

Epidemiological study of bovine neosporosis in Gyeonggi province

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Abstract

Neospora caninum (*N caninum*) is an intracellular protozoa parasite, and its infection is one of the important diseases because it can cause abortion in cattle. This study was conducted to gain epidemiological data for more effective control of the disease.

Bloods were collected from 2,162 cattle on 90 farms from February to September 2006. Serums were tested for antibodies to *N caninum* using ELISA (HerdCheck, IDEXX).

The dairy breed presented a higher proportion of seropositive results than that of Hanwoo. The estimated proportion of *N caninum*-positive dairy farms was 71.1%, but the overall seroprevalence was estimated as 22.8%. The seroprevalence of dairy cattle and Hanwoo were 31.0% and 4.9%, respectively.

There was no significant difference in the seroprevalence by age, but the not-intensive managed farms had a high seroprevalence (OR = 1.91, *p*-value < 0.01). The antibody rate of cattle with dog(s) was greater than that of those without dogs (OR = 2.13, *p*-value < 0.01). There was a significant difference in abortion rate between seropositive cattle and seronegative ones (OR = 6.2, *p*-value < 0.01).

Key words : *Neospora caninum*, Cattle, Prevalence, Risk factors, Abortion

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Introduction

Neospora caninum is an intracellular protozoan parasite reported to be the major cause of abortion in cattle worldwide. It was first described in dogs in 1984¹⁾ and later in calves with myeloencephalitis²⁾ but was not isolated and named until 1988³⁾. It may cause serious clinical illness in dogs and abortion in cattle and occasionally also in goats, deer and alpacas. Abortion is likely to take place more in subjects seropositive to this protozoa than in seronegative subjects⁴⁾.

Table 1. Number of cattle in test of Gyeonggi province

Area	Hanwoo	Dairy cattle	Total
Yongin	128	327	455
Pyeongtaek	119	322	441
Anseong	125	387	512
Yeoujoo	105	131	236
Kwangjoo	85	121	206
Icheon	46	119	165
Yangjoo	54	46	100
Hwaseong	15	32	47
Total	677	1,485	2,162

In Korea, *N. caninum* was first isolated from the tissues of an aborted bovine fetus and congenitally infected calf in 1997⁵⁾. However, serological evidence of its presence had been observed earlier in fetus of cattle⁶⁾. In Korea, the detection of antibodies to *N. caninum* has been reported in domestic animal at high seroprevalence.

There are many factors that can influence increasing infection of the disease. That is, a study of significant factors such as age could prove fruitful to better understand the epidemiology of bovine neospo-

rosis⁷⁾.

The horizontal route, for example, the post-natal infection which results from the ingestion of oocysts shed by dogs (only known definitive host) or perhaps other, so far unidentified, definitive hosts⁸⁾.

N. caninum was widely diffused as like above in Korea, but there were not many reports about epidemiology. The aims of this study were; 1) to examine the seroprevalence of dairy and Hanwoo cattle in Gyeonggi province, 2) to evaluate the relation between seropositiveness and abortion rate, 3) to identify the factors affecting the seropositive rate.

Materials and Methods

Collection of blood and study area

A total number of bloods collected from the 90 bovine farms was 2,162 (1,485 dairy cattle and 677 Hanwoo) in 8 area in Gyeonggi province (Table 1). All bloods were drawn from the jugular vein and marked with the identification number of the premise and animal type. They were centrifuged and the serum obtained was labeled and stored at -20°C until processing.

Sampling procedures and serological test

Each serum was tested for the presence of antibodies to *N. caninum* detected by a commercially available ELISA kit for bovine serum utilizing a whole tachyzoite lysate (HerdCheck® Anti-*Neospora* IDEXX Laboratories).

A microtitration format has been develo-

ped in which *Neospora* antigens are coated on 96 well plates. Upon incubation of the test sample in the coated well, antibody to *Neospora* forms a complex with the coated antigens. After washing away unbound material from the wells, an anti-bovine horse-radish peroxidase conjugate was added, which binds to any bovine antibody attached in the wells. In the final step of the assay, unbounded conjugate was washed away, and enzyme substrate (hydrogen peroxide) and a chromogen, 3,3', 5,5' tetra-methylbenzidine were added to the amount of antibody present in the test sample.

Interpretation of ELISA results

For the assay to be valid the difference (P-N) between the positive control mean (PCx) and the negative control mean (NCx) must be greater than or equal to 0.150. In addition, the NCx must be less than or equal to 0.20.

Checklist and statistical analysis

A brief checklist was distributed to each of 53 dairy farms, and made by staff in Gyeonggi Veterinary Service interviewing with farmers. It included four factors of farms : presence of dogs, farm management, herd size and reproductive perfor-

mance which may be significantly associated with *N caninum* infection.

Each data was analyzed with *t*-test and classified as significance when *p*-value was less than 0.01.

Results

The 90 farms were composed of 37 Hanwoo and 53 dairy farms. Serums were collected from 2,162 cows; Hanwoo 677, dairy cows 1,485. The mean total number of cows per farm was 24 (range: 12~192). The overall herd prevalence of *N caninum* antibodies among the 90 farms were 71.1%, (60.2-78.2%; CI: 95%) and 64 farms had at least one seropositive cattle (Table 2). The herd prevalence between Hanwoo and dairy was significantly different. Herd prevalence values were 43.2% (16/37) and 90.6% (48/53) in Hanwoo and Dairy herds, respectively. Positive farms were present in all the 8 areas studied. Of the 2,162 cattle tested by the ELISA, 493 animals (22.8%) were seropositive. Sample prevalence in the dairy cows was 31.0% (460/1485) and that in the Hanwoo was 4.9% (33/677).

According to the age, 27.8% (27/90) of cows from 12 to 24 months presented seropositive, seroprevalence of cattle from 25 to 60 months was 32.1% (295/919), 30.8% (105/340) in cows from 61 to 96 months, and

Table 2. Results of ELISA for the detection of antibodies to *N caninum* among 90 farms in Gyeonggi province

Breed	Farms			Cattle		
	Examined	Positive	%	Examined	Positive	%
Hanwoo	37	16	43.2	677	33	4.9
Dairy cattle	53	48	90.6	1,485	460	31.0
Total	90	64	71.1	2,162	493	22.8

28.5% (18/63) in cows from 97 to 156 months (Table 3).

The results of the logistic regression model of individual animal and farm manage-

ment data were shown in Table 4. The intensive management farm had a significantly lower seroprevalence than not-intensive management cows.

Table 3. Seroprevalence of *N caninum* in cattle according to their age

Age (month)	Tested animal	Negative	Positive	%	OR (odds ratio)	p-value
12-24	97	70	27	27.8	1*	
25-60	919	624	295	32.1	1.226	0.188
61-96	340	235	105	30.8	1.158	0.280
97-156	63	45	18	28.5	1.037	0.460
Total	1,419	974	445	31.3		

*: Baseline

Table 4. Relation between antibody rate to *N caninum* and farm management

Type of management	Tested animal	Negative	Positive	%	OR(odds ratio)	p-value
Intensive *	623	475	148	23.7%	1**	
Not	796	499	297	37.3%	1.91	<0.01

*: Intensive meant that there were some inspection or supervision by Animal Improvement Association. **: Baseline

Table 5. Rate of antibody to *N caninum* by herd size as a potential risk factors

Herd size	Tested animal	Negative	Positive	%	OR (odds ratio)	p-value
< 50	443	289	154	34.7%	1*	
50-100	624	420	204	32.6%	0.912	0.241
100-150	202	145	57	28.2%	0.738	0.047
> 150	150	120	30	20.0%	0.469	<0.01

In the prevalence by herd size (Table 5), the prevalence of small herd size (less than 50) was 34.7% (154/443), and that of the herds over 150 head was 20% (30/150).

The number of dogs per farm varied from one to five. Dogs were different breeds, and the age varied from three months to

11 years. Cattle raised on the farms having dog(s) showed a significantly higher seroprevalence than those on farms without dogs (Table 6).

The prevalence of antibodies to *N caninum* in cattle with abortion was significantly higher than that of non-experienced

cattle. Seropositive cows were 53.3% (72/135) of the 135 aborted cattle examined (Table 7).

Insemination times and repeated heating

rate were the independent variables that displayed significant association with seroprevalence by the variate analyzes (Table 8).

Table 6. Compare of seroprevalence in dairy cows with and without dogs

Dog (s)	Tested animal	Negative	Positive	%	OR (odds ratio)	p-value
Absent	369	294	75	20.3	1*	-
Present	1,050	680	370	35.2	2.133	<0.01

*: Baseline

Table 7. Compare of seroprevalence in cows with and without abortion

Abortion	Tested animal	Negative	Positive	%	OR (odds ratio)	p-value
Absence	471	398	73	15.5	1*	-
Presence	135	63	72	53.3	6.231	<0.01

*: Baseline

Table 8. The correlation of seroprevalence and insemination frequency

No of insemination	No. of tested	Negative	Positive	Percentage	OR(odds ratio)	p-value
1	111	92	19	17.1%	1*	-
>1.5	117	104	13	11.1%	0.605	0.097
>2.0	177	123	54	30.5%	2.126	<0.01
>2.5	73	53	20	27.4%	1.827	0.053
>3	105	74	31	29.5%	2.028	0.015

*: Baseline

Discussion

ELISA is an approved serological test⁹⁾ that has been used in epidemiological studies to estimate the prevalence of *N caninum* infection and to examine the relation between *N caninum* exposure and abortion, repeated heating in cattle.^{10),11)} The reported sensitivity and specificity of the ELISA tests used in these studies were 89~96% and 97~99%, respectively.

As like table 2, 71.1% of the dairy herds

had at least one animal seropositive to *N caninum*, which suggested that neosporosis was widespread among dairy herds in Gyeonggi province. The overall seroprevalence (22.8%) in Gyeonggi province was different to seroprevalence (35.6%) in other parts of Korea.¹²⁾ The high prevalence (31.0%) of *N caninum* antibodies in the dairy cow showed that the parasite was highly disseminated in the region despite only a small proportion of Hanwoo (4.9%).

From the results on the prevalence in

Hanwoo and Dairy cows, it could be suggested that the major transmission route may be transplacenta. If horizontal transmission was an important route of *N caninum* infection, seroprevalence in old cows would have been much higher than that in young cows because the chances of being exposed to the parasite become higher as the animal gets older. At the individual level, seropositivity was not significantly different according to age of the animal (Table 7). This result also suggested that horizontal transmission may be a low possibility and vertical transmission should be considered as the most important route of *N caninum* infection¹³⁾. Chronically infected cows can transmit infection to successive generations; therefore, an equal distribution among ages would be expected in the general population.⁴⁾

The occurrence of *N caninum* in cattle was statistically associated with number of cows, type of management, and presence of dogs in farms. Table 5 shows that lower seroprevalence was found in the larger herd. This result may result from being well and intensively managed. Intensively managed farms tended to have low seroprevalence (Table 4) because cows from the intensively managed farms were well inspected and low productive cows were weeded out by Animal Improvement Association.

The presence of dogs in cattle farms has been considered an important risk of cattle infection and abortion by *N caninum* in different countries. In this study, Table 6 showed that dogs presence farms were approximately 2.1 times higher seropre-

valence than the others. This result indicates that dogs can play an important role in *N caninum* transmission. Over a long period of time dogs shed oocysts in growing, repeated ingestion of oocysts by cattle, and leads to an increase of infected animals in herds. Most farms had more than one dog and many of them were free-roaming. However, no decisive conclusion could be drawn on the involvement of the canine species in the transmission of the bovine infection in Korea since no samples from the dogs in the targeted farms were available for *Neospora* antibodies in this study. We suspected that horizontal transmission of neosporosis between cattle and dogs may occur at affected farms.

Seroprevalence of *N caninum* was associated with reproductive problems in the present study. That is, Table 7 and 8 showed that seropositive cows had higher risk of abortion and stillbirth than seronegative cows, as like other reports.^{14,15)}

Studies in the United States have suggested a significant economic cost to producers due to losses associated with *N caninum* infections¹⁶⁾. It is unclear if the impacts will be same under the production conditions in Korea. However, it is needed that further investigation of the economic and production impacts of *N caninum* in Hanwoo and dairy cattle in Korea.

In conclusion, this study showed that *N caninum* infection was common among dairy cattle and Hanwoo through Gyeonggi province. Since the milk and beef industry are one of the key industries in Korea, the economic effect and risk factors of *N caninum* infection should be further

evaluated in the near future for the control of the disease.

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