TOXOPLASMOSIS AND NEOSPOROSIS AMONG BEEF CATTLE SLAUGHTERED FOR FOOD IN WESTERN THAILAND

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Abstract. Beef is a main type of meat consumed by Thais. The prevalences of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies were investigated among beef cattle slaughtered for food in western Thailand. A total of 389 blood samples obtained from beef cattle from 24 herds were collected at 3 slaughterhouses in 3 western provinces of Thailand: Kanchanaburi, Ratchaburi and Nakhon Pathom. An indirect immunofluorescent antibody test (IFAT) was performed using cut-off values of 1:128 for *T. gondii* and 1:200 for *N. caninum*. The antibodies to *T. gondii* were found in 100 samples (25.7%) and antibodies to *N.caninum* were found in 23 samples (5.9%) a significant difference ($p<0.001$) in prevalences, indicating the cattle tested had a greater exposure to *T.gondii* than *N.caninum*, and they should be regarded as a potential source of *T. gondii* infection to humans. The low prevalence of neosporosis in this study is still a risk for morbidity among cattle, including abortions. This is the first study in Thailand finding both *T. gondii* and *N. caninum* antibodies among beef cattle.

Keywords: *Neospora caninum, Toxoplasma gondii*, beef cattle, slaughter, Thailand

INTRODUCTION

Toxoplasma gondii and *Neospora caninum* are closely related protozoan parasites with a worldwide distribution (Payne and Ellis, 1996; Hemphill *et al*, 1999; Buxton *et al*, 2002; Dubey *et al*, 2002). *T. gondii* can affect many animal species, including humans, and can cause disease varying from subclinical to fatal infection depending on the host immune status and species (Innes, 1997). Toxoplasmosis is a major cause of abortion in sheep and goats but is considered uncommon in cattle (Tenter *et al*, 2000). Although *T. gondii* infection does not cause clinical disease in cattle, the tissue cysts may persist in muscles and visceral organs of infected hosts for years or even life (Esteban-Redondo and Innes, 1997; Tenter *et al*, 2000) and can be a source of toxoplasmosis among human who ingest undercooked meat. *T. gondii* infection in humans results in subclinical disease among immunocompetent hosts but is an important cause of morbidity and

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Fig 1–Map of Thailand showing study areas: 1) Kanchanaburi, 2) Nakhon Pathom, and 3) Ratchaburi Province.

mortality among immunodeficient and neonatal hosts (Montoya and Liesenfeld, 2004).

Contrary to *T. gondii*, *N. caninum* is one of the most causes of abortion and neonatal mortality in cattle (Anderson *et al*, 2000). *N. caninum* tissue cysts are harbored in the host's organs for life and can be repeatedly reactivated causing abortion in pregnant hosts (Anderson *et al*, 2000; Buxton *et al*, 2002). The effect of *N. caninum* infection among other domestic animals is unclear but there is no evidence that *N. caninum* can cause clinical illness in humans.

No information is available about the prevalence of *T. gondii* and *N. caninum* infections among cattle in Thailand, a major source of food. The objective of this study was to determine the prevalence of these diseases among beef cattle at slaughterhouses in western Thailand. This information may be used to combat food-borne zoonotic protozoa among humans and improve farm husbandry production.

MATERIALS AND METHODS

Study areas and samples

Blood samples were collected by veterinary practitioners from 389 beef cattle between July 2009 and December 2010 from 24 herds at 3 slaughterhouses in Kanchanaburi Province, Ratchburi Province and Nakhon Pathom Province, Thailand (Fig 1). The animals were of both sexes, 1-6 years old and originated from local farms in those areas. The reproductive backgrounds of those animals were not known. None of the cattle had overt symptoms of disease at sampling. All blood samples were collected in plain clot tube and stored at -20°C until analysis.

Serological examination

T. gondii- and *N. caninum*-specific IgG antibodies were detected using an indirect fluorescent antibody test (IFAT) as previously described by Wiengcharoen *et al* (2011), adopting 1:128 and 1:200 dilutions as cut-off points for diagnosing *T. gondii* and *N. caninum* infection, respectively. Goat anti-bovine IgG labeled with fluorescein isothiocyanate (FITC) was used as the conjugate. Negative and positive control sera were included in all tests.

Statistical analyses

The qualitative variables were des-

Table 1
Seroprevalence of *T. gondii* and *N. caninum* among beef cattle by sex and age groups.

Animals	Number of animals	*T. gondii*		*N. caninum*	
		Number positive	Seroprevalence (%)	Number positive	Seroprevalence (%)
Sex:					
Male	174	35	20.1	19	10.9
Female	215	65	30.2	4	1.9
Age:					
≤ 1 year	18	6	33.3	1	5.6
> 1 years	371	94	25.3	22	5.9
Total	389	100	25.7	23	5.9

cribed using frequencies and percentages. Comparison of *T. gondii* and *N. caninum* by sex and age group was performed using the χ^2-test. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

The prevalence results are shown in Table 1. For *T. gondii*, 100 of 389 beef cattle (25.7%) had titers equal to or exceeding 1:128. Among male beef cattle, the seroprevalence was 20.1% (35/174) and among females it was 30.2% (65/215) which was a significant difference (p=0.05). There were no significant differences by age group.

For *N. caninum*, 23 of 389 beef cattle (5.9%) had titers equal to or exceeding 1:200. Among male beef cattle, the seroprevalence was 10.9% (19/155) and amongs females it was 1.9% (4/215), a significant difference (p<0.001). There were no significant differences by age group.

T. gondii was statistically more prevalent than *N. caninum* (p<0.001).

DISCUSSION

Cattle, pigs and poultry are the main types of livestock in Thailand. There have been no previous published surveys of *T. gondii* and *N. caninum* infection among beef cattle in Thailand. We studied the seroprevalence of *T. gondii* and *N. caninum* infection among beef cattle in Thailand. The results show *T. gondii* and *N. caninum* infections are common in the studied region.

In our study 25.7% of beef cattle tested were infected with *T. gondii*, a lower seroprevalence than in chicken (64.0%) (Chumpolbanchorn *et al*, 2009) and goats (27.9%) (Jittapalapong *et al*, 2005) but higher than among dairy cattle (9.4%) (Inpankaew *et al*, 2010), humans (3.1-6.4%) (Maruyama *et al*, 2000; Sukthana *et al*, 2003), dogs (9.4%) (Jittapalapong *et al*, 2007) and cats (4.8-7.3%) (Sukthana *et al*, 2003; Jittapalapong *et al*, 2010) in Thailand.

The seroprevalence of *T. gondii* was higher in our study than that reported from beef cattle in Iran (0%) (Sharif *et al*, 2007) China (2.3%) (Yu *et al*, 2007) and Egypt (10.75%) (Ibrahim *et al*, 2009) but lower than that reported from Serbia (76.3%) (Klun *et al*, 2006) and Brazil (71.0%) (Santos *et al*, 2010). These differences may be explained by differences by regions, serological tests, cut-off values, sample sizes and sampling procedures (Faria *et al*, 2007).

Little is known about the best serological method to diagnose *T. gondii* infection in cattle, because even though the Sabin-Feldman dye test is considered the gold standard to diagnose toxoplasmosis in humans, it can give erratic results in cattle (Dubey *et al*, 1985). The IFAT is generally accepted as the most appropriate method to detect *T. gondii* antibodies among cattle because it gives fairly reliable results (Uggla and Nilsson, 1985; Esteban-Redondo and Innes, 1997). There is minimal information about the time course for *T. gondii* antibody levels to form naturally in infected cattle, but IgG antibodies appear 7-14 days after inoculation and persist for at least 98 days (Bevery and Henry, 1977; Wiengcharoen *et al*, 2011).

Cattle are considered unfavor hosts for *T. gondii* (Stalheim *et al*, 1980; Dubey, 1983, 1986; Esteban-Redondo *et al*, 1999; Masala *et al*, 2003). However, seropositive cattle can harbor *T. gondii* tissue cysts and once humans consume undercooked infected meat there is a potential risk for toxoplasmosis (Tenter *et al*, 2000; Kijlstra and Jongert, 2009; Tenter, 2009). There are reports of toxoplasmosis outbreaks from beef and dairy products (Kean and Kimball, 1969; Lord *et al*, 1975).

Fewer than 1% of humans and livestock acquire *T. gondii* infection transplacentally (Dubey and Jones, 2008). Most people and animals acquire *T. gondii* infection postnatally by ingestion of undercooked infected meat or ingestion of food or water contaminated with oocysts from felid excreta. The high prevalence of *T. gondii* among beef cattle indicates *T. gondii* oocysts are present in this region. This is important information for preventive programs because the sporulated oocysts of *T. gondii* are environmentally resistant. They may remain infectious in moist soil or sand for up to 18 months (Kuticic and Wikerhauser, 1996; Frenkel and Dubey, 2000). During that period, they may be spread mechanically by flies, cockroaches, beetles and earthworms (Dubey, 2004).

Due to the high seroprevalence of *T. gondii* infections among beef cattle, it is important to investigate infections among domestic and wild cats and people living in this region. This information would provide a clearer picture why there is a high prevalence of toxoplasmosis in this region.

The prevalence of *N. caninum* in our study (5.9%) is within the range (5.5-8%) found in dairy cattle from other parts of Thailand (Suteeraparp *et al*, 1999; Kyaw *et al*, 2004; Chanlun *et al*, 2007). These findings differ from those of Quintanilla-Gozalo *et al* (1999), Moore *et al* (2002) and Koiwai *et al* (2005), who found the prevalence of anti-*N. caninum* antibodies was significantly higher among dairy cattle than beef cattle.

The seroprevalence of *N. caninum* infection among beef cattle in the present study was higher than that detected in Sweden (2.8%) (Loobuyck *et al*, 2009) and Korea (4.1%) (Kim *et al*, 2002) but lower than Brazil (9.5%) (Aguiar *et al*, 2006).

Only 1.5% (6/389) of samples was positive for both *T. gondii* and *N. caninum*, indicating co-infection with these two parasites is uncommon (Panadero *et al*, 2010).

Alexander and Stinson (1988) found female animals were more susceptible to protozoan infections than males. There is also a cumulative effect of age on the prevalence of protozoan infections among many food animals (Tenter *et al*, 2000). In the present study the prevalence of *T. gondii* antibodies was more common among female beef cattle than males, but male beef cattle had a higher prevalence of

N. caninum antibodies. There was no cumulative effect of age on parasite prevalence in this study.

The high seroprevalence of anti-*T. gondii* antibodies among beef cattle in this study may be explained by the frequent presence of cats on cattle farms in Thailand. Most cats and dogs in Thailand are raised either outdoors or both outdoors and indoors. These animals have access to the water and food of the livestock and excrete around the farm. *T. gondii*-infected cats can release enormous numbers of oocysts of *T. gondii* (Dubey, 2004). The high prevalence of *T. gondii* infection supports the hypothesis that ingestion of oocysts shed by cats, may be the primary mode of infection among cattle (Gottstein *et al*, 1998). The low prevalence of *N. caninum* infections, despite the presence of dogs on the farm, supports the hypothesis that vertical transmission is the main route of transmission of *N. caninum* and horizontal transmission may be only minor route of transmission (Schares *et al*, 1998).

In conclusion, this study shows the presence of anti-*T. gondii* and anti-*N. caninum* specific antibodies among beef cattle in Thailand and reflects a wide distribution of *T. gondii* oocysts in the environment. Research on domestic and wild felids as a source of *T. gondii* oocysts in this region is a priority. It is also necessary to conduct further studies to determine the potential risk of toxoplasmosis transmission to humans through consumption of meat contaminated with tissue cysts and the impact of toxoplasmosis and neosporosis on the animal industry. This study shows the prevalence of *T. gondii* and *N. caninum* infection in Thailand in a specific region. This information can be used to assist in developing strategies for controlling these diseases and improve beef cattle husbandry production.

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