

***Neospora caninum* and *Toxoplasma gondii* Antibodies in Captive Elephants (*Elephas maximus indicus*) in Kanchanaburi Province**

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Abstract

Although neosporosis has long been detected in several wildlife species from different parts of the world, until now there has been no report of *Neospora caninum* infection in elephants in any country of the world. Serum samples of 115 captive elephant (*Elephas maximus indicus*) from the westernmost province of Kanchanaburi, Thailand were investigated for antibodies to *N. caninum* and *Toxoplasma gondii*. Antibodies of *N. caninum* were detected by the competitive ELISA (cELISA) test and *T. gondii* by the Latex agglutination (LAT) test. The prevalence of *T. gondii* antibodies was 13.04% (15/115), while anti-*N. caninum* was 33.04% (38/115). Only 7/115 (6.09%) were positive for both parasites. Our study showed a higher seroprevalence for *N. caninum* in elephants than the prevalence of *N. caninum*-infection in dairy cattle in Thailand from prior studies.

Keywords: elephant, *Neospora caninum*, Thailand, *Toxoplasma gondii*, seroprevalence

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บทคัดย่อ

แอนติบอดีต่อ *Neospora caninum* และ *Toxoplasma gondii* ในช้างเลี้ยงในจังหวัดกาญจนบุรีประเทศไทย

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ปัจจุบันมีรายงานการเกิดโรคโนอสปอริซในสัตว์ป่าหลายชนิดทั่วโลก แต่ยังไม่เคยมีรายงานการติดเชื้อ *Neospora caninum* ในช้าง การศึกษานี้จึงได้ตรวจหาแอนติบอดีต่อ *N. caninum* และ *Toxoplasma gondii* จากชิ้นรั่มของช้างเลี้ยงในจังหวัดกาญจนบุรี ทั้งหมด 115 ตัวอย่างโดยใช้ชุดตรวจ competitive ELISA (cELISA) และ Latex agglutination (LAT) test ตามลำดับ ผลการตรวจพบว่า ช้างตั้งกล้ามแอนติบอดีต่อ *N. caninum* ร้อยละ 33.04 (38/115) และมีแอนติบอดีต่อ *T. gondii* ร้อยละ 13.04 (15/115) ซึ่งอัตราอยู่ระหว่าง 6.09 (7/115) พbmีแอนติบอดีต่อเชื้อทั้งสองชนิด การศึกษาครั้งนี้พบว่าความชุกของแอนติบอดีต่อ *N. caninum* ในช้างสูงกว่าความชุกของโรคโนอสปอริซในโคนมที่เคยมีรายงานก่อนหน้านี้มาก

คำสำคัญ: ช้าง *Neospora caninum* ไทย *Toxoplasma gondii* ความชุก

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Introduction

Neospora caninum and *Toxoplasma gondii* are two closely related parasites (Buxton et al., 2002). *N. caninum* is a major pathogen of cattle with the Canidae acting as the definitive host (Anderson et al., 2000; Dubey, 2003). It is now recognized as a significant cause of economic loss in dairy and beef cattle herds worldwide due to abortion and reduced reproductive efficiency (Trees et al., 1999). The prevalence of *N. caninum* infection in cattle has been reported in many parts of the world (Gondim et al., 1999; Reichel, 2000; Koiwai et al., 2006; Yu et al., 2007).

T. gondii can infect a wide range of animal species in which the severity of the disease varies depending on the susceptibility of the species (Innes, 1997; Tenter et al., 2000). Felidae are the definitive hosts of *T. gondii* (Hegab and Mutawa, 2003; Montoya and Liesenfeld, 2004). There are many reports of clinical toxoplasmosis in some zoo species (Crawford et al., 2000; Epiphanio et al., 2000; Epiphanio et al., 2003; Sedlak et al., 2004; Spencer et al., 2004; Barrows, 2006; Harrison et al., 2007; Hartley et al., 2008; Pas and Dubey, 2008; Basso et al., 2009; Bermúdez et al., 2009; Dubey et al., 2009). Whether *T. gondii* can cause disease in elephants is not known, but to our knowledge there have been no previous reports of clinical toxoplasmosis in elephants anywhere in the world.

Little is known about the infection of *N. caninum* and *T. gondii* in elephants, which may serve as an intermediate host of these parasites. The aim of this study was to determine the seroprevalence of *N. caninum* and *T. gondii* in elephants in Kanchanaburi province, Thailand.

Materials and Methods

Blood samples were collected from the jugular vein of 115 elephants from 12 elephant camps located in 10 districts of Kanchanaburi province, between April 2009 and February 2010. All blood samples were allowed to clot before centrifugation and removal of serum which was then stored at -20°C until required for serological analysis.

Serological examination: *N. caninum* specific IgG antibodies were detected by a commercial cELISA test (VMRD, Pullman, USA) following the manufacturer's instructions. Briefly, the 96-well plates coated with *N. caninum* specific antigen provided in the kit were incubated with undiluted tested sera. Samples were tested in duplicate, and positive and negative control sera were included in each test series. The plates were washed after incubation and a conjugate was added. The plates were washed again, and a chromogenic enzyme substrate was added. The optical density (OD) at 620 nm was read using a photometer (Bio-Tek

Instruments, USA). The results were expressed as the percentage of inhibition. Sera were considered positive if the sample caused $\geq 30\%$ inhibition, as indicated by the manufacturer.

T. gondii specific IgG antibodies were detected by a commercial LAT test (TOXOCHECK-*MT*, Eiken, Japan). Briefly, the reactions were performed using 96 well U-bottom polystyrene microplate at two-fold dilution. The sera were screened at dilutions of 1:16 to 1:256 including positive and negative control sera provided in the kit. Twenty-five microlitre of *T. gondii* antigen-coated latex particles suspension was added to each well. The plates were incubated overnight at room temperature. An agglutination reaction at a dilution of 1:64 was considered positive according to the manufacturer's instructions.

Statistical analysis: The qualitative variables were described using frequencies and percentages. Comparison of *N. caninum* and *T. gondii* in different camps, sexes and age groups was performed by an χ^2 -test. The differences were considered statistically significant when $p \leq 0.05$.

Results and Discussion

The seroprevalence of antibodies against *N. caninum* and *T. gondii* in elephants by age and sex is shown in Table 1. Antibodies to *N. caninum* were found in 38 of 115 elephants (33.04%). The percentage of inhibition varied from 30.32-73.20%. There was no difference in seroprevalence of *N. caninum* between sexes and between age groups. Antibodies to *T. gondii* were detected in 15 of 115 elephants (13.04%). The antibody titers in positive samples varied from 1:64 (7 samples), 1:128 (4 samples), 1: 256 (3 samples) to 1:512 (1 sample). Significant differences in seroprevalence of *T. gondii* were found between age groups. The prevalence of *T. gondii* infection in elephants in age group between 21-40 years and more than 40 years were statistically significantly lower than the age group between 1-20 years ($p=0.005$), but there was no significant difference between sexes. The seroprevalence for *N. caninum* was significantly higher than *T. gondii* ($p<0.001$). All camps had at least one sample positive to either *N. caninum* or *T. gondii*. Seven samples (6.09%) were positive to both *N. caninum* and *T. gondii*.

Table 1 Seroprevalence of *N. caninum* and *T. gondii* antibodies in elephants in Thailand

Factor	No. examined	Positive serum (%)		
		<i>N. caninum</i>	<i>T. gondii</i>	Both
Age group (years)				
1-20	16	6 (37.5)	6 (37.5)	1 (6.25)
21-40	49	16 (32.65)	6 (12.24)	4 (8.16)
>41	37	12 (32.43)	3 (8.11)	2 (5.41)
No Data	13	4 (30.77)	0 (0)	0 (0)
Sex				
Male	30	6 (20)	3 (10)	0 (0)
Female	78	30 (38.46)	12 (15.38)	7 (8.97)
No Data	7	2 (28.57)	0 (0)	0 (0)
Total	115	38 (33.04)	15 (13.04)	7 (6.09)

Although the Indirect Fluorescent Antibody test (IFAT) was considered to be the reference method to detect *N. caninum* antibodies, it has the disadvantage of needing species-specific conjugates which are impossible to find in some species. As previous studies have reported that the ELISA test has a high correlation with the IFAT for detecting antibodies to *N. caninum* (Bjorkman and Uggla, 1999; Maley et al., 2001), thus the commercial ELISA test was chosen for use in this study. The kit has been validated for bovine sera; however, its principle of competition makes it theoretically possible to be used in other species (Almeria et al., 2007).

In Thailand, Suteeraparp et al. (1999) and Kyaw et al. (2004) showed that 6% of 904 dairy cattle and 5.5% of 549 dairy cattle from central Thailand had anti-*N. caninum* antibodies, respectively. Chanlun et al. (2007) showed that 8% of 424 dairy cattle from northeast Thailand had antibodies to *N. caninum*. Recently, Nam et al. (2012) showed that 4.5% of 532 swamp buffaloes from northeast Thailand had antibodies to *N. caninum*.

To our knowledge, this is the first evidence of presence of *N. caninum* antibodies in elephants. A serological study in elephants has not been done before in Thailand although *N. caninum* was isolated from aborted cattle (Kyaw et al., 2003) and recently, there was a case reported of systemic neosporosis in white rhinoceros (*Ceratotherium simum*) from Thailand (Sommanustweechai et al., 2010). The seroprevalence of *N. caninum* in elephants (33.04%) was statistically significantly higher than *T. gondii* (13.04%) ($p<0.001$) which indicated that elephants in this area had more exposure to *N. caninum* than to *T. gondii*. We did not find any differences in seroprevalence of *N. caninum* between females and males and between any age groups. As the prevalence of *N. caninum* infection in cattle in this area has not been reported, the high prevalence of *N. caninum* in elephants may reflect a high rate of infection in cattle in this region and suggest that elephants may have frequent contact with *N. caninum*. Further studies are needed to determine the potential role of this protozoan infection as a cause of abortion and for its effect on the reproductive efficiency of elephants in Thailand.

Elephants are herbivores and their life spans are similar to that of humans. They probably become infected by *N. caninum* due to the ingestion of large amounts of food or water contaminated with oocysts excreted by dogs in this area (McAllister et al., 1998; Lindsay et al. 1999). There was one study which reported that dogs from this area were 1.2% seropositive to *N. caninum* (Kyaw et al., 2004). In Thai rural culture, pet owners usually free their pets, including dogs, outside the house, which can increase the risk of oocyst shedding in the environment. Besides this horizontal transmission, vertical transmission, which is considered to be the major route of neosporosis transmission in cattle (Schares et al., 1998; Fioretti et al., 2003) might be possible in elephants due to the fact that the number of *N. caninum* oocysts shed in canine feces is low (Lindsay et al., 1999; Dijkstra et al., 2001). A long-term study of elephants would be needed to confirm this hypothesis.

LAT was used as a screening serological test for *T. gondii* infection in animals (Jittapalapong et al., 2005). Antibodies against *T. gondii* were present in 13.04% of elephants in this study. Our results were lower than the previous report by Tantasawan et al. (2001), who reported 25.6% seropositive to *T. gondii* by LAT in elephants in Thailand, and Dangolla et al. (2006) who found 32% seropositivity in elephants (*Elephas maximus maximus*) in Sri Lanka. Among serological surveys of *T. gondii* infection in animals and humans in Thailand, *T. gondii* antibodies were found in 27.9% of goats (Jittapalapong et al., 2005), 7.3% of cats (Sukthana et al., 2003), 15.4% of wild felids (Thiangtum et al., 2006) and 3.1% of healthy individuals (Maruyama et al., 2000). Only 7 of 115 elephants (6.09%) had antibodies to both *N. caninum* and *T. gondii*, indicating that cross reactions between these parasites are rare (Panadero et al., 2010).

In conclusion, the results of this study indicate that antibodies reacting with *N. caninum* and *T. gondii* are present in Thai elephants. Further studies are necessary to determine the possible potential role of these parasites as causes of abortion, stillbirth, resorption or congenital infection in elephants.

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