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Supplementary

Prevalence of *Trypanosoma evansi* infections in buffaloes, beef and dairy cattle in Sakon Nakhon province using molecular and serological assays

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Table S1 Details of primer sets used in this study

Name	Reference	Primer sequence	PCR product size (bp)
TRYP1	Milocco et al., 2011	TRYP1 S: 5'– CGT CCC TGC CAT TTG TAC ACA C –3' TRYP1 R: 5'– GGA AGC CAA GTC ATC CAT CG –3'	520
RoTat1.2	Claes et al., 2004	RotTat1.2 F: 5'– GCG GGG TGT TTA AAG CAA TA –3' RoTat1.2 R: 5'– ATT AGT GCT GCG TGT GTT CG –3'	205
LEW1	Desquesnes et al., 2011	LEW1 S: 5'– ACC ACC ACA CGC TCT CTT CT –3' LEW1 R: 5'– TGT ATG TGC GTG CTT GTT CA –3'	220
TCZ	Moser et al., 1989	TCZ1: 5'– CGA GCT CTT GCC CAC ACG GGT GCT –3' TCZ2: 5'– CCT CCA AGC AGC GGA TAG TTC AGG –3'	188

PCR = polymerase chain reaction

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References

Claes, F., Radwanska, M., Urakawa, T., Majiwa, P.A., Goddeeris, B., Büscher, P. 2004. Variable Surface Glycoprotein RoTat 1.2 PCR as a specific diagnostic tool for the detection of *Trypanosoma evansi* infections. Kinetoplastid Biol. Dis. 3: 3. doi.org/10.1186/1475-9292-3-3

Marc, D., Ketsarin, K., Sarawut, Y., et al. 2011. Specific primers for PCR amplification of the ITS1 (ribosomal DNA) of *Trypanosoma lewisi*. Infect. Genet. Evol. 11: 1361–1367. doi.org/10.1016/j.meegid.2011.04.030

Milocco, C., Kamyingkird, K., Desquesnes, M., Jittapalapong, S., Herbreteau, V., Chaval, Y., Douangboupha, B., Morand, S. 2013. Molecular demonstration of *Trypanosoma evansi* and *Trypanosoma lewisi* DNA in wild rodents from Cambodia, Lao PDR and Thailand. Transbound. Emerg. Dis. 60: 17–26. doi.org/10.1111/j.1865-1682.2012.01314.x

Moser, D.R., Kirchhoff, L.V., Donelson, J.E. 1989. Detection of *Trypanosoma cruzi* by DNA amplification using the polymerase chain reaction. J. Clin. Microbiol. 27: 1477–1482. doi.org/10.1128/jcm.27.7.1477-1482.1989

Table S2 Number and percentage of positive-*T. evansi* samples based on (ELISA) and polymerase chain reaction (PCR) for beef cattle, buffalo and dairy cattle

Factor	Total animal (% Pos)	PCR			ELISA			Total farm (% pos farm)	Total animal in pos farm (% Pos Ani)
		Pos BC (%)	Pos BF (%)	Pos DC (%)	Pos BC (%)	Pos BF (%)	Pos DC (%)		
Sex									
Female	1,528 (4.84)	2 (0.13)	6 (0.39)	0 (0.00)	30 (1.96)	34 (2.22)	4 (0.26)	-	-
Male	231 (3.90)	0 (0.00)	0 (0.00)	0 (0.00)	3 (1.30)	6 (2.60)	0 (0.00)	-	-
Age									
≤ 1 yr	180 (1.11)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.56)	1 (0.56)	-	-
> 1–3 yr	777 (6.82)	1 (0.13)	4 (0.51)	0 (0.00)	19 (2.45)	27 (3.47)	1 (0.13)	-	-
> 3 yr	802 (3.49)	1 (0.13)	2 (0.25)	0 (0.00)	14 (1.75)	12 (1.50)	2 (0.25)	-	-
Type									
Beef Cattle	1077 (3.25)	2 (0.19)	-	-	33 (3.06)	-	-	-	-
Buffalo	455 (9.67)	-	6 (1.32)	-	-	40 (8.79)	-	-	-
Dairy cattle	227 (1.76)	-	-	0 (0.00)	-	-	4 (1.76)	-	-
Farm unit									
Small (<10)	1,093 (5.40)	2 (0.18)	6 (0.55)	0 (0.00)	18 (1.65)	34 (3.11)	1 (0.09)	342 (10.23)	129 (45.75)
Medium (11–50)	217 (5.99)	0 (0.00)	0 (0.00)	0 (0.00)	6 (2.76)	6 (2.76)	1 (0.46)	11 (36.36)	57 (22.81)
Large (>50)	449 (2.45)	0 (0.00)	0 (0.00)	0 (0.00)	9 (2.00)	0 (0.00)	2 (0.44)	3 (100.00)	449 (2.45)
District									
Waritchaphum	145 (1.38)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (1.38)	12 (16.67)	20 (10.00)
Akat Amnuai	75 (1.33)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.33)	0 (0.00)	20 (5.00)	6 (16.67)
Khok Sri Suphan	199 (0.50)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.50)	0 (0.00)	0 (0.00)	90 (1.11)	1 (100.00)
Kusuman	325 (4.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.61)	11 (3.38)	0 (0.00)	90 (7.78)	27 (48.15)
Mueang	566 (1.94)	0 (0.00)	0 (0.00)	0 (0.00)	9 (1.59)	0 (0.00)	2 (0.35)	24 (12.50)	449 (2.45)
Phanna Nikhom	150 (21.33)	0 (0.00)	6 (4.00)	0 (0.00)	0 (0.00)	28 (18.67)	0 (0.00)	54 (33.33)	63 (50.79)
Sawang Daen Din	100 (9.00)	0 (0.00)	0 (0.00)	0 (0.00)	9 (9.00)	0 (0.00)	0 (0.00)	19 (21.05)	43 (20.93)
Wanon Niwat	199 (7.03)	2 (1.00)	0 (0.00)	0 (0.00)	12 (6.03)	0 (0.00)	0 (0.00)	47 (12.77)	26 (53.85)
Prevalence			8 (0.45)		77 (4.38)			-	-

Pos = Positive, BC = Beef cattle, BF = Buffalo, DC = Dairy cattle, Ani = Animal

Table S3 Hematological values of positive and negative animals, classified in three levels based on comparison with normal range values (low, normal and high)

Blood parameter	Positive animals (n = 83)			Negative animals (n = 552)		
	Low	Normal	High	Low	Normal	High
RBC* (x 10^6/μL)	10 [4.20 ± 0.68]	73 [6.71 ± 1.03]	-	39 [4.57 ± 0.45]	489 [7.21 ± 1.20]	24 [10.61 ± 0.76]
HGB (g/dL)	6 [6.67 ± 1.53]	76 [11.26 ± 1.75]	1 [15.60]	23 [7.21 ± 0.59]	509 [11.41 ± 1.68]	20 [15.83 ± 0.99]
HCT (%)	3 [17.60 ± 5.53]	79 [33.89 ± 5.76]	1 [47.10]	19 [22.01 ± 1.01]	512 [34.96 ± 5.30]	21 [49.27 ± 2.51]
MCV* (fL)	4 [38.02 ± 1.34]	66 [51.57 ± 5.31]	13 [63.25 ± 2.17]	45 [37.28 ± 2.12]	463 [49.47 ± 4.57]	44 [63.25 ± 3.20]
MCH* (pg)	5 [12.86 ± 0.76]	46 [16.16 ± 1.17]	32 [19.66 ± 0.89]	69 [12.61 ± 0.76]	397 [15.98 ± 1.16]	86 [19.72 ± 1.47]
MCHC (g/dL)	3 [29.55 ± 0.35]	79 [32.86 ± 1.16]	1 [36.20]	32 [28.97 ± 0.69]	516 [32.74 ± 1.26]	4 [40.40 ± 7.21]
PLT* (x 10^4/μL)	48 [5.97 ± 2.27]	35 [16.78 ± 4.74]	-	89 [6.64 ± 2.38]	463 [21.42 ± 9.16]	-
WBC (x 10^3/μL)	2 [2.24 ± 1.07]	41 [9.35 ± 1.84]	40 [15.96 ± 3.11]	4 [3.74 ± 0.01]	341 [9.23 ± 1.74]	207 [16.08 ± 8.61]
BandNEU (%)	-	83 [0.01 ± 0.05]	-	-	551 [0.01 ± 0.09]	1 [9.00]
SegNEU* (%)	52 [7.98 ± 3.83]	31 [22.39 ± 5.24]	-	283 [8.97 ± 3.69]	262 [23.36 ± 6.99]	7 [55.50 ± 13.07]
LYMP* (%)	-	31 [67.16 ± 6.87]	52 [83.78 ± 5.03]	17 [36.67 ± 11.08]	299 [65.54 ± 7.22]	236 [82.76 ± 5.18]
MONO* (%)	65 [0.30 ± 0.46]	17 [3.53 ± 1.29]	1 [10.00]	484 [0.22 ± 0.38]	62 [3.76 ± 1.56]	6 [10.17 ± 1.40]
EOSIN* (%)	-	79 [7.09 ± 4.10]	4 [22.25 ± 2.84]	-	520 [9.52 ± 5.03]	32 [25.67 ± 4.92]
BASO* (%)	-	83 [0.16 ± 0.35]	-	-	550 [0.24 ± 0.40]	2 [2.50 ± 0.00]

RBC = red blood cell, HGB = hemoglobin; HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT= platelets, WBC = white blood cells, BandNEU = band neutrophils, SegNEU = segmented neutrophils, LYMP = lymphocytes, MONO = monocytes, EOSIN = eosinophils, BASO = basophils. * Significant at $p < 0.05$ (between positive and negative animals). Numbers in [] = mean ± SD of each hematological value.

(A)

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BUFFALO_PHANNANIKOM-045  CGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTG  60
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BUFFALO_PHANNANIKOM-080  CGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTG  60
BEEF_WANONNIWAT-121      CGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTG  60
U75507_T_evasi           CGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTG  60
BUFFALO_PHANNANIKOM-048  CGTCCCTGCCCTTTGTACACACCGCCCGTCGTTGTTTCCGATGATGGTGCAATGCAGGTG  60
BEEF_WANONNIWAT-098      CGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCGATGATGGTGCAATACAGGTG  60
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BUFFALO_PHANNANIKOM-046  CGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCGATGATGGTGCAATGCAGGTG  60
BUFFALO_PHANNANIKOM-047  CGTCCCTGCCATTTGTACACACCGCCCGTCGTTGTTTCCGATGATGGTGCAATGCAGGTG  60
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BUFFALO_PHANNANIKOM-080  ATCGGACCGTCGCTCGTCTCGGGCGACCGAAAGTTCACCGATATTGCTTCAATAGAGGAA  120
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BUFFALO_PHANNANIKOM-080  GCAAAAGTCGTAACAAGGTAGCTGTAGGTGAACCTGCAGCTGGATCATTTTCTGATATCC  180
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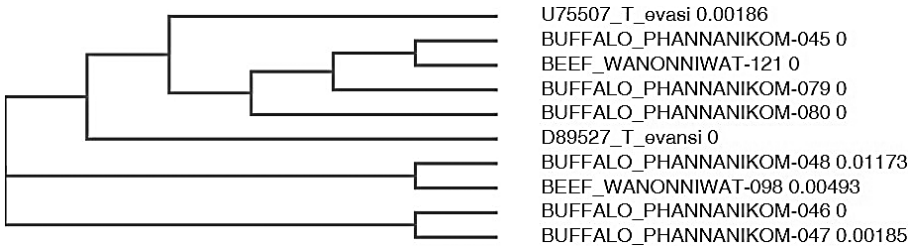

Fig. S1 Sequencing analysis: (A) multiple sequence alignment revealing 97.22–100% sequence identities; (B) phylogram divided into two groups of *T. evansi* Tansui-Taiwan (D89527.1) and Batongtani-Thailand (U75507.1) strains