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Development and Validation of Indirect Enzyme-Linked Immunosorbent Assays for Detecting Antibodies to SARS-CoV-2 in Cattle, Swine, and Chicken

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SARS-CoV-2 Spike Pseudovirus Production and Neutralization Assay

SARS-CoV-2 pseudo viruses were produced as described previously by Crawford et al [55,56]. Plasmids encoding spike proteins of SARS-CoV-2 Wuhan strain and Alpha, Beta, Gamma, Delta, and Omicron VOCs were co-transfected with lentiviral backbone plasmid expressing firefly luciferase and lentiviral helper plasmids expressing HIV Gag-pol, Tat and Rev. 293T cells were transfected with the plasmids using jetPRIME® transfection reagent (Polyplus®). Supernatants containing pseudo-typed particles were collected at 48 hr post-transfection and filtered with a 0.45 µM filter. For neutralization assays, HIS produced against RBD in cattle, swine and chicken was 3-fold serially diluted prior to incubation with pseudo viruses for one hr at 37°C. Pseudovirus/HIS mixtures were then added to 96-wells pre-seeded with 293T-hACE2/TMPRSS2 cells (NR-55293, BEI resources). Luciferase activity was measured at 48hr post infection to quantify the neutralization titer of tested sera.

Table S1. Characteristics of species-specific iELISAs developed for detecting antibodies against SARS-CoV-2.

Characteristic	Value (95% Confidence Interval)
Cattle	
Sensitivity	100.00% (75.29% to 100.00%)
Specificity	100.00% (97.57% to 100.00%)
Accuracy	100.00% (97.76% to 100.00%)
Swine	
Sensitivity	100.00% (54.07% to 100.00%)
Specificity	100.00% (97.57% to 100.00%)
Accuracy	100.00% (97.66% to 100.00%)
Chicken	
Sensitivity	100.00% (85.75% to 100.00%)
Specificity	100.00% (97.57% to 100.00%)
Accuracy	100.00% (97.90% to 100.00%)

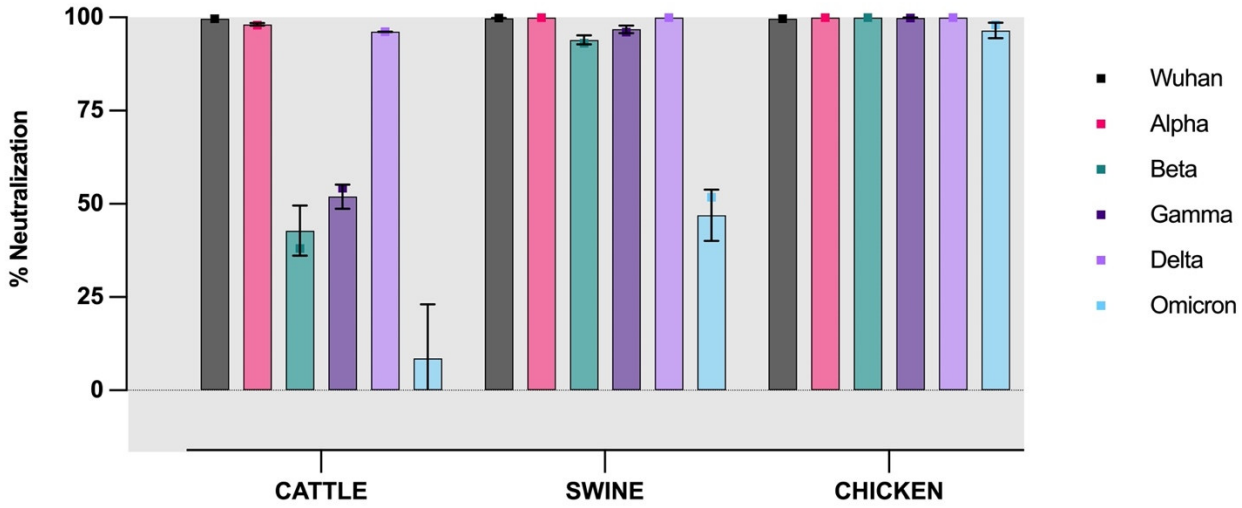

Figure S1. Hyperimmune serum raised with Wuhan RBD cross reacts with SARS-CoV-2 VOCs. Cattle, swine, and chicken hyperimmune sera raised against SARS-CoV-2 RBD were tested for the ability to neutralize SARS-CoV-2 pseudoviruses expressing spike protein of Wuhan and Alpha, Beta, Gamma, Delta and Omicron VOCs. The hyperimmune serum from all three species broadly cross-neutralized all the tested SARS-CoV-2 VOCs. Height of bar represents the percent (%) neutralization. Error bars represent the standard deviation.