Standardisation of an ELISA for *Trypanosoma evansi* and Its Application to Dairy Cattle in Thailand

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Introduction

*Trypanosoma evansi* is a parasite of camels and horse, originating from Africa, mechanically transmitted by biting insects (1). Due to healthy carriers, “surra” is a neglected disease which easily spreaded into Latin America, Asia and even recently in Spain (2) and France where it can be considered as an emerging disease (3). In South-East Asia, it is present from Myanmar to Indonesia (4, 5) where it affects horse, water buffalo, but also pig and cattle, inducing fever, loss of weight, nervous symptoms and abortion (6, 7). It is also responsible for failure in vaccination campaign against foot and mouth disease, Hemorragic septicaemia and classical swine fever (8). More recently the zoonotic potential of *T. evansi* has been stressed by several cases reported in humans in India (9); a serological survey demonstrated a significant contact between humans and parasite (10). Contamination in humans could be due to insects, transcutaneous or by peroral route. Epidemiological studies should then be carried out in South-East Asia with comparable tools in order to increase the knowledge on this neglected and emerging disease. In the present study, we describe standardisation of an ELISA for *T. evansi* in an epidemiological survey organised by random sampling of 500 dairy cattle in Thailand.

Materials and Methods

A cryopreserved *T. evansi* isolated from camel in France and previously confirmed for species identification (3), was inoculated intraperitoneally to 2 wistar rats. At the peak of parasitaemia, parasites were separated by DEAE-cellulose (11) and soluble antigens were prepared as previously described (12) in 1mg/ml protein concentration, stored at -80°C and transferred on dry ice to Thailand.

Five hundred cattle were sampled from 59 farms in 4 regions of Thailand: Udon Thani, Khon Kaen, Sakon Nakhon and Chiang Mai. Blood was collected at the jugular vein in dry and citrated tubes, respectively for serology and PCR examinations; serum and blood were kept at -20°C until processing. The ELISA procedure derives from a technique previously described (12) and result were first expressed in optical densities (OD). OD < 0.250 was considered as negative. A pool of 300 presumed “negative samples” was constituted with samples from farms presenting only negative samples. The mean OD of these negative samples (MeanN) was calculated and 3 negative control (NC) were selected such as their OD values were equal to the MeanN +/- 10% such as: Similarly, OD>0.500 was considered as positive. The mean OD of the positive samples (MeanP) was calculated and 3 positive control (PC) were selected such as their OD values were equal to the MeanP +/- 10%.

ELISA were proceeded again for all samples in duplicate with 3 PC and 3 NC on each plate. The OD value of blank was automatically deduced from the mean OD of the sample -  mean OD of NC

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\text{COV} \%: \text{mean RPPN} \% + 3 \text{ standard deviation} \%
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Sample statuts was positive when the RPP of a sample, together with the 6 control sera and the 300 negative samples (Mean OD of PC -  mean OD of NC

Results and Discussion

Standardisation of the ELISA: 39 farms (306 samples) out of 61 were presumed as non infected (all samples < 0.250). Their MeanN OD=0.136. Negative controls were then selected for their values close to: NC1= 0.136; NC2=0.122; NC3=0.149.

23 positive samples (OD>0.500) had a MeanP of 0.834. Positive control were selected as: PC1=0.834; PC2=0.750; PC3=0.917.

All ELISA were carried out in duplicate for each sample, together with the 6 control sera and the results expressed in RPP.

The COV was determined based on the mean RPP of the 300 negative samples: COV (%): mean RPPN (%) + 3 standard deviation (%). Sample statuts was positive when the RPP of a sample was > the COV.

100µl of 20 seropositive and 20 seronegative blood samples were submitted to DNA preparation with Flexigene DNA kit (Quiagen®) and PCR analysis with specific primers for Trypanozoon (13).

Serological survey: Amongst 500 samples, a total of 49 samples were positive for ELISA-*T. evansi* (10%). In infected farms, the seroprevalence was nil in animals less than 1 year, while it was of 39% between 1-5 years, and 34% above.

A total of 15 farms were infected amongst 61 (25%). At the farm leve,l the prevalence rate was from 0 to
67%, with a mean of 33% in infected farms. In these farms, 150 animals were exposed to infected animals, which represents 30% of the population screened. At the regional level, prevalence rate was lower in Khon Kaen, while it was very close in the other regions with 15% in Chiang Mai, 16% in Sakon Nakon and 17% in Udon Thani. Amongst 20 seropositive and 20 seronegative samples tested, respectively 12 and 0 were positive by PCR confirming the specificity of the ELISA. Among this preliminary survey, all region of Thailand were investigated and proved to be infected by T. evansi at various levels, and 30% of the animals are exposed to the infection. The fact that animals less than 1 year were not infected is generally described in disease mechanically transmitted by insects, due to the fact that young animals are less attractive than older ones, and since adults may act as a protective barrier between insects and calves. Trypanosomosis can still be one of the explicative factor for abortion in and/or unfertility in dairy cattle, together with Neospora and Toxoplasma.

Standardisation of the ELISA was done with dairy cattle samples from Thailand; however the soluble antigen used in this study was produced in France with a parasite isolated in camel from Canary Islands. It is now on work to produce a local antigen with a T. evansi isolated from Rusa deer in 2001, in Ratchaburi, Thailand. The further step of this regional project is to distribute the test inside Thailand via a number of workshop aiming at protocol training and standardisation. Trypanosomose due to T. evansi has been little studied due to poorly characteristic symptoms. For these reasons, T. evansi is rarely looked for and then, rarely detected. The study of its geographical distribution, host and vector range and relative prevalence, other ways of transmission and medical and economical impacts, are necessary preliminary research to be developed to generate a better knowledge of this disease, its importance and to develop potential means of control. The zoonotic potential of T. evansi should also be explored.

The ELISA developed in this study had been already proved with high efficient in heterologous system for detection of Chagas disease in humans. It would be even more efficient in the present case (homologous system) to evaluate the contact between humans and parasite in Asia. The risk for human contamination should be considered in professional activities such as farmers and veterinary technicians, but it should also be explored for people exposed to infection by manipulating or eating raw meat from pig or buffaloes (Fig. 2).

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References


Fig. 1: traditional slaughtering & distribution of raw porc’s meat in North of Chiang Mai, Thailand; risk for percutaneous transmission

Fig. 2: Traditional preparation of raw porc’s blood & meat; risk for percutaneous and periorale transmission.