

## High Occurrence of *Neospora caninum* Antibodies in Buffaloes from the Central, Thailand

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**Abstract:** *Neospora caninum* is now recognized as a major cause of abortion in cattle worldwide. The prevalence of *N. caninum* infection in buffaloes reported worldwide caused the buffalo considered to be an important intermediate host of *N. caninum* even though the role of *N. caninum* infection in buffalo is still unclear. At present, there was only few reports concerned *N. caninum* infection in buffaloes from Thailand. We examined the seroprevalence of *N. caninum* antibodies in 112 clinical healthy buffaloes from the central, Thailand using a commercial competitive ELISA (VMRD, Pullman, USA). Antibodies to *N. caninum* were found in 78 out of 112 buffaloes (69.6%). Our study showed the high seroprevalence of *N. caninum* infection in buffaloes from Thailand. To our knowledge, this is the first report of *N. caninum* infection in Murah, river buffaloes and crossbred buffaloes in Thailand.

## INTRODUCTION

*Neospora caninum*, an intracellular protozoa is recognized as an important cause of abortion and neonatal death in cattle worldwide. In addition, it can also cause congenital disease in calves and milk yield reduction in the infected cows<sup>[1]</sup>. The major route of *N. caninum* infection is transplacental transmission from infected cows to their off springs<sup>[2]</sup>. The buffaloes (*Bubalus bubalis*) are also an important natural host for *N. caninum*<sup>[3, 2]</sup>. Although, the role and consequence of *N. caninum* infection in buffaloes is still unclear but natural congenital infection of *N. caninum* in buffalo and

successful isolation of the pathogen from buffalo tissues were reported<sup>[3-5]</sup>. In Thailand, *N. caninum* infection has been reported in dairy cattle and beef cattle; however, there is few information about *N. caninum* infection in buffaloes. Buffaloes in Thailand have been classified as swamp buffalo, river buffalo and crossbred. Traditionally, they were raised by small farm holders for multipurpose roles as complementary with their crop production and meat or milk production.

We study the seroprevalence of *N. caninum* infection in buffaloes from large 4 herds in Nakorn Nayok and Chacheong-Sao province, the central part of Thailand.

## MATERIALS AND METHODS

**Animals and serum samples:** Serum samples were randomly collected by veterinary practitioners from 112 clinically healthy buffaloes originated from four largest herds of Nakorn Nayok province and Chacheong-Sao province in central Thailand from February to December in 2015. Serum samples were stored at -20°C until being tested for the presence of antibodies against *N. caninum*. Ethical approval for the collection of the blood samples was obtained from the Institutional Animal Care and Use Committee, Mahanakorn University of Technology. Informed consent was obtained from the owners of those farms.

**Serology:** *N. caninum* specific IgG antibodies were detected by a commercial cELISA (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. The plates were washed 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 (I&S, Italy). 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.

**Statistical analysis:** The qualitative variables were described using frequencies and percentages. Comparison of seroprevalence in different sexes, breed and age groups was performed by using Odd Ratio (OR) and 95% CI of OR.

## RESULTS AND DISCUSSION

*N. caninum* antibodies were detected in 78 (69.6%) out of 112 buffalo samples. The proportions of seropositive animals by farm, sex, breed and age group were shown in Table 1.

*N. caninum* antibodies were detected in 100% (7/7) male buffaloes and 66.7% (52/78) female buffaloes. With regard to breed of the animals, the *N. caninum* antibodies were detected in 77.8% (21/27) of river buffaloes or Murah, 70% (7/10) of swamp buffaloes, 58.5% (24/41) of crossbred buffaloes while regard to age group, the *N. caninum* antibodies were detected in 70.3% (26/37) of animals younger than 3 year old and 65.0% (26/40) of animals older than 3 year old.

Buffalo is an importance source of meat and milk for human in Thailand. Nowadays, the role of *N. caninum* as a causal agent of abortion and economic loss in buffalo is still unclear. The abortion rate caused by *N. caninum* reported in buffalo at present was low which may caused by lower immune response in the placentomes of buffalo after getting *N. caninum* infection during geatation comparing to cattle<sup>[6]</sup>. Although, the abortion rate caused by *N. caninum* in buffalo was low but the seroprevalence of *N. caninum* infection in buffalo worldwide was three or four times higher than the seroprevalence in cattle<sup>[7]</sup>. Rezvan *et al.*<sup>[8]</sup> indicated that the buffalo is more sensitive to *N. caninum* due to its life style which is different to cattle. On the other hand, some studies showed that the buffalo had lower *N. caninum* seroprevalence compared to cattle when reared in the same condition which resulted to the prospect that buffalo may be resistant to *N. caninum* infection<sup>[9, 10]</sup>.

The seroprevalence of *N. caninum* in buffaloes varies among countries and tests; for instance, 44% in Brazil by IFAT<sup>[10]</sup>, 68.5% in Romania by PCR<sup>[11]</sup>, 88.3% in

Table 1: The occurrence of *N. caninum* antibodies by cELISA in buffaloes from the central Thailand

Factor	Samples number	Positive number (%)	OR	95% CI of OR
<b>Farm</b>				
A	28	22(78.6)	2.64	0.83-8.65
B	18	15(83.3)	3.59	0.83-17.72
C	11	9(81.8)	3.23	0.56-24.04
D	55	32(58.2)	1	-
Total	112	78(69.6)	-	-
<b>Sex</b>				
Male	7	7(100)	Undefined	-
Female	78	52(66.7)	1	-
Total	85	59(69.6)	-	-
<b>Breed</b>				
River buffaloes (Murah)	27	21(77.8)	2.48	0.73-8.65
Swamp buffaloes	10	7(70)		
Crossbred buffaloes	41	24(58.5)	1.65	0.3-9.55
Total	78	52(66.7)	-	-
<b>Age group</b>				
$\leq 3$ years	37	26(70.3)	1.27	0.44-3.7
$> 3$ years	40	26(65.0)	1	-
Total	77	52(67.5)	-	-

Australia by ELISA<sup>[12]</sup>, 43.3% in Northeast of Argentina by IFAT<sup>[13]</sup>, 25.4% in Northeast of Argentina by IFAT<sup>[14]</sup>, 54.7% in Pakistan by ELISA<sup>[15]</sup>, 37% in Iran by ELISA<sup>[16]</sup>, 55.5% in Brazil by ELISA<sup>[17]</sup>, 24.3% in Mexico by ELISA<sup>[18]</sup>.

To our knowledge, there was few study concerned the infection of *N. caninum* in buffaloes from Thailand. This present study showed evidence for the first time that *N. caninum* infection in swamp buffaloes, river buffaloes and crossbred buffaloes in central part of Thailand is a highly endemic which was higher than the previous studies conducted in the northeast part. Nam *et al.*<sup>[9]</sup> found that 6.4% of 532 swamp buffaloes in the Northeast of Thailand had *N. caninum* antibodies while 43.6% of 78 beef cattle from the same area were seropositive to *N. caninum*. Kengradomkij *et al.*<sup>[19]</sup> showed that 9.1% of 628 water buffaloes in the northeast of Thailand were seropositive to *N. caninum*.

Although, our study showed that there was no association between the seropositive and the sex, the breed, the farm and the age group of buffalo but many studies showed that the seropositive for *N. caninum* infection in buffalo increased with age as in cattle and suggested that horizontal transmission from dogs was an important route of exposure to *N. caninum*<sup>[3, 15, 20, 13, 19, 18, 11]</sup>. However, there were many studies showed that transplacental transmission can also occur in buffaloes as in cattle<sup>[21, 4, 14, 5, 22, 23]</sup>. The major route of *N. caninum* transmission in buffaloes should be more investigated.

## CONCLUSION

According to the result of the present study, the high prevalence of *N. caninum* infection in buffaloes in this area from Thailand suggested that further study on the influence of this agent in buffaloes as the intermediate host of *N. caninum* are needed and the management and control strategies should be determined.

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