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

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Abstract

Conventional piglets were inoculated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) through different routes, including intranasal, intratracheal, 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).

KEYWORDS
immunogenicity model, inoculation routes, lack of susceptibility, pig, SARS-CoV-2

Piglets inoculated by different routes are not susceptible to SARS-CoV-2, but those inoculated parenterally were immunized against the virus.

The coronavirus disease 2019 (COVID-19) is an infectious disease that has caused a global pandemic with more than 36 million infected people from around 200 countries or territories, with more than 1 million deaths to date (World Health Organization (WHO), 2020). 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 (Andersen et al., 2020; Zhou et al., 2020). Several species have been studied to determine their potential role as intermediate hosts (Shi et al., 2020). Moreover, animal models to recapitulate a COVID-19-like disease are considered a major research line and required for the development of therapeutic drugs and prophylactic compounds.

Besides several modelling studies proposing potential animal species susceptible to SARS-CoV-2 (Damas et al., 2020; Qiu et al., 2020; Veljkovic et al., 2020), 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 (Bao et al., 2020; Halfmann et al., 2020; Kim et al., 2020; Rockx et al., 2020; Shi et al., 2020; Yu et al., 2020). From an experimental point of view, dog susceptibility to SARS-CoV-2 is limited, since inoculated animals can partly seroconvert (Shi et al., 2020). In contrast, the intranasal inoculation of chicken, duck and pig resulted in no evidence of infection (Schlottau et al., 2020; Shi et al., 2020).

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 (Meurens et al., 2012). However, since pigs are not susceptible to SARS-CoV-2 infection when inoculated intranasally (Schlottau et al., 2020; Shi et al., 2020), 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) (Zhou et al., 2020) or that pseudoparticles with the S protein of SARS-CoV-2 are able to infect swine kidney cells (Letko et al., 2020). Furthermore, the ACE2 protein is expressed in all major tissues of pigs as assessed by immunohistochemistry (Xiao et al., 2020). 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 × Large White) were selected and inoculated by means of different routes: intranasal (IN, 1.5 ml/nostril; total volume of 3 ml), intratracheal (IT, 3 ml) as previously described (Garcia-Morante et al., 2016), 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_510689) per each animal. The IT and IV groups were anaesthetized with 10 mg/kg of ketamine and 0.8 mg/kg xylazine prior inoculation. A passage-2 SARS-CoV-2 was propagated and titrated in Vero E6 cells (ATCC CRL-1586), following the same protocol as for other coronaviruses (Rodon et al., 2019). Two extra pigs were used as negative controls.

All animals were seropositive against porcine respiratory coronavirus, as determined by a commercial ELISA (INgezim Corona Diferencial 2.0 [TGEV/PRCV]). Taking into account that no antibody cross-reactivity between alpha- and beta-coronaviruses has been described (Okba et al., 2020), the animals were kept into the study. Initial reactivity against PRCV was expected since this virus is ubiquitous in the European swine livestock (Saif et al., 2012; Vidal et al., 2019).

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 (Shi et al., 2020), 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. Samples were collected and processed as previously described (Vergara-Alert et al., 2017). Briefly, 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 haematoxylin 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 (Corman et al., 2020). Nasal and rectal swabs were also taken (daily during the first week and at 14 and 22 dpi) to analyse them for the presence of viral RNA by means of the above-mentioned 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 (Institut de Recerca de la sida (Irsicaixa), 2020). Also, a

FIGURE 1 (a) 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 intratracheally (No. 8–10, IT group), intramuscularly (No. 13–15, IM group) and intravenously (No. 18–20, IV group). (b) Antibody detection of pigs experimentally inoculated with SARS-CoV-2. Detection of neutralizing antibodies (days 0 and 22 pi) in sera from animals inoculated intratracheally (No. 8–10, IT group), intramuscularly (No. 13–15, IM group) and intravenously (No. 18–20, IV group). The graph shows the reciprocal serum dilution showing neutralization activity versus dpi. Dotted line indicates limit of detection of the assay (1/20 serum dilution). A value of 5 was assigned to undetectable neutralization activity. Unpaired Student's *t* tests were performed to assess whether neutralizing antibodies significantly increased at 22 dpi. ns, not significant; *p-value < .05
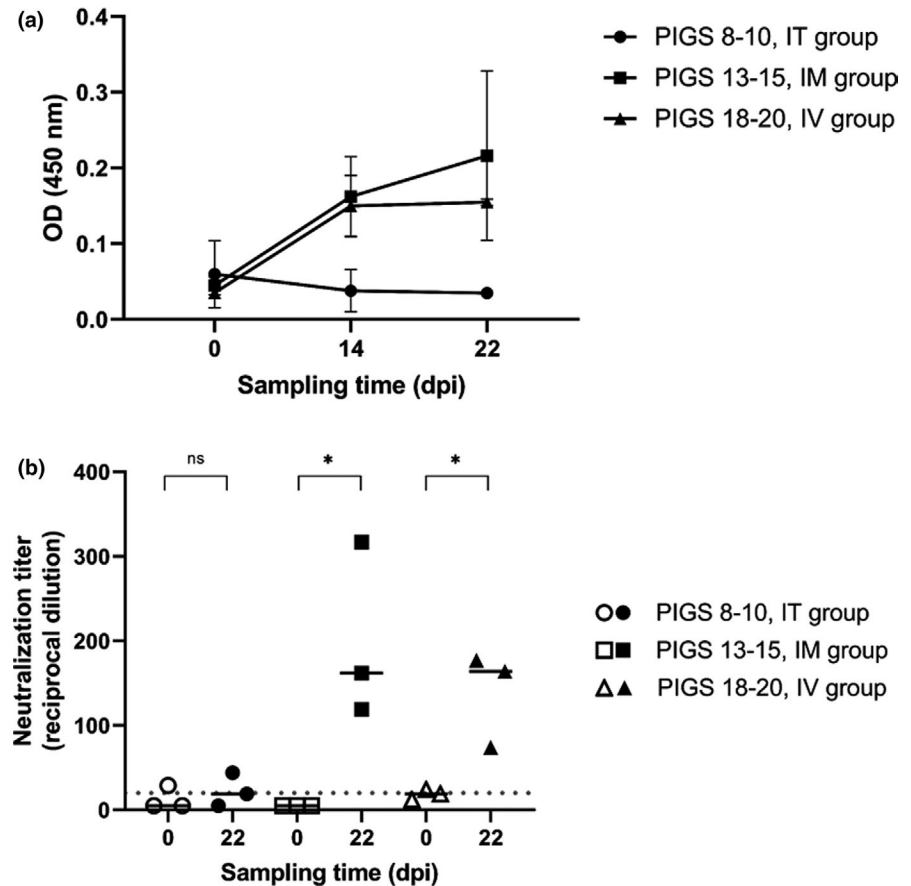
virus neutralization assay was performed following a previous protocol with a minor modification (Rodon et al., 2020), the serial dilutions of sera and SARS-CoV-2 were incubated for 1 hr at 37°C prior the plate assay performance.

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 (data not shown).

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 (Cq = 24.36). The remaining tissues from this animal and the rest of pigs resulted negative for RT-qPCR (qPCR detection limit of 38.6 cycles).

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 titres at 22 dpi (ranging from 74 to 317 SNT_{50} reciprocal dilution titre) (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 N protein as well as neutralizing titres (SNT_{50} reciprocal dilution titre of 29) at day 0 pi, which might suggest a potential cross-reaction with

another coronavirus infecting swine. Of note, these antibodies against the N protein decreased by the time the experiment finished, suggesting they were of maternal origin. In addition, this animal did not show seroneutralizing antibodies at the 22 dpi (Figure 1b).

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 (Schlottau et al., 2020; Shi et al., 2020), 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 (Rothkötter, 2009).

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.

DATA AVAILABILITY STATEMENT

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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