VETBIONET

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

Deliverable D27.1

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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
In vivo study of ciprofloxacin-resistant *Campylobacter jejuni* fitness: comparison of the capacity of different clonal complexes to acquire resistance	Noel McCarthy	University of Warwick	UK	service Seleac	Sep 2021	Dec 2021	1
In vivo study of ciprofloxacin-resistant *Campylobacter jejuni* fitness: comparison of the competitiveness of FQS strains of different ST and their resistant mutants generated during TNA1	Noel McCarthy	University of Warwick	UK	service Seleac	Feb 2022	Aug 2022	1
Use of extracellular vesicles as immunogens for African swine fever virus: preparation of two batches of extracellular vesicles free of ASF virus	Maria Montoya	Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC), Madrid	ES	service SPPAE	May 2020	Jul 2020	1
Use of extracellular vesicles as immunogens for African swine fever virus: Evaluation of the immunogenicity of the ExVes and their efficacy towards a virulent ASF virus challenge	Maria Montoya	Centro de Investigaciones Biológicas Margarita Salas (CIB-CSIC), Madrid	ES	service SPPAE	Feb 2022	Apr 2022	1

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2. Final reports of each TNA provided

2.1 TNA 1

In vivo study of ciprofloxacin-resistant Campylobacter jejuni fitness (comparison of the capacity of different clonal complexes to acquire resistance)

2.2 TNA 2

In vivo study of ciprofloxacin-resistant Campylobacter jejuni fitness (comparison of the competitiveness of FQS strains of different ST and their resistant mutants generated in the first study)

Context (TNA 1 and TNA 2)

Campylobacter is the most common cause of food related bacterial gastroenteritis. The main vector for human infection is chicken. Campylobacter costs the EU economy an estimated €2.4 million per year. Fluoroquinolones are an important broad-spectrum antibiotic, used to treat a number of different bacterial infections. They work by inhibiting the activity of DNA gyrase (typically in Gram negative bacteria) and topoisomerase IV (typically in Gram positive bacteria), which are responsible for supercoiling during replication. The most common mechanism for fluoroquinolone resistance in Campylobacter is a single point mutation on the target site of DNA gyrase therefore rendering fluoroquinolones ineffective. Fluoroquinolones have historically been used as a prophylaxis to treat Salmonella in the rearing of poultry and despite a significant reduction in the use of fluoroquinolones in agriculture resistance is still rising. In Europe, in 2018, more than 70% of chicken Campylobacter jejuni strains were resistant to fluoroquinolones, which leads to a high risk of therapeutic failures when treating human infections and increased expression of virulence factors. However, a thorough analysis of the genomes of C. jejuni strains shows that some MLST profiles of C. jejuni strains are almost always found to be resistant, while others are almost always found to be susceptible.

The aim of the project was to evaluate and compare the ability of different MLST profiles to acquire resistance to fluoroquinolones, and to compare the competitiveness of susceptible strains of different sequence types and their resistant mutants in chicken.

Work performed

The aim of the first experiment (TNA 1) was to compare the capacity of different clonal complexes to acquire resistance. Five groups of specific pathogen-free (SPF) leghorn chickens (n=15) were housed in separate isolators. Four groups were inoculated with fluoroquinolone-susceptible (FQS) strains of *Campylobacter* belonging to different MLST (from clonal complexes CC-464, CC-354, CC-45 and CC-443) and one group served as an untreated control group. On day 14, birds were treated for five days with enrofloxacin. The resistant mutants of *C. jejuni* selected by the treatment were isolated by culture and characterized.

The aim of the second experiment (TNA 2) was to compare the competitiveness of FQS strains of different ST and their resistant mutants generated in the first study. Thus, for each pair of strains (the FQS strain and its resistant mutant), three batches of 15 birds, were housed in separate isolators (total of 180 birds, 12 isolators). For each ST, birds were dosed with the FQS *Campylobacter* strain, or its fluoroquinolone resistant (FQR) mutant or a 50/50 mix of

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both FQS and FQR mutant. The proportions of FQS and FQR strains were assessed by standard *Campylobacter* culture techniques on all fecal samples, to compare the establishment of strains and their mutants, when present singly or in competition in the chicken gut.

Results

During the first experiment, *in vivo* FQR mutants belonging to each studied MLST could be obtained. In the next experiment, both FQR and FQS isolates were able to persist throughout the trial at similar levels in mono-inoculated chicks. Competition assays showed that FQR variants of isolates CC354, CC573 and even CC45 appear to have a competitive advantage in mixed cultures *in vivo* over their FQS counterparts, whereas CC443 FQR becomes more dominant over time, but did not dominate its FQS counterpart up to day 34 (last sampling day).

Publications

Hanford, Taylor, Noel McCarthy, Isabelle Kempf, Katell Rivoal, Shaun Cawthraw, Muna Anjum, Manal Abu Oun et John Rodgers. 2022. "Determining the fitness of fluoroquinolone resistant *Campylobacter* using in vivo competition models in chickens." 20th Congress of the International Society for Animal Hygiene (ISAH), Berlin, Germany, 5-7 October

2.3 TNA 3:

Use of extracellular vesicles as immunogens for African Swine fever Virus (ASFV): preparation of two batches of extracellular vesicles free of ASF virus

2.4 TNA 4:

Use of extracellular vesicles as immunogens for African Swine fever Virus (ASFV): Evaluation of the immunogenicity of the ExVes and their efficacy towards a virulent ASF virus challenge

Context (TNA 3 and TNA 4)

African swine fever is a devastating hemorrhagic infectious disease, which affects domestic and wild suids of all breeds and ages, with high lethality up to 90-100% in naïve animals. The causative agent, African swine fever virus (ASFV), is a large and complex double-stranded DNA arbovirus, only member of the Asfarviridae family, genus Asfivirus (Alonso et al., 2018). There is no treatment or effective vaccine commercially available. After the re-introduction of ASFV genotype II isolates into Georgia in 2007, the disease spread from Eastern to Western Europe and then jumped up to the world's largest pig producer, China, in August 2018. Since then, ASF has spread out of control to several countries in Southeast Asia until reaching in September 2019 the very doors of the Australian continent (OIE, 2019). As a result, million pigs have been culled and more than 500 million pigs are under threat, a scenario that has generated uncertainty in markets and pork industry. The objectives of these TNA were to prepare extracellular vesicles of ASF virus and to evaluate their immunogenicity and efficacy towards a virulent ASF virus challenge

Work performed

In 2020, in a first experiment (TNA 3), a group of piglets were immunized by intramuscular inoculation with the naturally attenuated ASFV strain OURT88/3, and another was not immunized. On D31 pi, their sera were collected in large volumes, at euthanasia, to prepare

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extracellular vesicles (ExVes) that were purified and concentrated in Maria Montoya's laboratory. Therefore, two batches of ExVes were available, one prepared from the serum of negative piglets (Mock) and one prepared from the serum of ASFV inoculated piglets. These two batches of vesicles were free of ASF virus (verification made by PCR upon reception at Ploufragan,UVIP).

The aim of this second experiment (TNA 4) carried out in 2022, was to evaluate the immunogenicity of the ExVes and their efficacy towards a virulent ASF virus challenge

These ExVes were then inoculated intramuscularly to piglets as part of the 2nd procedure (22PPA01) according to a prime-boost vaccination protocol, 15 days apart. Then the piglets were challenged with the virulent ASFV strain OURT88/1, two weeks post-booster.

Twelve piglets were distributed in three groups . The group E was not immunized nor challenged (negative control). The group D was inoculated with Mock ExVes, and the group C with ASFV-ExVes, both were challenged with the ASFV OURT88/1, as described in the following table

Group (facilities)	Treatment	Nb of pigs	Prime D0	Boost D14	Challenge D 28
E (B4)	Negative control	3	/	/	/
D (B2)	Mock-ExVes)	3	Intramuscular	Intramuscular	Intramuscular
C (B3)	ASFV-ExVes	6	Intramuscular	Intramuscular	Intramuscular

Piglets were followed clinically on a daily basis, and clinical scores were recorded from the day of the 1st inoculation until the end of the experiment. Blood was collected at D0, 7, 14, 21 and 27 post immunization and at euthanasia for serological and virological analysis.

Results

No adverse reactions were seen after the inoculation of extracellular vesicles. ASFV-ExVec did not have negative impact on the growth performance of animals before Day 28 (881 g vs 793 g on average for animals of the other two groups Mock and Negative control, during the period D0-D27). However, they did not have a protective effect against the African swine fever virus challenge. Piglets form the two groups ASFV ExVes and Mock displayed a characteristic African swine fever three days after challenge, with hyperthermia, further confirmed by PCR. Moreover, the two intramuscular inoculations of ASFV-ExVes did not induce either a serological response (ELISA) nor a cellular response (INFγ ELISPOT).

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