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Prevalence of *Trypanosoma evansi* Infection Causing Abortion in Dairy Cows in Central Thailand

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ABSTRACT

Abortion in dairy cows is the major factor affecting livestock development in Thailand and is caused by many diseases. Trypanosomosis is one of these factors and also results in an immunosuppressive effect in cattle. The objective of this study was to investigate the prevalence of trypanosomosis in dairy cows in central Thailand. From March to September 2007, 544 samples were collected from 105 farms in the five major dairy provinces of Kanchanaburi, Ratchaburi, Nakhon Pathom, Saraburi and Lop Buri. ELISA was performed to test all sera. The overall prevalence of *T. evansi* infection in dairy cows was 8.1% (44/544) and herd prevalence was 19.2% (20/105). The highest individual prevalence was found at Saraburi (17.4%, 21/121) but the highest number of herd infections was at Nakhon Pathom (30%, 6/20). The parity-four and four-plus cows were 3.7 times more likely to be infected than heifers and parity-one cows (P<0.034). Large herds (40 milking cows) were found to be 5.4 times more infected than small herds (P<0.021). The results found that trypanosome infection might be the predisposing cause of other diseases and is a barrier to productivity gains in dairy herds.

Key words: Trypanosomosis, dairy cow, prevalence, central Thailand

INTRODUCTION

Trypanosoma evansi is a parasite of camels and horses, originating from Africa, which is mechanically transmitted by biting insects (Aradaib and Majid, 2006). Due to healthy carriers, "surra" is a neglected disease which easily spread into Latin America, Asia and more recently to Spain (Gutierrez *et al.,* 1998) and France (Desquesnes *et al.*, 2008) where it can be considered as an emerging disease (Davison *et al.*, 1999).

T. evansi infection is normally a subclinical disease in cattle; however, its pathogenicity seems to be diverse among Southeast Asian countries, where it is present from Myanmar to Indonesia (Kaewthamasorn and Wongsamee, 2006; Luckins, 1988) affecting

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horses, water buffaloes, pigs and cattle, inducing fever, loss of weight, nervous symptoms and abortion (Lohr *et al*., 1986; Lun *et al*., 1993). It is also responsible for failures in vaccination campaigns against foot and mouth disease, hemorrhgic septicaemia and classical swine fever (Payne *et al*., 1993). More recently, the zoonotic potential of *T. evansi* has been stressed by several cases reported in humans in India (Powar *et al*., 2006); a serological survey demonstrated a significant contact between humans and the parasite. Contamination in humans could be due to insects, by either a transcutaneous or peroral route.

Few data are available for the study of trypanosomosis with *T. evansi*, which is due to poorly characterized 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 economic impacts, are necessary preliminary research to be developed to generate a better knowledge of this disease. It is important to develop potential means of control. The zoonotic potential of *T. evansi* should also be explored.

Trypanosomosis caused by *T. evansi* is a significant constraint to livestock productivity and health in major areas of Thailand. The most important symptom of the infection in cattle is abortion, which occurs suddenly in the late stage of pregnancy without any clinical signs (Lohr *et al*., 1986; Lun *et al*., 1993; Kashiwazaki *et al*., 1998).

The best way to diagnose trypanosomosis is to isolate the parasite in the blood but the reliability of this parasitological technique is frequently questioned because of the paucity and cyclical fluctuation of parasitaemia. Serological diagnosis for antibody detection is often hampered by the inability to distinguish a current infection from past exposure and lack of specificity.

Epidemiological studies should be carried out in Southeast Asia with comparable tools in order to increase the knowledge on this neglected but emerging disease. The present study described standardization of an ELISA for *T. evansi* through an epidemiological survey in a random sample of 544 dairy cattle in Thailand.

MATERIALS AND METHODS

Cryopreserved *T. evansi* isolated from camels in France and previously confirmed for species identification, was inoculated intraperitonally to two wistar rats. At the peak of parasitaemia, parasites were separated by DEAE-cellulose (Reid, 2002) and soluble antigens were prepared as previously described (Desquesnes *et al.,* 2007) in 1mg/ml protein concentration, stored at -80°C and transferred on dry ice to Thailand. From March to September 2007, 544 dairy cows were sampled from 105 farms in the central region of Thailand including Kanchanaburi, Ratchaburi, Nakhon Pathom, Saraburi, and Lop Buri provinces. A questionnaire was used to collect data on the cow and herd characteristics at each farm visit. Blood was collected from the jugular vein in sterile and citrated tubes, for serology and PCR examinations, respectively. Sera and whole blood were kept at -20°C until processing. The ELISA procedure was derived from a technique previously described (Desquesnes *et al*., 2007) and results were expressed in optical densities (OD), with OD < 0.250 considered as negative. Statistical analysis of descriptive data was performed using logistic regression to determine unconditional associations between disease status and each variable that were significant at $p < 0.05$ (two-sided), based on the likelihood ratio Chi-square test. All analyses was conducted using the STATA statistical software package (version 8.2, Stata Corp, 2003, College Station, TX).

RESULTS

The overall prevalence of *T. evansi* was 8.1%. Cows with more than four lactations had the highest seroprevalence (18.6%). A total of 19.1% (20/105) of dairy farms were infected. The highest endemic area for *T. evansi* infections in dairy cows was Nakhon Pathom (30%). However, Saraburi had the highest number of cows infected (17.4%) (Table 1). The parity-four and four-plus cows were 3.7 times more likely to be infected than heifers and parity-one cows ($P<0.034$). Large herds (40 milking cows) were 5.4 times significantly more infected than small herds (5-10 miking cows ($p<0.021$).

DISCUSSION

From this preliminary survey, the central region of Thailand was infected by *T. evansi* at various levels, and 8.1% of the animals were seropositive, which differed from the result of 25% by Kashiwasaki *et al.* (1998) in Loei province using a haematocrit centrifuge technique, and the result of 40% by Pholpark *et al.* (1999) using ELISA. The different results were due to variations

Table 1 Factors associated with *T. evansi* infection of dairy cows in central Thailand (CI = confidence interval at 95%).

Factors	Category	Number of examined	Number of positive (% and CI)	P-value
Lactation	0	25	4 (16.0 ± 14.4)	
	1	121	6 (4.9 ± 3.9)	
	2	121	10 (8.3 ± 4.9)	
	3	112	3 (2.7 ± 3.0)	
	4	**70**	**13 (18.6 ± 9.1)**	**0.034**
	5	39	3 (7.7 ± 8.4)	
	6	26	4 (5.0 ± 13.9)	
	7	16	0	
	8	8	0	
	9	2	1	
	10	1	0	
	11	1	0	
	12	2	0	
Farm holders	Kanchanaburi	21	1 (4.8 ± 9)	**0.024**
	Ratchaburi	21	5 (23.8 ± 18.2)	0.076
	Nakhon Pathom	20	6 (30 ± 20.1)	**0.009**
	Saraburi	24	7 (29.2 ± 18.2)	0.947
	Lop Buri	19	1 (5.3 ± 10.0)	
	Total	**105**	**20 (19.1 ± 7.5)**	
Dairy cows	Kanchanaburi	112	1 (0.9 ± 1.7)	
	Ratchaburi	112	11 (9.8 ± 5.5)	
	Nakhon Pathom	103	10 (9.7 ± 5.7)	
	Saraburi	121	21 (17.4 ± 6.7)	
	Lop Buri	96	1 (1.04 ± 2.03)	
	Total	**544**	**44 (8.1 ± 2.3)**	

in sample regions, the season of sampling, and the method of detection. The rainy season should provide the optimal climatic conditions for fly activity and thus the best timing for mechanical transmission in dairy cows.

The results indicated the degree of infertility in dairy cows in the central provinces of Thailand, especially in larger herds (> 40 milking cows) which were 5.4 times more infected than small herds (P<0.021). No treatment for the successful elimination of the infection has been reported recently; therefore, screening tests are the only way to isolate negative animals from the positives. The highest infection was statistically significant in cows with more than 4 lactations (P<0.034), which might suggest some kinds of cumulative effect.

Kashiwasaki and Thammasart (1998) used ELISA for detection of *T. evansi* infection of dairy cattle in Loei; the ELISA was developed and proved highly efficient in a heterologous system for the detection of trypanosomosis in dairy cows, compared to other technique, such as PCR. It would be even more efficient in the present case (homologous system) to evaluate the contact between humans and parasites in Asia (Desquesnes, 1997).

The risk for human contamination should be considered for individuals working as farmers and veterinary technicians, but the risk of infection should also be explored in people handling or eating raw pork or buffalo meat.

Trypanosomosis could be an explanatory factor for abotion and/or infertility in dairy cows, together with *Neospora* spp. and *Toxoplasma* spp. To prevent farmers from experiencing economic loss caused by trypanosomosis, a systematic control programme using diagnostic tools, trypanocidal drugs and repellents to protect animals from the vector should be established.

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