

Supplemental figure legends

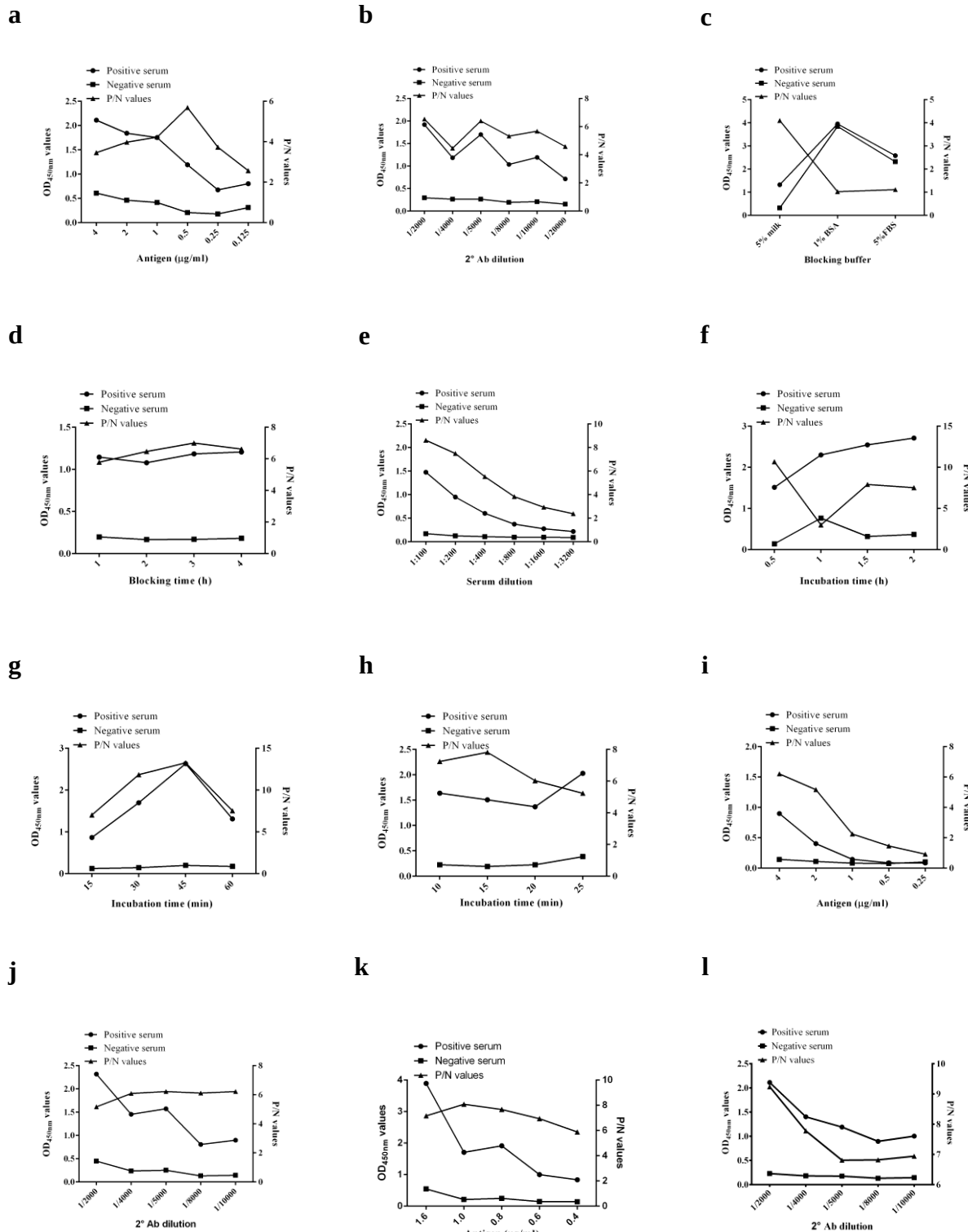
Supplemental Figure S1. Optimization of indirect ELISA methods.

(a-b) Determination of the optimal concentration of NS6 protein and 2° Ab dilution for detecting anti-PDCoV IgG by checkerboard titration, NS6 protein concentration gradually decreased from 4 µg/mL to 0.125 µg/mL, 2° Ab dilution was gradually increased from 1:2000 to 1:20000. P/N: the ratio of positive to negative serum OD450nm values. (c-d) Determination of the optimal blocking buffer and blocking time, blocking buffer was 5% nonfat powdered milk, 5% fetal bovine serum and 1% bovine serum albumin; incubation time with blocking buffer was 1 h, 2 h, 3 h and 4 h respectively. (e) Determination of the optimal serum sample dilution. Serum was diluted from 1:100 to 1:2000. (f-g) Determination of the optimal incubation times of serum sample and HRP-goat anti-pig. Incubation time for serum sample was 0.5 h, 1 h, 1.5 h and 2 h, incubation time for HRP-goat anti-pig was 15 min, 30 min, 45 min and 60 min. (h) Determination of the optimal color development time. TMB color development time was 10 min, 15 min, 20 min and 25 min, respectively. (i-j) Determination of the optimal concentration of NS6 protein and 2° Ab dilution for detecting anti-PDCoV IgA by checkerboard titration. NS6 protein concentration gradually decreased from 4 µg/mL to 0.25 µg/mL, 2° Ab dilution was gradually increased from 1:2000 to 1:10000. (k-l) Determination of the optimal concentration of S1 protein and 2° Ab dilution for detecting anti-PDCoV IgA. S1 protein concentration gradually decreased from 1.6 µg/mL to 0.4 µg/mL, 2° Ab dilution was gradually increased from 1:2000 to 1:10000.

Supplemental Figure S2. Phylogenetic analysis between different avian coronaviruses and PDCoV.

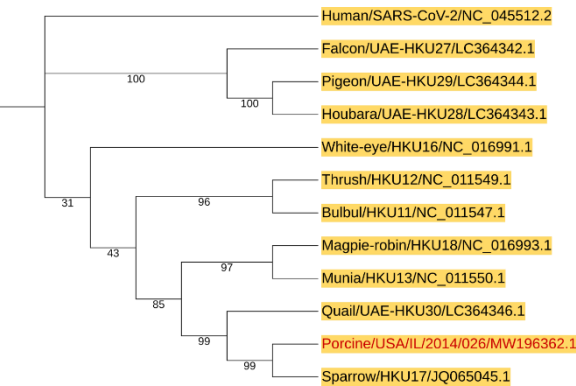
(a) Phylogenetic analysis of the RNA-dependent RNA polymerase (RdRp) gene of PDCoV and avian CoVs. The tree was constructed by the maximum likelihood method using the JTT+G+F model in MEGA x software. (b) Phylogenetic analysis of the NS6 gene of PDCoV and avian CoVs. The tree was constructed by the maximum likelihood method using the LG+G model in MEGA x software. Bootstrap values are indicated for each node from 1000 re-samplings.

Supplemental Figure S1



Supplemental Figure S2

a



b

