CHAPTER IV
RESULTS

1. Investigation of prevalence of *N. caninum* infection in swamp buffaloes in Northeast Thailand

Table 1 Prevalence of *N. caninum* infection in swamp buffaloes in 5 provinces in Northeast Thailand

Provinces	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)

Antibodies to *N. caninum* were found in 24 out of 532 swamp buffaloes accounting for 4.5%. Particularly, 6.4% (12/187), 5.0% (3/60), 0% (0/31), 5.1% (4/78) and 2.8% (5/176) tested buffaloes in Khon Kaen, Maha Sarakham, Nong Khai, Sakon Nakhon and Udon Thani were seropositive, respectively. Chi-square test conferred no significant difference in proportions of positive buffaloes among different provinces (P=0.40).

A total of 318 swamp buffaloes from Khon Kaen and Udon Thani were determined their age basing on their permanent teeth. Distribution of *N. caninum* positive swamp buffaloes in different groups of age was demonstrated in Table 2.

Swamp buffaloes were categorized into 5 groups of age including buffaloes less than 2 years old, from 2 to 3 years old, from 3 to 3.5 years old, from 3.5 to 4.5 years old and older than 4.5 years old. Antibodies to *N. caninum* were detected in all groups of buffaloes older than 2 years old. By contrast, no buffaloes under 2 years old were positive.

Table 2 Distribution of *N. caninum* positive swamp buffaloes in different groups of age

Age (year)	Number of samples	Number of positive samples (%)
≥4.5	117	6 (5.1)
3.5-4.5	40	4 (10.0)
3-3.5	21	1 (4.8)
2-3	52	2 (3.8)
<2	88	0 (0.0)

No significant difference in prevalence of *N. caninum* infection among 5 age groups of buffaloes was uncovered (P=0.09). However, there was a trend that older buffaloes having higher risk of being infected than the younger ones in which the buffaloes at 3.5-4.5 years old were likely to be at the highest danger. As the buffaloes were classified into two groups, i.e. buffaloes younger than 3.5 years old and buffaloes older than 3.5 years old, the older group was at significantly higher risk of being infected than the younger group (P=0.04). Totally, 59 pairs of dams-calves were specified in which 3 dams were positive while their 3 calves were negative. One calf was at about 5 months old and other two calves were at approximately 1 year old.

Table 3 Distribution of antibodies to *N. caninum* in different age groups of swamp buffaloes in Khon Kaen and Udon Thani

	Khon Kaen		Udon Thani	
Age	Number of samples	Number of positive samples (%)	Number of samples	Number of positive samples (%)
≥4.5	66	5 (7.6)	51	1 (2.0)
3.5-4.5	24	3 (12.5)	16	1 (6.3)
3-3.5	13	1 (7.7)	8	0 (0.0)
2-3	32	2 (6.3)	20	0 (0.0)
<2	52	0 (0.0)	36	0 (0.0)
Total	187	12 (6.4)	132	2 (1.5)

Prevalence of infection in different age groups of swamp buffaloes in Khon Kaen and Udon Thani was shown in Table 3. In Khon Kaen, except the absence of infection in buffaloes under 2 years old, antibodies were found in all other categories. On the other hand, infection was only detected in buffaloes older than 3.5 years old in Udon Thani. The prevalence of infection in all the according age groups of swamp buffaloes in two provinces had no significant difference ($P>0.05$).

Prevalence of *N. caninum* infection in female and male buffaloes was depicted in Table 4. One hundred and eighty-seven swamp buffaloes in Khon Kaen were identified their gender. Ten out of 148 female and two out of 39 male buffaloes were positive with *N. caninum* proportioning 6.8% and 5.1%, respectively. Gender did not significantly affect the infection status in swamp buffaloes ($P=0.71$).

Table 4 Prevalence *N. caninum* infection in female and male swamp buffaloes

Gender	Number of samples	Number of positive samples (%)
Female	148	10 (6.8)
Male	39	2 (5.1)

2. Investigation of the effects of *N. caninum* infection on fertility of artificially inseminated swamp buffaloes

Conception rates of swamp buffaloes in different hormone programs, provinces and serostatus were shown in the Table 5. Out of 115 swamp buffaloes, 11 of them were pregnant accounting for 9.6%. Among three hormone programs, the fertility rate was highest in the program 1 (13.6%) while that in program 2 was 4.8%. None of the swamp buffaloes were pregnant in the program 3. There was no significant difference in the conception rates of swamp buffaloes among three hormone programs ($P=0.21$).

Among three provinces, the proportion of pregnant buffaloes in Maha Sarakham, Nong Khai and Sakon Nakhon were 10.8%, 13.6% and 7.1%, respectively. Chi-square test provided no significant difference in pregnancy rate of swamp buffaloes among three provinces ($P=0.65$).

Conception rate of *N. caninum* positive buffaloes was 16.7% while that of *N. caninum* negative buffaloes was 9.2%. Effects of *N. caninum* infection on the pregnancy rate of swamp buffaloes at either day 45 or 60 post artificial insemination was not found (P=0.54).

Association of the pregnancy rate of the swamp buffaloes with other factors was also examined by using logistic regression models. There was no statistically significant effect of the hormone programs, location and serostatus of the swamp buffaloes on the conception rate (P>0.05).

Table 5 Conception rate of swamp buffaloes in different hormone programs, location and serostatus.

	Number of synchronized buffaloes	Number of pregnant buffaloes	Conception rate (%)
Hormone program			
Program 1	66	9	13.6
Program 2	42	2	4.8
Program 3	7	0	0.0
Location			
Maha Sarakham	37	4	10.8
Nong Khai	22	3	13.6
Sakon Nakhon	56	4	7.1
Serostatus			
Seronegative	109	10	9.2
Seropositive	6	1	16.7
Total	115	11	9.6

3. Prevalence of *N. caninum* infection in swamp buffaloes and beef cattle in Phu Wiang, Khon Kaen

The infection was found in all categories of beef cattle. Animals over three years old seemed to have equal risk of being positive (50%). The result was also similar to the beef cattle under three years old. There was a trend that cattle older than three years old had higher change of being positive than those younger. Nonetheless, since the number of

surveyed animals was limited, no significant difference was observed (P=0.17).

Table 6 Prevalence of *N. caninum* infection in different age groups of beef cattle

Age (year)	Number of beef cattle	Number of positive (%)
≥4.5	32	16 (50.0)
3.5-4.5	6	3 (50.0)
3-3.5	8	4 (50.0)
2-3	11	4 (36.4)
<2	19	7 (36.8)
Total	78	34 (43.6)

Male and female beef cattle had an equal risk of being positive. The prevalence of *N. caninum* infection was 42.9% in both of two sexes of the beef cattle.

Table 7 Prevalence of *N. caninum* infection in male and female beef cattle

Gender	Number of beef cattle	Number of positive (%)
Female	56	24 (42.9)
Male	21	9 (42.9)

Prevalences of antibodies to *N. caninum* in swamp buffaloes and beef cattle were 6.4% and 43.6%, respectively. Chi square test resulted in a significantly higher prevalence of infection in beef cattle than that in swamp buffaloes (P<0.001).

Table 8 Comparison of prevalences of *N. caninum* infection in swamp buffaloes and beef cattle in Phu Wiang, Khon Kaen

Types of animals	Number of tested animals	Number of positive animals (%)
Swamp buffaloes	187	12 (6.4)
Beef cattle	78	34 (43.6)

4. Demonstration of *N. caninum* DNA in swamp buffalo whole blood

The results of PCR and serological test in the second collection from Phu Wiang Khon Kaen were shown in the Table 9.

Table 9 Serological and Nested-PCR results in swamp buffaloes from the second collection in Phu Wiang, Khon Kaen.

Types of tests	Number of tested samples	Number of positive samples (%)
Iscom-ELISA	81	2 (2.5)
Nested-PCR	81	0 (0.0)

In the second sample collection, although we focused on 9 farms those had at least one positive buffaloes in the first collection. Some farmers had sold some of their buffaloes, and one sold all his animals. Therefore, only 2 of the 12 positive swamp buffaloes in the first sample collection were identified. One of them was still serologically positive and the other was negative. Two positive buffaloes in the second collection had their calves of 3-4 months old. All of these two calves were also negative. Presence of *N. caninum* DNA was not detected in any of 81 samples used in the Nested-PCR. In both primary and secondary amplifications, neither fragments of 328 bp nor 224 bp was demonstrated (Figure 9).

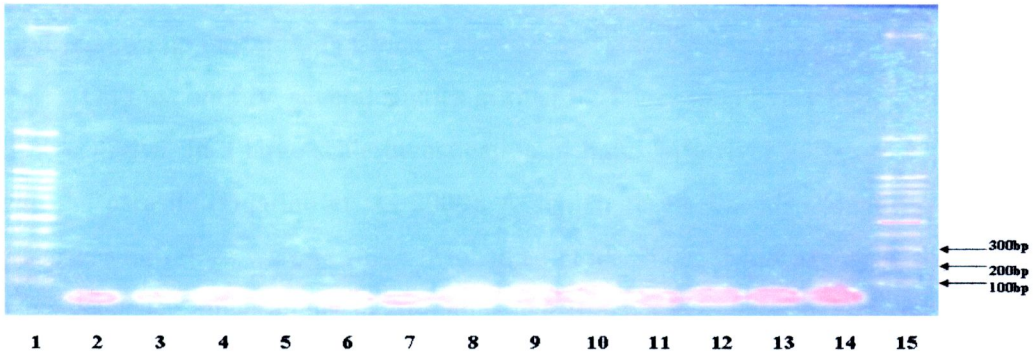

Figure 9 PCR products amplified using primer pairs $Np21^{+}/Np6^{+}$ and Np9/Np10. Lane 1 and 15: ladder 100 bp, lane 2: negative control, lanes 3-4: seropositive samples, lanes 5-14 seronegative samples.