JIP06-COVID19-COVRIN-D.2.2.3

Workpackage 2

Responsible Partners:

APHA(21), IZSAM (28), FLI (P10)

Contributing partners:

FLI (P10), WBVR (P31), APHA(21), IZSAM (28)

GENERAL INFORMATION

European Joint Programme full title	**Promoting One Health in Europe through joint actions on foodborne zoonoses, antimicrobial resistance and emerging microbiological hazards**
European Joint Programme acronym	**One Health EJP**
Funding	**This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 773830.**
Grant Agreement	**Grant agreement n° 773830**
Start Date	**01/01/2018**
Duration	**60 Months**

DOCUMENT MANAGEMENT

Title OHEJP deliverable	**D-COVRIN.2.2.3 Report on complex culture viral characterisation**
WP and task	JIP06-WP2-T2.2 In vitro and ex vivo biological characterization of SARS-CoV2 / ST2.2.3 Report on complex culture viral characterisation
Leader	T2.2 Alessio Lorusso, IZSAM (P28) and Sharon Brookes, APHA (P21)/ ST2.2.3 Stefan Finke, FLI (P10)
Other contributors	WBVR (P31)
Due month of the deliverable	57
Actual submission month	63
Type *R: Document, report* *DEC: Websites, patent filings, videos, etc.; OTHER*	R, DEC **Save date**: 23-Mar-23
Dissemination level *PU: Public (default)* *CO: confidential, only for members of the consortium (including the Commission Services).*	PU
Dissemination *Author's suggestion to inform the following possible interested parties.*	OHEJP WP 1 ☐ OHEJP WP 2 ☐ OHEJP WP 3 ☐ OHEJP WP 4 ☐ OHEJP WP 5 ☐ OHEJP WP 6 ☐ OHEJP WP 7 ☐ Project Management Team ☒ Communication Team ☒ Scientific Steering Board ☐ National Stakeholders/Program Owners Committee ☐ EFSA ☐ ECDC ☐ EEA ☐ EMA ☐ FAO ☐ WHO ☐ OIE ☐ Other international stakeholder(s): …… Social Media: ..... **Other recipient(s):** .....

JIP06-COVID19-COVRIN-D.2.2.3

JIP06-WP2-T2.2 Analyses of virus traits related to zoonotic and/or reverse zoonotic transmission
JIP06-WP2 sub-task ST2.2 In vitro and ex vivo biological characterization of SARS-CoV2
D2.2.3 Report on complex culture viral characterisation.

Description of the task

The three main objectives of COVRIN WP2 are:

1) Characterization of SARS-CoV-2 variants through the mapping of evolutionary changes of SARS-CoV-2 viruses isolated within and across human and different animal species

2) Virus surveillance to assess the risk of zoonotic and reverse zoonotic transmission

This deliverable describes the activities and first outcomes of the **sub-task ST2.2.3** (Report on complex culture viral characterisation)

Description of the deliverable

A notable feature of SARS-CoV-2 is a polybasic cleavage site (RRAR) at the junction between S1 and S2, which is flanked by a leading proline (PRRAR). This allows effective cleavage by furin and other proteases and has a role in determining viral infectivity and host range. However, it was demonstrated that a chimeric SARS-CoV-2 virus without the PRRA motif is less virulent in experimentally infected hamster and transgenic K18-hACE2 mice with respect to the parental strain. The presence of an intact furin cleavage site has been demonstrated to be critical also for transmission in ferrets. We observed a deletion in the spike protein of a SARS-CoV-2 isolate spanning the furin cleavage site (Δfcs). In this concern, we tested the replication kinetics of this virus in ex vivo organ cultures (EVOCs) from mink lungs.
Lung tissue samples were collected from three 1 year-old American minks (Neovison vison). Additionally, respiratory and rectal swabs were collected before slaughtering and were tested for SARS-CoV-2 RNA (TaqManTM 2019-nCoV Assay Kit v2, Thermo Fisher,Waltham, MA, USA), canine distemper virus (CDV) (SuperScript III Platinum One-Step Quantitative RT-PCR System, Invitrogen, Waltham, MA, USA) and Aleutian mink disease virus DNA (AMDV) (Real-time PCR detection kit for Aleutian disease virus, Genesig, Primerdesign Ltd, York House, UK) with negative results. Two SARS-CoV-2 isolates were selected for the experiments: SARS-CoV-2/INMI1- Isolate/2020/Italy, showing an in-frame 10-amino acid deletion (Δ_{680}SPRAARSVAS$_{689}$; Δfcs) in the spike protein encompassing the furin cleavage between S1 and S2 and SARS-CoV-2/IZSAM/46419. Both SARS-CoV-2 strains had been isolated and propagated on Vero E6 cells using MEM supplemented with 10% FBS. The high-throughput sequencing on both isolates showed that, with respect to the reference sequence of SARS-CoV-2 isolate Wuhan-Hu-1, the original SARS-CoV-2/INMI1-Isolate/2020/Italy shows G251V in the NS3, and SARS-CoV-2/IZSAM/46419 shows D614G in the spike protein. SARSCoV-2/INMI1 Isolate/2020/Italy is, indeed, representative of the early Chinese isolates, whereas SARS-CoV-2/IZSAM/46419 is representative of the early Italian strains characterizing the so-called “first wave” of the Italian pandemic in late winter 2020. The deletion Δ_{680}SPRAARSVAS$_{689}$ spontaneously emerged starting from passage 4 on Vero E6 as all viral passages were monitored either by partial or whole

genome sequencing before stocking. Cellular tropism of both SARS-CoV-2 isolates was investigated by immunohistochemistry (IHC).
Both SARS-CoV-2 isolates, during the replication in mink lung EVOCs, displayed increase of viral titers on cell cultures and of the number of RNA copies over time. Titer values and number of RNA copies were significantly higher at 24, 48 and 72 h post infection (hpi). G614 replicated in mink lung EVOCs at higher magnitude at 48 hpi with respect to D614 Δfcs in both titrations ($p < 0.05$). IHC demonstrated SARS-CoV-2 antigens of both isolates in the cytoplasm of bronchial ciliated epithelial cells. Immunoreactivity was not observed in mock-infected EVOCs. No differences of antigens distribution were observed between the two isolates and no mutations were evidenced in the genome of viruses sequenced out of explant supernatants with respect to viruses used for infection.
In a previous study, we demonstrated that early SARS-CoV-2 variants were able to replicate in respiratory explants of cattle and sheep and that the isolate showing G614 in the spike protein exhibited enhanced replicative capabilities compared to the earlier D614 strain, originally collected from a Chinese tourist who visited Rome, Italy, in January 2020. However, after four passages on Vero E6 cells of the D614 virus, a 10 amino acid deletion, encompassing the furin cleavage site of the spike protein, was demonstrated by whole genome sequencing. This deletion was not observed on G614. The deletion was noticed as we planned to reproduce, by infecting mink lung explants, the experimental approach used previously on ruminant tissues.
However, this finding was already evidenced between D614 and G614 in cattle lung tissues suggesting that this slight difference obtained in this round of experiments could be related to the presence of D614G rather than Δfcs. D614 Δfcs and G614 also exhibited the same immune reactivity in lung tissues of minks. Neutralization was not significantly hampered by the deletion under investigation. However, this study highlights the importance of the preliminary phenotypic characterization in ex vivo tissues and shows that mink lung tissues were permissive to the replication of a mutant form of SARS-CoV-2 showing a deletion spanning the furin cleavage site at the S1/S2 junction. Furthermore, we also showed that this deletion did not alter the neutralization capabilities of human SARS-CoV-2 convalescent serum samples. This study has been published on *Pathogens* (Valleriani *et al*., 2022).

At FLI, established air-liquid-interphase (ALI)-cultures from pigs (protocol: Müller et al., 2023) and ferrets were cultivated and demonstrated to be not susceptible to SARS-CoV-2 infection, whereas some porcine cell lines (ST 2.2.1) could be efficiently infected with SARS-CoV-2, incl. the alpha, delta and omicron variants of concern. Also primary stone marten bronchial epithel cells successfully cultivated at as ALI cultures could not be infected. Intensive work was performed on hamster bronchial epithel cells and precision lung cut slices (PCLS) from the hamster lung, since it is a highly susceptible model for SARS-CoV-2. As control, PCLS from K18 mice were used. Though control infections with influenza A virus revealed focal infection of bronchial epithelia, SARS-CoV-2 infections (inc. the alpha, delta and omicron variants) revealed only limited or no infection. Overall, these experiments revealed that in contrast to mink ex vivo tissue cultures, where SARS-CoV-2 replication was demonstrated by IZSAM, organotypic cultures from many other animal species have to be considered critically. Althought further refinement of preparation/cuture conditions may increase suceptibiltiy in some cases (e.g. hamster PCLS or ALI-cultures), and some infection of hamster PCLS could be demonstrated by WBRV (Gerhards et al., 2021), it appears unlike that those cultures represent models of SARS-CoV-2 infection that really mimic the in vivo situation in the animal lung. This is demonstrated by an infection and host response dynamics study in hamsters at FLI, where SARS-CoV-2 unable to infect hamster PCLS exhibited strong infection of bronchial epithelia and alveoles in a 7 days time course (Bagato et al., 2023). The in vivo study revealed that virus elimination was associated with the influx of a macrophage wave and we speculate that presence of resident lung macrophages in the PCLS may limit infection and virus spread in the PCLS.

At WBRV protocols for multiple organoid-derived air liquid interface (ALI) culturs of human, cattle, pig, dog, sheep, ferret, rabbit, hamster, goat origin have been generated. These works are expected to provide further complex culture models for downstream analysis of SARS-CoV-2 replication in in-vivo like cell culture models and to clarify issues raise by the hamster models described above.

References

Bagato O, Balkema-Buschmann A, Todt D, Weber S, Gömer A Qu B, Miskey C, Ivics Z., Mettenleiter TC, Finke S, Brown RJP, Breithaupt G., Dmitry S. Ushakov DS. Spatiotemporal analysis of SARS-CoV-2 infection in hamster lungs reveals an expansive wave of monocyte-derived macrophages associated with vascular damage and virus clearance. biorxiv 2023. DOI: 10.1101/2023.03.22.533759.

Di Teodoro G, Valleriani F, Puglia I, Monaco F, Di Pancrazio C, Luciani M, Krasteva I, Petrini A, Marcacci M, D'Alterio N, Curini V, Iorio M, Migliorati G, Di Domenico M, Morelli D, Calistri P, Savini G, Decaro N, Holmes EC, Lorusso A. SARS-CoV-2 replicates in respiratory ex vivo organ cultures of domestic ruminant species. Vet Microbiol. 2021 Jan;252:108933. doi: 10.1016/j.vetmic.2020.108933. Epub 2020 Nov 24. PMID: 33278734; PMCID: PMC7685048.

Gerhards NM, Cornelissen JBWJ, van Keulen LJM, Harders-Westerveen J, Vloet R, Smid B, Vastenhouw S, van Oort S, Hakze-van der Honing RW, Gonzales JL, Stockhofe-Zurwieden N, de Jong R, van der Poel WHM, Vreman S, Kortekaas J, Wichgers Schreur PJ, Oreshkova N. Predictive Value of Precision-Cut Lung Slices for the Susceptibility of Three Animal Species for SARS-CoV-2 and Validation in a Refined Hamster Model Predictive Value of Precision-Cut Lung Slices for the Susceptibility of Three Animal Species for SARS-CoV-2 and Validation in a Refined Hamster Model Pathogens. 2021 Jun 30;10(7):824. doi: 10.3390/pathogens10070824.

Müller M, Fischer K, Woehnke E, Zaeck ML, Prönnecke C, Knittler RM, Karger A, Diederich S, Finke S. Analysis of Nipah virus replication and host proteome response patterns in differentiated porcine airway epithelial cells cultured at the air-liquid interface. Preprints.org 2023. DOI: 10.20944/preprints202303.0241.v1.

Valleriani F, Jurisic L, Di Pancrazio C, Irelli R, Ciarrocchi E, Martino M, Cocco A, Di Felice E, Colaianni ML, Decaro N, Bonfini B, Lorusso A, Di Teodoro G. A Deletion Encompassing the Furin Cleavage Site in the Spike Encoding Gene Does Not Alter SARS-CoV-2 Replication in Lung Tissues of Mink and Neutralization by Convalescent Human Serum Samples. Pathogens. 2022 Oct 6;11(10):1152. doi: 10.3390/pathogens11101152. PMID: 36297209; PMCID: PMC9609486.