Pigs are not susceptible to SARS-CoV-2 infection but are a model for viral immunogenicity studies

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July 16, 2020

Abstract

Conventional piglets were inoculated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) through different routes, including intranasal, endotracheal, intramuscular and intravenous ones. Although piglets were not susceptible to SARS-CoV-2 and lacked lesions or viral RNA in tissues/swabs, seroconversion was observed in pigs inoculated parenterally (intramuscularly or intravenously).

Text – 1258 words

The coronavirus disease 2019 (COVID-19) is an infectious disease that has caused a global pandemic with more than 7.5 million infected people from around 200 countries or territories, with more than 425,000 deaths to date[1]. The causative agent of COVID-19, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is assumed to be originated in bats, since the bat-borne coronavirus RaTG13 is the closest genetic relative to date[2]. Several species have been studied to determine their potential role as intermediate hosts. Moreover, animal models to recapitulate a COVID-19-like disease have focused another major research line and are required for the development of therapeutic drugs and prophylactic compounds.

Besides several modelling studies proposing potential animal species susceptible to SARS-CoV-2[3–5], multiple experimental infections have already shown a broad range of susceptible animals. Specifically, Egyptian fruit bat, ferret, golden Syrian hamster, cat, mice expressing humanized angiotensin-converting enzyme 2 (ACE2), BALB/c mice (using a mutated SARS-CoV-2 by several cell culture passages) and some non-human primate species are permissive to viral infection, developing from subclinical to mild-to-moderate respiratory disease[6–11]. From an experimental point of view, dog susceptibility to SARS-CoV-2 is limited, since inoculated animals can partly seroconvert[8]. In contrast, the intranasal inoculation of chicken, duck and pig resulted in no evidence of infection[8].

Pig is commonly used in research because of the similarities existing with humans in terms of anatomy, genetics, physiology and, also, immunology. Indeed, experiments in pigs are likely to be more predictive of therapeutic and preventive treatments in humans than experiments in rodents[12]. However, since pigs are

not susceptible to SARS-CoV-2 infection when inoculated intranasally[8], the possibility to develop a swine infection model with this virus using other potential inoculation routes deserves investigation. The main rational to test pigs is that the ACE2 receptor of this species is functional either by transfecting swine ACE2 in HeLa cells (which do not express constitutively the human ACE2)[2] or that pseudoparticles with the S protein of SARS-CoV-2 are able to infect swine kidney cells[13]. In consequence, to set up a putative COVID-19 pig model, we investigated the effect of different natural and non-natural routes of SARS-CoV-2 inoculation in domestic pigs (*Sus scrofa domesticus*).

For the purpose, four groups of five 5-to-6-week-old conventional piglets (Landrace x Large White) were selected and inoculated by means of different routes: intranasal (IN, 1.5 mL/nostril; total volume of 3 mL), endotracheal (IT, 3 mL), intramuscular (IM, 1 mL in each side of the neck muscles; total volume 2 mL) or intravenous (IV, 2 mL), with a final dose of $10^{5.8}$ tissue culture infectious dose ($TCID_{50}$) of the SARS-CoV-2 isolate (GISAID ID EPI_ISL_418268) per each animal. SARS-CoV-2 was produced and titrated in Vero E6 cells (ATCC CRL-1586). Two extra pigs were used as negative controls. Animal experiments were approved by the Institutional Animal Welfare Committee of the *Institut de Recerca i Tecnologia Agroalimentàries* (CEEA-IRTA) and by the Ethical Commission of Animal Experimentation of the Autonomous Government of Catalonia and conducted by certified staff. Experiments with SARS-CoV-2 were performed at the Biosafety Level-3 (BSL-3) facilities of the Biocontainment Unit of IRTA-CReSA (Barcelona, Spain).

On 2- and 22-days post-inoculation (dpi), two and three animals/group (IT, IM and IV), respectively, were euthanized. Since IN inoculation was already demonstrated as non-effective to cause SARS-CoV-2 infection[8], pigs inoculated by this route were euthanized on days 1 and 2 pi to assess evidence of a possible transient early infection in tissues. Negative control animals were euthanized prior to the start of the experiment. Complete necropsies were performed in all animals. Several tissues (frontal, medial and caudal turbinates; proximal, medial and distal trachea; large and small bronchus, left cranial, mediodorsal and caudal lung areas; kidney; liver; heart; and spleen) were taken, fixed by immersion in 10% neutral-buffered formalin, embedded in paraffin and sectioned at 3 µm to prepare slides. Histology slides were stained with hematoxylin and eosin (HE) to assess potential microscopic lesions. Besides, the same tissues plus ileum, cervical lymph node (LN), mediastinal LN, mesenteric LN, olfactory bulb, tonsil, thymus, parotid salivary gland, adrenal, pancreas, brainstem, eyelids, and bone marrow were also taken in Dulbecco's modified Eagle medium (DMEM) in tubes with beads to perform SARS-CoV-2 UpE gene detection by RT-qPCR[14]. Nasal and rectal swabs were also taken (daily during the first week and at 14 and 22 dpi) to analyze them for the presence of viral RNA by means of the abovementioned RT-qPCR. Serum samples collected on days 0, 14 and 22 pi were tested for the presence of antibodies against SARS-CoV-2 spike S1+S2 and nucleocapsid (N) proteins by in-house ELISAs (Rodríguez de la Concepción et al., 2020). Also, a virus neutralization assay was performed following a previous protocol with a minor modification[15], the serial dilutions of sera and SARS-CoV-2 were incubated for 1 h at 37°C prior the plate assay performance.

All animals were daily monitored but none of them showed clinical signs after SARS-CoV-2 inoculation. Also, no gross or microscopic lesions attributable to SARS-CoV-2 infection were found in any of the studied animals from all inoculation groups as well as control ones.

None of the pigs had nasal or rectal shedding of viral RNA. Proximal trachea from one IN-inoculated animal was positive at 1 dpi for viral RNA. The remaining tissues from this animal and the rest of pigs resulted negative for RT-qPCR.

By 14 and 22 dpi, low levels of antibodies directed against the Spike protein could be detected in all animals from IM and IV groups (Figure 1A). Furthermore, these pigs also showed neutralizing antibody titers at 22 dpi (ranging from 40-160 reciprocal dilutions) (Figure 1B). Also, low antibody levels targeting the N protein were found in one out of three IM and all IV inoculated animals by the end of the experiment (data not shown). Importantly, one single animal from the IT group did not show antibodies against the S but had antibodies against the nucleocapsid protein as well as neutralizing titers (1:20-1:40) at day 0 pi, which might suggest a potential cross-reaction with another coronavirus infecting swine. Of note, these antibodies waned by the time the experiment finished, suggesting they were of maternal origin or their expression was down

regulated during experimental procedure. In addition, this animal did not show seroneutralizing antibodies at the 22 dpi.

Present data indicate that SARS-CoV-2 was not able to infect pigs by any of the tested routes, namely IN, IT, IM and IV. Therefore, our efforts confirm earlier experiments indicating lack of susceptibility of infection by the pig[8] Schlottau et al., 2020; Juergen Richt, Kansas State University, USA, personal communication), although it can be used for assessing the immunogenicity of the upcoming vaccine candidates.

Importantly, the current study goes beyond other studies with SARS-CoV-2 and pigs since we tested a broader number of inoculation routes. However, none of them resulted in a productive infection in piglets. A significant outcome of this study was the evidence of seroconversion against the Spike glycoprotein at days 14 and 22 pi and presence of neutralizing antibodies at day 22 pi in pigs inoculated by parenteral routes (IM and IV). Considering the short duration of the experiment (22 days), such seroconversion emphasizes the potential interest of the pig to be used in immunogenicity studies for SARS-CoV-2. In fact, the interest of swine as a suitable animal model for immunology, as well as physiology, pharmacology and surgery, applicable to human medicine is widely acknowledged[16].

In conclusion, the present study confirms that piglets are not a suitable animal model for COVID-19, but its potential usefulness as a model for immunogenicity in preclinical vaccine development studies deserves further investigation.

Acknowledgments

The authors thank the IRTA-CReSA staff for the care and handling of the animals. Also special thanks to Nuria Navarro, Mónica Pérez, Rosa Valle and Marta Muñoz for their technical assistance. The CBIG Consortium (constituted by IRTA-CReSA, BSC, & IrsiCaixa) is supported by Grifols pharmaceutical.

Conflict of interest

The authors declare that there is no conflict of interest.

Data availability

The data that support the findings of this study are openly available in GISAID (ID EPI_ISL_418268). Other data that support the findings of this study are mentioned in the manuscrit and/or available from the corresponding author upon reasonable request.

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Figure 1A. Antibody detection of pigs experimentally inoculated with SARS-CoV-2.

Detection of antibodies against the spike protein (days 0, 14 and 22 pi) by ELISA in sera from animals inoculated endotracheally (No. 8-10), intramuscularly (No. 13-15) and intravenously (No. 18-20).

Figure 1B. Antibody detection of pigs experimentally inoculated with SARS-CoV-2.

Detection of neutralizing antibodies (days 0 and 22 pi) in sera from animals inoculated endotracheally (No. 8-10), intramuscularly (No. 13-15) and intravenously (No. 18-20). The graph shows the logarithm of the reciprocal serum dilution vs dpi. Dotted line indicates limit of detection of the assay (1/20 serum dilution). Undetectable neutralization activity was assigned a value of 5.