NOTE *Parasitology*

Prevalence of Antibodies to *Neospora caninum* in Korean Native Beef Cattle

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ABSTRACT. A total of 438 sera from Korean native beef cattle in 9 provinces were tested for *Neospora caninum* antibodies using an immunofluorescent antibody test (IFAT). Eighteen (4.1%) cattle were positive by IFAT. The titers ranged from 1:200 (10 animals), 1:400 (5 animals), 1:800 (2 animals) to 1:1,600 (1 animal). Although the seroprevalence was slightly higher in Chungnam (8.9%), this was not significantly different from those noted in Kyunggi, Kangwon, Kyungbuk, Kyungnam, and Cheju provinces. Sera obtained from beef cattle in the provinces of Chungbuk, Jeonbuk and Jeonnam were all negative. Neospora positive sera were also tested for anti-*Toxoplasma gondii* antibodies using a commercial latex agglutination test (LAT). Antibody to *T. gondii* was detected in only 1 (5.6%) of 18 *N. caninum* positive sera. These results indicate that *N. caninum* and *T. gondii* infection are present at a low level in the Korean native beef cattle.

KEY WORDS: immunofluorescent antibody test, Korean native beef cattle, *Neospora caninum*.

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Neospora caninum is a coccidian protozoan parasite of livestock and companion animals such as cattle, sheep, goats, horses, and dogs [10]. It has been regarded as the major cause of bovine abortion with great economical impact in dairy cattle industry. Numerous sporadic and endemic abortion and neonatal mortality associated with *N. caninum* have been reported almost worldwide [10]. It was first recognized in puppies in 1984 [3] and in bovine in 1988 [8]. On retrospective study of archival materials, *N. caninum* was identified in canine tissue dating from 1957 [9] and in bovine tissue from 1974 [6]. Exposure to *N. caninum* causes decrease in milk production in dairy cattle [19] and reduction in post weaning weight gain, carcass weight in beef cattle [2]. Recently, dogs have been documented as a definite host of *N. caninum* [16].

In Korea, bovine neosporosis was first reported in 1997 [13] and a nationwide serosurvey revealed 20% of the Korean dairy cattle positive for *N. caninum* by indirect fluorescent antibody test (IFAT) [12]. In addition, 24 of the 144 (16.7 per cent) aborted bovine fetuses were diagnosed as *N. caninum*-associated abortion by histopathology and PCR [15]. Studies however on neosporosis in Korea have been limited to dairy cattle. Although several *N. caninum* infections have also been reported in beef cattle [4,7], information on the prevalence of *Neospora* infection or abortion in Korean native beef cattle is not available. The purpose of this study was to determine the prevalence of antibodies to *N. caninum* in Korean native beef cattle.

A total of 438 blood samples were randomly collected from Korean native beef cattle in Korea within a 2-year period, from January 1999 to December 2000. They were from 78 beef herds in 9 provinces (Table 1). The ages of cattle were from 2 to 8 years old, and the mean age was 4 years old. Sera were separated by centrifugation and kept at –20°C until analysis.

The antigen slides of the isolate KBA-1 [14] was used and IFAT was performed as previously described [12, 20]. A commercial fluorescein-labeled goat anti-bovine IgG (Cappel, Durham, North Carolina, U.S.A.) at a dilution of 1:200 was used as the secondary antibody. According to previous work [17], only a complete peripheral fluorescence of tachyzoite at a dilution of 1:200 was considered positive. Positive sera were further titrated using two-fold dilutions starting from 1:200 to 12,800. The end point titer was the last serum dilution showing distinct, whole parasite fluorescence. Negative and positive sera generously provided by the National Institute of Animal Health, Japan.

A commercial latex agglutination test kit (Eiken Chemical Co., Tokyo, Japan) was used to test for anti- *Toxoplasma gondii* antibodies to the *Neospora* positive sera. The reactions were performed using 96 well U-bottom polystyrene

Table 1. Seroprevalence of *Neospora* antibodies in Korean native beef cattle in nine provinces in Korea

Province	Sample size	Positives (IFA titers)				Total (%)
		200	400	800	1600	
Kyunggi	44	2	0	0	0	2 (4.6)
Kangwon	36	1	1	0	0	2 (5.6)
Chungbuk	21	0	0	0	0	0
Chungnam	123	5	3	2	1	11 (8.9)
Kyungbuk	34	0	1	0	0	1 (2.9)
Kyungnam	24	1	0	0	0	1 (4.2)
Jeonbuk	78	0	0	0	0	0
Jeonnam	21	0	0	0	0	0
Cheju	57	1	0	0	0	1 (1.8)
Total	438	10	5	2	1	18 (4.1)

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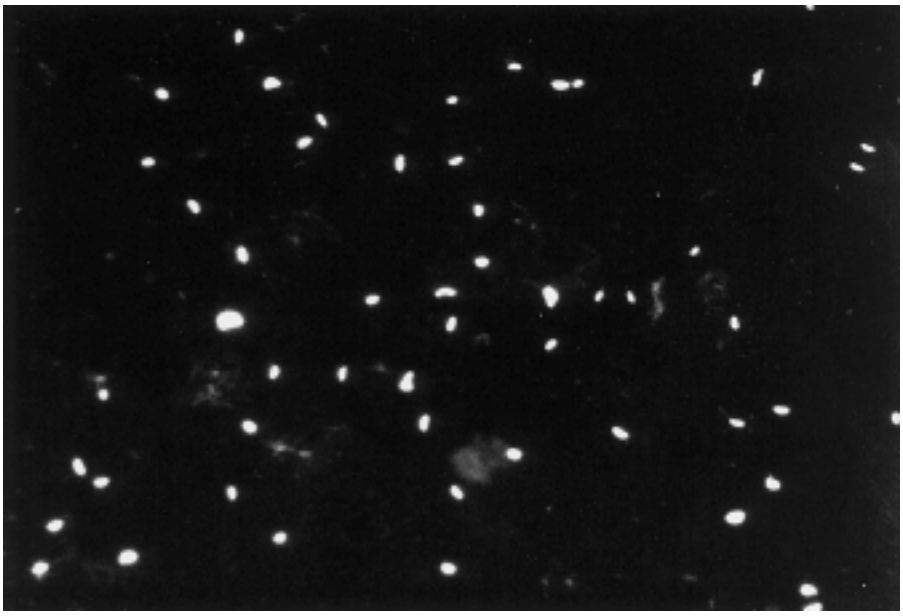

Fig. 1. Immunofluorescence of *Neospora caninum* tachyzoites using the IFAT of a cattle. Positive titer with tachyzoite presenting complete peripheral fluorescence.

microplate at two dilution. The sera were screened at dilutions of 1:16 to 1:256. To each well was then added 25 μl of *T. gondii* antigen-coated latex particles suspension and incubated overnight at room temperature. An agglutination titer at a 1:64 dilution was considered positive. Positive and negative controls (VMRD, Pullman, Washington, U.S.A.) were performed as well.

Of the 438 sera tested (Table 1), eighteen (4.1 per cent) were found positive by IFAT: titers of 1:200 (10 animals), 1:400 (5 animals), 1:800 (2 animals) and 1:1600 (1 animal). Sera obtained from beef cattle in the provinces of Chungbuk, Jeonbuk and Jeonnam were all negative. However, there were no marked differences in seroprevalence among the remaining six provinces. Although the seroprevalence was slightly higher in Chungnam, this was not significantly different from those noted in Kyunggi, Kangwon, Kyungbuk, Kyungnam, and Cheju provinces. Of the 18 samples positive for *Neospora* antibody, only 1 with titer of 1:400, was also positive for antibody to *T. gondii* at a titer of 1:64.

An earlier nationwide serosurvey showed 49% of the sera obtained from dairy herds with abortion rates higher than the national average were positive for *N. caninum* [12]. The mean seropositive rate of those herds was 2.5 times higher than that of herds with no abortion problem, which suggests a correlation between *N. caninum* seropositivity and the abortion rate. Although *N. caninum* has been demonstrated to be the major cause of bovine abortion in dairy cattle in Korea, its abortifacient effect in Korean native beef cattle is still to be established. We have not found a single case of abortion due to *N. caninum* in Korean native beef cattle (unpublished data).

The 4.1% seroprevalence of *N. caninum* in the present study is similar level to that report in New Zealand [5]. The detection of only one seropositive case of *T. gondii* in 18 *N. caninum* positive samples, indicates toxoplasmosis seems not to be a major problem in Korean native beef cattle. IFAT and enzyme-linked immunosorbent assay (ELISA) were mainly used for the serological diagnosis of toxoplasmosis. However, these methods were difficult for standardization and had very complex procedures. The diagnostic kit for toxoplasmosis using latex agglutination test was developed and compared with Toxo-MT kit (Eiken, Japan) [1]. According to this previous survey, 3.5% of random sampled cattle were seropositive to *T. gondii* in Korea [1].

The differences in the seroprevalence of *N. caninum* between dairy and beef cattle depend largely on several major factors. Nevertheless, the low seroprevalence (4.1 per cent) noted in the present study compared to dairy cattle in Korea may be indicative of the difference in the epidemiology of neosporosis between dairy and beef cattle. Prevalence of *N. caninum* is higher in dairy cattle than in beef cattle in New Zealand [5] and Spain [11]. This may be related to different production systems for dairy and beef cattle rather than just breed differences. Through epidemiological studies, Gozalo *et al.* [11] found a significant association between *N. caninum* infection and herd size in beef cattle. In the northwestern United States, exposure to *N. caninum* is common in beef cow-calf herds, and the risk factors for horizontal transmission of *N. caninum* may be related to cow density [18]. The population of Korean native beef cattle (1,590,000 cattle, Ministry of Agriculture and Forestry Report, 2000) is higher than that of dairy cattle (544,000) in Korea. But, the mean herd size of beef cattle (5.5 heads) is much lower than that of dairy cattle (40.7

heads). This small herd size of beef cattle may be related with the lower seroprevalence to *N. caninum* in Korea. In addition, Korean native beef cattle are generally raised under less stressful conditions such as winter stocking density, more regular stock movement, and herd management than dairy cattle. More supplemental feeding practices in the farm with a high stocking density of cattle and frequent regular stock movement on dairy farms may increase the risk of horizontal transmission through definitive host. So neosporosis may not spread easily by contaminated feed with fecal oocysts in beef farms.

The result of our study suggest that further investigation using larger and well-characterized cohorts of beef cattle would be warranted to better delineate the significance of *N. caninum* as a abortifacient in Korean beef cattle. However, it appears that neosporosis might not be a big problem to beef cattle compared to that of dairy cattle in Korea.

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