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Seroprevalence of *Neospora caninum* in Swamp Buffaloes and Beef Cattle in the North-east of Thailand

Nguyen Hoai Nam^{1,4} Aran Chanlun² Kwankate Kanistanon³ Suneerat Aiumlamai^{1*}

Abstract

A seroepidemiological survey was carried out to investigate seroprevalence of *Neospora caninum* in swamp buffaloes from five provinces in north-eastern Thailand, and to compare infection status in swamp buffaloes and beef cattle at one area of Thailand. A single serum sample was collected from each of 532 swamp buffaloes and 78 beef cattle during January and July 2009. Antibodies against *N. caninum* were analyzed by a commercial ELISA test. Chi-square tests were used to compare the seroprevalence between species, gender and age groups in Khon Kaen. Result showed that infection prevalence in swamp buffaloes in northeastern Thailand was 4.5%. At one area in Khon Kaen province, proportions of seropositive swamp buffaloes and beef cattle were 6.4% and 43.6%, respectively, with significant difference ($p < 0.001$). In both species, gender and age had no significant effects on the *N. caninum* infection status. The present study indicated that seroprevalence of *N. caninum* infection in swamp buffaloes in northeastern Thailand was low, and also suggested that swamp buffaloes were more resistant to *N. caninum* than beef cattle.

Keywords: Beef cattle, *Neospora caninum*, seroprevalence, swamp buffaloes, Thailand

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บทคัดย่อ

ความชุกของการติดเชื้อ นีโอสปอรา เคโนนุมา ของกระบือปลักและโคเนื้อ ในภาคตะวันออกเฉียงเหนือประเทศไทย

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ทำการศึกษาความชุกของการติดเชื้อ นีโอสปอรา เคโนนุมา ของกระบือปลักในพื้นที่ 5 จังหวัดในภาคตะวันออกเฉียงเหนือของประเทศไทย และเปรียบเทียบความชุกการติดเชื้อดังกล่าวในกระบือปลักและโคเนื้อที่เลี้ยงในพื้นที่เดียวกันในจังหวัดขอนแก่น ตัวอย่างซีรัมจากกระบือปลักและโคเนื้อจำนวน 532 ตัวและ 78 ตัว ถูกเก็บในช่วงมกราคม-กรกฎาคม 2552 และถูกทดสอบหาแอนติบอดีที่จำเพาะต่อเชื้อดังกล่าวด้วยชุดทดสอบสำเร็จรูปไอโซชา ในจังหวัดขอนแก่นศึกษาความแตกต่างทางสถิติของความชุกของโรคในชนิดสัตว์ เพศ และอายุ ด้วยวิธีโคสแคร์ว ผลการศึกษาพบว่าความชุกของการติดเชื้อ นีโอสปอรา เคโนนุมา ในกระบือปลักในภาคตะวันออกเฉียงเหนือเป็นร้อยละ 4.5 ในจังหวัดขอนแก่นพบความแตกต่างอย่างมีนัยยะสำคัญของความชุกการติดเชื้อในกระบือปลักและโคเนื้อที่เลี้ยงในพื้นที่เดียวกันคือร้อยละ 6.4 และ 43.6 ตามลำดับ ($p < 0.001$) แต่ไม่พบความแตกต่างของการติดเชื้อระหว่างเพศและอายุ การศึกษานี้แสดงให้เห็นว่าความชุกการติดเชื้อ นีโอสปอรา เคโนนุมา ในกระบือปลักมีระดับต่ำ และกระบือปลักมีความทนต่อการติดเชื้อนี้มากกว่าโคเนื้อ

คำสำคัญ: นีโอสปอรา เคโนนุมา ความชุกการติดเชื้อ กระบือปลัก โคเนื้อ ประเทศไทย

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Introduction

Neospora caninum is an obligate intracellular parasite which can infect a wide range of animals (Dubey et al., 2007). Parasite infection can induce bovine abortion, and is responsible for tremendous economic loss in the cattle farming industry (Barling et al., 2000; Reichel, 2000; Chi et al., 2002; Hasler et al., 2006). Serological evidence of *N. caninum* infection in dairy and beef cattle has been reported worldwide (Dubey et al., 2007). In the buffalo, seroprevalence ranged from 0% to 70.9% (Gennari et al., 2005; Yu et al., 2007).

In Thailand, infection of *N. caninum* was first reported in dairy cattle (Suteeraparp et al., 1999). Since then, several epidemiological studies on the disease have been carried out. Prevalence of infection in dairy cattle at individual level was from 5.5% to 70% (Kashiwazaki et al., 2001; Kyaw et al., 2004; Chanlun et al., 2007) and that at herd level varied from 25% to 57% (Chanlun et al., 2002; 2006). In north-eastern region, *Neospora*-associated abortion in both dairy and beef cattle has also been indicated (Charoenchai et al., 2000).

According to Thai national record, there were around 1 million buffaloes and 4 million beef cattle in Thailand (DLD, 2009). However, no information about *N. caninum* infection in swamp buffaloes and only limited information about that in

beef cattle has been reported. The aim of the present study was to investigate seroprevalence of *N. caninum* in swamp buffaloes, and to compare seroprevalence in these two species at one area of Thailand.

Materials and Methods

Blood samples were collected from either jugular or coccygeal vein of 532 swamp buffaloes from 5 provinces: Khon Kaen, Maha Sarakham, Nong Khai, Sakon Nakhon and Udon Thani, during January to June 2009. Seventy-eight beef cattle in the same villages in Phu Wiang district, Khon Kaen province were also sampled to compare prevalence of infection in these two species.

In Khon Kaen province, the study was conducted on 30 and 19 swamp buffalo and beef cattle farms, respectively, during June, 2009. Herd sizes of both buffaloes and beef cattle were usually smaller than 20 animals. From 1 to 27 swamp buffaloes were sampled from each herd and that of beef cattle was from 1 to 9. Cattle enrolled into the study were Thai beef and Thai beef cross-breeds. Animals were at various age. Buffaloes and cattle were usually kept in loose pens, or tethered during rice planting season and let wander and graze on fields after harvesting season. Cut green grass and rice straw were also

provided during confinement. Contact between swamp buffaloes and beef cattle was apparent, and dog presence was also available. Investigated animals were checked for their gender and number of permanent teeth. Animals' age was estimated according to Bragulla et al. (2007) by which animals had no, one, two, three or four pairs of permanent teeth were judged to be less than 2, from 2 to 3, from 3 to 3.5, from 3.5 to 4.5 or above 4.5 years old, respectively. Relationship of animals were recorded through which 59 and 14 pairs of buffalo and beef cattle dams-calves were indicated, respectively. Sera were stored at -20°C until analysis.

Presence of antibodies against *N. caninum* in sera was examined by *N. caninum* iscom ELISA (SVANOVIR Neospora-Ab iscom ELISA, Svanova Biotech AB, Sweden) following the manufacturer's instruction. Briefly, sera were analysed at dilution of 1:100. Anti-bovine conjugate was used in all tests. The optical density (OD) was measured in Microplate Reader, model 3550, Bio-Rad, at 450 nm interference filter. Percent positivity (PP) was calculated by correlating OD values of samples to that of the positive control. A sample was classified positive if PP ≥ 20.

The difference in proportions of *N. caninum* positive animals by species, gender and age groups was analyzed by a Chi-square association test with α = 0.05 in SPSS version 17.

Results and Discussion

In this study, antibodies to *N. caninum* in swamp buffaloes and beef cattle were tested by an iscom ELISA which was used to study the infection of this parasite in dairy cattle and swamp buffaloes (Bjorkman et al., 1997; Huong et al., 1998). This study demonstrated a low seroprevalence of *N. caninum* infection in swamp buffaloes in the north-eastern of Thailand as showed in Table 1. The overall seroprevalence of *N. caninum* was only 4.5% (24/532), ranged from 0% to 6.4% among five provinces. The result was consistent with those previously reported in swamp buffaloes from Vietnam (1.5%) (Huong et al., 1998) and the Philippines (2%) (Konnai et al., 2008). By contrast, results observed in river buffaloes were from 34.6% to 70.9% (Dubey et al., 1998; Guarino et al., 2000; Fujii et al., 2001; Gennari et al., 2005; Rodrigues et al., 2005; Campero et al., 2007; Meenakshi et al., 2007; Mohamad et al., 2007). The difference in results between this study and others conducted on the river buffaloes could be due to the different tests used, age groups, geography,

management and/or breeds. Different susceptibilities to other diseases between river and swamp buffaloes were also reported. Frisch and Vercoe (1979) found that swamp buffaloes were highly resistant to *Oesophogostomum radiatum* which could cause heavy infection in Italian river buffaloes. Anaplasmosis, Babesiosis and Trypanosomiasis also more heavily infected Murrah buffaloes than swamp buffaloes in southern Vietnam (cited from Mingala et al. (2006)). Kumar et al. (2007) suggested that river and swamp buffaloes belong to two different clades, and were independently domesticated. River buffaloes are usually kept under intensive conditions which may promote more horizontal transmission than that in swamp buffaloes those are often under extensive conditions. Furthermore, the difference in their genomes in which the river type has 50 diploid chromosomes and swamp type has 48 also merits a possible reason.

Proportions of seropositive animals in Khon Kaen province by gender, age and species groups are shown in Table 2. Of the two species, seroprevalence of *N. caninum* infection in swamp buffaloes (6.4%) was significantly lower than that in beef cattle (43.6%) ($p < 0.001$). Prevalence of infection was 42.9% in both male and female beef cattle and that in swamp buffaloes were 5.1% and 6.8%, respectively ($p > 0.05$). Antibodies were detected in all the age groups of beef cattle but not in buffaloes under 2 years old. No apparent associations between age or gender of both swamp buffaloes and beef cattle and serological status to *N. caninum* was observed. In Iran, female buffaloes were reported to be more susceptible than male buffaloes to *N. caninum* (Mohamad et al., 2007). Associations between seroprevalence and age of buffaloes were also found in Brazil (Fujii et al., 2001), Italy (Guarino et al., 2000) and Iran (Mohamad et al., 2007). Hornok et al. (2006) found that seroprevalence was higher in the older than in the younger beef cattle. No effects of gender on the infection status of beef cattle were previously reported (Rajkhowa et al., 2008; Damriyasa et al., 2010).

A Brazilian study showed a very high rate of transplacental infection in river buffaloes (73.9%) (Rodrigues et al., 2005). In this study, none of the 5 buffalo calves and only 3 beef cattle calves born to 5 and 9 positive dams, respectively, were positive. It is not known if dams got infected before or after delivery. In the case that infection existed in dams before parturition, frequency of vertical transmission in swamp buffaloes and beef cattle in this study was

Table 1: Seroprevalence of *N. caninum* infection in swamp buffaloes from five provinces in the North-east of Thailand

Province	Number of samples	Number of positive samples (%)
Khon Kaen	187	12 (6.4)
Maha Sarakham	60	3 (5.0)
Nong Khai	31	0 (0.0)
Sakon Nakhon	78	4 (5.1)
Udon Thani	176	5 (2.8)
Total	532	24 (4.5)

Table 2: Distribution of seropositive swamp buffaloes and beef cattle in Khon Kaen province by age and gender groups

	Swamp buffalo		Beef cattle	
	Number of swamp buffaloes	Number of positive swamp buffaloes (%)	Number of beef cattle	Number of positive beef cattle (%)
Age (year)				
≥ 4.5	65	5 (7.7)	32	16 (50.0)
3.5 - 4.5	24	3 (12.5)	6	3 (50.0)
3 - 3.5	13	1(7.7)	8	4 (50.0)
2 - 3	32	2(6.3)	11	4 (36.4)
< 2	52	0 (0.0)	19	7 (36.8)
Gender				
Female	148	10 (6.8)	56	24 (42.9)
Male	39	2 (5.1)	21	9 (42.9)
Total	187	12 (6.4)	78	34 (43.6)

Note: 186 swamp buffaloes and 77 beef cattle had age and gender records, respectively.

suggested to be low. Small rate of transplacental transmission was also found in dairy cattle in Thailand in which 3 out of 4 calves born to infected dams were negative (Kyaw et al., 2005). Inconsistently, a high vertical transmission rate of 58% in dairy cattle from Khon Kaen province was reported (Chanlun et al., 2007).

The present study showed a high prevalence of *N. caninum* infection in beef cattle at one area in Khon Kaen province, Thailand (43.6%). The result was consistent with reports from other provinces in Thailand in which prevalence in dairy and beef cattle was from 37.5% to 70% (Charoenchai et al., 2000; Kashiwazaki et al., 2001). In different fashion, prevalence of infection in dairy cattle from Khon Kaen fluctuated from 10% to 13% in a 4-year longitudinal study (Chanlun et al., 2007). Other studies in dairy cattle from Thailand conferred lower positive proportions, from 5.5-6.0% (Suteeraparp et al., 1999; Kyaw et al., 2004).

It is very interesting that the prevalence of the infection in swamp buffaloes (6.4%) was significantly lower than that in beef cattle (43.6%). Both of the two species were raised at the same villages in Khon Kaen province and contact between them was obvious. Management and feeding were similar as well. Presence of the dogs at those villages was also common. There was difference in probability of being positive with *N. caninum* between dairy breeds (Romero et al., 2002), beef breeds (Armengol et al., 2007) and between dairy and beef cattle breeds (Quintanilla and Gozalo et al., 1999). Different serological status to *N. caninum* in swamp buffaloes from that in beef cattle in this study may be due to their different susceptibility to the parasite.

This first study on *N. caninum* infection in swamp buffaloes from Thailand demonstrates a low seroprevalence status, and imply that swamp buffaloes are less susceptible than beef cattle to *N. caninum*. Further studies are required to investigate the effects of this parasite on reproductive performance of both swamp buffaloes and beef cattle.

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