Adaptation and evaluation of an ELISA for *Trypanosoma evansi* infection (surra) in elephants and its application to a serological survey in Thailand

MARGOT CAMOIN[1,2], ARTHUR KOCHER[1,2,3], PIANGJAI CHALERMWONG[2], SARAWUT YANGTARRA[2], NIKORN THONGTIP[4], SATHAPORN JITTAPALAPONG[2,5]* *and* MARC DESQUESNES[1,2]

[1] *Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), UMR Intertryp, F-34398 Montpellier, France*
[2] *Faculty of Veterinary Medicine, Kasetsart University, Chatuchak, Bangkok 10900, Thailand*
[3] *CNRS, Université Toulouse III Paul Sabatier, ENFA; UMR5174 EDB (Laboratoire Evolution et Diversité Biologique), Toulouse, France*
[4] *Kasetsart University Veterinary Teaching Hospital, Kamphaeng Saen, Faculty of Veterinary Medicine, Kasetsart University, Nakhon Pathom, Thailand*
[5] *Faculty of Veterinary Technology, Kasetsart University, Chatuchak, Bangkok 10900, Thailand*

(*Received 1 February 2017; revised 10 August 2017; accepted 15 August 2017; first published online 25 September 2017*)

SUMMARY

Trypanosoma evansi, the causative agent of surra, is widespread in domestic livestock and wildlife in South East Asia. Surra can affect cattle, buffaloes, horses and also Asian elephants (*Elephas maximus*). Despite the 'threatened to extinction' CITES status of elephant, surra's impact has not been thoroughly assessed yet in this species. This work offers to adapt an antibody enzyme-linked immunosorbent assay (ELISA) protocol, to detect *Trypanosoma evansi* antibodies in elephant serum. The test was validated with 365 negative-reference samples, which allowed the determination of a 16% positive threshold. The test was applied to a serological survey including 375 individuals. The estimated global seroprevalence was 2·1% (95% CI 1·1–4·2%). Therefore, surra does not appear to be endemic in Thai domestic elephants, but occasional outbreaks were reported to our laboratory during the survey period. These outbreaks seemed to be linked to close proximity to cattle or buffaloes, and led to severe clinical signs in elephants. Frequent relapses were observed after treatment with diminazene aceturate, the only trypanocide drug currently available in Thailand. Therefore, care should be taken to keep elephants away from bovine reservoirs, and to monitor the disease in this endangered species. ELISA proved to be reliable for screening purposes as well as for post-treatment monitoring.

Key words: Elephant, *Trypanosoma evansi*, surra, ELISA, application.

INTRODUCTION

Trypanosoma evansi (Kinetoplastida, Trypanosomatidae) is the causative agent of surra, a widespread vector-borne, multispecies disease characterized by the polymorphism of its clinical effects depending on the host species and geographic area (Singh and Singla, 2013). It can affect camels, horses and small ruminants in Africa; Bactrian camels, horses, cattle, buffaloes and dogs in Asia; horses and dogs in South America (Desquesnes *et al*. 2013). Many wildlife species can also develop the disease, such as rhinoceros, deer and wild pig; the Asian elephant is one of them. *Trypanosoma evansi* is not considered as a zoonotic agent since humans are thought to be naturally resistant, but a case in a livestock farmer was reported in India (Joshi *et al*. 2005; Powar *et al*. 2006) and another one in a Vietnamese villager (Van Vinh Chau *et al*. 2016). Epidemiological surveys of surra in Asia focused on bovines (cattle and buffaloes), horses or camels (Sarataphan *et al*. 1989; Payne *et al*. 1991; Thu *et al*. 1998; Reid and Copeman, 2002; Dobson *et al*. 2007; Desquesnes *et al*. 2009; Kocher *et al*. 2015). In bovines, the main reservoir species, the disease undergoes a chronic evolution with depression, reluctance to work and abortion. On the contrary, horses develop an acute and often lethal form of the disease, so that they do not maintain the disease (Luckins, 1988; Silva *et al*. 1995; Berlin *et al*. 2009).

Surra, or 'Thut' in elephants, has been carefully studied and described by G. H. Evans in Burma (Myanmar) (Evans, 1910). Unfortunately, from that time, information regarding the epidemiology of the disease in Asian elephants has been very scarce. Occasional cases were reported in India, Burma and Thailand (Hin-On *et al*. 2004), and usually occurred in animals that were kept in close proximity with other livestock species. Symptoms in elephants are similar to those in horses (Evans, 1910): they develop acute form with hyperthermia, anaemia, lower part oedema and even nervous signs when the parasites pass through the blood–

* Corresponding author. Faculty of Veterinary Technology, Kasetsart University, Chatuchak, Bangkok 10900, Thailand. E-mail: fvetspj@ku.ac.th

Parasitology (2018), **145**, 371–377.
doi:10.1017/S0031182017001585

brain barrier; however, the clinical evolution does not systematically lead to death (Luckins, 1988; Silva *et al*. 1995; Berlin *et al*. 2009). Surra also causes reluctance to work in elephants but all signs are non-pathognomonic and thus the disease is not frequently noticed nor reported by the owner or 'mahout'. In Thailand, although they are rare, cases of surra in elephants are regularly observed. In the recent times, cases were described in 1 elephant in Chiang Mai (Northern Thailand), in 2003, and in 5 elephants in Nakhon Si Thammarat (Southern Thailand), in 2010 (Hin-On *et al*. 2004; Arjkumpa *et al*. 2012). Thin stained blood smear examination, Hematocrit Centrifuge Technic, serology and/or polymerase chain reaction (PCR) allowed case confirmations. The animals were treated with diminazene aceturate (5 mg kg^{-1}), but they relapsed 14 days later, suggesting a lack of efficacy of this treatment.

In the framework of a research project supported by the Thailand International Cooperation Agency (TICA), surra is currently investigated in Thailand, in a number of host species including dairy and beef cattle, buffaloes, horses and dogs. As Asian elephant is currently threatened to extinction (cites.org), it is of value to evaluate surra's potential impact on its decreasing population in Thailand. The antibody enzyme-linked immunosorbent assay (ELISA) – an OIE recommended diagnosis method (OIE, 2012) – has already been used for the detection of surra in several host species including cattle buffaloes and horses (Monzon *et al*. 2003; Desquesnes *et al*. 2009; Kocher *et al*. 2015). Here, we adapted and validated this test in elephants and conducted a seroprevalence survey in Thai domestic elephants from various parts of Thailand.

MATERIAL AND METHODS

Sample collection

The Department of Livestock Development, Ministry of Agriculture (DLD) closely monitors the elephant population, notably through the National Institute of Elephant Research and Health Service (NIERH); their regional Veterinary Research and Development Centers, are responsible for the routine health check, disease control and identification of all domestic elephants. Surin and Lampang elephant hospitals (Regional Veterinary Research and Development Centers no 6 & 3) have mobile teams visiting all camps in Thailand twice a year (mainly tourist camps, because timber activity has been forbidden by the government). During those visits, they collect blood samples from a small number of elephants in each camp to be used for different studies. Blood was collected on both plain and EDTA tubes. Blood in plain tubes was allowed to clot in the shadow for 2–4 h, and transferred in an icebox until serum was separated, aliquoted and stored at −20 °C for serological tests. EDTA tubes were kept on ice and used for parasitological and DNA-based tests. Those samples were the ones used for our epidemiological survey on surra.

On the other hand, for the purpose of laboratory diagnosis, private and public veterinaries use to refer suspicions of surra to Kasetsart University and CIRAD, known to investigate such parasitoses in Thailand. Such samples, proved to be positive by parasitological method, were used as positive controls in this study.

The total domestic elephant population in Thailand was estimated to 4435 in 2013 (Pintavong *et al*. 2014). Because there were no data on expected seroprevalence (less than 10 cases have been recorded in the last 15 years) this survey aimed at collecting samples from 327 elephants all over the country, to be able to estimate an expected seroprevalence of 10% (close to values found in cattle and buffaloes) with a 95% confidence level and a 5% precision (assuming our tests had 90% specificity and 90% sensitivity). Sample size calculations were done using Epitools website (http://eitools.ausvet.com.au).

Parasitological examination, PCR and CATT/ T. evansi

EDTA tubes were centrifuged within less than 3 h after collection in a micro haematocrit tube (Hirschmann® Laborgeräte, Eberstadt, Allemagne) for direct microscopic observation of the buffy coat and measurement of packed cell volume (PCV), according to the Haematocrit Centrifuge Technique (HCT) (Woo, 1970).

Buffy coats were collected from the micro haematocrit tubes, transferred in microtubes and prepared using Chelex® for PCR analyses using Tepan primers, according to protocols previously published (Pruvot *et al*. 2010). In the first half of the sample collection, PCR was systematically performed; further on, because all samples had been negative, it was performed only in case of suspicion or positivity using parasitological examination or card agglutination test. In some cases, only serum samples were obtained from a partner institute, thus only serological tests could be performed.

The card agglutination test for trypanosomosis/ *T. evansi* (CATT/*T. evansi*®, Institute of Tropical Medicine "Prince Leopold", Laboratory of Serology, Nationalstraat 155, B-2000 Antwerp, Belgium) is a direct agglutination test detecting the presence of circulating anti-trypanosome antibodies, mainly immunoglobulin type M (OIE, 2012). It was performed according to manufacturer's instructions (Bajyana Songa and Hamers, 1988) using a serum dilution of 1:4.

https://doi.org/10.1017/S0031182017001585 Published online by Cambridge University Press

ELISA T. evansi

Protocol. The indirect ELISA *T. evansi*, as described by the OIE (OIE, 2012) has been used in several host species and was already validated for cattle and buffaloes in Thailand (Desquesnes *et al*. 2009; Kocher *et al*. 2015). The test used in this study is derived from previously published protocols with slight modifications and adaptation to elephant samples. Briefly, Microtest 96-well Polysorp Nunc® (Nunc®, Roskilde, Denmark) were coated with 100 μL well^{-1} of *T. evansi* soluble antigen at a high concentration of 15 μg mL^{-1} in carbonate buffer (0·05 M, pH 9·6) and incubated for 2 h at 37 °C. The plates were blocked with 150 μL well^{-1} of blocking buffer (BB) [phosphate saline buffer (PBS) with 5% skim milk powder (ref: 190–12865, Wako Pure® Chemical Industries Ltd., Osaka, Japan] with permanent shaking (200 rpm) for 60 min at 37 °C. The BB was discarded. Sera diluted 1:50 in BB were transferred in duplicate on the ELISA plate. After incubating 30 min at 37 °C, plates were washed 5 times with washing buffer (WB) (PBS – 0·1% Tween 20). Then, 100 μL of Protein A-peroxidase conjugated (ref: P8651, Sigma®–Aldrich, USA), diluted 1:2000 in BB, was added and the plates incubated for 30 min at 37 °C with permanent shaking (200 rpm). After washing 5 times with WB, 100 μL of the complex substrate/chromogen 3,3′,5,5′-tetramethylbenzidine (TMB) (SureBlue™ TMB, KPL®, Maryland, USA) was added. The plates were incubated, without shaking, in a dark room for 30 min. Optical density (OD) was measured at 620 nm in an ELISA reader (Dynex Technologies®, VA, USA).

Selection of negative and positive controls and expression of test results. In a preliminary assay, the ELISA was performed once in the absence of reference samples to determine the mean optical density of the samples. Then, all the animals that exhibited an optical density below 0·400 and tested negative to the CATT/*T. evansi* and, when available, negative to HCT and PCR, were used to constitute a pool of non-infected reference samples. Three samples, representative of the mean optical density of these non-infected samples (±10%) were selected as negative controls.

Since no active infection was detected during this survey, 3 sick animals reported to our laboratory, proved to be infected by parasitological examination and PCR during an outbreak of surra in an elephant camp in Chiang Mai were used as positive controls.

ELISAs were performed again, in duplicate for each sample (controls and test), with the 3 positive controls and the 3 negative controls (selected as indicated above) on each plate. The blank OD value was systematically subtracted from the average OD of each sample and the results were expressed as a relative percentage of positivity (RPP) as previously described (Desquesnes, 1997) and as follows:

$$\text{RPP sample} = \frac{\text{mean OD sample} - \text{mean OD of C}-}{\text{mean OD of C} + - \text{mean OD of C}-}.$$

Determination of the cut-off value. The normality of ELISA's RPP value distribution obtained with the pool of 'negative samples' was checked using a Shapiro–Wilk test using the stats package in *R*. A cut-off value was then determined as: mean RPP + 4 standard deviations (S.D.). Subsequently, the samples exhibiting a RPP value below or above this cut-off were classified as negative or positive respectively. The specificity of the test was estimated as the proportion of non-infected reference samples classified as negative and the Wilson score method was used for the computation of the confidence interval.

Seroprevalence estimations. Prevalence estimations where inferred directly from the ELISA results and the Wilson score method was used for the computation of confidence intervals. All statistics were computed using R software.

RESULTS

Sampling

A total of 375 samples were collected from 46 camps distributed in the 4 regions throughout 7 provinces between 2010 and 2014: Chiang Mai, Chiang Rai, Kanchanaburi, Mae Hong Son, Phang Nga, Surin, Ubon-Ratchathani (Fig. 1). The elephant population in those provinces amounts to 2134 and represents 53% of the total domesticated elephant population of Thailand. In 336 individuals for which the information was available, the age ranged from 1 to 68 years with an average of 29·8 years (S.D. = 16·0). Females represented 70·9% of the animals. The mean size camp was 8·2 (S.D. = 8·7). More than half of them were composed of less than 5 individuals and nearly 15% were composed of more than 20 individuals. None of these animals presented clinical signs of surra at the time of sampling.

Eighteen extra samples collected in 2 camps facing surra outbreaks were reported to our laboratory; the first in Sakhon Nakhon Province occurred in November 2013 in a group of 3 animals amongst which 2 exhibited clinical signs and were positive by parasitology 3 days prior to our sampling; and the second in Chiang Mai Province occurred in September 2014 in a group of 15 animals amongst which 3 exhibited clinical signs and proved to be positive by microscopic observation of the blood during our sampling. These 18 samples were not included in the serological survey but they were

https://doi.org/10.1017/S0031182017001585 Published online by Cambridge University Press

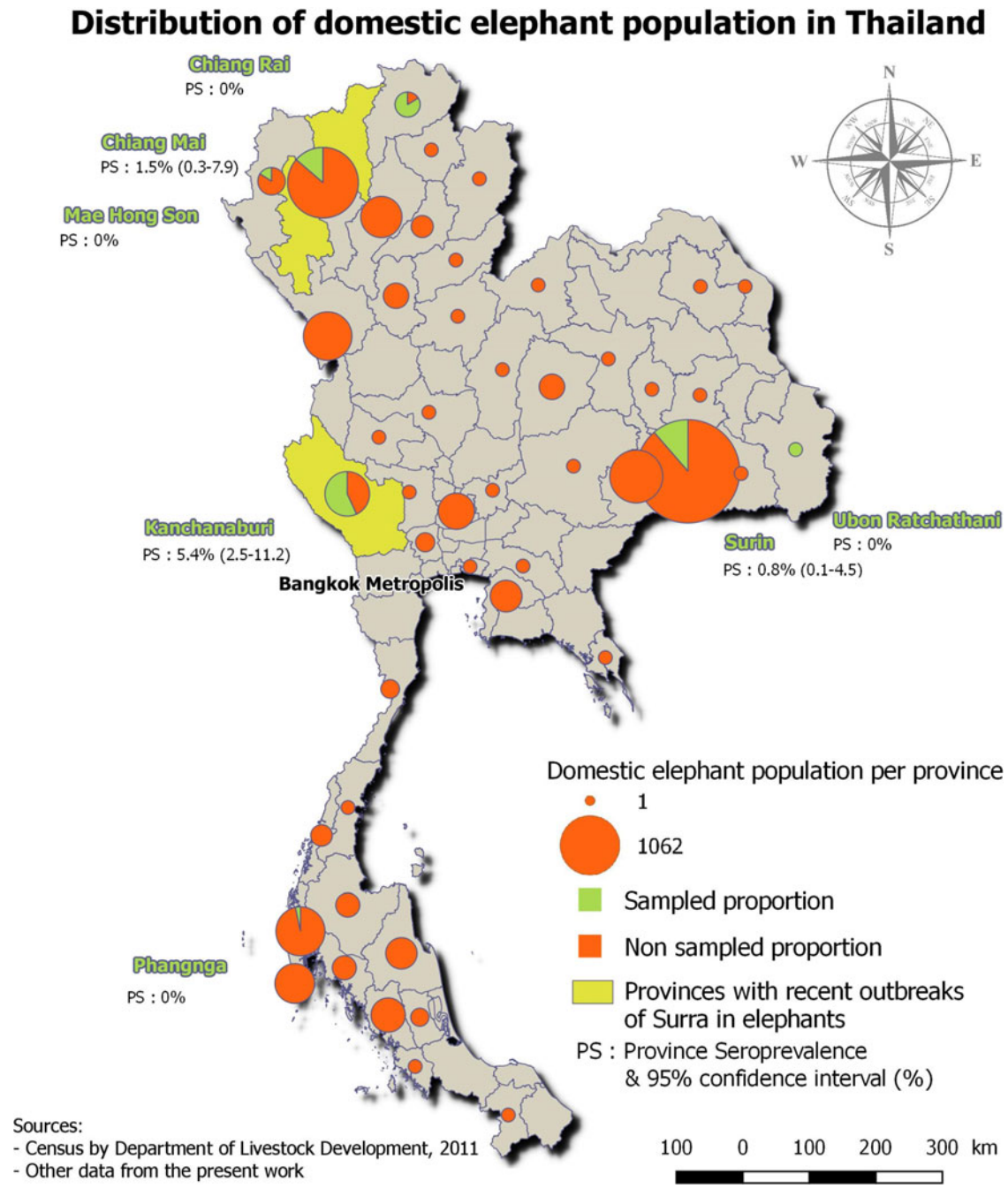

Fig. 1. Distribution of domestic elephant population in Thailand, sampled proportion and seroprevalence results.

used to evidence the sensitivity of the test, and the serum samples from the 3 animals proved to be actively infected were used as positive control for the ELISA.

ELISA standardization

Out of 375 serum samples collected for the survey, none was positive by parasitological examination, 9 were positive by CATT and 2 had OD >0·400. The pool of non-infected reference samples was thus constituted of 365 samples.

As indicated in 'material and methods', the 3 selected positive samples for the computation of ELISA RPP exhibited mean OD values of 2·540, 1·006 and 0·740 (after deduction of the mean plate optical density: 0·034 ±0·004 OD). The 3 negative controls exhibited mean OD values of 0·005, 0·006 and 0·022. The 365 samples selected as non-infected reference pool, following the criteria described above exhibited a mean optical density of 0·036 (S.D. = 0·049), which converted into RPP value of 1·82% (S.D. = 3·56%). The distribution of ELISA's RPP values in non-infected animals is presented in Fig. 2. The normality of negative ELISA's RPP values was further assessed with a Shapiro–Wilk test using the stats package in *R* ($W = 0{\cdot}\ 66\,285$, P value $< 10^{-15}$). Consequently, the cut-off value

https://doi.org/10.1017/S0031182017001585 Published online by Cambridge University Press

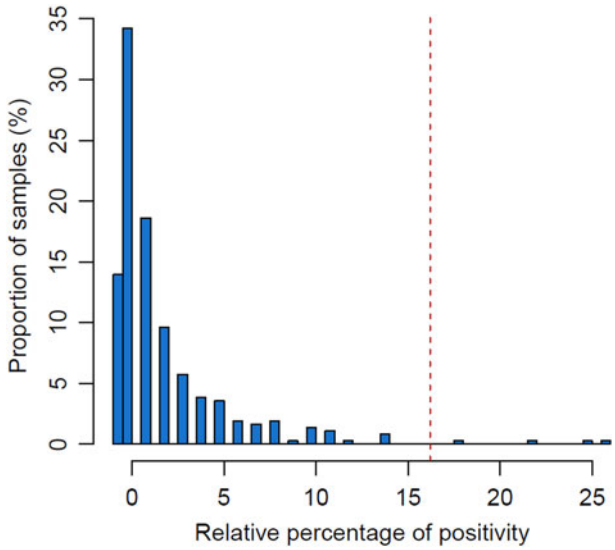

Fig. 2. Distribution of ELISA's RPP values in non-infected animals. ELISA, enzyme-linked immunosorbent assay; RPP, relative percentage of positivity.

(COV) was set at 16% (mean + 4S.D.). Applying this COV to the pool of non-infected animals resulted in 4 positive samples, leading to a test specificity of 98·9% (95% CI 96·2–99·6%).

In the absence of sufficient positive reference samples, it was not possible to precisely quantify sensitivity, however it was evaluated by verifying ELISA results of confirmed infected animals.

Serological survey

Amongst the 375-survey samples, 8 individuals were found positive with the indirect-ELISA for an estimated global seroprevalence of 2·1% (95% CI 1·1–4·2%). Estimations by province ranged from 0·0% in Chiang Rai, Mae Hong Son, Ubon-Ratchathani and Phang Nga to 5·4% (95% CI 2·5–11·2%) in Kanchanaburi (Table 1).

The mean age of positives elephants was 38·2 years and all elephants were aged more than 25 years but no significant effect of age was found on the probability of infection when considering these 2 age classes ($\geqslant$ or $<$ to 25 years old; $\chi^2 = 1{\cdot}9$, $P = 0{\cdot}16$). Among the animals for which the sex information was available, 1% of males (1/100) and 2·5% of females (6/244) were positives, but no significant effect of sex on infection probability was found ($\chi^2 = 0{\cdot}20$, $P = 0{\cdot}65$).

Outbreaks

In the recent case of Sakhon Nakhon Province, the 2 animals (out of 3) exhibiting clinical signs and being positive by parasitology 3 days prior to our sampling were positive by ELISA (RPP of 25 and 32%); the last animal, ever negative, was also negative by ELISA. The 3 animals were negative by CATT and PCR.

In the case of Chiang Mai Province, 3 animals (out of 15) had previously been found positive and treated using 5 mg kg^{-1} of diminazene aceturate some months before. These 3 animals exhibiting again clinical signs were sampled and found positive by microscopic observation (3/3), CATT (3/3), PCR (1/3) and ELISA (3/3), with RPP of 44, 71 and 213%; these animals had already been detected positive by parasitology respectively 4, 4 and 7 months before. The other 12 animals were all negative with the 3 tests.

DISCUSSION

The ELISA *T. evansi* protocol adapted to elephant samples proved to be very robust since the optical densities of non-infected animals were very low (0·036 ± 0·049) while those of confirmed infected animals were always above 0·200 and reached 1·500 and even 2·540 in 1 case. In elephants, the rate of ELISA's optical densities in non-infected *vs.* infected animals was 11/1428 (0·007) compared with 60/401 (0·149) in buffaloes, and 136/833 (0·163) in cattle (Desquesnes *et al.* 2009; Kocher *et al.* 2015). This confirms the expected robustness of the test in elephants.

Sensitivity and specificity of parasitological detection and PCR in elephant are unknown; however, their specificity is thought to be very high since *T. evansi* is the only *Trypanosoma* species that can infect elephants in Asia. Therefore, these tests are adapted to define positive samples. However, the quasi-absence of infected individuals in our dataset (as identified by these tests) hampers precise calculation of ELISA's sensitivity with any statistical method. Nevertheless, the 5 animals in which infection was confirmed by parasitological and PCR methods were all positive by ELISA, which suggests a good sensitivity of the test. Of note, only 3 of these samples were positive by CATT. On the other hand, specificity was estimated classically, based on a large batch of negative reference samples defined using all available tests (PCR, HCT, CATT and ELISA optical density <0·4).

RPP values of both animals recently found infected in Sakhon Nakhon Province were quite low (25 and 32%) while RPP values of animals from Chiang Mai Province, known to be infected for a long time and exhibiting recent clinical relapse, were very high (44, 71 and 213%); these results are consistent with an increasing sensitivity along the infection, thus a promising capacity to detect healthy carriers using ELISA, while the CATT exhibited a lower capacity for such detection.

All elephants found infected in the 2 outbreaks have shown clinical signs at least in the early stage of their infection, and no seropositive was found

https://doi.org/10.1017/S0031182017001585 Published online by Cambridge University Press

Table 1. Results of the seroprevalence survey by ELISA *T. evansi* in elephants in Thailand, detailed by Province

Provinces	Number of samples tested	Number of positive samples	Sero-prevalence (%)	95% CI−	95% CI+
Surin	121	1	0.8	0.1	4·5
Chiang Rai	52	0	0·0	0·0	6·9
Mae Hong Son	12	0	0·0	0·0	24·2
Chiang Mai	68	1	1·5	0·3	7·9
Kanchanaburi	112	6	5·4	2·5	11·2
Ubon-Ratchathani	2	0	0·0	0·0	65·8
Phang Nga	8	0	0·0	0·0	32·4
Total	375	8	2·1	1·1	4·2

amongst the animals remaining apparently healthy; this suggests that elephants would rather exhibit clinical signs when primo-infected by *T. evansi*. Conversely, post treatment, 3 elephants remained subclinical for several months before they all relapsed, which suggest that immune animals might remain unapparent carriers. The very high score of these 3 animals in ELISA, when relapsing, demonstrates the worth of the test to detect such carriers.

The estimated seroprevalence observed in this survey was lower than expected. Sample size calculations thus overestimated the number of elephant to include in the survey. Out of the 77 Thai provinces, 48 have elephant population; only 7 were sampled, but they amount for 53% of the total elephant population in Thailand. Still, provinces with large elephant population such as Buriram, Lampang and Tak are missing from our samples.

Taking into consideration the estimated specificity of this ELISA (estimated >98·9%), 1·1% of negative samples should give false positive results. Therefore, with the very low level of seroprevalence observed (2·1%), around half of positive samples from the survey might be false positives. However this result is consistent with a prevalence of 1·4% previously observed in Kanchanaburi and Chiang Mai Provinces (Pintavong *et al*. 2014)

Although carried out with a limited number of samples from infected animals, these results suggest that ELISA is robust, specific and sensitive enough to detect unapparent carriers and could then be used for screening of animals prior to exportation.

In the case of Chiang Mai where 3 amongst 15 elephants had been detected infected 4–7 months ago and had received 5 mg kg^{-1} diminazene aceturate treatment, the relapse observed in all 3 animals demonstrates the inefficacy of the treatment. Such observations were already made in the past (Hin-On *et al*. 2004). Due to the toxicity of diminazene aceturate, it is risky to suggest increasing the dose to 7–8 mg kg^{-1}; side effects might be of concerns, or even fatal. However, in an attempt to control such relapse, a treatment using 8 mg kg^{-1} of dimiazene aceturate was successfully applied to an infected elephant in Chiang Mai (Rodtian *et al*. 2012). A possible alternative to such treatment might be the use of melarsamine hydrochloride (Cymelarsan®) at a dose related to the large size of elephant, which might be 0·2 mg kg^{-1}.

In Chiang Mai and Sakhon Nakhon Province, as well as in the previous cases reported, all elephant cases detected in Thailand so far have been associated with the occasional and unusual proximity of bovines (cattle and/or buffaloes) acting as a source of infection for elephants. It is then strongly recommended to keep them apart from these host species. As observed when a flooding occurs, it is sometime difficult or impossible to follow such rules; however, elephant and horse keepers should always have a 'B plan' to keep these highly sensitive animals in safe place and separated from the bovine reservoir.

This survey indicated that surra is rare in Thai elephants, but that occasional outbreaks may occur, especially when animals are kept close to bovines that act as source of infection. The manifestation of severe clinical signs, the uncertain issue of the treatment, and the potential for clinical relapses after a period of unapparent infection call for great caution in the management of surra in these endangered animals. ELISA proved to be a useful tool in this regard, as it is especially efficient for the follow up of sick animals post-treatment and could be easily used for disease screening before exportation.

ACKNOWLEDGEMENT

We would like to thank the National Research and Health Service Institute, Department of Livestock Development, and Wildlife Unit, Kasetsart Kamphaeng Saen Veterinary Teaching Hospital, Kasetsart University, for providing some elephant blood samples.

FINANCIAL SUPPORT

We would like to thank the Thailand International Cooperation Agency (TICA) for diplomatic and financial support to the project.

REFERENCES

Arjkumpa, O., Cheewasereechon, M., Suksaithaichana, P., Worasing, R., Anan, S., Khongo, P., Homkong, P. and Vongpakorn, M. (2012). Surra in timber elephant in Thungsong district, Nakhonsrithammarat Province, Thailand: Case investigation. Proceedings of the first Regional Conference of the Society for Tropical Veterinary Medicine (STVM): A change in global environment, biodiversity, diseases and health, 18–21 June 2012, Phuket, Thailand, p. 26.

Bajyana Songa, E. and Hamers, R. (1988). A card agglutination test (CATT) for veterinary use based on an early VAT RoTat 1/2 of *Trypanosoma evansi*. *Annales de la Société Belge de Médecine Tropicale* **68**, 233–240.

Berlin, D., Loeb, E. and Baneth, G. (2009). Disseminated central nervous system disease caused by *Trypanosoma evansi* in a horse. *Veterinary Parasitology* **161**, 316–319.

Desquesnes, M. (1997). International and regional standardization of immunoenzyme tests: methods, concerns and limitations. *Revue Scientifique Et Technique* (International Office of Epizootics) **16**, 809–823.

Desquesnes, M., Kamyingkird, K., Pruvot, M., Kengradomkij, C., Bossard, G., Sarataphan, N. and Jittapalapong, S. (2009). Antibody-ELISA for *Trypanosoma evansi*: application in a serological survey of dairy cattle, Thailand, and validation of a locally produced antigen. *Preventive Veterinary Medicine* **90**, 233–241.

Desquesnes, M., Holzmuller, P., Lai, D. H., Dargantes, A., Lun, Z. R. and Jittaplapong, S. (2013). *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *BioMed Research International* **2013**, 194176.

Dobson, R. J., Dargantes, A. P., Reid, S. A., Hood, G. M. and Mercado, P. (2007). Estimating the impact of *Trypanosoma evansi* (surra) on buffalo populations of the Philippines using data from cross-sectional surveys. Proceedings of 21st Int. Conf. WAAVP, Gent, 2007 198, p. 229. DOI: 10.1016/j.ijpara.2009.02.012.

Evans, G. H. (1910). *Elephants and Their Diseases: A Treatise on Elephants*. Government Press, Rangoo, Burma, 344 pp.

Hin-On, W., Rodtian, P., Nuangmek, A., Uthaiwan, V. and Muangyai, M. (2004). Surra in timber elephant from Northern Thailand; Diagnosis and treatment; A case study. In: proceedings of the 30th annual conf. Thai Vet Med Assoc., Bangkok, Thailand, 10–12 November 2004, pp. 373–385.

Joshi, P. P., Shegokar, V. R., Powar, R. M., Herder, S., Katti, R., Salkar, H. R., Dani, V. S., Bhargava, A., Jannin, J. and Truc, P. (2005). Human trypanosomiasis caused by *Trypanosoma evansi* in India: the first case report. *The American Journal of Tropical Medicine and Hygiene* **73**, 491–495.

Kocher, A., Desquesnes, M., Kamyingkird, K., Yangtara, S., Leboucher, E., Rodtian, P., Dargantes, A. and Jittapalapong, S. (2015). Evaluation of an indirect-ELISA test for *Trypanosoma evansi* infection (surra) in buffaloes and Its application to a serological survey in Thailand. *BioMed Research International* **2015**, 361037.

Luckins, A. G. (1988). *Trypanosoma evansi* in Asia. *Parasitology Today* **4**, 137–142.

Monzon, C. M., Mancebo, O. A. and Russo, A. M. (2003). Antibody levels by indirect ELISA test in *Trypanosoma evansi* infected horses following treatment with quinapyramine sulphate. *Veterinary Parasitology* **111**, 59–63.

OIE (2012). Chapitre 2.1.17. Trypanosoma evansi infection (Surra). OIE Terrestrial Manual 2012, 1–15.

Payne, R. C., Sukanto, I. P., Djauhari, D., Partoutomo, S., Wilson, A. J., Jones, T. W., Boid, R. and Luckins, A. G. (1991). *Trypanosoma evansi* infection in cattle, buffaloes and horses in Indonesia. *Veterinary Parasitology* **38**, 109–119.

Pintavong, W., Chueplaivej, P., Boonyasart, B., Kidyhoo, S., Pravai, W., TRattanakunuprakam, J., Ounsiri, S., Lorsanyaluck, B., Sunyathitiseree, P., Jittapalapong, S., Wajjwalku, W. and Thongtip, N. (2014). Domestic elephant population structure and health status in Thailand. *Journal of Kasetsart Veterinarians* **24**, 16–24.

Powar, R. M., Shegokar, V. R., Joshi, P. P., Dani, V. S., Tankhiwale, N. S., Truc, P., Jannin, J. and Bhargava, A. (2006). A rare case of human trypanosomiasis caused by *Trypanosoma evansi*. *Indian Journal of Medical Microbiology* **24**, 72–74.

Pruvot, M., Kamyingkird, K., Desquesnes, M., Sarataphan, N. and Jittapalapong, S. (2010). A comparison of six primer sets for detection of *Trypanosoma evansi* by polymerase chain reaction in rodents and Thai livestock. *Veterinary Parasitology* **171**, 185–193.

Reid, S. A. and Copeman, D. B. (2002). Evaluation of an antibody-ELISA using five crude antigen preparations for the diagnosis of *Trypanosoma evansi* infection in cattle. *Veterinary Parasitology* **104**, 79–84.

Rodtian, P., Hin-on, W. and Muangyai, M. (2012). A Success Dose of Eight mg per kg of Diminazene Aceturate in a Timber Elephant Surra Treatment: Case Study. Proceedings of the first Regional Conference of the Society for Tropical Veterinary Medicine (STVM): A change in global environment, biodiversity, diseases and health; 18–21 June 2012, Phuket, Thailand, p. 24.

Sarataphan, N., Nishikawa, H., Tuntasuvan, D., Sukhapesna, V., Markvishitr, K. and Satjapitak, S. (1989). Effects of natural *Trypanosoma evansi* infection on milk yield of dairy cattle in Thailand. Proceeding of 1st Symposium on Ruminant Reproduction and Parasitology, Chiang Mai, Thailand, 27–29 November 1989, pp. 331–343.

Silva, R. A. M. S., Barros, A. T. M. and Herera, H. M. (1995). Trypanosomiasis outbreacks due to *Trypanosoma evansi* in the pantanal, Brazil. Apreliminary approach on risk factors. *Revue d'Elevage et de Médecine Vétérinaires des Pays Tropicaux* **48**, 315–319.

Singh, V. and Singla, L. D. (2013). Trypanosomiasis (surra) in livestock (Chapter 11). In *Veterinary Parasitology in Indian Perspective*. (ed. Katoch, R., Godara, R. and Yadav, A.), pp. 305–330. Serial Publishing House, Dehli. DOI: 10.1007/s11250-009-9461-1.

Thu, L., My, L., Lan, P., Lang, P., Phuc, D. and Doanh, P. (1998). Epidemiological survey on *Trypanosoma evansi* infection in Vietnam. *Journal of Protozooly Research* **8**, 177–181.

Van Vinh Chau, N., Buu Chau, L., Desquesnes, M., Herder, S., Phu Huong Lan, N., Campbell, J. I., Van Cuong, N., Yimming, B., Chalermwong, P., Jittapalapong, S., Ramon Franco, J., Tri Tue, N., Rabaa, M. A., Carrique-Mas, J., Pham Thi Thanh, T., Tran Vu Thieu, N., Berto, A., Thi Hoa, N., Van Minh Hoang, N., Canh Tu, N., Khac Chuyen, N., Wills, B., Tinh Hien, T., Thwaites, G. E., Yacoub, S. and Baker, S. (2016). A Clinical and epidemiological investigation of the first reported human infection with the zoonotic parasite *Trypanosoma evansi* in Southeast Asia. *Clinical Infectious Diseases* **62**, 1002–1008.

Woo, P. T. K. (1970). The heamatocrit centrifuge technique for diagnosis of African trypanosomiasis. *Acta Tropica* **27**, 384–386.