



VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

Deliverable D18.1

Quantity of access provided over the duration of the project to IRTA Animal facility

Due date of deliverable: M72

Actual submission date: M72

Start date of the project: March 1st, 2017 Duration: 72 months

Organisation name of lead contractor: Your organisation

Revision: V1

Dissemination level	
Public	X
Confidential, only for members of the consortium (including Commission Services)	
Classified, as referred to in Commission Decision 2001/844/EC	

Table of contents

1. TNA Provided	3
2. Final reports of each TNA provided.....	4
2.1 TNA 1	4
2.2 TNA 2	4
2.3 TNA 3	5
2.4 TNA 4	6

1. TNA Provided

Name of the TNA project	Name of TNA user	Organisation of TNA user	Country of TNA user	Installation from the RI	Start date	End date	Number of units of access provided
Experimental infection of ewes and pigs with a novel pestivirus closely related to classical swine fever virus	Ana Maria Moreno	Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna - IZSLER	Italy	Animal facility	19/07/2019	01/03/2020	1.8
Characterization of Cuban CSFV strains as possible escape variants to vaccination	Carmen Perera	Centro Nacional de Sanidad Agropecuaria - CENSA	Cuba	Animal facility	07/05/2021	01/03/2022	2.4
Comparative pathobiology and transmission parameters of clade 2.3.4.4b H5N8 HPAIVs from the European 2017 and 2020 epizootics in mule ducks	Jean-Luc Guerin	Ecole Nationale Vétérinaire de Toulouse - ENVT	France	Animal facility	05/07/2021	23/07/2021	0.75
Comparison of follicular B-cell maturation during PRRSV and Influenza virus (IAV) infections in swine	Nicolas Bertho	Ecole Nationale Vétérinaire Agroalimentaire et de l'alimentation - ONIRIS	France	Animal facility	14/09/2021	24/11/2021	0.375

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

2. Final reports of each TNA provided

2.1 TNA 1

Experimental infection of ewes and pigs with a novel pestivirus closely related to classical swine fever virus

The aim of the project was to study in two different animal species, the host range, clinical disease and pathogenic role of a new pestivirus detected in 2017 in Italy. First, we investigated the effects of this pestivirus in its natural hosts (sheep), with particular attention to the abortogenic role of this pestivirus and therefore to the effects in pregnant ewes and its offspring. Secondly, we studied its ability to infect pigs and to induce disease. Finally, we investigated the ability of this virus to induce an immunological response in pigs capable of protecting animals against a challenge with a highly virulent classical swine fever virus (CSFV) strain, as well as the possible serological cross-reactions between the new pestivirus and CSFV.

The abortogenic role of the Italian pestivirus was demonstrated by the abortions, stillbirths or weak lamb births recorded in all inoculated ewes and by the high viral RNA load detected in all foetal tissues. This pestivirus also demonstrated the ability to infect pigs, but with mild clinical signs. Interestingly, pigs experimentally infected with the new pestivirus developed immunological responses capable of protecting them from infection with a highly virulent strain of bluetongue virus (challenge). These animals showed only mild CSF symptoms compared to the infected control group, and a low viral RNA load was detected in one pig compared to the high viral titres observed in all control pigs.

Finally, a close serological and genomic relationship between the new ovine pestivirus and CSFV was evidenced using serological and RNA detection methods. Further studies are needed to investigate the prevalence of this new pestivirus in sheep and pigs.

Ana Maria Moreno and Enrica Sozzi from IZSLER stayed 3 days at CReSA in December 2019 to discuss and analyse the results obtained from this TNA. Biological material was sent to IZSLER.

From the results provided by this TNA, 2 publications were produced: doi: 10.3390/v12070775 and doi: 10.1111/tbed.14119.

NB: Due to the complexity and length of this TNA and the need for animal and laboratory BSL3 facilities, this work required 1 and 1.5 access units for Animal and BSL3 laboratories facilities and BSL3/2 laboratories, respectively (see D19.1 and D20.1).

2.2 TNA 2

Characterization of Cuban CSFV strains as possible escape variants to vaccination

Classical swine fever (CSF) is a highly contagious viral disease affecting domestic and wild swine worldwide. In Cuba, CSF is an endemic disease despite extensive vaccination efforts

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

with the C strain vaccine for more than 20 years. Previous molecular studies suggest the emergence of new strains that have evolved under the positive selection pressure of the ineffective immune response induced by the current vaccine. This viral evolution has led to the circulation of strains of low and moderate virulence with changes in the genome that could be related to the viral evasion of the immune response caused by the vaccine.

The objective of this project was to evaluate the capacity of two classical swine fever virus (CSFV) strains, CSF1058/2010 and CSF1057/2011, recovered from the CSF endemic in Cuba, to escape the immune response generated by vaccination with the C-strain vaccine.

For this purpose, 10 pigs per strain were challenged, five of them having been previously vaccinated with the C-strain. None of the animals included in the experiment showed CSF clinical symptoms. Pigs challenged with the CSFV strain CSF1057/2011 showed a delay in the antibody-enhancing effect expected after virus challenge (boosting effect).

In general, CSFV RNA was barely detected in serum samples and nasal and rectal swabs of the experimental animals, confirming the presence of low and moderate virulence strains. The sequences of the virus recovered in the experimental groups are being processed, and we are currently analysing the results.

Liani Coronado from CENSA spend a complete month in November 2021 in the BSL3 laboratories of CReSA to analyse the biological material from experimentally infected pigs. A publication is in preparation.

NB: At the beginning of the experiment (May 7, 2021), all purchased pigs tested positive for PRRS one week after their arrival at CReSA. They had to be sacrificed one week later. The experiment was rescheduled to July 19 with new pigs. This added 0.5 units of access to the 1.9 AU originally planned. This TNA required 1 AU for analyses in the BSL3/2 laboratories (see D20.1).

2.3 TNA 3

Comparative pathobiology and transmission parameters of clade 2.3.4.4b H5N8 HPAIVs from the European 2017 and 2020 epizootics in mule ducks

Avian influenza is a highly contagious infectious disease caused by influenza A virus belonging to the *Orthomyxoviridae* family. Wild birds are considered the natural reservoir that contributes to the spread of the virus, occasionally generating panzootics that result in massive death of wild and domestic birds and significant economic losses. In recent years, outbreaks of highly pathogenic avian influenza (HPAI) have occurred with increasing frequency in Europe. To date, the 2016-2017 epizootic is considered the largest epizootic caused by a HPAI virus (HPAIV) in the continent in terms of number of birds and countries affected by H5 clade 2.3.4.4b HPAIV. However, the winter 2020-2021 epizootic had also caused hundreds of outbreaks in wild birds and poultry flocks in Europe. Field observations and literature for the past epizootics suggest potential evolution in the pathogenicity of H5 HPAIVs and the key role of mule duck farming.

In this context, this project aimed to compare the transmission parameters and disease picture of clades 2.3.4.4b H5N8 HPAIV from the 2016-17 and 2020-21 epizootics. To do so,

5-week-old mule ducks were experimentally inoculated with H5N8 HPAIV (2017) or H5N8 HPAIV (2020) and followed for 14 days post-challenge to compare clinical signs, mortality, lesions, viral titration and host immune response.

Mortality and clinical profiles:

- Before challenge, all acclimated birds were confirmed AIV-serologically negative and were clinically healthy.
- Clinical signs started at 3 days post challenge (dpc) and 4 dpc for H5 (2017)- and H5 (2020)-inoculated birds, respectively. Clinical signs included non-specific depression to prostration and inability to stand upright, neurological signs (ataxia, head tremor, head tilt), and unresponsiveness to visual stimuli.

For inoculated birds, mortality started at 5 and 6 dpc for H5 (2017)- and H5 (2020)-inoculated groups, respectively, and lasted for 4 and 2 days, respectively. Overall, mortality rates of inoculated birds were 30% (with H5/2017) and 20% (with H5 /2020), with MDTs of 7 days (with H5/2017) and 6 days (with H5/2020). Percentage of survival was not statistically different between groups ($p>0.05$).

For contact birds, mortality started at 7 and 8 dpc for H5 (2017)- and H5 (2020)-contact groups, respectively, and lasted for 5 days. Percentage of survival was significantly higher for H5 (2017) group ($p<0.05$). Overall, mortality rates of contact birds were 100% (with H5 (2017)) and 33% (with H5 (2020)), with MDTs of 8.2 days (with H5 (2017)) and 10 days (with H5 (2020)).

All inoculated and contact birds seroconverted following challenge.

Biological material was sent to ENVT for further characterization and for the study of the immune response of the different batches of animals.

Two publications are in preparation.

2.4 TNA 4

Comparison of follicular B-cell maturation during PRRSV and Influenza virus (IAV) infections in swine

Porcine reproductive and respiratory syndrome virus (PRRSV) infections are responsible of serious respiratory problems and their persistence in herds has resulted in an estimated cost of 1,503 million € per year in Europe. While porcine influenza infections last 1 week, PRRSV infections can persist for several weeks/months, hence the economic impact.

It is hypothesized that this persistence is due to a delay in the appearance of neutralizing antibodies, which should appear one week after infection, as is observed in influenza challenges. Neutralizing antibodies are produced through a maturation process of antibody-producing cells, B lymphocytes, within the lymph nodes (LN), but so far, no information has been published on how PRRSV interferes with this process. In order to clarify this point, we infected animals with influenza virus or PRRSV and compared the maturation process of B cells for 6 days post-infection. This required intensive use of the cytometer/cell sorter in CReSA BSL3 facilities.

Nicolas Bertho stayed at CReSA for the entire duration of the experiment, with the exception of one week corresponding to extension of acclimatisation of pigs (see below). Caroline Hervet

from INRAE-ONIRIS stayed one week at BSL3 laboratories of CReSA to help sorting of the immune cells and conditioning them for further analysis.

The collected samples were sent to the project participants:

- Gaëlle SIMON and Olivier BOURRY (ANSES, Ploufragan) received the sera for neutralizing antibodies assays.
- Daniel DORY (ANSES, Ploufragan) received PBMC in RLT Buffer to analyse antibody diversity by CDR3 sequencing.
- Nicolas BERTHO received the frozen PBMC, the rest of the frozen lymph node cells as well as the whole lymph nodes in Tissuetek/OCT for further analysis of differentiation of blood and LN B cells upon PRRSV and swine influenza virus infection.

Comprehensive laboratory analyses were conducted in 2022 and extended into 2023, with additional experiments needed to complete the initial results.

NB: Pigs were purchased in Andalusia from a farm that did not practice vaccination against PRRS or influenza virus. Upon arrival at the CReSA animal facilities, they presented mild respiratory symptoms. Viral and severe bacterial infections were ruled out after laboratory diagnostic. After an additional week of acclimatisation, pigs recovered a healthy status and infections could be performed according to the initial protocol. This additional week of acclimatisation involved the use of 0.375 AU for the Animal facility (reported here) in addition to the 1 AU initially required for Animal and BSL3 laboratories facilities (see deliverable D19.1). Additional 0.5 AU for BSL3/2 laboratories were also required to perform cytometry and cell sorting experiments on the various samples generated in this TNA project.