VETBIONET

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

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Classified, as referred to in Commission Decision 2001/844/EC	

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

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
PPRV sheep vaccination with bovine Herpesvirus-4-Based vector delivering PPRV-H protein	Gaetano Donofrio	Università di Parma	IT	INIA-CISA BSL3	15/01/2020	15/03/2020	2
Role of scavenger endothelial cells in elimination of viruses	Karen K. Sørensen	The Arctic University of Norway (UiT)	NO	INIA-CISA BSL3	02/01/2023	28/02/2023	2

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2. Final reports of each TNA provided

2.1 TNA 1- PPRV sheep vaccination with bovine Herpesvirus-4-Based vector delivering PPRV-H protein.

The Morbillivirus peste des petits ruminants' virus (PPRV) is the causal agent of a highly contagious disease that mostly affects sheep and goats and produces considerable losses in developing countries. Current PPRV control strategies rely on live-attenuated vaccines, which are not ideal, as they cannot differentiate infected from vaccinated animals (DIVA). Recombinant vector-based vaccines expressing viral subunits can provide an alternative to conventional vaccines, as they can be easily paired with DIVA diagnostic tools. In the performed work, we used the bovine herpesvirus-4-based vector (BoHV-4-A) to deliver PPRV hemagglutinin H antigen (BoHV-4-A-PPRV-H-DTK). Vaccination with BoHV-4-A-PPRV-H-DTK protected sheep from virulent PPRV challenge and prevented virus shedding. Protection correlated with anti-PPRV IgGs, neutralizing antibodies and IFN-g producing cells induced by the vaccine. Detection of antibodies exclusively against H-PPRV in animal sera and not against other PPRV viral proteins such as F or N could serve as a DIVA diagnostic test when using BoHV-4-A-PPRV-H-DTK as vaccine. Our data indicate that BoHV-4-A-PPRV-H-DTK could be a promising new approach for PPRV eradication programs.

TNA 1- General information

1. User-Project Title: **PPRV sheep vaccination with bovine Herpesvirus-4-Based vector delivering PPRV-H protein**
2. User Name: **Dr. Gaetano Donofrío**
3. User Institute: **University of Parma, Italy**
4. Service Provider Institute: **Dra. Verónica Martín (CISA-INIA)**
5. Starting date of the Access: **15/01/2020**
6. Finishing date of the Access: **15/03/2020**
7. Biosafety level (BSL) / Containment of the Access: **BSL3**
8. Details of users involved in the research project, including users who did not stay at the Service provider:

	User1	User2	User3	User4
Family_Name	Macchi	Franceschi	Russo	Donofrío
First_Name	Francesca	Valentina	Luca	Gaetano

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Gender (M/F)	F	M	M	M
Birth_year				
Nationality	ITALY	ITALY	ITALY	ITALY
Researcher_status (1)				Professor
Scientific field 1 (main)	Virus	Veterinary Epidemiology	Infectious diseases	Animal Infectious Disease
Scientific field 2 (optional)	Animal models	Virus	Viral Vectors	Viral vectors
Scientific field 3 (optional)				Vaccines
Home_Institution_Type (2)	UNI	UNI	UNI	UNI
Home_Institution_Name	Univ. of Parma	Univ. of Parma	Univ. of Parma	Univ. of Parma
Home_Institution_Town	PARMA	PARMA	PARMA	PARMA
Home_Institution_Country	ITALY	ITALY	ITALY	ITALY
Email (3)				gaetano.donofrio@unipr.it
New_User (Y/N)	y	y	y	y
Group_leader (Y/N)	N	N	N	Y
Remote_user (Y/N) (4)	Y	Y	Y	Y
Number_of_visits (5)	0	0	0	0
Duration_of_stay (6)	0	0	0	0
Travel_and_Subsistence reimbursed (Y/N) (7)	N	N	N	N
Additional_Information				

Introduction & Aim of project:

Peste des Petits Ruminants Virus (PPRV) is an extremely infective morbillivirus that primarily affects domestic and wild ruminants. PPRV causes considerable economic losses, predominantly in developing countries where livestock are the main economical resource, being the causal agent of Peste des Petits Ruminants (PPR), notifiable to the World Organization for the Animal Health (OIE). PPRV, like other Morbilliviruses, induces an immune-suppression during the acute phase of the disease, which favors the establishment and aggravates the progression of secondary infections.

Protective current PPRV vaccines are extensively used in countries where PPRV is endemic. They are based on attenuation of various live PPRV strains. Single immunization with live PPRV vaccines has been able to maintain protective levels of serum antibody for up to three

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years, undistinguishable from the antibody pattern generated after a natural infection. These live vaccines are however thermosensitive and require an efficacious cold chain to remain active, which can be problematic in PPRV endemic countries usually situated in warm climates areas. Effective vaccines that distinguish between vaccinated and naturally infected animals would also be required for PPRV control and eradication programs in PPRV free countries, that are at risk, or endemic countries after finishing vaccination campaigns.

An effective vaccine candidate viral vector should present the expressed antigen as an immune target and should remain in the host long enough to stimulate an effective response, acting both as adjuvant and delivery system. The molecular and biological characteristics of Bovine herpes virus 4 (BoHV-4) make it an attractive candidate vaccine vector.

Therefore, we have proposed to evaluate the effectiveness of a DIVA vaccine in the natural host, the sheep, in the CISA facilities, a specialised centre prepared for these experiments, under the VetBionet Project. We analysed the immunogenicity and protection of a recombinant BoHV-4 vector expressing the protective PPRV-H antigen (BoHV-4-A-PPRV-H-ΔTK) directly in the natural target species (sheep).

Materials & Methods of experiments performed:

For the purpose afore mentioned, 11 nine month old-female sheep from the ‘‘Colmenareña’’ breed from a certified provider, randomly divided them into 3 groups, with 5 sheep per group, and housed in separate rooms with controlled temperature and light/dark cycles were used. Food and water were provided ad libitum. An acclimatization period of two weeks was observed, during which animals were daily monitored for general health status prior to the beginning of the experiment.

Animals were inoculated intramuscularly (im) with PBS (group 1; n= 5); with 106 tissue culture infectious dose 50 (TCID50) of BoHV-4-A-PPRV-H-ΔTK (group 2; n= 4); or with 106 TCID50 of BoHV-4-A-ΔTK (group 3; n= 5). One booster inoculation was performed with the same amount of recombinant virus vaccine after 21 days.

Challenge was performed at day 42 post-immunization by intravenous inoculation of 106 plaque forming units (pfu) of virulent ICV’89 PPRV strain. Animals will be bled at days 0 (naïve), 7, 21, 28, 42 (prechallenge) and 3, 5, 7, 11, 13 (post-challenge), and sacrificed at 13 days post-challenge (pc).

Euthanasia was performed by intravenous administration of T61 (4–6 ml/50 kg bw) following intramuscular intramuscular xylazine (0.3 mg/kg bw) inoculation to minimize suffering of sheep.

Ocular, nasal and oral swabs were collected at different days pre and post-challenge for detection of PPRV.

Animals were daily examined for clinical signs of infection and their rectal temperatures were recorded to ensure that any animal found to be suffering could be given appropriate veterinary care in accordance with standard veterinary practice.

Scores from 0 to 4 for each animal were calculated based on the severity of ocular, oral and nasal congestion and discharge as well as signs of apathy, anorexia, diarrhoea and loss of appetite, using a slightly modified version of the scoring method used in (Rojas et al., 2014). Briefly, scores were assigned for each of the following categories: general clinical signs,

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pyrexic response, ocular/nasal discharge, and gastrointestinal and respiratory signs. The final score obtained for each animal and day was the sum of these, ranging from 0 for a healthy animal to a possible maximum of 20.

We performed different experiments analyzing IFN-γ response, anti-PPRV antibody production, neutralising antibody titer or viral RNA detection. Thus, the samples obtained allowed us to analyse both PPRV-specific B- and T-cell responses, PPRV viral RNA levels, and to evaluate the protection conferred with the potential vaccine, BoHV-4-A-PPRV-H-ΔTK.

Ethical Clearance & Justification of experiments performed

All animal experiments were carried out in a disease-secure isolation facility (BSL·3) at the Centro de Investigación en Sanidad Animal (CISA), in strict accordance with the recommendations in the guidelines of the Code for Methods and Welfare Considerations in Behavioural Research with Animals (Directive 86/609EC; RD 1201/2005), and all efforts were made to minimeze suffering. Experiments were approved by the Committee on the Ethics of Animal Experiments were approved of the Spanish Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and the National Animal Welfare Committee (PROEX 032/19).

Results:

Vaccination with BoHV-4-A-PPRV-H-ΔTK protected sheep from virulent PPRV challenge, overcoming immune suppression and prevented virus shedding. Protection correlated with anti-PPRV IgGs, neutralizing antibodies that cross-reacted with lineages I and IV; and IFN-γ producing cells induced by the vaccine. Moreover, the rapid expansion of CD8+ and CD4+ T cells after PPRV challenge (detected from day 2 post-challenge) suggests that BoHV-4-A-PPRV-H-ΔTK immunization led to differentiation of memory anti-PPRV T cells. An expansion of γδ T cells, important portion of circulating T cells that can be implicated in antiviral immunity, in vaccinated animals at day 4 post-challenge was also detected. Detection of antibodies exclusively against H-PPRV in animal sera and not against other PPRV viral proteins such as F or N, could serve as a DIVA diagnostic test.

Conclusions:

Our data showed the potential of BoHV-4-A-PPRV-HDTK as a DIVA multistrain vaccine and confirm the versatility of recombinant BoHV-4 as a vaccination platform. This is the first time that sheep have been immunized with a BoHV-4-based vector vaccine candidate. BoHV-4-A-PPRV-H-DTK is a novel PPRV vaccine candidate that provides protection against virulent PPRV challenge, overcoming immune suppression and may be combined with other constructs such as adenoviruses if needed.

The performed work highlights the potency of BoHV-4 recombinant viral vector as an antigen delivery system that could eventually help in PPRV eradication programs.

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Reporting of financial aspects

Number of accesses used: 2

The User requested access to the BSL3 facility, available at CISA to conduct the evaluation of the immunogenicity of recombinant BoHV-4 vector expressing PPRV-H antigen in sheep and the protection conferred after the challenge with virulent PPRV'89 ICV. It has been necessary to finance the purchase and transport of the sheep, as well as feeding, cleaning, experimental material, publications fares, etc for a total amount of 10.554,28€.

Problems, delays, and deviations, their consequences and corresponding corrective actions:

The sheep experiment started on January 2020 and ended at the same time that mobility restrictions were applied in Spain due to the SARS-CoV2 pandemic. Therefore, since the laboratory and animal facilities were closed, the analyses of the samples collected during the experiment were delayed more than expected. However, interesting results were achieved and published in ***Front Immunol 2021:705539. DOI: 10.3389/fimmu.2021.705539*.**

2.2 TNA 2- "Role of scavenger endothelial cells in elimination of viruses".

The liver is a fundamental organ for the body's defense against external agents, and contains the largest number of resident macrophages (Kupffer cells) and the highest concentration of natural killer (NK) cells and T cells in the body. In addition, liver sinusoidal endothelial cells (LSECs) constitute the entire lining of the millions of liver sinusoids, and at the same time are highly active sequestering cells. LSECs daily remove large amounts of waste macromolecules and colloids from the blood thanks to their high endocytic capacity, which is considered highly specific and efficient, and their intracellular catabolism. These features suggest that LSECs have a potentially important role in virus clearance from the bloodstream, since viruses are nanometer-sized colloidal particles. The role of LSECs in the elimination of viruses in the body is the focus of an ongoing project in our research group funded by the Research Council of Norway (RCN) entitled: "Role of scavenger endothelial cells in elimination of viruses (SECVIR)". In regard of previous results achieved analyzing pathogens BSL2, in this TNA project three bloodborne viruses have been used : bluetongue virus (BTV), Rift Valley fever virus (RVFV) and West Nile virus (WNV)) to investigate how LSECs interact with this type of virus. By investigating the interaction of mouse pathogenic viruses (RVFV and WNV) with the equivalent of a mouse non-infectious virus (BTV), we will be able to see whether pathogenic viruses are cleared from circulation in the same way as particles. The experiments have been carried out in female C57BL/6J mice. The main objective of this project is to increase the knowledge that exists about the role of LSECs in the general defense of the body against viral infections. Since there is currently no alternative to the use of animals when studying the half-life of the virus in the blood, nor for the study of the distribution of the virus in organs, there is a need to perform these experiments in mice.

TNA 2- General information

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

9. User-Project Title: **Role of scavenger endothelial cells in elimination of viruses**

10. User Name: **Dr. Katerine Kristine Sorensen**

11. User Institute: **Artic University, Tromso. Norway**

12. Service Provider Institute: **Dr. Esther Blanco (CISA-INIA)**

13. Starting date of the Access: **2/01/2023 started the animal experiments, but Javier Sanchez the researcher from K.Sorensen group spent 1 week at INIA (14th to 18th November 2022) to organize the animal experiments, material, procedures, etc.**

14. Finishing date of the Access: **28/03/2023**

15. Biosafety level (BSL) / Containment of the Access: **CISA-BSL3 INIA-CSIC**

16. Details of users involved in the research project, including users who did not stay at the Service provider:

	User1	User2
Family_Name	Sorensen	Sanchez-Romano
First_Name	K.Kristine	Javier
Gender (M/F)	F	M
Birth_year		
Nationality	NORWAY	NORWAY
Researcher_status (1)	Professor. Head of the Vascular Biology.	Postdoctoral Researcher
Scientific field 1 (main)	Vascular Biology	Veterinary Science
Scientific field 2 (optional)	Histology	Virology
Scientific field 3 (optional)	Cell biology	Zoonotic disease
Home_Institution_Type (2)	UNI	UNI
Home_Institution_Name	UiT The Artic University of Norway	UiT The Artic University of Norway
Home_Institution_Town	TROMSO	TROMSO
Home_Institution_Country	NORWAY	NORWAY
Email (3)	karen.sorensen@uit.no	javier.s.romano@uit.no
New_User (Y/N)	y	Y
Group_leader (Y/N)	y	N

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Remote_user (Y/N) (4)	**y**	**N**
Number_of_visits (5)	**0**	**2**
Duration_of_stay (6)	**0**	**1,5 months**
Travel_and_Subsistence reimbursed (Y/N) (7)	**N**	**Y**
Additional_Information		

Introduction & Aim of project:

Background:

The liver is an important organ in host defense and contains the single largest number of resident macrophages (i.e., Kupffer cells) and the highest concentration of natural killer cells and natural killer T cells in the body. In addition, the liver sinusoidal endothelial cells (LSECs), which make up the entire lining of the millions of hepatic sinusoids, are highly active scavenger cells (Sørensen et al. 2012; Bhandari et al. 2021) and are involved in liver immune functions (Shetty et al. 2018). Based on work by us and others we have launched the term scavenger endothelial cells to describe this unique function of LSECs in elimination of many waste macromolecules, exogenous and endogenous toxins, and nano particles from the circulation (Seternes et al. 2002; Sørensen et al. 2012). As opposed to Kupffer cells which are professional phagocytes, LSECs are essentially non-phagocytic and mostly operates via clathrin-mediated endocytosis. The LSECs express many endocytosis receptors and pattern recognition receptors not normally expressed in other endothelial cells, including many scavenger receptors and C-type lectins (e.g., MRC1, L-SIGN, LSECtin), and the Fc□RIIb2 that mediates removal of small, soluble immune complexes (Sørensen et al. 2012, 2015, Pandey et al. 2020; Bhandari et al. 2020). The cells daily remove large amounts of waste macromolecules and colloids from the blood by rapid (within few minutes), specific and highly efficient endocytosis, and intracellular catabolism (Bhandari et al., 2021). This suggests a potentially important role of LSECs in the elimination of virus from the blood circulation, as viruses are nanosized colloidal particles. The role of LSECs in virus elimination is the focus of an ongoing project in our research group, funded by the Research Council of Norway (RCN), entitled: Role of scavenger endothelial cells in elimination of virus (see the section "Approaches, hypothesis, and choice of methods" for more information about the RCN project).

We are constantly exposed to a host of non-pathogenic and pathogenic viruses. While some viruses are quickly annihilated already at their entry site, other viruses reach the circulation, but here most of them are eliminated by the liver (Zhang et al., 1999; 2002). Most reports on hepatic clearance of blood-borne viruses have focused on the role of phagocytosis in Kupffer cells, which are frequently and mistakenly understood as the only player in the liver reticuloendothelial system (Seternes et al. 2002; Sørensen et al., 2012; Elvevold et al., 2008). Several studies have shown that a variety of viruses rapidly disappear from the circulation, e.g. polyomavirus BK, and BK and JC polyomavirus(PyV)-like particles (Funk et al., 2006; Simon-Santamaria et al., 2014), simian immunodeficiency virus (SIV) (Zhang et al., 1999; 2002), human immunodeficiency virus-like particles (Mates et al., 2017) or wild-type and recombinant human adenovirus type 5 (Alemany et al., 2000; Ganesan et al., 2011), with the liver being the main uptake organ and the LSEC being singled-out as the cell type mainly responsible for the

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rapid blood clearance (Mates et al., 2017; Ganesan et al., 2011; Simon-Santamaria et al., 2014). Experiments in our research group have shown rapid clearance of blood-borne BK and JC PyV-like particles in mouse LSECs in vivo (Simon-Santamaria et al., 2014), and that LSECs contribute to the uptake and degradation of Enterobacteria phage T4 (Øie et al., 2020; rat LSEC in vitro experiments). We are currently working with in vivo and in vitro inoculations with bacteriophage K1F (unpublished results) and human betaherpesvirus 5 (human cytomegalovirus; hCMV) and murid betaherpesvirus 1 (murine cytomegalovirus; mCMV) in mouse models (unpublished results). LSECs have a very well-developed endocytic apparatus and high content of lysosomal enzymes, and rapidly degrade endocytosed material following internalization (Bhandari et al. 2021, Sørensen et al. 2015). Our leading hypothesis is therefore that many viruses that are taken up by LSECs will be efficiently degraded after uptake, and that LSECs may act as part of the defense mechanism against blood-borne viruses. Some viruses have, however, been reported to have the ability to infect LSECs - examples are Dengue virus (Zellweger et al., 2010) and murine cytomegalovirus (Seckert et al., 2020). Recently, evidence was presented that SARS-Cov2 bind to L-SIGN (Kondo et al., 2021), which is expressed by LSECs, suggesting that LSECs may also act as a site of entry of virus infection in human liver.

Although it is common to not distinguish between cellular uptake of viruses and viral infection, this view does not make sense in the case of virus uptake in LSECs, since most of the virus types that are known or presumed to be taken up by the LSECs, are non-pathogenic and will likely be eliminated through the very effective degradative endolysosomal apparatus of the LSEC. Accordingly, it makes more sense to ask how pathogenic viruses escape elimination in LSECs, enabling productive infection of hepatocytes or other cell types.

The interaction of viruses with LSECs is little described in the literature. However, such interactions may lead to: i) Elimination, i.e., uptake and degradation. ii) Productive infection, defined as virus replication and often cell death following uptake of virus. iii) Transcytosis. iv) Shedding of viral nucleic acids or proteins in exosomes. v) Latency, meaning that virus may enter a host cell and stay dormant in the cell for various lengths of time, until certain conditions (e.g., immune suppression after transplantation) allow the virus to initiate replication and/or escape the host cell and initiate infection in other cells.

Based on this we have identified a need to determine on a broader scale the mechanism of, and to what extent the LSEC plays a role as scavengers of circulating viruses and how LSECs interact with different viruses.

Approaches, hypotheses, and choice of method:

This application is part of a larger research initiative funded by the Research Council of Norway (RCN): “Role of scavenger endothelial cells in elimination of virus” (SECVIR - RCN Project No 262538). The hypothesis underpinning the SECVIR project is that the LSECs represent an important player in the cellular arm of the anti-viral innate immune system.

By studying different types of viral particles (naked and enveloped viruses, as well as bacteriophages) the SECVIR project aims to determine the general viral scavenging activity of LSECs. The exact cellular site(s) and modes of uptake, the receptor(s) involved, and the intracellular processing of virus is investigated using both in vivo and in vitro experiments

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together with cutting edge optical imaging techniques. To this end, we have brought together interdisciplinary expertise in virology, optical nanoscopy, cell and molecular biology, and hepatology with the aim to study interaction of virus with LSECs.

In the project described in this TNA application, we aim to investigate to what extent the liver, and more specifically the LSECs, is involved in uptake of important zoonotic blood-borne viruses from the circulation and interactions between these viruses and the liver scavenger cells. We will inoculate mice i.v. with the viruses and measure the rate of virus blood clearance, and organ and cellular distribution of the injected viruses. Our studies are expected to reveal the role of LSECs in viral clearance from blood, the mechanisms of how the viruses enter these cells, as well as the intracellular fate of the viruses.

Relation to VetBioNet objectives:

Through the VetBioNet Transnational Access Activities (TNA) grant, we aim to study the organ and cellular distribution of important bloodborne and zoonotic BSL3 viruses (e.g., West Nile Virus or Rift Valley Fever Virus), which are viruses of veterinary and human interest that cannot be studied in our university due to lack of adequate facilities and virus models.

Our proposal will be in line with the main objectives of VetBioNet. Through TNA, we will have the possibility to access the infrastructures available through VetBioNet, i.e., BSL3 animal experimental facilities and laboratories, while forging a cooperative relationship between UiT The Arctic University of Norway (Tromsø, Norway) and INIA-CISA (Madrid, Spain). Through this project collaboration we will strengthen our competence and capacity to do research on zoonotic viruses. At the same time the project will lay a solid fundament for future collaborations between the two laboratories.

Materials & Methods of experiments performed:

For the purpose afore mentioned, 30 mice of strain C57BL/6J provider by Harlam (Envigo), randomly divided them into 6 groups, with 5 mice per group, and housed in BSL3 room conditioned for mice experiments with controlled temperature and light/dark cycles was used. Food and water were provided ad libitum. An acclimatization period of two weeks was observed, during which animals were daily monitored for general health status prior to the beginning of the experiment.

Two different procedures have been carried out. In the fist one the aim was to determine the time at which the virus is eliminated from blood and it is distributed in the organs. The mice were inoculated intravenous (iv) with WNV, strain NY99, RVFV and BTV serotype with 10^8 tissue culture infectious dose 50 (TCID50). Blood samples were collected at 1,3,5,7,10 and 20 minutes post-inoculation and the virus detected by RT-PCR Real-Time. The mice were slaughter at 20 minutes and tissue samples collected and fixed in for histology or keep in trizol for RNA extraction. The second experiment had as main objective to identify the virus distribution in organ tissues just when the virus is not present in the blood. The mice were inoculated as has been explained above and slaughtered at time detected for each virus as the end of viraemia period. At this moment the animals were perfunded with a tissue fixation buffer and the tissues were stored for immunohistological and microscopical analyses.

Challenge was performed at day 42 post-immunization by intravenous inoculation of 106 plaque forming units (pfu) of virulent ICV'89 PPRV strain. Animals will be bled at days 0 (naïve),

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7, 21, 28, 42 (prechallenge) and 3, 5, 7, 11, 13 (post-challenge), and sacrificed at 13 days post-challenge (pc).

Ethical Clearance & Justification of experiments performed

All animal experiments were carried out in a disease-secure isolation facility (BSL·3) at the Centro de Investigación en Sanidad Animal (CISA), in strict accordance with the recommendations in the guidelines of the Code for Methods and Welfare Considerations in Behavioural Research with Animals (Directive 86/609EC; RD 1201/2005), and all efforts were made to minimeze suffering. Experiments were approved by the Committee on the Ethics of Animal Experiments were approved of the Spanish Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) and the National Animal Welfare Committee (CEEA 2022-12) , by CSIC Ethical committee (193/2022) and finally by Goverment Agriculture organism (PROEX 280-5/22).

Results:

The samples collected are being tested at present. We have identified the time at which each of the viruses tested are eliminated from blood: WNV (20 minutes), RVFV (10 minutes) and BTV (20 minutes).

Reporting of financial aspects

Number of accesses used: 2

BSL3 facility, available at CISA to conduct mice experiments with WNV, RVFV and BTV used during all the experiment. The box used have an individual laminar flow to manage the viruses avoiding risk of infection, in particular for WNV and RVFV both zoonotic viruses. It has been necessary to finance the purchase and transport of the mice, as well as feeding, cleaning, experimental material, RT-PCR reagents, cell culture medium, general plastic, delivery of some reagents from Artic University (payment of custom fees), biosecurity material (Tyvek suits, gloves, etc). In addition, the TNA-Access have provided Javier Sanchez-Romano payment of allocation, subsistence and travel to go back to Tromso (Norway) the next 25th of February.

Problems, delays, and deviations, their consequences and corresponding corrective actions:

The obtention of ethical authorizations were delayed due to a change in the normative and procedures to be followed after the integration of INIA in the CSIC institution. This required us to delay the begin of the experiment to January of 2023.

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