# VETBIONET

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

## Deliverable D12.1

***Quantity of access provided over the duration of the project to CVI, Wageningen Bioveterinary Research (WBVR)***

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| **Public** | **X** |
| **Confidential, only for members of the consortium (including Commission Services)** | |
| **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 |
|---|---|---|---|---|---|---|---|
| Novel glycoconjugate vaccine against Actinobacillus pleuropneumoniae | Irene Schiller | Malcisbo b.v. | CH | WBVR | 15-12-2018 | 15-09-2019 | 350 |
| Development of vaccines against Hepatitis E virus based on an infectious molecular DNA clone using the pig as experimental model | Lars E. Larsen | DTU Copenhagen | DK | WBVR | 25-01-2021 | 28-02-2023 | 480 |
| Novel glycoconjugate vaccine against Actinobacillus pleuropneumoniae - Follow up study | Christine Neupert | Malcisbo b.v. | CH | WBVR | 15-11-2020 | 01-09-2021 | 250 |
| Innovative adjunct therapies as alternative strategies to antimicrobial treatment of swine respiratory tract infections | Jean-Claude Sirard | INSERM | FR | WBVR | 15-05-2020 | 24-02-2022 | 495 |

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# 2. Final reports of each TNA provided

## 2.1 TNA 1: Novel glycoconjugate vaccine against Actinobacillus pleuropneumoniae

Actinobacillus pleuropneumoniae (APP) is the major cause of porcine pleuropneumonia, a highly contagious respiratory disease in pigs responsible for major economic losses in swine industry. Transmission occurs through aerosol or close contact with infected animals or asymptomatic carriers. To date the protective effect induced by all commercialized vaccines is not satisfactory. The goal of the project was to develop a novel carbohydrate-based vaccine against APP conferring protection against all APP serotypes. We developed glycoconjugate vaccine strains against APP consisting of avirulent vector bacteria expressing (1) APP lipopolysaccharides and (2) neutralizing epitopes of APP toxoids on their surface. For the evaluation of the efficacy of APP vaccine candidates it is crucial to test them in a challenge model. The goal of a pre-study was to establish an infection model with an APP 2 strain which was either administered intranasal or by an aerosol in three increasing doses. In a vaccination efficacy study the immune responses and protective efficacy of two recombinant S. typhimurium constructs encoding the O-antigen cluster of APP2 were tested. Results showed that vaccine candidates were highly efficacious and significantly reduced lung lesions. Western blot analysis of sera revealed APP2 LPS-specific IgG responses in some, but not all pigs. These IgG responses indicate the recognition/processing of the vaccine bacteria by the immune system and the development of a systemic immune response and are interpreted to be vaccination-induced immune responses.

## 2.2 TNA 2: Development of vaccines against Hepatitis E virus based on an infectious molecular DNA clone using the pig as experimental model

Hepatitis E virus (HEV), a single-stranded RNA virus is an important cause of outbreaks and of sporadic cases of viral hepatitis in humans in many developing tropical and subtropical countries, mostly caused by HEV genotype 2. It also spreads zoonotically in industrialized countries, but here, mostly HEV genotype 3 and genotype 4 (HEV3/HEV4). The virulence of HEV 3 for humans is very low, however, in immuno-compromised individuals HEV can persist and cause chronic liver disease. Developments in molecular virology have paved the way to use gene copies as infectious materials other than viable virus particles. This technique has been successfully used for various viruses and has also been used in one study with HEV genotype 4. In a first study (study 1), the infectivity of the infectious clone was determined and in a second study (study 2), the model was applied by using HEV positive liver material as infectious material. The goal was to provide an in vivo proof of concept for the use of an infectious clone of HEV3 and derivates thereof in challenge experiments. Furthermore, the challenge experiments generated basic knowledge of the pathogenesis of HEV in pigs under controlled conditions. The study successfully provided proof of concept for the infectiousness of the molecular HEV clone, but the onset of infection was delayed compared to the positive control group, and only one out of eight pigs got infected. The pigs were killed at 21 days after inoculation, and it is possible that more pigs would have become infected if the duration of the study were extended.

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Although viral RNA load was rather low (Ct 28), using the material from this one pig resulted in infection of all intravenously infected pigs with a similar course of infection in terms of virus excretion in feces as in those pigs which were infected with naturally infected liver tissue supernatant.

Thus, the outcome of the study strongly indicated that establishment of a porcine model for HEV by the use of molecular clones is possible. This work paves the way for examining virulence differences between circulating HEV strains and strains which have led to pathology in human patients.

### 2.3 TNA 3: Novel glycoconjugate vaccine against Actinobacillus pleuropneumoniae - Follow up study

Actinobacillus pleuropneumoniae (APP) causes a highly contagious pleuropneumonia in pigs and is responsible for major economic losses in the swine industry. To date, available vaccines reduce the symptoms but do not prevent APP colonisation.

In this project we tested a bacterial vector vaccine presenting the APP serotype 8 (APP8) O-antigen (lipopolysaccharide glycan repeats subunit) on the surface of the vaccine strain. In addition, Apx toxin epitopes of APP were added. These toxins play a strong role in the pathogenicity of APP in pigs.

For the evaluation of the efficacy of APP vaccine candidates it is crucial to test them in a challenge model providing preferentially moderate clinical symptoms and/or pulmonary lesions in a statistically significant number of animals. Therefore, prior to the vaccine efficacy study, a virulence titration study was performed with two isolates, i.e. APP 6 and APP 8. Based on clinical features and pathology results an appropriate dose could be defined for the use in 8 APP challenge studies. The used APP6 strain did not induce clinical or pathological alterations.

The vaccine candidates were applied to the pigs either by oral/nasal administration or intramuscular injection. Following a challenge with either APP8 or APP6, which has a similar O-antigen structure as APP8, the development of clinical symptoms was monitored. In this homologous and heterologous challenge, a cross-protectivity of the vaccine was tested.

Results showed that injecting the APP8 vaccine presenting the Apx toxin epitopes on the cell surface resulted in a tendency of lung lesion reduction and a significant reduction of reisolated challenge bacteria in pigs. Applying the APP8 vaccine oral/nasally and injecting the toxin epitopes did not result in any reduction of lung lesions and APP re-isolation. Immune responses against the APP8 O-antigen were measurable in vaccinated animals. Antibody generation against the Apx toxins were mainly detected in animals with oral/nasal APP8 vaccine application and Apx toxin epitope injection. Unfortunately, the APP6 challenge infection, using a recent clinical field isolate was not successful. In the control group only one animal developed lung lesions which made the evaluation of the vaccine efficacy under these conditions impossible.

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## 2.4 TNA 4: Innovative adjunct therapies as alternative strategies to antimicrobial treatment of swine respiratory tract infections

The global objective of the proposal was to build the proof-of-concept that targeted immune-stimulation can provide protection against bacterial pneumonia of major relevance for animal health and to use these essential data later on for transposition to human health. To enhance the protection against infectious pneumonia, an immunostimulatory molecule, the bacterial flagellin, an agonist of Toll-like receptor 5 (TLR5) has been selected as the protective biologic product. The primary add-in value of using TLR agonists as immuno-prophylactic and immune-therapeutic drugs is the universality and the conservation of the signaling receptors and defense effectors, thus allowing intervention in any animals and diseases. This project assessed the impact of respiratory delivery of flagellin on the respiratory tract and on the control of Actinobacillus pleuropneumoniae (App) respiratory infections in pigs. To this aim, two sets of experiments were performed:

1. The evaluation of respiratory aerosol-mediated delivery of flagellin in pigs to trigger innate immune defenses in the respiratory tract and in the systemic compartment.

2. The efficacy analysis of preventive respiratory administration of flagellin against pleuropneumonia.

We found that respiratory delivery of flagellin was highly effective at stimulating immunity within the respiratory tract. We also observed that pre-exposure to nebulized flagellin 24h before App infection does not prevent infection.

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