemistry for detection of viral antigen in tissues.
- ➔ Serological techniques (ELISA, IHA) to detect humoral immune responses against AIV.
- ➔ Infection model for highly pathogenic avian influenza in chicken.
- ➔ Model of spread of avian influenza in wild birds.
- ➔ Scenario Tree Model to evaluate the sensitivity of the different surveillance components of the actual system used in domestic poultry.

Knowledge generated:

- ➔ Dynamics of highly pathogenic AIV infection in chickens:
 - Clinical manifestations
 - Viral distribution and associated lesions
 - Viral shedding
 - Immune response
- ➔ Persistence of AIV in the environment (*in vitro* studies):
 - Waters
 - Fomites
 - Chicken carcasses
- ➔ Descriptive spatio-temporal study of the surveillance data (2005-2008) in Catalonia.
- ➔ Risk analysis of the introduction of AI into Spain.

PROJECTS

EUROFLU. Molecular Factors and Mechanisms of Transmission and Pathogenicity of Highly Pathogenic Avian Influenza Virus

Funding organization: 6th Framework Programme (FP6)
Code: SPB5-CT-2007-044098
2007-2010
Contact: Dr Ayub Darji

The ultimate objective is to fill the gaps in knowledge concerning the molecular factors and mechanisms of HPAIV transmission and pathogenesis. This objective will be reached through the integration of interdisciplinary experimental research approaches and bioinformatics analyses.

Tests of environmental persistence of the virus and study of immunopathogeny in birds raised in non-intensive regimes

Funding organization: National Institute for Agricultural and Food Research and Technology (INIA)
Code: FAU2006-00007-C02-02
2007-2010
Contact: Dr Natalia Majó

The main objectives are studying the persistence of the AI virus in different environments and the main factors influencing its persistence, assessing the involvement of other wild birds and mammals or animals bred in semi-extensive regimes in the epidemiology of AI viruses and determining the dynamics of viral infection and the efficacy of the vaccines currently registered in wild ducks and birds reared in semi-extensive regimes.

Role of the innate immunity of the host on protection and pathogenesis of the avian influenza virus infection

Funding organization: Ministry of Education and Science (MEC)
Code: AGL2007-60434
2007-2010
Contact: Dr Ayub Darji

The main objective is to characterize the virulence of an HPAIV strain and to determine the host factors in particular factors involved in innate immune mechanism, influenced by AIV infection during the very early phase of infection. The ultimate goal of the project is to better understand disease pathogenesis and host-pathogen interactions of AIV infection and to define the molecular basis for host specificity in chickens and mice.

COST Action B28 on Array Technologies for BSL3 and BSL4 pathogens

Funding organization: EU
Code COST B28
2007-2010
Contact: Dr Francesc Xavier Abad and Dr Ayub Darji

The main objective is to increase knowledge on BSL3 and BSL4 agents in order to support the development of more accurate diagnostics, vaccines and therapeutics, and to better understand the epidemiology of these highly pathogenic microorganisms that can be potentially used as biological weapons.

SURVEILLANCE PROGRAMS

Avian influenza surveillance programme in wild birds in Catalonia

Funding organization: Department of Agriculture, Food and Rural Action (DAR) and Department of the Environment and Housing (DMAiH)
Contact: Dr Natàlia Majó

In 2005, as part of the European initiative, an Avian Influenza (AI) surveillance system in wild birds was established in Spain. The purposes of this programme were to detect the circulation of influenza A viruses in wild birds, to improve the knowledge of their pathogenesis in wildlife, and to provide an early warning system to prevent the transmission of these viruses from wild birds to domestic poultry. CReSA participates, along with the DAR and DMAiH, in co-ordinating and applying the vigilance programme for avian influenza in wild birds in Catalonia.



TECHNOLOGICAL OFFER

CReSA centralizes its activity in the facilities of the **CReSA** building, which incorporates a wide technological offer with capability for virological, immunological, molecular biology and microbiological studies, and constitutes a reference centre for research and giving support to administrations, as well as to other institutions and companies. This technological offer consists of infrastructures, equipment and highly specialized services inside a building that includes conventional laboratories, biocontainment laboratories of level-3, and level-3 biocontainment facilities for animals (BSL3).

Studies of *in vitro* and *in vivo* infection in level-3 biosafety facilities (BSL-3)

Development of new virus infection models

Immunological studies

- Measurement of innate and acquired humoral or antibody responses
 - ELISA
 - Assess for virus neutralization and hemagglutination inhibition
 - Lysis by complement (in progress)
- Measurement of the cellular response
 - ELISPOT for measuring cytokines (in progress)
 - ELISAs for measuring cytokines
 - Lymphoproliferation
 - Studies of flow cytometry
 - Study of cell surface markers
 - Study of intracellular cytokines
 - Obtaining and purification of cell subpopulations of the immunological system (macrophages, dendritic cells, T cells, etc.) by flow cytometry ("sorting")

Generation of biological reagents

- Monoclonal antibodies
- Polyclonal antibodies

Development and setting up of diagnosis techniques

- Serological techniques
 - ELISA
 - Western blot (in progress)
 - Microscopy (optical, fluorescence)
- Molecular techniques
 - RNA extraction and RT-PCR development
 - Real time PCR
 - *In situ* hybridization

Study and development of new vaccine strategies

- Development of vaccines (in progress)
- Assessment of possible adjuvants and vaccine immunomodulators using *in vitro* and *in vivo* models (in progress)

Viral studies

- Isolation, identification, culture and titration of animal virus
- Inactivation studies in waters, foods, cosmetics and blood-derivatives
- Molecular studies
 - Sequencing of virus and phylogenetical comparisons

Epidemiological studies

- Observational epidemiological studies and surveys
 - transversal studies
 - case-control studies
 - prospective studies (cohorts)
- Modeling
- Risk analysis
- Space-time analysis

PEOPLE

Experimental infections and pathology

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