

Supplementary Materials

Supplementary Table S1. The number and percentage of 260 sera from five dog groups that tested positive by modified ELISAs (antibody detection) using other four local isolates of leptospiral serovars and four common *Leptospira* serovars used in the leptospirosis vaccine for dogs.

Groups	Group 1	Group 2	Group 3		Group 4	Group 5	Total
	Infected dogs from Nan province confirmed by positive PCR and isolation	Unvaccinated dogs from Nan province	Vaccinated dogs from Bangkok		Unvaccinated dogs from non-endemic areas	Unvaccinated puppies from non-endemic areas	
Number of samples	6	21	23*	89**	108	13	260
Methods (modified ELISAs), protein preparation, and local isolates of serovars used in the ELISAs							
WCP-Paidjan/IgG-ELISA	6 (6/6; 100%)	13 (13/21; 62%)	5 (5/23; 22%)	18 (18/89; 20%)	0 (0/108; 0%)	0 (0/13; 0%)	42 (42/260; 16%)
WCP-Bataviae/IgG-ELISA	6 (6/6; 100%)	14 (14/21; 67%)	23 (23/112; 21%)	5 (5/89; 6%)	0 (0/108; 0%)	0 (0/13; 0%)	26 (26/260; 10%)
WCP-Mini06/IgG-ELISA	6 (6/6; 100%)	13 (13/21; 62%)	1 (1/23; 4%)	4 (4/89; 4%)	0 (0/108; 0%)	0 (0/13; 0%)	24 (24/260; 9%)
WCP-Mini13/IgG-ELISA	6 (6/6; 100%)	15 (15/21; 71%)	5 (5/112; 4%)	4 (4/89; 4%)	0 (0/108; 0%)	0 (0/13; 0%)	26 (26/260; 10%)
TMP-Paidjan/IgG-ELISA	6 (6/6; 100%)	13 (13/21; 62%)	2 (2/23; 9%)	8 (8/89; 9%)	0 (0/108; 0%)	0 (0/13; 0%)	29 (29/260; 11%)
TMP-Bataviae/IgG-ELISA	6 (6/6; 100%)	12 (12/21; 57%)	10 (10/112; 9%)	9 (9/89; 10%)	0 (0/108; 0%)	0 (0/13; 0%)	29 (29/260; 11%)
TMP-Mini06/IgG-ELISA	6 (6/6; 100%)	11 (11/21; 52%)	0 (0/23; 0%)	0 (0/89; 0%)	0 (0/108; 0%)	0 (0/13; 0%)	17 (17/260; 7%)
TMP-Mini13/IgG-ELISA	6 (6/6; 100%)	12 (12/21; 57%)	0 (0/23; 0%)	0 (0/89; 0%)	0 (0/108; 0%)	0 (0/13; 0%)	18 (18/260; 7%)
OMP-Paidjan/IgG-ELISA	6 (6/6; 100%)	13 (13/21; 62%)	0	0	0 (0/108; 0%)	0 (0/13; 0%)	19 (19/260; 7%)

			(0/23; 0%)	(0/89; 0%)			
			0 (0/112; 0%)				
OMP-Bataviae/IgG-ELISA	6 (6/6; 100%)	9 (9/21; 43%)	0 (0/23; 0%)	0 (0/89; 0%)	0 (0/108; 0%)	0 (0/13; 0%)	15 (15/260; 6%)
			0 (0/112; 0%)				
OMP-Mini06/IgG-ELISA	6 (6/6; 100%)	10 (10/21; 48%)	0 (0/23; 0%)	0 (0/89; 0%)	0 (0/108; 0%)	0 (0/13; 0%)	16 (16/260; 6%)
			0 (0/112; 0%)				
OMP-Mini13/IgG-ELISA	6 (6/6; 100%)	12 (12/21; 57%)	0 (0/23; 0%)	0 (0/89; 0%)	0 (0/108; 0%)	0 (0/13; 0%)	18 (18/260; 7%)
			0 (0/112; 0%)				
Methods (modified ELISAs), protein preparation, and common <i>Leptospira</i> serovars in the canine leptospirosis vaccine used in the ELISAs							
WCP-Ictero/IgG-ELISA	0 (0/6; 0%)	0 (0/21; 0%)	23 (23/23; 100%)	74 (74/89; 83%)	31 (31/108; 29%)	0 (0/13; 0%)	128 (128/260; 49%)
			97 (97/112; 87%)				
WCP-Pom/IgG-ELISA	0 (0/6; 0%)	0 (0/21; 0%)	23 (23/23; 100%)	67 (67/89; 75%)	28 (28/108; 26%)	0 (0/13; 0%)	118 (118/260; 43%)
			90 (90/112; 80%)				
WCP-Grip/IgG-ELISA	0 (0/6; 0%)	0 (0/21; 0%)	23 (23/23; 100%)	70 (70/89; 79%)	29 (29/108; 27%)	0 (0/13; 0%)	122 (122/260; 47%)
			93 (93/112; 83%)				
WCP-Can/IgG-ELISA	0 (0/6; 0%)	0 (0/21; 0%)	23 (23/23; 100%)	77 (77/89; 87%)	34 (34/108; 31%)	0 (0/13; 0%)	134 (134/260; 52%)
			100 (100/112; 89%)				
TMP-Ictero/IgG-ELISA	0 (0/6; 0%)	0 (0/21; 0%)	20 (20/23; 87%)	69 (69/89; 78%)	29 (29/108; 27%)	0 (0/13; 0%)	118 (118/260; 43%)
			89 (89/112; 79%)				
TMP-Pom/IgG-ELISA	0 (0/6; 0%)	0 (0/21; 0%)	18 (18/23; 78%)	64 (64/89; 72%)	25 (25/108; 23%)	0 (0/13; 0%)	107 (107/260; 41%)
			82 (82/112; 73%)				
TMP-Grip/IgG-ELISA	0 (0/6; 0%)	0 (0/21; 0%)	19 (19/23; 83%)	66 (66/89; 74%)	27 (27/108; 25%)	0 (0/13; 0%)	112 (112/260; 43%)
			85 (85/112; 76%)				
TMP-Can/IgG-ELISA	0 (0/6; 0%)	0 (0/21; 0%)	20 (20/23; 87%)	72 (72/89; 81%)	31 (31/108; 29%)	0 (0/13; 0%)	123 (123/260; 47%)
			92 (92/112; 82%)				
OMP-Ictero/IgG-ELISA	0 (0/6; 0%)	0 (0/21; 0%)	17 (17/23; 74%)	64 (64/89; 72%)	26 (26/108; 24%)	0 (0/13; 0%)	107 (107/260; 41%)
			81 (81/112; 72%)				
OMP-Pom/IgG-ELISA	0 (0/6; 0%)	0 (0/21; 0%)	15 (15/23; 65%)	59 (74/89; 66%)	23 (23/108; 21%)	0 (0/13; 0%)	97 (97/260; 37%)
			74 (74/112; 66%)				
OMP-Grip/IgG-ELISA	0 (0/6; 0%)	0 (0/21; 0%)	16 (16/23; 70%)	61 (61/89; 69%)	24 (24/108; 22%)	0 (0/13; 0%)	101 (101/260; 39%)

			77 (77/112; 69%)				
			17 (17/23;	68 (68/89;			
			74%)	76%)			
			85 (85/112; 76%)				
OMP-Can/IgG-ELISA	0 (0/6; 0%)	0 (0/21; 0%)			29 (29/108; 27%)	0 (0/13; 0%)	114 (114/260; 44%)

N/A; Not applicable. WCP; whole-cell protein, TMP; total membrane protein, OMP; outer membrane protein. Ictero; Icterohaemorrhagiae, Pom; Pomona, Grip; Grippotyphosa, Can; Canicola. *; Twenty-three serum samples from group 3 were examined by both MAT and ELISAs and used for preliminary evaluation of the effectiveness of diagnostic performance of the modified ELISAs. **; Another 89 serum samples were examined only by ELISAs and were used to screen for leptospiral IgG antibody and confirm the usefulness of the modified ELISAs.

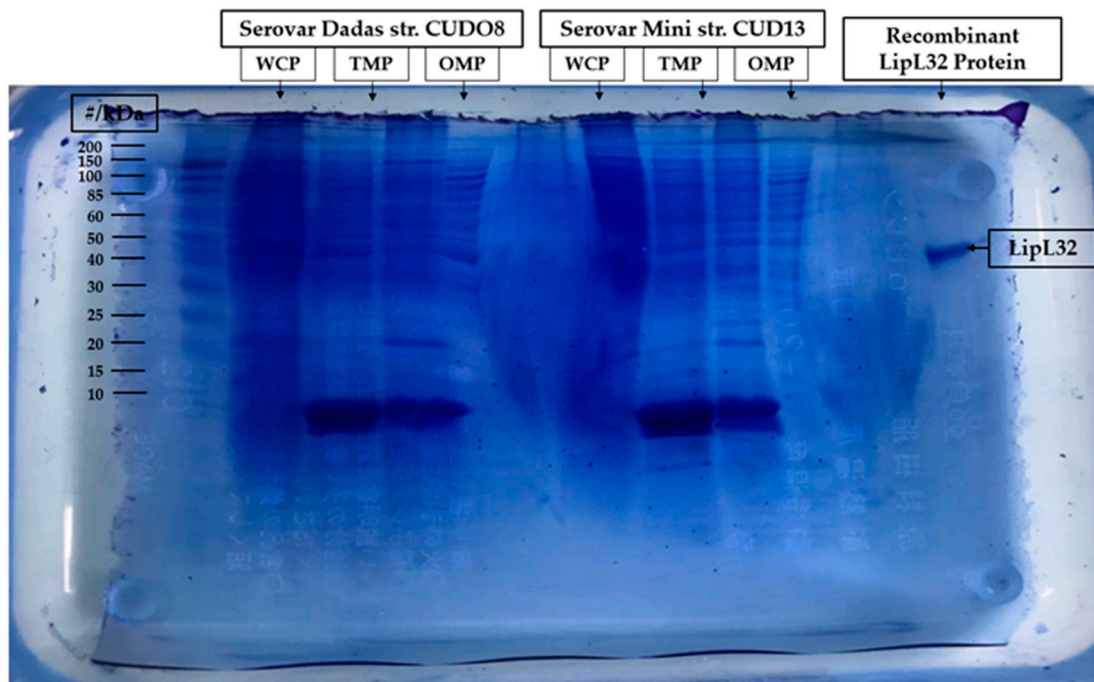
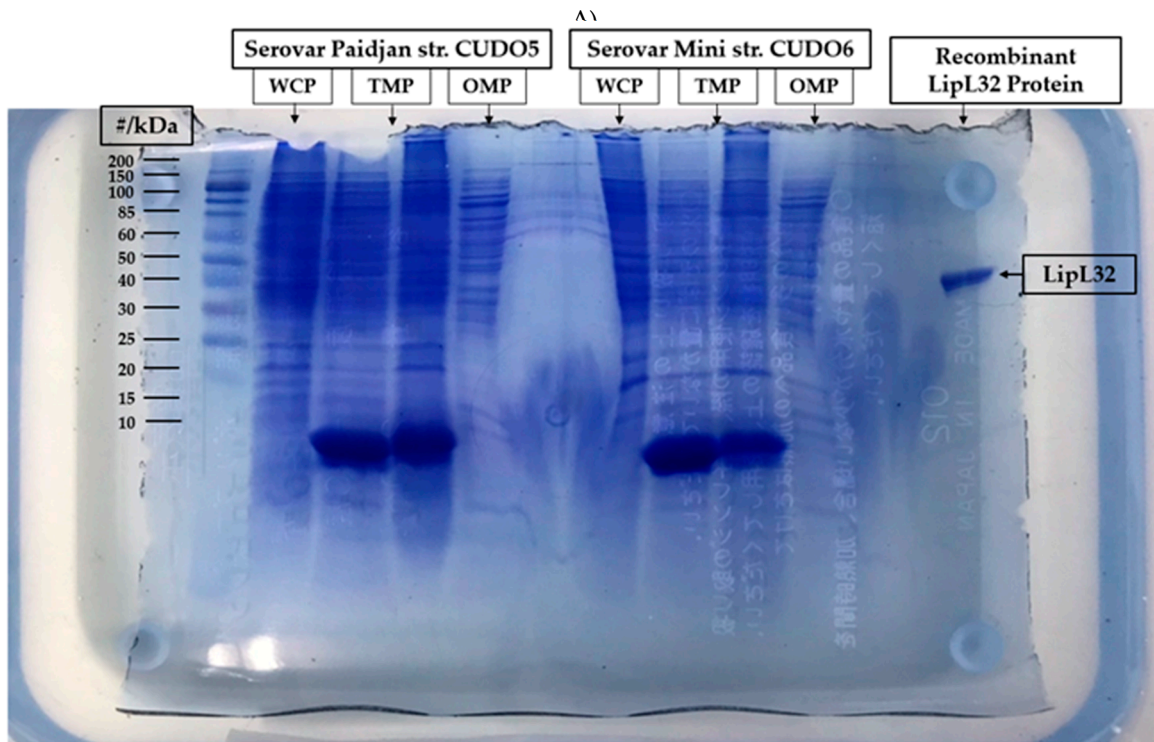
Supplementary Table S2. Evaluation of diagnostic tests, including sensitivity, specificity, PPV, NPV, accuracy and repeatability, and reproducibility (intra- & inter-assay), among the modified ELISAs using the five local Thai isolates of *Leptospira* serovars with 50 sera from three groups of dogs (groups 1, 2 and 3) compared to serum PCR and MAT results.

IgG ELISAs	Evaluation of diagnostic tests														
	Comparison with PCR from urine (n = 27)					Comparison with PCR from sera (n = 50)					Comparison with MAT from sera (n = 50)				
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
WCP-Paidjan	100	38.1	31.6	100	51.9	100	59.1	25.0	100	64.0	57.1	55.6	33.3	76.9	56.0
WCP-Dadas	100	33.3	30	100	48.1	100	40.9	18.8	100	48.0	85.7	44.4	37.5	88.9	56.0
WCP-Bataviae	100	33.3	30	100	48.1	100	65.9	28.6	100	70.0	57.1	63.9	38.1	79.3	62.0
WCP-Mini06	100	38.1	31.6	100	51.8	100	68.2	30.0	100	72.0	57.1	66.7	40.0	80.0	64.0
WCP-Mini13	100	28.6	28.6	100	44.4	100	63.6	27.3	100	68.0	64.3	63.9	40.9	82.1	64.0
TMP-Paidjan	100	38.1	31.6	100	51.9	100	65.9	28.6	100	70.0	64.3	66.7	42.9	82.8	58.0
TMP-Dadas	100	38.1	31.6	100	51.9	100	56.8	24.0	100	62.0	64.3	55.6	36.0	80.0	66.0
TMP-Bataviae	100	42.9	33.3	100	55.6	100	68.9	30.0	100	72.0	57.1	66.7	40.0	80.0	64.0
TMP-Mini06	100	47.6	35.3	100	59.3	100	75.0	35.3	100	78.0	42.9	69.4	35.3	75.8	62.0
TMP-Mini13	100	42.9	33.3	100	55.6	100	72.7	33.3	100	76.0	50.0	69.4	38.9	78.1	64.0
OMP-Paidjan	100	38.1	31.6	100	51.9	100	70.5	31.6	100	74.0	57.1	69.4	42.1	80.7	66.0
OMP-Dadas	100	28.6	28.6	100	44.4	100	65.9	28.6	100	70.0	64.3	66.7	42.9	82.8	66.0
OMP-Bataviae	100	57.1	40	100	54.5	100	79.6	40.0	100	82.0	35.7	72.2	33.3	74.3	62.0
OMP-Mini06	100	52.4	37.5	100	63.0	100	77.3	37.5	100	80.0	42.9	72.2	37.5	76.5	64.0
OMP-Mini13	100	42.9	33.3	100	55.6	100	72.7	33.3	100	76.0	50.0	69.4	38.9	78.1	64.0

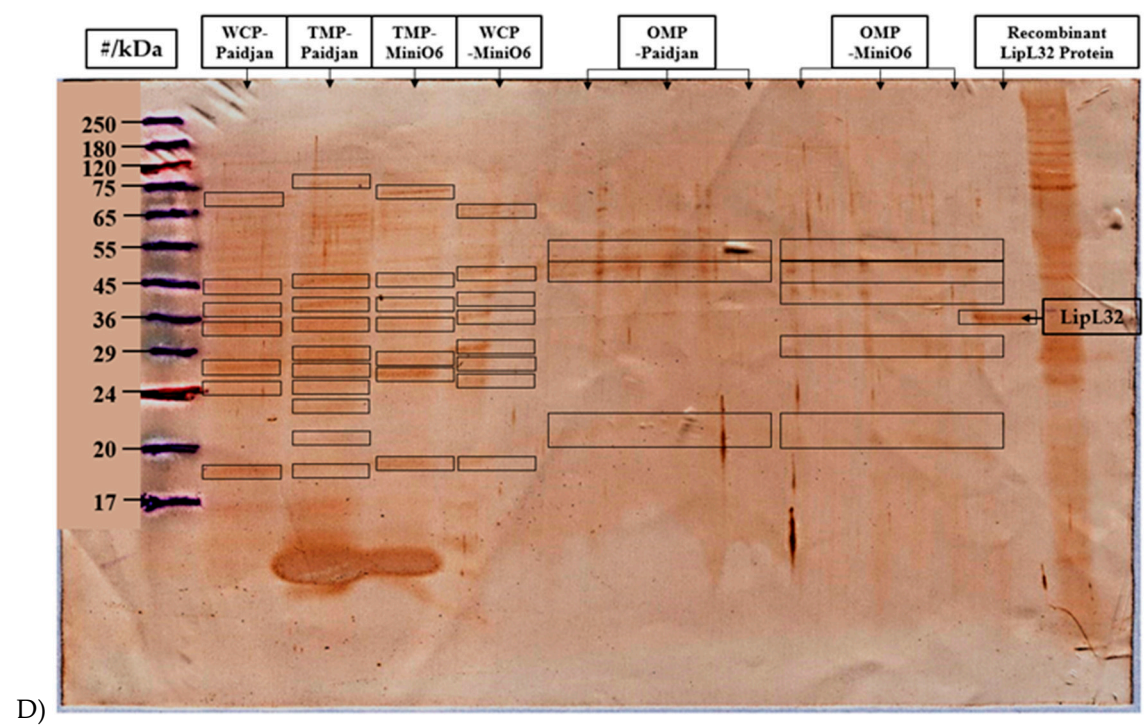
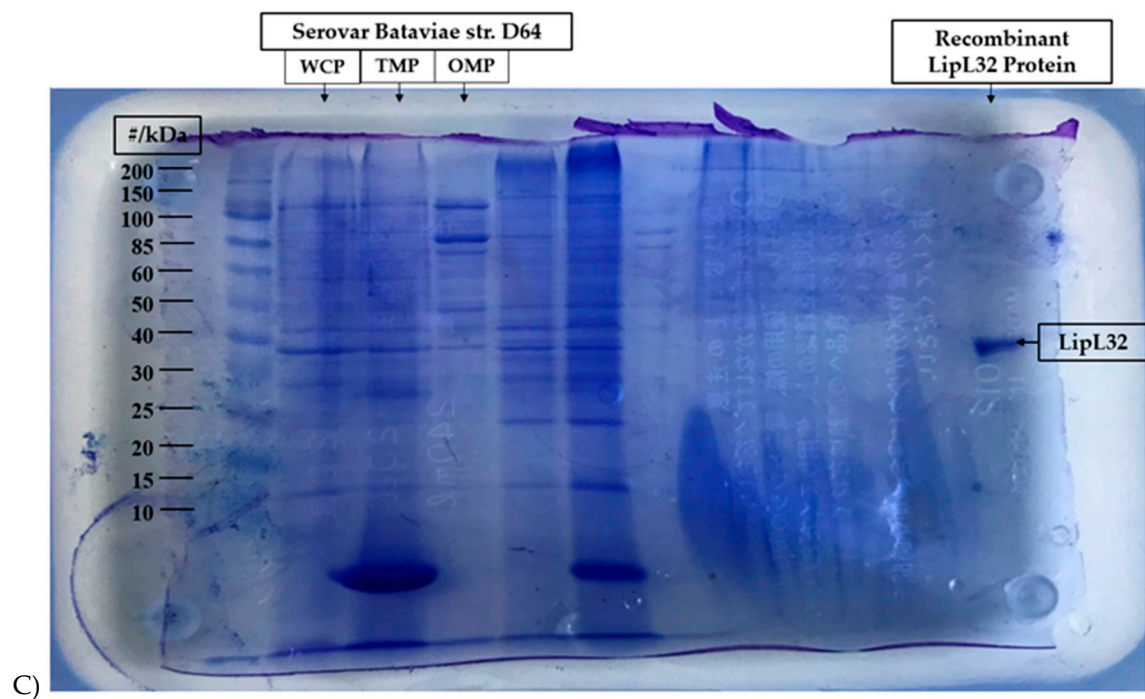
IgG-ELISA assays	Diagnostic precision	
	Intra-assay/repeatability (%)	Inter-assay/reproducibility (%)
WCP/IgG-ELISAs	6.3	11.0
TMP/IgG-ELISAs	4.1	9.3
OMP/IgG-ELISAs	2.6	8.5

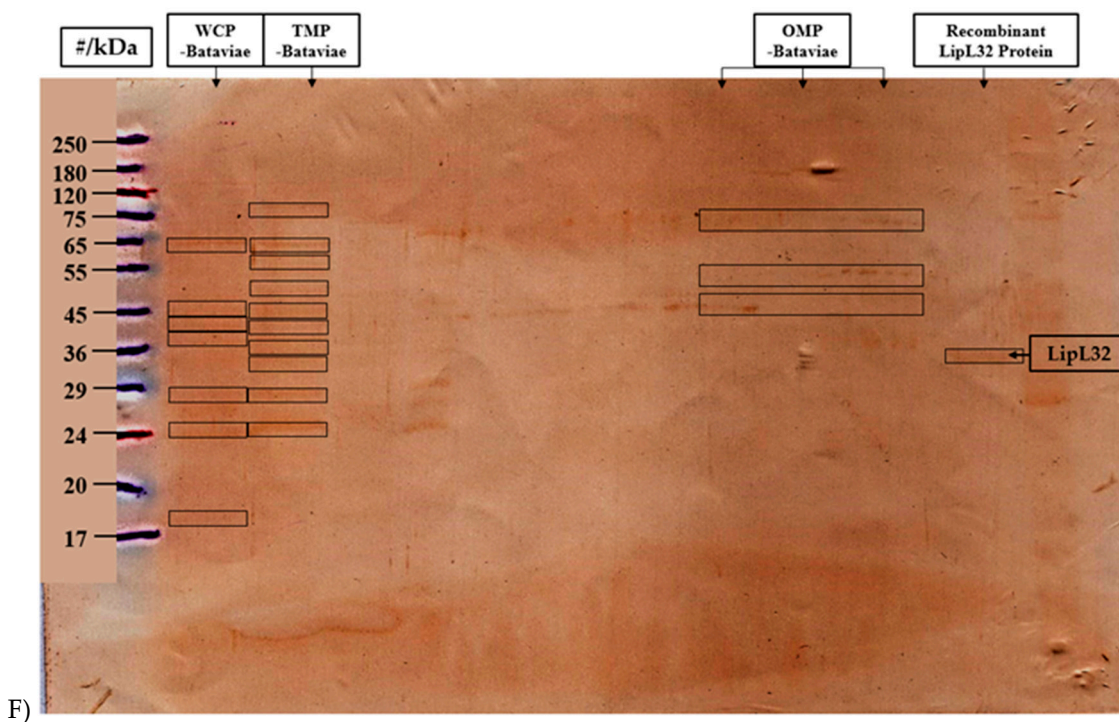
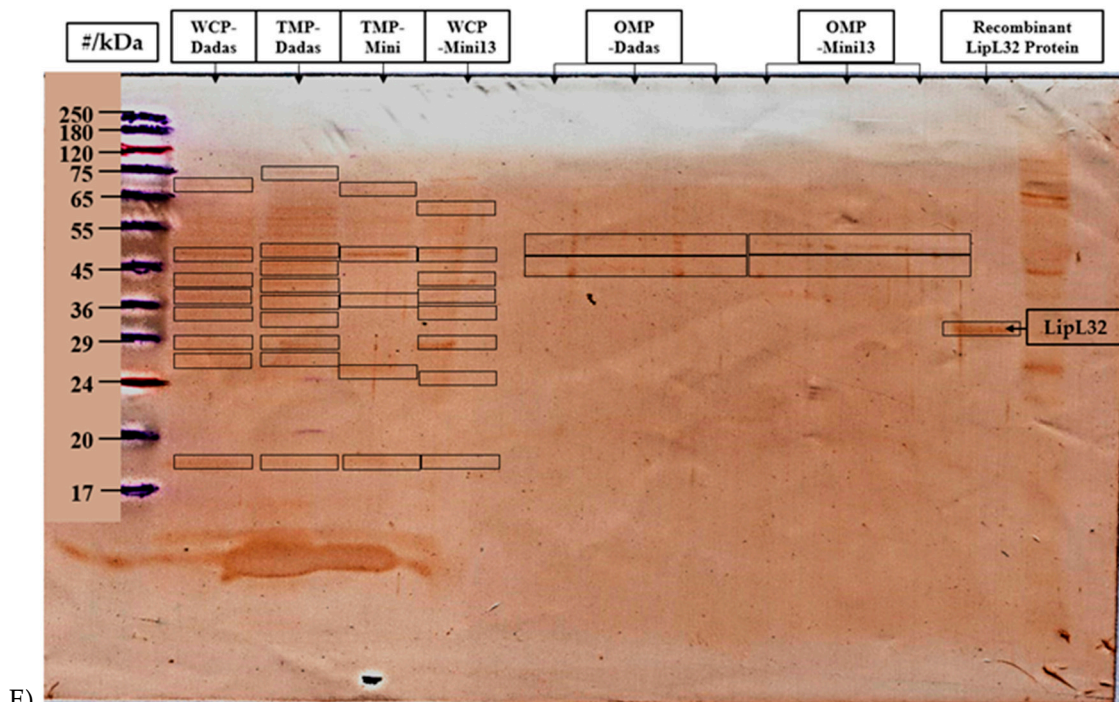
Supplementary Table S3. Cohen's kappa statistics of modified ELISAs against PCR (agent detection) and MAT (antibody detection) for 50 sera from three dog groups (groups 1, 2, and 3).

ELISAs	Cohen's kappa index		
	Comparison with PCR from urine (n=27)	Comparison with PCR from sera (n=50)	Comparison with MAT from sera (n=50)
WCP-Paidjan/IgG-ELISA	0.215	0.257	0.104
WCP-Dadas/IgG-ELISA	0.182	0.142	0.217
WCP-Bataviae/IgG-ELISA	0.182	0.317	0.182
WCP-Mini06/IgG-ELISA	0.215	0.340	0.211
WCP-Mini13/IgG-ELISA	0.151	0.300	0.240
TMP-Paidjan/IgG-ELISA	0.215	0.317	0.269
TMP-Dadas/IgG-ELISA	0.215	0.240	0.160
TMP-Bataviae/IgG-ELISA	0.250	0.340	0.211
TMP-Mini06/IgG-ELISA	0.288	0.419	0.115
TMP-Mini13/IgG-ELISA	0.250	0.390	0.179
OMP-Paidjan/IgG-ELISA	0.215	0.364	0.240
OMP-Dadas/IgG-ELISA	0.151	0.317	0.269
OMP-Bataviae/IgG-ELISA	0.372	0.483	0.078
OMP-Mini06/IgG-ELISA	0.328	0.449	0.144
OMP-Mini13/IgG-ELISA	0.250	0.390	0.179

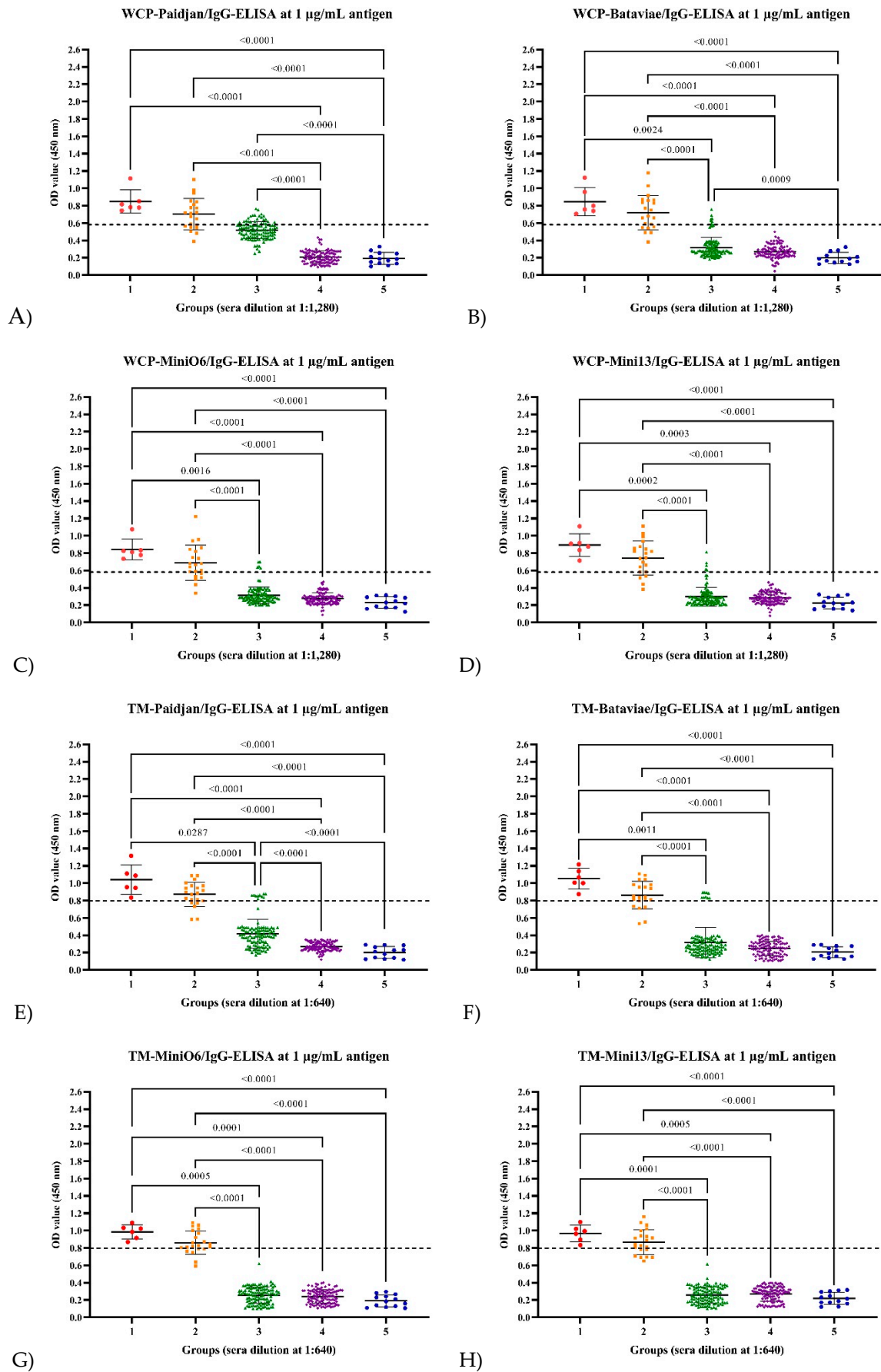


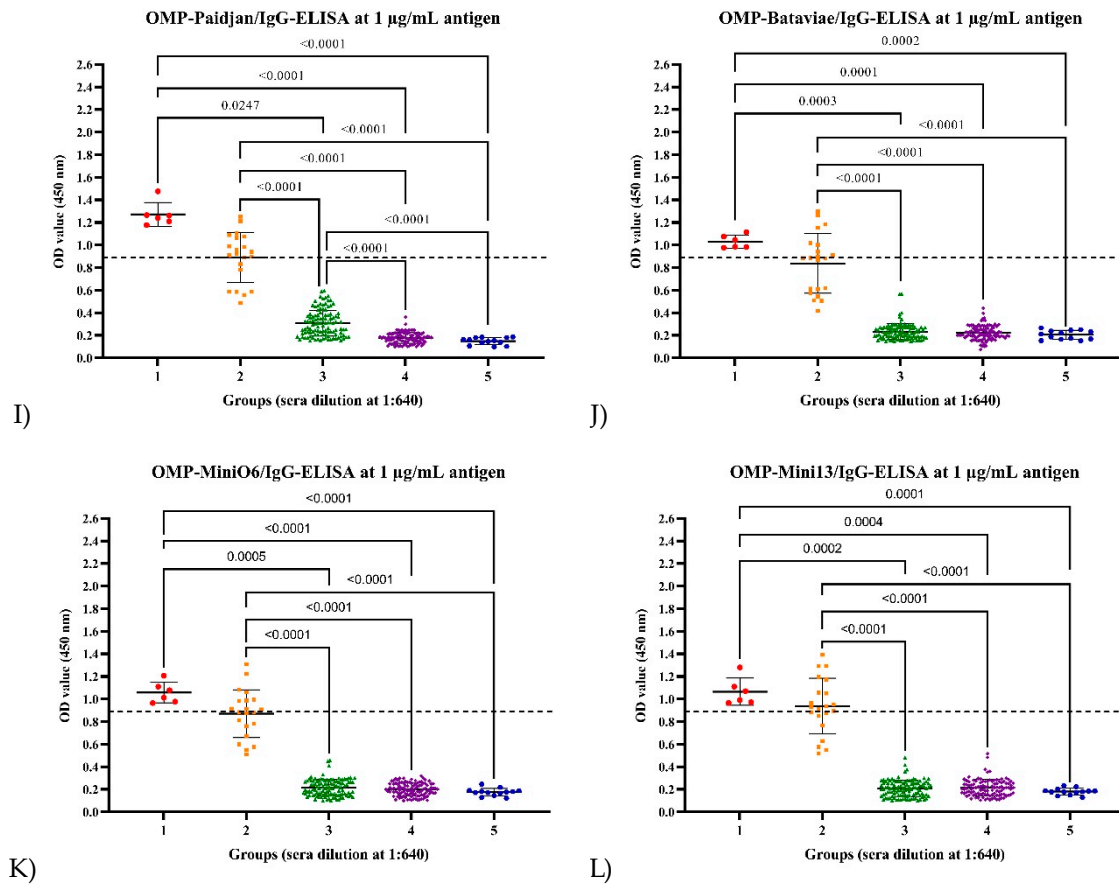
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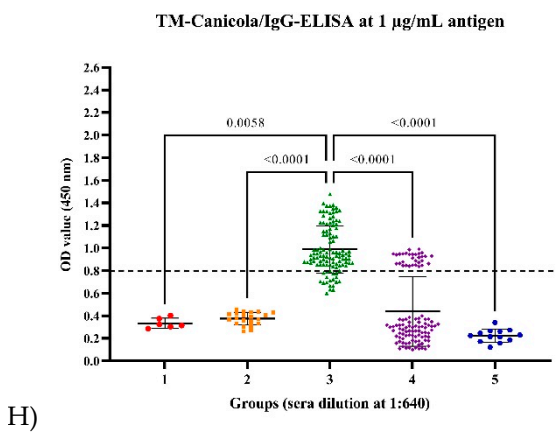
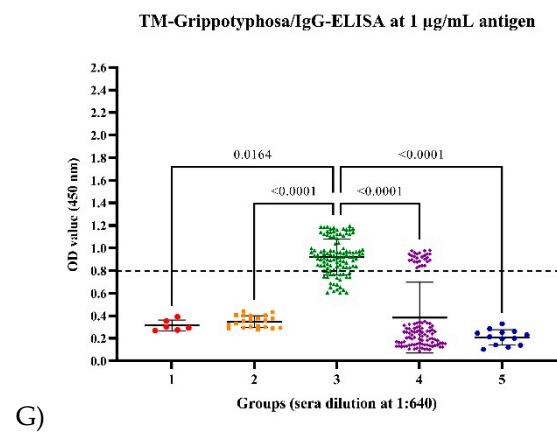
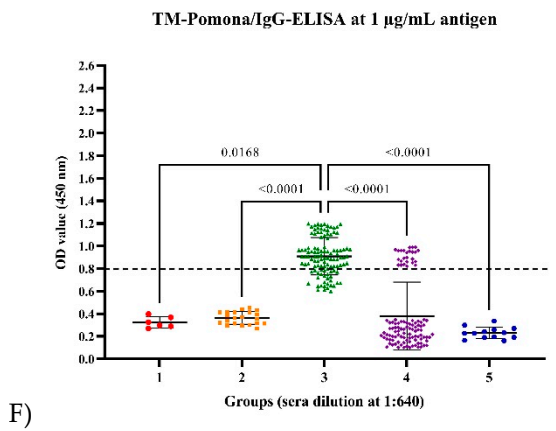
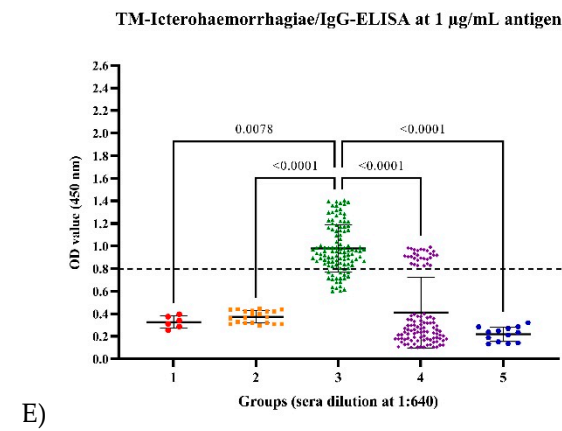
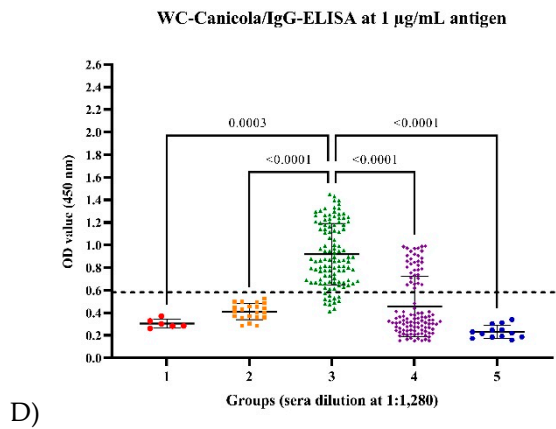
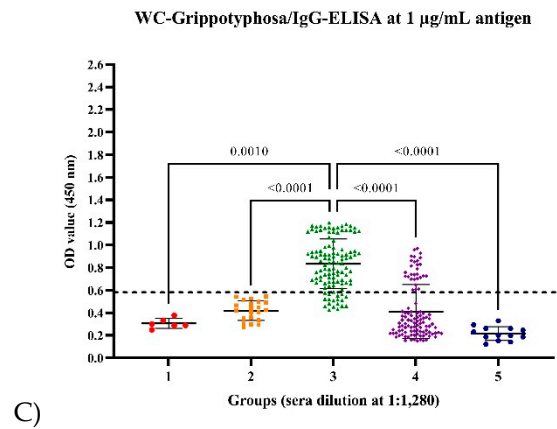
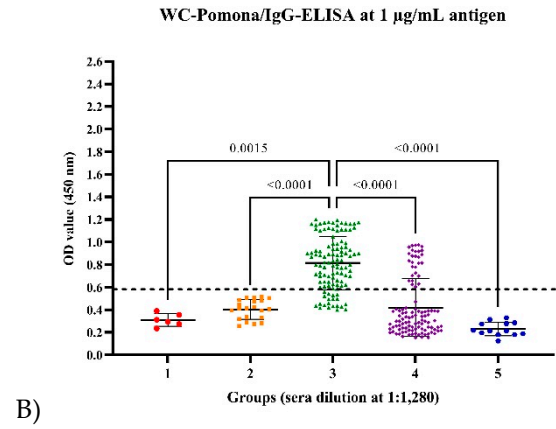
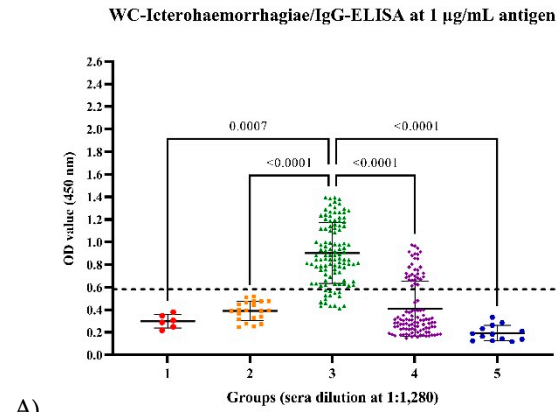


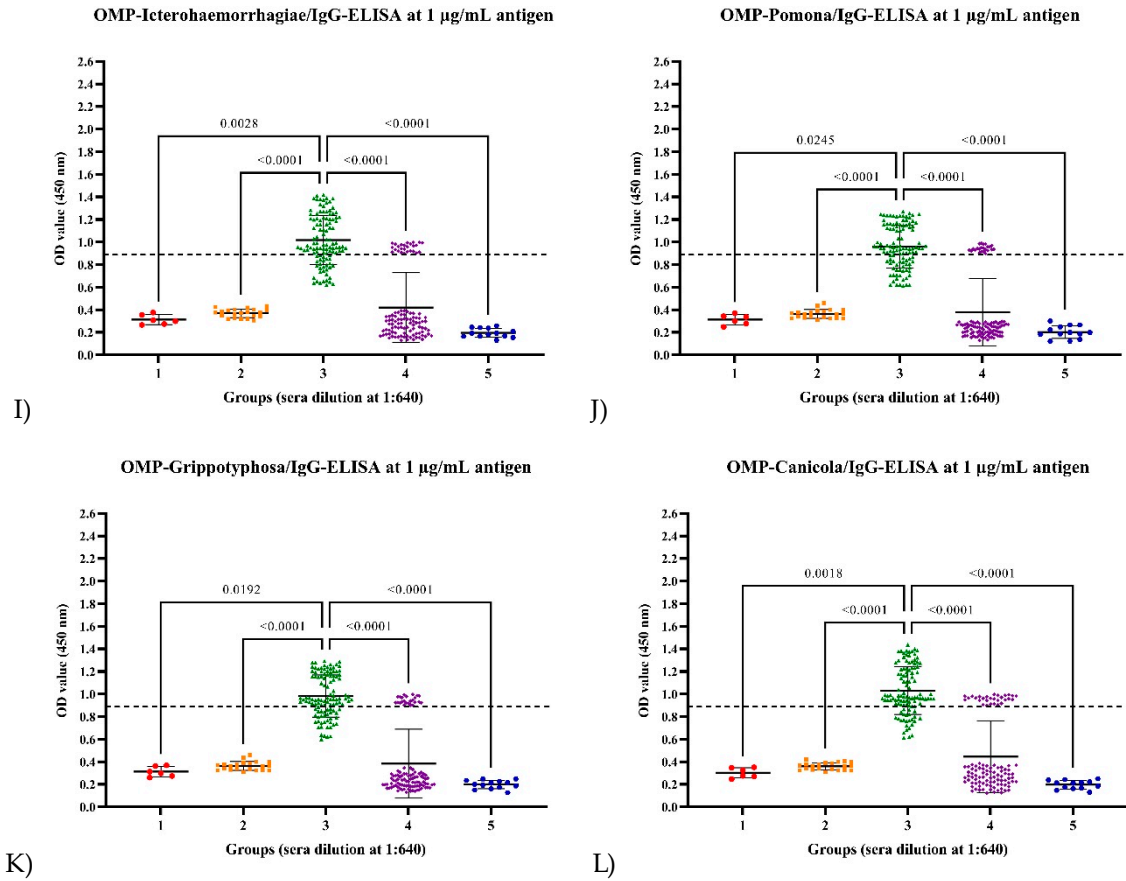
Supplementary Figure S1. Protein component confirmation of the five local Thai isolates of *Leptospira interrogans* and *Leptospira weilii* serovars, including serovars Paidjan, Dadas, Bataviae, Mini06, and Mini13 by 12.5% SDS-PAGE with Coomassie Brilliant Blue R-250 (A, B, and C) and Western blotting with serum from a dog confirmed as positive for leptospirosis by isolation and PCR, which was followed by horseradish peroxidase (HRP) conjugated goat anti-dog IgG antibody and visualized by adding DAB substrate (D, E and F).



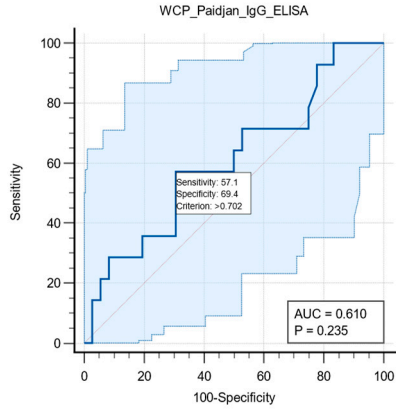


Supplementary Figure S2. The levels of IgG antibody detected in modified ELISAs against whole-cell protein (WCP), total membrane protein (TMP), and outer membrane protein (OMP) from the other four local Thai isolates of *Leptospira* serovars, including Paidjan, Bataviae, Mini06, and Mini13, at 1:1,280, 1:640 and 1:640 sera dilutions. Comparisons among 260 sera from five groups consisting of dogs from Nan province confirmed as infected by PCR and isolation (Group 1), unvaccinated dogs from Nan province (Group 2), vaccinated dogs from Bangkok (Group 3), unvaccinated dogs from non-endemic areas (Group 4), and unvaccinated puppies from non-endemic areas (Group 5).

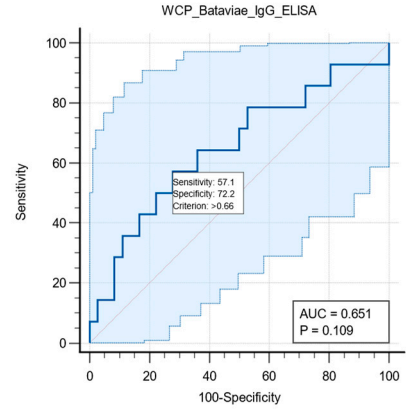




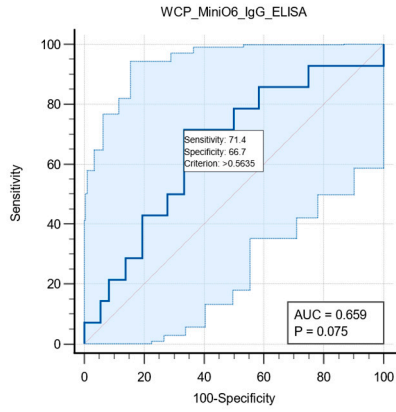
Supplementary Figure S3. The levels of IgG antibody in modified ELISAs against whole-cell protein (WCP), total membrane proteins (TMP), and outer membrane protein (OMP) preparations from four common *Leptospira* serovars used in the leptospirosis vaccine for dogs, including Icterohaemorrhagiae, Pomona, Grippotyphosa and Canicola at 1:1,280, 1:640 and 1:640 sera dilutions. Comparisons among 260 sera from five groups consisting of dogs from Nan province confirmed as infected by PCR and isolation (Group 1), unvaccinated dogs from Nan province (Group 2), vaccinated dogs from Bangkok (Group 3), unvaccinated dogs from the non-endemic areas (Group 4), and unvaccinated puppies from non-endemic areas (Group 5).



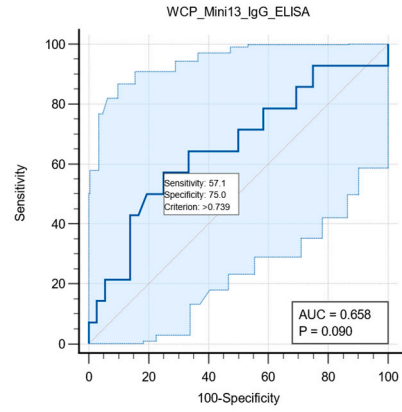
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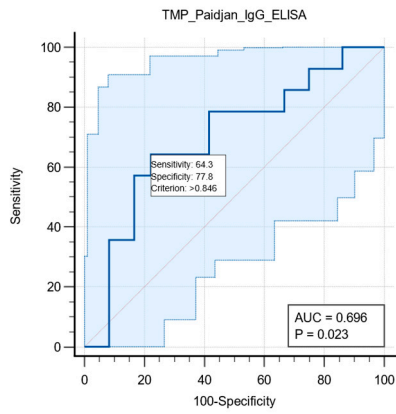
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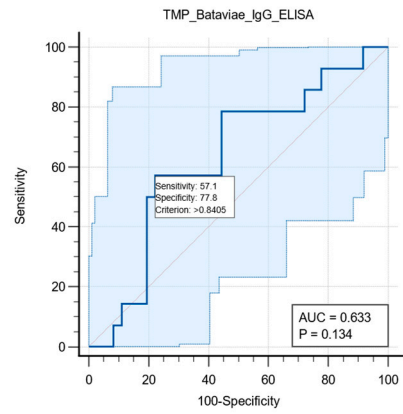
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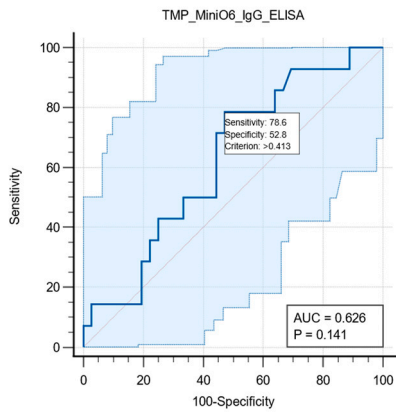
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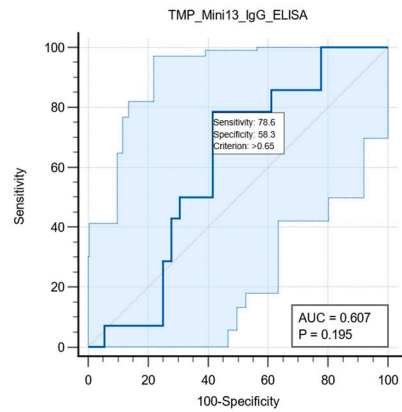
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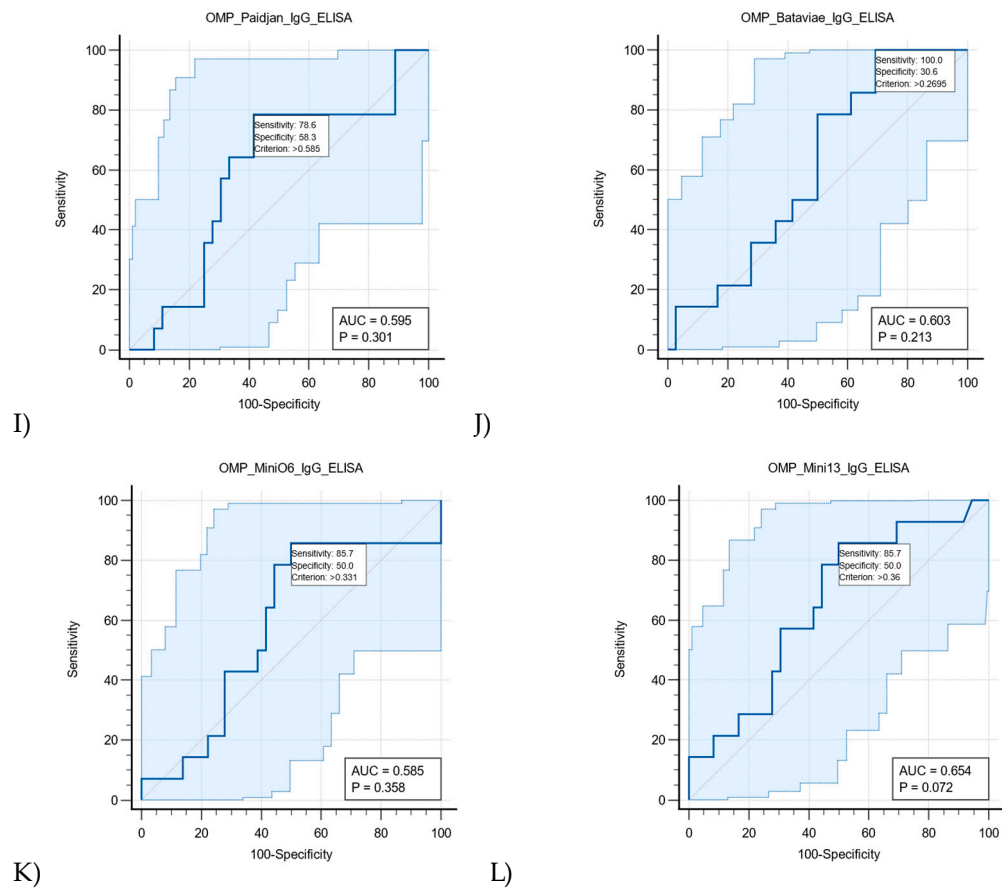
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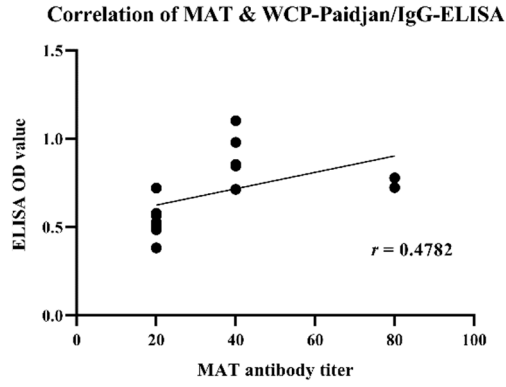
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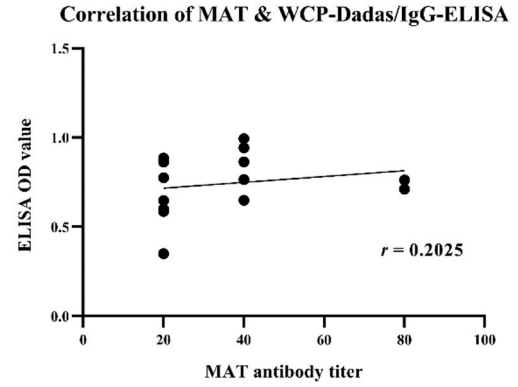
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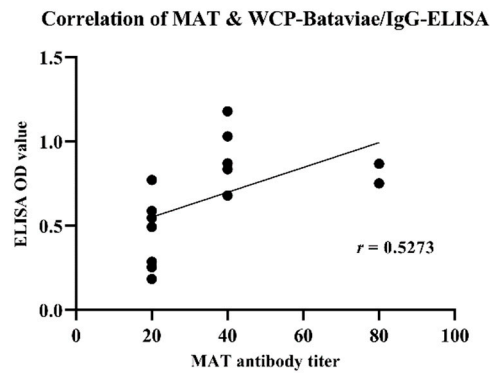
Supplementary Figure S4. Receiver–operator curve (ROC) and area under the curve of ROC (AUC) of modified ELISAs against whole-cell protein (WCP), total membrane protein (TMP), and outer membrane protein (OMP) preparations from the four local Thai isolates of *Leptospira* serovars, including Paidjan, Bataviae, Mini06, and Mini13, at 1:1,280, 1:640 and 1:640 sera dilutions, with 50 sera from three groups consisting of dogs from Nan province confirmed as infected by PCR and isolation (Group 1), unvaccinated dogs from Nan province (Group 2), and vaccinated dogs from Bangkok (Group 3).



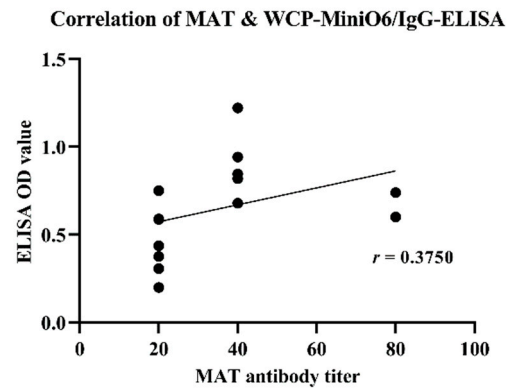
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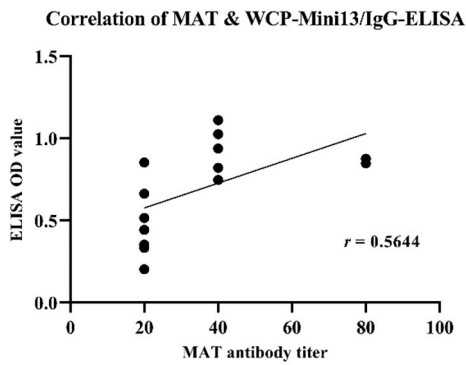
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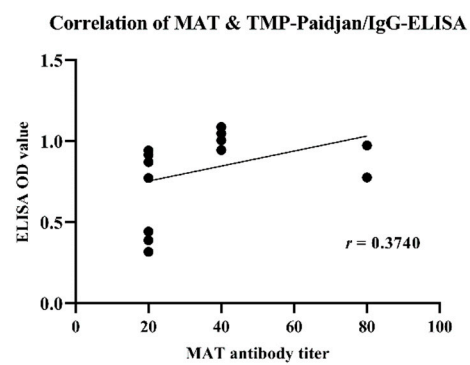
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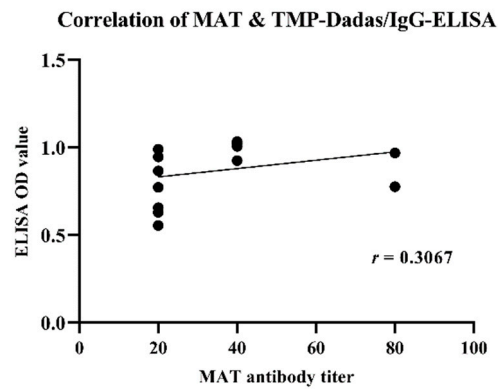
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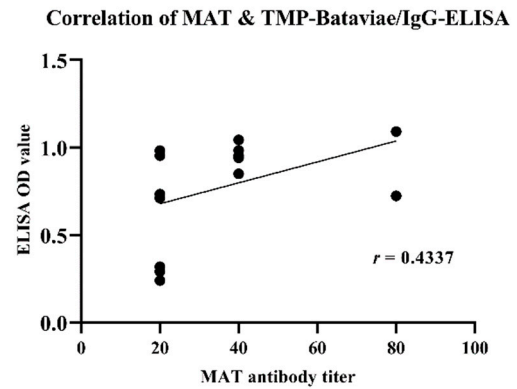
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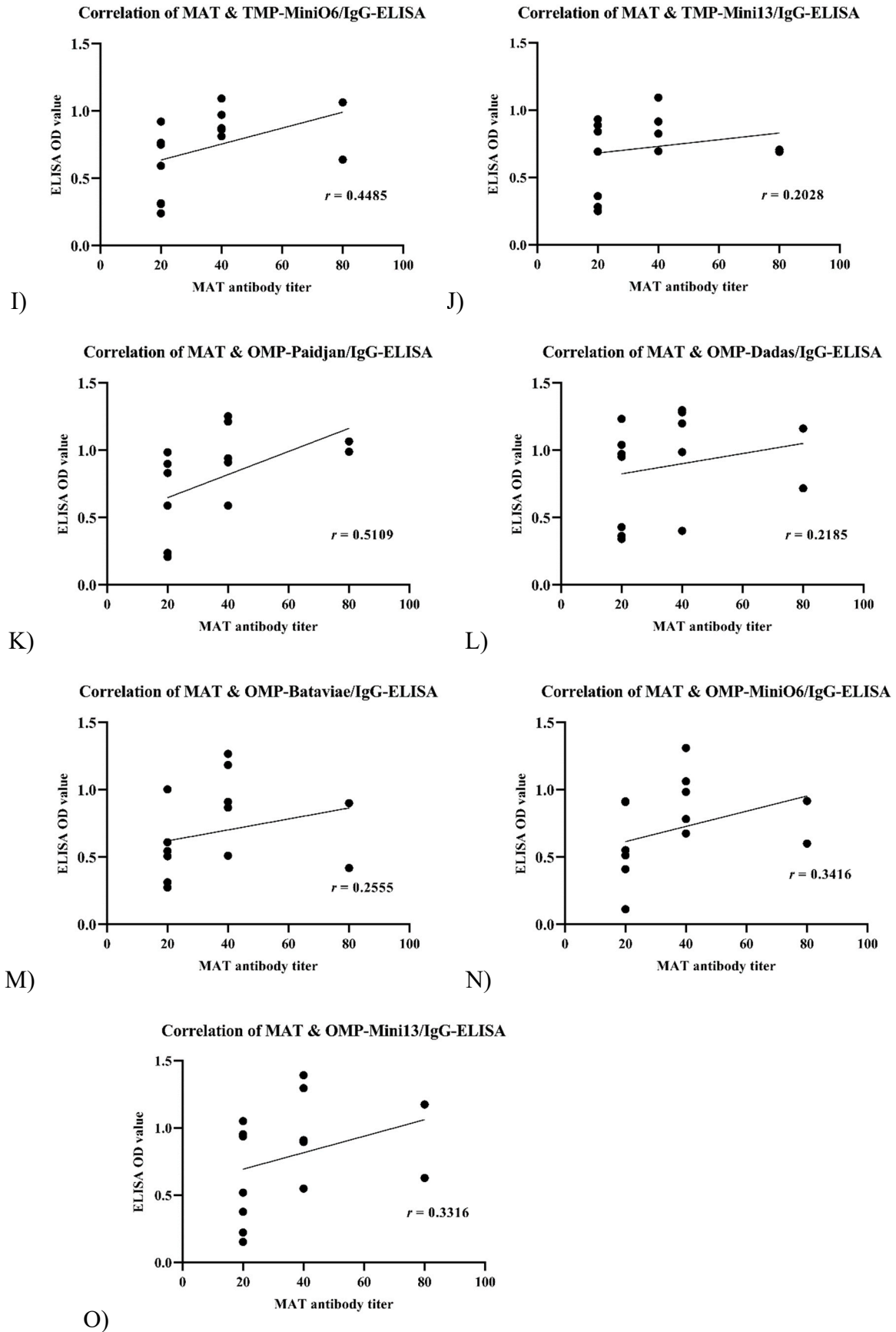
F)



G)



H)



Supplementary Figure S5. Pearson correlation of antibody titer in MAT and antibody level in modified ELISAs using 14 sera with MAT titers above 1:20 dilution obtained from dogs in groups 1, 2, and 3.