**Table S1.** Evaluation of difference among sex in the Ossabaw Island Hog tissues infected by *B. suis* and the intensity of infection using Fisher's exact probability test of binomial proportions (number of tissues infected) and the mean bacterial count and number of tissues infected using Fisher's exact Poisson test. Results that are significant at the 0.05 level are noted in bold

| Tissue | Tissues Infected | | | | | Bacterial Count | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Male | $n$ | Female | $n$ | P–Value | Male | $n$ | Female | $n$ | P–Value |
| Mandibular lymph node | 9 | 11 | 7 | 12 | 0.221 | 113.8 | 11 | 70.5 | 12 | **$9.20 \times 10^{-28}$** |
| Retropharyngeal lymph node | 7 | 11 | 9 | 12 | 0.445 | 103.2 | 11 | 38.8 | 12 | **$4.37 \times 10^{-78}$** |
| Parotid lymph node | 9 | 11 | 9 | 12 | 0.500 | 105.6 | 11 | 7.9 | 12 | **$6.34 \times 10^{-255}$** |
| Mediastinal lymph node | 8 | 11 | 6 | 12 | 0.246 | 1.9 | 11 | 87.2 | 12 | **$3.43 \times 10^{-259}$** |
| Mesenteric lymph node | 4 | 11 | 5 | 12 | 0.500 | 38.9 | 11 | 18.5 | 12 | **$1.63 \times 10^{-20}$** |
| Mammary/Inguinal lymph node | 7 | 11 | 7 | 12 | 0.500 | 27.6 | 11 | 50.2 | 12 | **$1.81 \times 10^{-18}$** |
| Spleen | 6 | 11 | 7 | 12 | 0.500 | 61.0 | 11 | 88.2 | 12 | **$2.70 \times 10^{-14}$** |
| Liver | 7 | 11 | 5 | 12 | 0.262 | 8.4 | 11 | 4.2 | 12 | **$3.46 \times 10^{-05}$** |
| Lung | 7 | 11 | 5 | 12 | 0.262 | 10.4 | 11 | 4.8 | 12 | **$5.17 \times 10^{-07}$** |
| Peripheral blood mononuclear cell | 4 | 11 | 5 | 12 | 0.500 | 2.3 | 11 | 6.0 | 12 | **$7.07 \times 10^{-06}$** |
| All Tissues | 6.2 | 11 | 5.8 | 12 | 0.665 | 473.1 | 11 | 377.2 | 12 | **$3.34 \times 10^{-29}$** |

**Table S2.** Evaluation of difference among age in the Ossabaw Island Hog tissues infected by *B. suis* and the intensity of infection using Fisher's exact probability test of binomial proportions (number of tissues infected) and the mean bacterial count and number of tissues infected using Fisher's exact Poisson test. Results that are significant at the 0.05 level are noted in bold.

| Tissue | Tissues Infected | | | | | Bacterial Count | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Adult | $n$ | Subadult | $n$ | P–Value | Adult | $n$ | Subadult | $n$ | P–Value |
| Mandibular lymph node | 12 | 18 | 4 | 5 | 0.510 | 83.8 | 18 | 117.8 | 5 | 1 |
| Retropharyngeal lymph node | 13 | 18 | 3 | 5 | 0.500 | 41.3 | 18 | 171.4 | 5 | **$2.82 \times 10^{-169}$** |
| Parotid lymph node | 14 | 18 | 4 | 5 | 0.500 | 22.2 | 18 | 171.4 | 5 | **$2.26 \times 10^{-271}$** |
| Mediastinal lymph node | 11 | 18 | 3 | 5 | 0.500 | 58.9 | 18 | 1.2 | 5 | **$2.45 \times 10^{-102}$** |
| Mesenteric lymph node | 8 | 18 | 1 | 5 | 0.318 | 32.1 | 18 | 14.4 | 5 | **$9.39 \times 10^{-13}$** |
| Mammary/Inguinal lymph node | 10 | 18 | 4 | 5 | 0.682 | 39.1 | 18 | 40.8 | 5 | 0.724 |
| Endometrium | 3 | 11 | 0 | 1 | 0.500 | 0.4 | 11 | 0 | 1 | 0.706 |
| Vaginal swab | 2 | 11 | 0 | 1 | 0.500 | 0.6 | 11 | 0 | 1 | 0.544 |
| Spleen | 11 | 18 | 2 | 5 | 0.370 | 66 | 18 | 108.2 | 5 | 1 |
| Liver | 9 | 18 | 3 | 5 | 0.500 | 5.7 | 18 | 7.8 | 5 | 0.957 |
| Lung | 9 | 18 | 3 | 5 | 0.500 | 7.3 | 18 | 7.8 | 5 | 0.672 |
| Peripheral blood mononuclear cell | 8 | 18 | 1 | 5 | 0.318 | 4.6 | 18 | 3 | 5 | 0.0807 |
| All Tissues | 6.1 | 18 | 5.6 | 5 | 0.385 | 361.7 | 18 | 643.8 | 5 | 1 |

**Table S3.** Evaluation of difference among strain in the Ossabaw Island Hog tissues infected by *B. suis* and the intensity of infection using Fisher's exact probability test of binomial proportions (number of tissues infected) and the mean bacterial count and number of tissues infected using Fisher's exact Poisson test. Results that are significant at the 0.05 level are noted in bold.

| Tissue | Tissues Infected | | | | | Bacterial Count | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1330 | *n* | 294 | *n* | *P*–Value | 1330 | *n* | 294 | *n* | *P*–Value |
| Mandibular lymph node | 8 | 12 | 8 | 11 | 0.500 | 133.8 | 12 | 44.7 | 11 | **$2.44 \times 10^{-117}$** |
| Retropharyngeal lymph node | 8 | 12 | 8 | 11 | 0.500 | 96.5 | 12 | 40.3 | 11 | **$3.83 \times 10^{-61}$** |
| Parotid lymph node | 9 | 12 | 9 | 11 | 0.500 | 74 | 12 | 33.5 | 11 | **$9.76 \times 10^{-41}$** |
| Mediastinal lymph node | 8 | 12 | 6 | 11 | 0.434 | 4.4 | 12 | 92.2 | 11 | **$3.30 \times 10^{-250}$** |
| Mesenteric lymph node | 4 | 12 | 5 | 11 | 0.434 | 6.7 | 12 | 51.8 | 11 | **$6.92 \times 10^{-102}$** |
| Mammary/Inguinal lymph node | 8 | 12 | 6 | 11 | 0.434 | 23.6 | 12 | 56.7 | 11 | **$2.17 \times 10^{-37}$** |
| Endometrium | 3 | 7 | 0 | 5 | 0.155 | 0.6 | 7 | 0 | 5 | 0.116 |
| Vaginal swab | 0 | 7 | 2 | 5 | 0.147 | 0 | 7 | 1.4 | 5 | **0.00218** |
| Spleen | 8 | 12 | 5 | 11 | 0.273 | 49.9 | 12 | 102.7 | 11 | **$7.21 \times 10^{-49}$** |
| Liver | 8 | 12 | 4 | 11 | 0.150 | 6.3 | 12 | 6 | 11 | 0.407 |
| Lung | 8 | 12 | 4 | 11 | 0.150 | 5.8 | 12 | 9.2 | 11 | **0.00207** |
| Peripheral blood mononuclear cell | 5 | 12 | 4 | 11 | 0.500 | 6.3 | 12 | 1.9 | 11 | **$8.87 \times 10^{-08}$** |
| All Tissues | 6.4 | 12 | 5.5 | 11 | 0.222 | 407.8 | 12 | 439.7 | 11 | 1 |

**Table S4.** Diagnostic assay performance using a single assay.

| Assay | Category | Sensitivity | Specificity | Positive Predictive Value | Negative Predictive Value | Diagnostic Odds Ratio |
|---|---|---|---|---|---|---|
| Buffered Acidified Plate Antigen | All Animals | 0.980 (0.909–0.984) | 0.833 (0.636–0.969) | 0.891 (0.754–0.981) | 0.967 (0.857–0.977) | 8.2 (3.1–51.0) |
| Card Test (8%) | All Animals | 0.917 (0.784–0.982) | 0.935 (0.786–0.979) | 0.953 (0.833–0.983) | 0.891 (0.719–0.977) | 20.0 (5.0–58.0) |
| Complement Fixation (Cold) | All Animals | 0.970 (0.941–0.980) | 0.730 (0.511–0.900) | 0.836 (0.688–0.945) | 0.964 (0.941–0.975) | 5.1 (2.2–17.7) |
| Fluorescent Polarization Assay | All Animals | 0.564 (0.429–0.692) | 0.701 (0.548–0.835) | 0.723 (0.578–0.850) | 0.539 (0.402–0.674) | 2.6 (1.4–5.5) |
| Buffered Acidified Plate Antigen | Adult | 0.976 (0.894–0.981) | 0.885 (0.636–1.000) | 0.946 (0.810–1.000) | 0.944 (0.769–0.967) | 17.0 (4.4–Inf) |
| Card Test (8%) | Adult | 0.919 (0.767–0.979) | 0.957 (0.923–1.000) | 0.975 (0.966–1.000) | 0.864 (0.625–0.967) | 39.0 (28.0–Inf) |
| Complement Fixation (Cold) | Adult | 0.963 (0.929–0.974) | 0.840 (0.577–1.000) | 0.925 (0.784–1.000) | 0.947 (0.900–0.966) | 12.7 (3.6–Inf) |
| Fluorescent Polarization Assay | Adult | 0.578 (0.430–0.722) | 0.745 (0.550–0.906) | 0.817 (0.661–0.934) | 0.472 (0.307–0.635) | 4.5 (2.0–14.2) |
| Buffered Acidified Plate Antigen | Subadult | 0.900 (0.750–0.941) | 0.778 (0.444–1.000) | 0.727 (0.333–1.000) | 0.923 (0.857–0.947) | 2.7 (0.5–Inf) |
| Card Test (8%) | Subadult | 0.875 (0.500–0.938) | 0.846 (0.526–1.000) | 0.769 (0.333–1.000) | 0.923 (0.667–0.950) | 3.3 (0.5–Inf) |
| Complement Fixation (Cold) | Subadult | 0.900 (0.800–0.938) | 0.583 (0.267–0.905) | 0.579 (0.263–0.909) | 0.900 (0.800–0.941) | 1.4 (0.3–10.0) |
| Fluorescent Polarization Assay | Subadult | 0.500 (0.190–0.812) | 0.654 (0.406–0.857) | 0.450 (0.167–0.750) | 0.694 (0.441–0.897) | 0.8 (0.2–3.0) |
| Buffered Acidified Plate Antigen | Female | 0.964 (0.848–0.973) | 0.846 (0.526–1.000) | 0.920 (0.731–1.000) | 0.923 (0.692–0.957) | 11.5 (2.8–Inf) |
| Card Test (8%) | Female | 0.879 (0.667–0.970) | 0.941 (0.889–1.000) | 0.962 (0.944–1.000) | 0.824 (0.538–0.957) | 25.0 (16.0–Inf) |
| Complement Fixation (Cold) | Female | 0.947 (0.875–1.000) | 0.720 (0.375–1.000) | 0.867 (0.667–1.000) | 0.917 (0.800–0.952) | 6.6 (2.0–Inf) |
| Fluorescent Polarization Assay | Female | 0.614 (0.435–0.780) | 0.651 (0.406–0.865) | 0.766 (0.579–0.911) | 0.475 (0.279–0.686) | 3.3 (1.4–10.2) |
| Buffered Acidified Plate Antigen | Male | 0.957 (0.933–0.969) | 0.840 (0.571–1.000) | 0.870 (0.633–1.000) | 0.947 (0.900–0.963) | 6.7 (1.8–Inf) |
| Card Test (8%) | Male | 0.952 (0.800–0.968) | 0.889 (0.625–1.000) | 0.900 (0.667–1.000) | 0.944 (0.773–0.964) | 9.3 (2.0–Inf) |
| Complement Fixation (Cold) | Male | 0.929 (0.889–0.952) | 0.750 (0.450–0.955) | 0.806 (0.562–0.968) | 0.941 (0.889–0.960) | 4.2 (1.3–30.0) |
| Fluorescent Polarization Assay | Male | 0.500 (0.297–0.700) | 0.744 (0.543–0.902) | 0.667 (0.436–0.872) | 0.586 (0.400–0.763) | 2.0 (0.8–6.8) |

**Table S5.** Diagnostic assay performance when two assays are interpreted in parallel.

| Assay | Assay | Sensitivity | Specificity | Positive Predictive Value | Negative Predictive Value | Diagnostic Odds Ratio | Area Under Curve |
|---|---|---|---|---|---|---|---|
| Buffered Acidified Plate Antigen | Complement Fixation (Cold) | 0.980 (0.914–0.984) | 0.833 (0.635–0.969) | 0.891 (0.754–0.981) | 0.967 (0.857–0.977) | 8.3 (3.1–50.0) | 0.981 (0.884–0.999) |
| Card Test (8%) | Buffered Acidified Plate Antigen | 0.898 (0.753–0.981) | 0.936 (0.780–0.978) | 0.952 (0.832–0.983) | 0.869 (0.692–0.975) | 19.5 (4.9–57.0) | 0.977 (0.874–0.999) |
| Card Test (8%) | Complement Fixation (Cold) | 0.917 (0.782–0.982) | 0.937 (0.781–0.979) | 0.952 (0.830–0.983) | 0.890 (0.716–0.976) | 19.8 (5.0–57.0) | 0.984 (0.889–0.999) |
| Fluorescent Polarization Assay | Buffered Acidified Plate Antigen | 0.556 (0.421–0.685) | 0.829 (0.693–0.932) | 0.818 (0.676–0.927) | 0.573 (0.442–0.700) | 4.5 (2.1–12.2) | 0.731 (0.616–0.836) |
| Fluorescent Polarization Assay | Card Test (8%) | 0.480 (0.347–0.617) | 0.931 (0.828–0.987) | 0.905 (0.771–0.982) | 0.565 (0.444–0.684) | 9.6 (3.3–53.0) | 0.767 (0.663–0.854) |
| Fluorescent Polarization Assay | Complement Fixation (Cold) | 0.564 (0.431–0.694) | 0.753 (0.605–0.876) | 0.759 (0.615–0.880) | 0.556 (0.421–0.689) | 3.2 (1.6–7.3) | 0.685 (0.570–0.796) |
| Buffered Acidified Plate Antigen | Complement Fixation (Cold) | 0.980 (0.910–0.984) | 0.833 (0.635–0.970) | 0.890 (0.752–0.981) | 0.967 (0.855–0.977) | 8.2 (3.0–49.0) | 0.950 (0.848–0.999) |
| Card Test (8%) | Buffered Acidified Plate Antigen | 0.898 (0.757–0.981) | 0.936 (0.778–0.979) | 0.952 (0.831–0.983) | 0.869 (0.690–0.975) | 19.8 (4.9–56.0) | 0.987 (0.907–0.999) |
| Card Test (8%) | Complement Fixation (Cold) | 0.918 (0.782–0.982) | 0.936 (0.780–0.978) | 0.952 (0.835–0.983) | 0.890 (0.714–0.976) | 20.0 (5.0–57.5) | 0.950 (0.848–0.999) |
| Fluorescent Polarization Assay | Buffered Acidified Plate Antigen | 0.556 (0.423–0.685) | 0.831 (0.696–0.930) | 0.817 (0.675–0.926) | 0.573 (0.444–0.700) | 4.5 (2.1–12.7) | 0.931 (0.855–0.985) |
| Fluorescent Polarization Assay | Card Test (8%) | 0.481 (0.347–0.616) | 0.930 (0.828–0.987) | 0.905 (0.772–0.982) | 0.566 (0.444–0.685) | 9.5 (3.3–55.0) | 0.932 (0.854–0.985) |
| Fluorescent Polarization Assay | Complement Fixation (Cold) | 0.564 (0.430–0.695) | 0.753 (0.605–0.877) | 0.759 (0.615–0.879) | 0.556 (0.423–0.687) | 3.2 (1.6–7.3) | 0.926 (0.853–0.981) |

**Table S6.** Diagnostic assay performance when two assays are interpreted in series.

| Assay | Assay | Sensitivity | Specificity | Positive Predictive Value | Negative Predictive Value | Diagnostic Odds Ratio | Area Under Curve |
|---|---|---|---|---|---|---|---|
| Buffered Acidified Plate Antigen | Complement Fixation (Cold) | 0.980 (0.914–0.984) | 0.833 (0.635–0.969) | 0.891 (0.754–0.981) | 0.967 (0.857–0.977) | 8.3 (3.1–50.0) | 0.981 (0.884–0.999) |
| Card Test (8%) | Buffered Acidified Plate Antigen | 0.898 (0.753–0.981) | 0.936 (0.780–0.978) | 0.952 (0.832–0.983) | 0.869 (0.692–0.975) | 19.5 (4.9–57.0) | 0.977 (0.874–0.999) |
| Card Test (8%) | Complement Fixation (Cold) | 0.917 (0.782–0.982) | 0.937 (0.781–0.979) | 0.952 (0.830–0.983) | 0.890 (0.716–0.976) | 19.8 (5.0–57.0) | 0.984 (0.889–0.999) |
| Fluorescent Polarization Assay | Buffered Acidified Plate Antigen | 0.556 (0.421–0.685) | 0.829 (0.693–0.932) | 0.818 (0.676–0.927) | 0.573 (0.442–0.700) | 4.5 (2.1–12.2) | 0.731 (0.616–0.836) |
| Fluorescent Polarization Assay | Card Test (8%) | 0.480 (0.347–0.617) | 0.931 (0.828–0.987) | 0.905 (0.771–0.982) | 0.565 (0.444–0.684) | 9.6 (3.3–53.0) | 0.767 (0.663–0.854) |
| Fluorescent Polarization Assay | Complement Fixation (Cold) | 0.564 (0.431–0.694) | 0.753 (0.605–0.876) | 0.759 (0.615–0.880) | 0.556 (0.421–0.689) | 3.2 (1.6–7.3) | 0.685 (0.570–0.796) |
| Buffered Acidified Plate Antigen | Complement Fixation (Cold) | 0.980 (0.910–0.984) | 0.833 (0.635–0.970) | 0.890 (0.752–0.981) | 0.967 (0.855–0.977) | 8.2 (3.0–49.0) | 0.950 (0.848–0.999) |
| Card Test (8%) | Buffered Acidified Plate Antigen | 0.898 (0.757–0.981) | 0.936 (0.778–0.979) | 0.952 (0.831–0.983) | 0.869 (0.690–0.975) | 19.8 (4.9–56.0) | 0.987 (0.907–0.999) |
| Card Test (8%) | Complement Fixation (Cold) | 0.918 (0.782–0.982) | 0.936 (0.780–0.978) | 0.952 (0.835–0.983) | 0.890 (0.714–0.976) | 20.0 (5.0–57.5) | 0.950 (0.848–0.999) |
| Fluorescent Polarization Assay | Buffered Acidified Plate Antigen | 0.556 (0.423–0.685) | 0.831 (0.696–0.930) | 0.817 (0.675–0.926) | 0.573 (0.444–0.700) | 4.5 (2.1–12.7) | 0.931 (0.855–0.985) |
| Fluorescent Polarization Assay | Card Test (8%) | 0.481 (0.347–0.616) | 0.930 (0.828–0.987) | 0.905 (0.772–0.982) | 0.566 (0.444–0.685) | 9.5 (3.3–55.0) | 0.932 (0.854–0.985) |
| Fluorescent Polarization Assay | Complement Fixation (Cold) | 0.564 (0.430–0.695) | 0.753 (0.605–0.877) | 0.759 (0.615–0.879) | 0.556 (0.423–0.687) | 3.2 (1.6–7.3) | 0.926 (0.853–0.981) |

**Table S7.** Logistic model performance and Bayesian *p*–values for predictors included in the models.

| Assay | Bayesian R² | LOO Adjusted R² | Sex (Male) | Age (Subadult) | Strain (1330) |
|---|---|---|---|---|---|
| Buffered Acidified Plate Antigen | 0.87 | 0.92 | 0.0665 | 0.2754 | 0.3431 |
| Card Test (8%) | 0.63 | 0.63 | 0.1897 | 0.0779 | 0.1586 |
| Complement Fixation (Cold) | 0.65 | 0.59 | 0.0398 | 0.0580 | 0.4530 |
| Fluorescent Polarization Assay | 0.87 | 0.88 | 0.0524 | 0.3367 | 0.3629 |
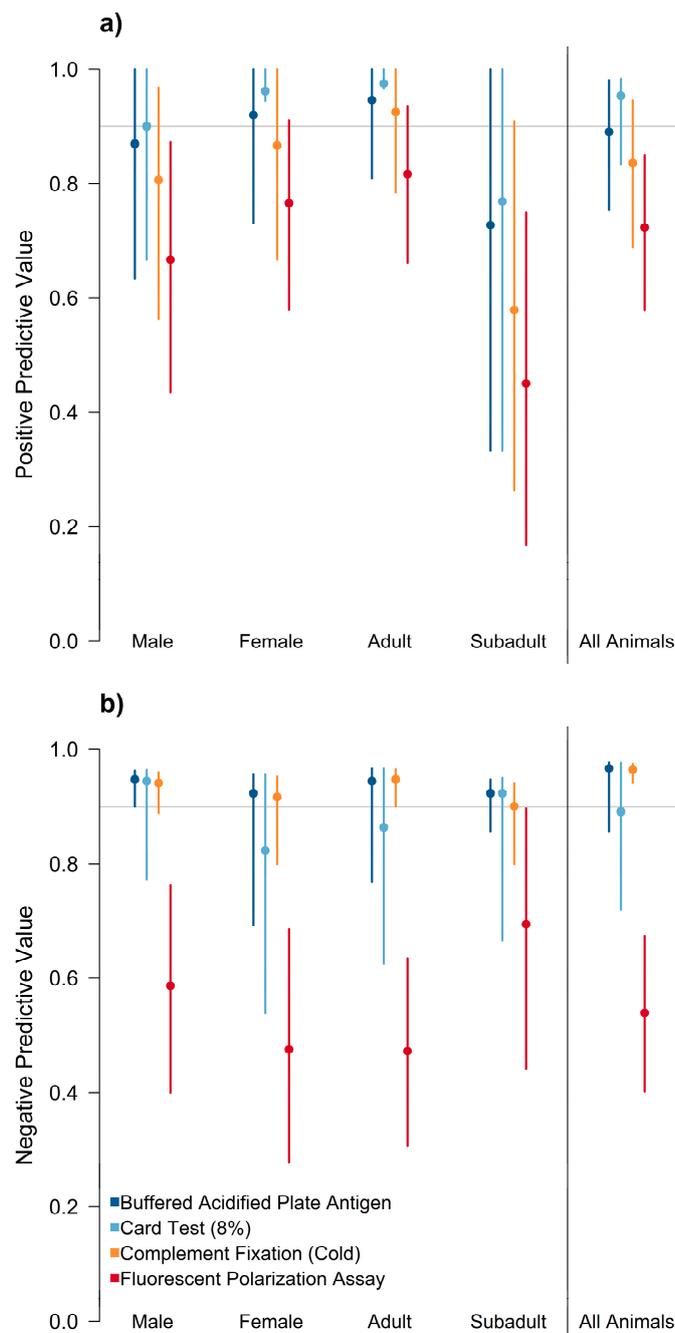


**Figure S1.** Positive and negative predictive values for the buffered acidified plate antigen test (BAPA), the 8% card agglutination test (card test), the fluorescence polarization assay (FPA) in tube format, and the *Brucella abortus/suis* complement fixation (CF) test. (**a**) Positive predictive value had generally large variation among age and sex except for the card test which had median above 0.95. All assays appeared to have the lowest positive predictive value for subadults although variance around the estimates was large. (**b**) Negative predictive value for three of the assays, BAPA, card

test, and CF, was generally above 0.90 with all having the highest negative predictive value for males.
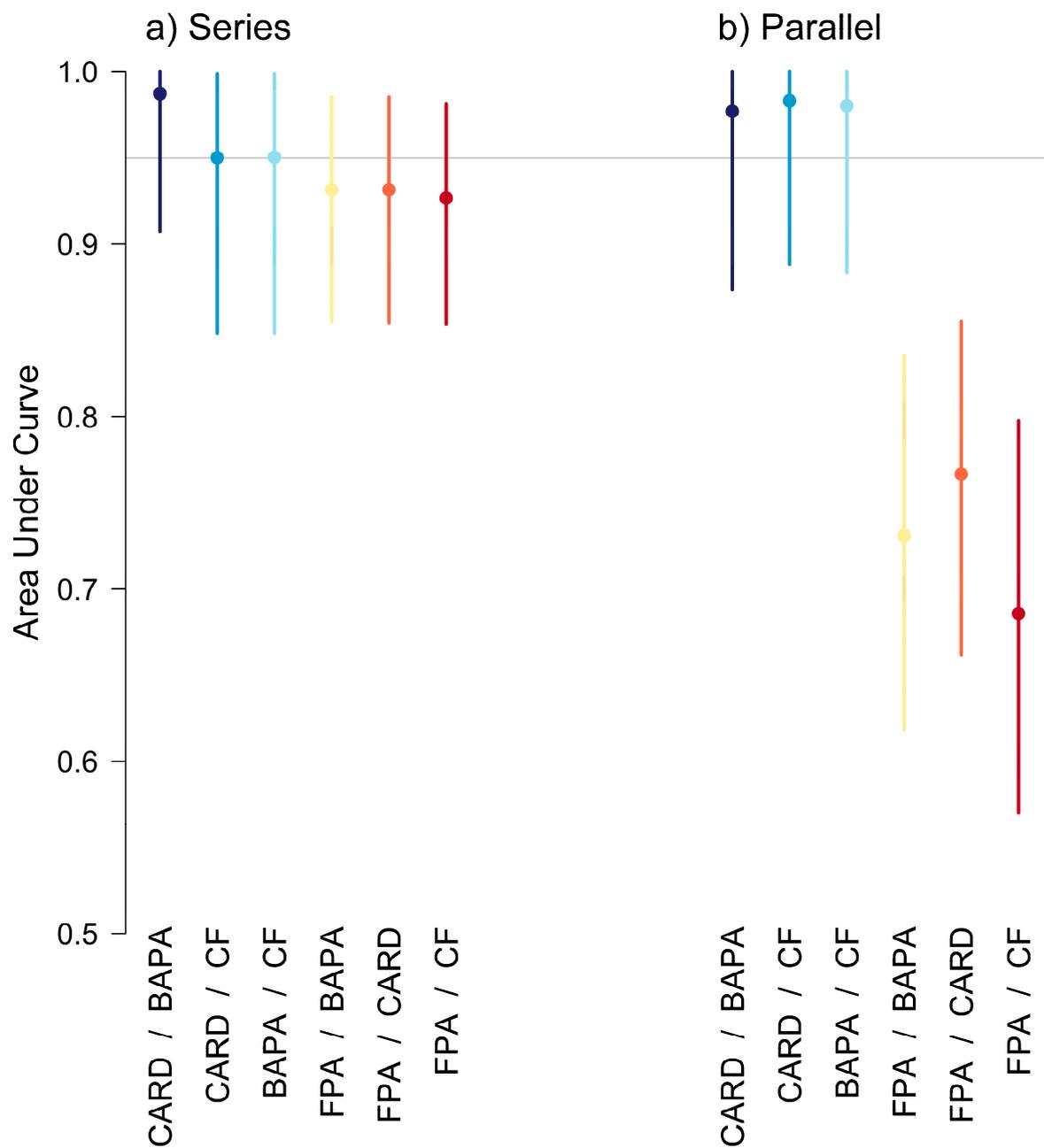


**Figure S2.** (**a**,**b**) The predictive capacity measured using area under the curve of the buffered acidified plate antigen test (BAPA), the 8% card agglutination test (card test), the fluorescence polarization assay (FPA) in tube format, and the *Brucella abortus/suis* complement fixation (CF) test when interpreted in parallel or series. The card test interpreted in series with BAPA had the highest area under the curve. However, the card test interpreted in parallel with BAPA or CF and BAPA interpreted in parallel also have high predictive capacity.
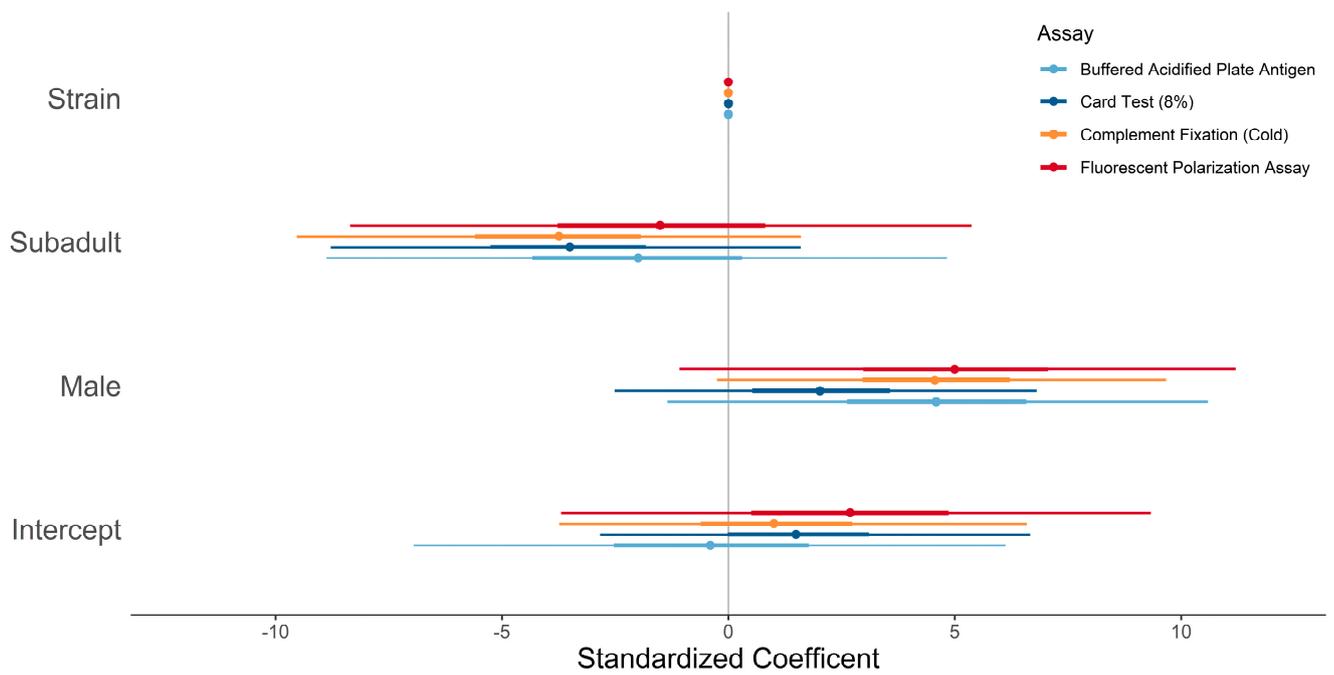
**Figure S3.** Predicted posterior distributions of standardized coefficients (median) with 50% credible interval (thick line) and 95% credible interval (thin line) for predictors used in the logistic models accounting for repeated measures. Coefficient values for subadult are relative to adult animals and coefficient values for male are relative to female animals.