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Zoonotic Exposure to Porcine Deltacoronavirus, United States, 2019–2021

Appendix

Appendix Materials and Methods

Serum

A total of 200 human serum samples were procured from a commercial supplier (Innovative Research). Samples were collected from de-identified blood donors at FDA-approved collection facilities in the Midwestern and Eastern United States during 2021 and tested for standard FDA-required infectious disease markers. The adult donor population included both sexes and multiple racial and ethnic groups (Appendix Table 1). Donors ranged from 18–67 years of age (mean age 40 years). Samples were aliquoted, heat-inactivated for 30 min at 56°C, and stored at –80°C until testing. Juvenile swine exhibitors (5–19 years of age) participating in county and state agricultural exhibitions during 2019–2021 were enrolled through an ongoing cross-sectional influenza surveillance study at 2 national and 1 state swine jackpot exhibition during 2019–2021. Jackpot exhibitions are competitive youth livestock events in which exhibitors present swine that are raised specifically for exhibition purposes. To be eligible, exhibitors reported daily or near-daily contact with swine. Exhibition swine are typically housed and managed separately from commercial production swine, they are fed and cared for by the exhibitors at their local venue and the exhibition venue and they are transported among exhibition venues, creating distinct opportunities for human–animal contact. Blood samples were collected by venipuncture, processed into serum, aliquoted, and stored at –80°C. Serum samples were obtained from 11 states. Adult and juvenile serum samples were thawed and tested side-by-side by a single research associate using identical laboratory protocols. Serologic analyses were

performed in 2024–2025. Juvenile exhibitor serum samples collected during 2019–2021 and adult donor serum samples collected in 2021 were maintained at −80°C until testing.

ELISA

All serum samples were tested with a whole virion PDCoV ELISA assay (*1*,*2*) adapted and optimized for human samples. Briefly 50 µL of serum diluted in (phosphate buffered saline tween 20 (PBST)/2% nonfat dried milk (NFDM) (1:400) were added to the PDCoV antigen-coated wells (1 ng/µl in carbonate buffer. This was followed by 50 µL of horseradish peroxidase-labeled Goat-anti-Human IgG (Invitrogen), at a dilution of 1:2000. Plates were read at an absorbance of 450 nm using a SpectraMax plate reader (Molecular Devices, Union City, CA). Samples were tested in triplicate, and repeated testing was performed for positive results. Uncoated wells and no serum wells were included on each plate. The secondary antibody goat anti-Swine IgG (H&L) (MilliporeSigma, Rockville, MD, USA) was used at 1:2000 for OH-DC 97 hyperimmune swine serum positive control. A negative cut off of OD: 0.2794 was established using the Frey method (*3*).

ELISA Cross Reactivity

Coronavirus serum samples (Appendix Table 2,(4)) were also tested with the PDCoV ELISA assay for cross reactivity to the antigen. All hyperimmune serum samples (*4*,*5*) were from inactivated whole virus lysates inoculated intramuscularly with Freund's incomplete adjuvant into either swine or guinea pigs (*6*). The PEDV serum sample was provided by Q. Wang. The SARS-CoV-2 antibody positive human plasma sample was purchased (Innovative Research) and is a whole blood derived product processed using EDTA as the anticoagulant. Samples were collected from a single donor with COVID-19 positive status confirmed via SARS-CoV-2 IgG antibody assay. Briefly, 50 µL of serum diluted in PBST/2% NFDM (1:100 swine, 1:200 guinea pig, 1:400 human) was added to the PDCoV antigen coated wells (1 ng/µl in carbonate buffer). This was followed by 50 µL of horseradish peroxidase labeled Goat-anti-Swine IgG (Invitrogen) or rabbit anti-guinea pig (Seracare). Plates were read at an absorbance of 450 nm using a SpectraMax plate reader (Molecular Devices, Union City, CA). Samples were tested in triplicate and repeated twice for a total of three independent replicates. Uncoated wells and no serum wells were included on each plate.

Virus Neutralization Test (VNT)

Neutralizing antibodies in the sera were assessed using an optimized protocol using ST cells in 96-well plates. The swine hyperimmune antiserum OH-DC 97 and human serum were used as positive and negative serum controls, respectively. All sera were inactivated at 56°C for 30 minutes before testing and prepared as 2-fold serial dilutions in maintenance media (MM: Advanced Minimum Essential Medium (MEM) with 1% Anti-anti and 1% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). Each sample was mixed with an equal volume of TC-PDCoV (OH-FD22-P100) at 100 $TCID_{50}$ per well. The mixture was incubated at 37°C for 60 min. on a separate plate. The growth medium for ST cells was replaced with MM once the cells reached 100% confluence. Following 1 h of incubation at 37°C, cells were washed once with PBS. The virus-serum mixtures were added to the ST cells. The positive virus control wells had no serum and the negative control wells received MM only. After adsorption for 1 h at 37°C in an atmosphere of 5% CO_2, 100 µl of MM containing the addition of 1% pancreatin (Sigma Aldrich) was added to each well. The plates were incubated for 3 days in an incubator (37°C, 5% CO_2), and the plates were observed for cytopathic effect (CPE). The media was removed, and cells were washed and fixed with 4% paraformaldehyde before adding Amido Black solution (Bio-World). Pig hyperimmune serum against PDCoV (positive control) and PDCoV antibody negative (negative control) gnotobiotic pig serum were used for experimental optimization due to limited volume of the human serum samples for testing.

Statistical analysis

Seropositivity rates were compared between groups using Fisher exact test. Relative measures of association, including odds ratios (OR) and risk ratios (RR) with 95% confidence intervals (CI), were calculated. The absolute risk difference (ARD) and its 95% CI were estimated using the Wald method for the difference in proportions. ARD = p_{youth} $-p_{adults}$ = 0.09−0.005 = 0.085. $SE = \sqrt{((p_youth(1 - p_youth) / n_youth) + (p_adults(1 - p_adults) / n_adults))}$. 95% CI = ARD±1.96×SE→ 8.5% (95% CI: 2.8%–14.2%)

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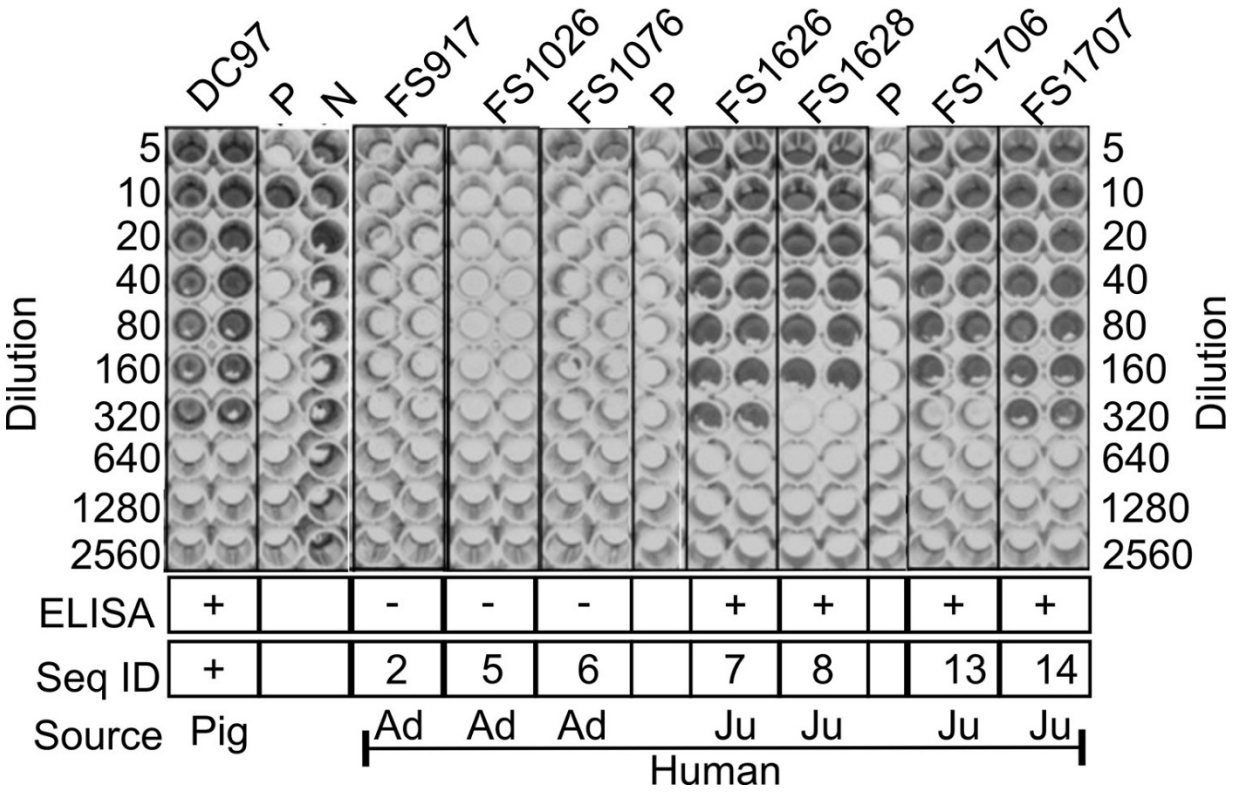
Appendix Table 1. Demographic Summary of Commercially Acquired Random Adult Serum Samples

Demographics	No.	%
Gender		
Male	93	46.5
Female	107	53.5
Race		
Caucasian	23	11.5
Hispanic	84	42
African American	91	45.5
Undetermined	2	1.0
Age (y)		
<21	5	2.5
21–30	54	27.0
31–40	46	23.0
41–50	36	18.0
51–60	32	16.0
>60	27	13.5

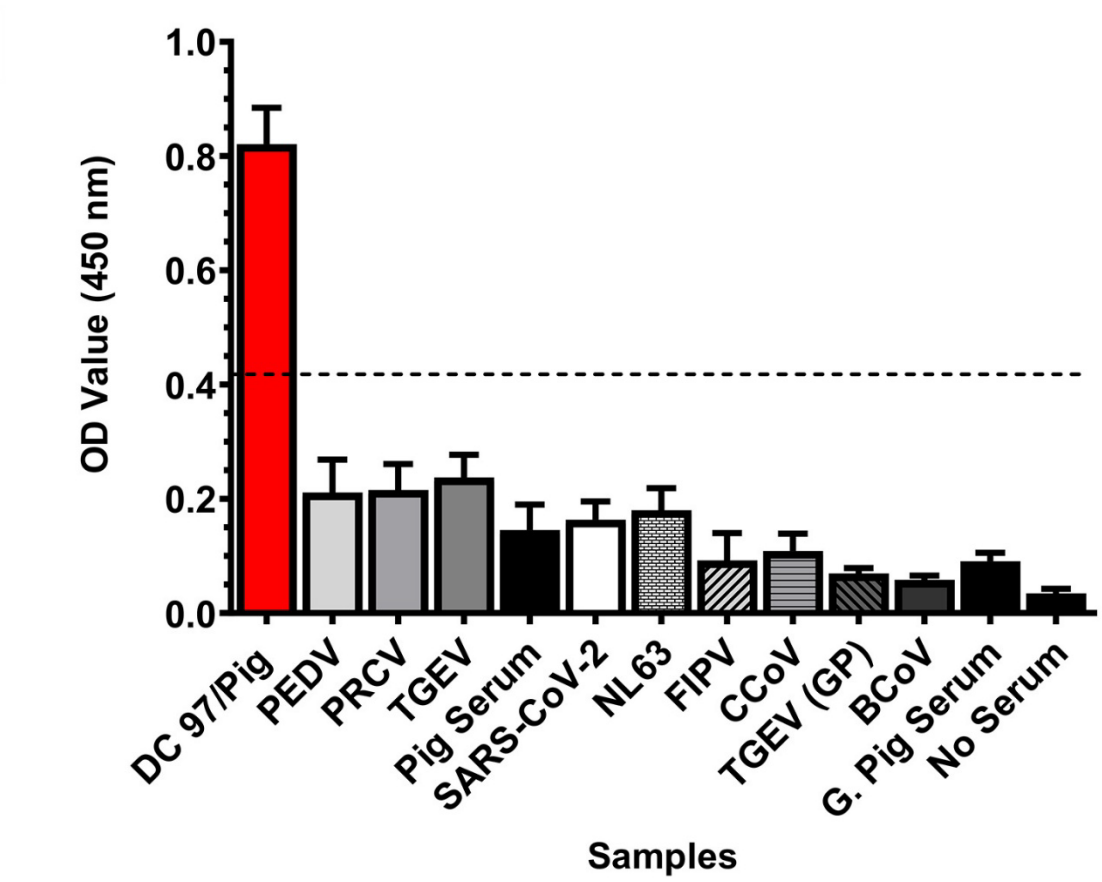
Appendix Table 2. Serum Samples Tested for Cross Reactivity with PDCoV in ELISA*

Disease	Host	Type
Porcine Epidemic Diarrhea Virus	Pig	Serum
Porcine Respiratory Coronavirus	Pig	HI Serum
Transmissible Gastroenteritis	Pig	HI Serum
Severe Acute Respiratory Syndrome	Human	Plasma
Human Coronavirus NL-63	GP	HI Serum
Feline Infectious Peritonitis Virus	GP	HI Serum
Canine Coronavirus	GP	HI Serum
Transmissible Gastroenteritis	GP	HI Serum
Bovine Coronavirus	GP	HI Serum

*GP, guinea pig; HI, heat inactivated



Appendix Figure 1. VNT titers for commercially acquired adult and juvenile swine exhibitor serum samples with potential exposure to porcine deltacoronavirus, United States, 2019–2021. VNT titers for selected adult and juvenile serum samples. Samples positive using ELISA are indicated by the (+) sign and samples negative using ELISA are indicated by the (–) sign. Ad, adult; ELISA, enzyme linked immunosorbent assay; Ju, juvenile; N, negative control; OD, optical density; VNT, virus neutralizing test.



Appendix Figure 2. ELISA OD values for commercially acquired adult and juvenile swine exhibitor serum samples with potential exposure to porcine deltacoronavirus, United States, 2019–2021. Cross-reactivity of serum samples to other coronaviruses. Bars indicate the mean OD value with error bars representing the SEM from 3 independent experiments. Dashed horizontal line indicates the ELISA positivity cutoff (OD 0.42). BCoV, bovine coronavirus; CCoV, canine coronavirus; ELISA, enzyme linked immunosorbent assay; FIPV, feline infectious peritonitis virus; GP, glycoprotein; NL63, human coronavirus NL63; OD, optical density; PDCoV, porcine deltacoronavirus; PEDV, porcine epidemic diarrhea virus; PRCV, porcine respiratory coronavirus; SARS-CoV2, sudden acute respiratory syndrome coronavirus 2; TGEV, transmissible gastroenteritis virus.