Available online at www.sciencedirect.com
SCIENCE DIRECT
Veterinary Parasitology 135 (2006) 175–179
veterinary parasitology
www.elsevier.com/locate/vetpar

Short communication

Nationwide seroprevalence of *Neospora caninum* among dairy cattle in Japan

Masahiro Koiwai [a,*], Takafumi Hamaoka [b], Makoto Haritani [c], Shinya Shimizu [d], Yumie Zeniya [e], Mariko Eto [a], Rieko Yokoyama [a], Toshiyuki Tsutsui [e], Kumiko Kimura [c], Itsuro Yamane [e]

[a] *Department of Diagnosis and Research, Animal Quarantine Service, Ministry of Agriculture, Forestry and Fisheries, Haramachi 11-1, Yokohama 235-0008, Japan*
[b] *Shichinohe Research Unit, Department of Epidemiology, National Institute of Animal Health, Uminai 31, Shichinohe 039-2586, Japan*
[c] *Department of Infectious Diseases, National Institute of Animal Health, Kannondai 3-1-5, Tsukuba 305-0856, Japan*
[d] *Department of Immunology, National Institute of Animal Health, Kannondai 3-1-5, Tsukuba 305-0856, Japan*
[e] *Department of Epidemiology, National Institute of Animal Health, Kannondai 3-1-5, Tsukuba 305-0856, Japan*

Received 25 April 2005; received in revised form 15 August 2005; accepted 30 August 2005

Abstract

Serum samples from 2420 clinically healthy dairy cattle, randomly selected from stored sera in 18 districts of Japan, were tested for the presence of *Neospora caninum* antibodies using an indirect fluorescent antibody test (titer $\geq$ 1:200). Nationwide seroprevalence is estimated at 5.7% (139/2420). Seropositive cattle were detected in all surveyed districts despite the evidence of confirmed case reports of bovine neosporosis, showing that *N. caninum* is widely distributed throughout Japan. Age-specific seroprevalence did not increase with cattle age, suggesting that *Neospora* infection is likely to be transmitted vertically rather than horizontally in Japan. Considering that *N. caninum* seropositive cows are thought to be more likely to abort, substantial fetal losses may be induced by *N. caninum* infection in Japan. Devising strategies are needed to reduce the economic impact on the Japanese dairy industry. This is the first study to investigate the nationwide seroprevalence of *N. caninum* in cattle in Asia.

Keywords: *Neospora caninum*; Nationwide seroprevalence; Japan; Dairy cattle

1. Introduction

Neospora caninum, a protozoan parasite, is recognized as a major cause of bovine abortion and subsequent economic loss especially in the dairy industry (Dubey and Lindsay, 1996; Dubey, 1999). In

* Corresponding author. Tel.: +81 45 751 5921; fax: +81 45 752 5466.
E-mail address: koiwaima@maff-aqs.go.jp (M. Koiwai).

0304-4017/$ – see front matter
doi:10.1016/j.vetpar.2005.08.014

Japan, bovine neosporosis was first reported in dairy cattle in 1992 (Ogino et al., 1992), since then, many cases have been reported (Haritani, 1997). National disease control strategies are usually evaluated on the basis of economic impacts (Smith, 1995); nevertheless, limited information on the prevalence of bovine neosporosis in Japan is available. In addition, risk factors such as age of the animals may also be important epidemiological information, particularly to estimate the modes of transmission. Therefore, we decided to investigate the distribution of *N. caninum* among dairy cattle at the national level combined with analysis of some factors associated with seroprevalence.

2. Materials and methods

2.1. Targeted areas for sampling

At the time this study was conducted, bovine neosporosis had been confirmed in 22 of 47 districts in Japan based on histopathology and immunohistochemical examination (Haritani, 1997). Overall, 11 of the 22 districts with confirmed case reports of bovine neosporosis (Table 1) and 7 of 25 districts without any reports (Table 2) were selected throughout Japan, as shown in Fig. 1. This total of 18 district administrations volunteered for participating in the National Research Project for Livestock Reproduction (MAFF, 1997).

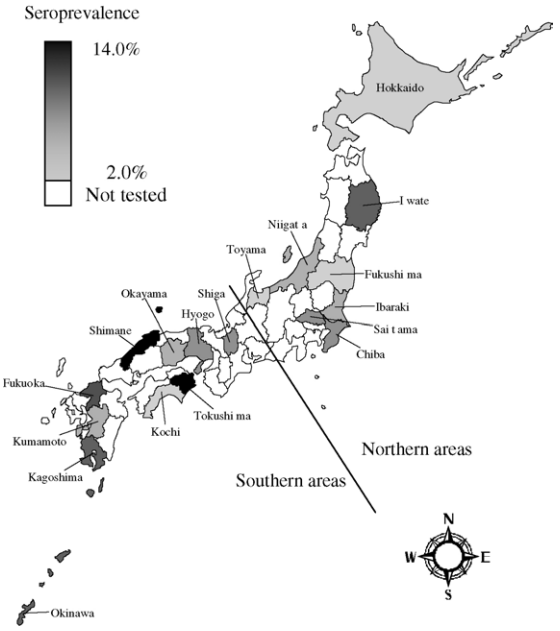

Fig. 1. Distribution of *Neospora caninum* seropositive reactions in clinically healthy dairy cattle in Japan.

2.2. Stored serum samples from dairy cattle

In Japan, serum samples from 752,033 dairy cattle were previously obtained from clinically healthy dairy cattle for the purpose of the National Brucellosis Eradication Program in 1997 (MAFF, 1998). All serum samples had been stored at each of the Livestock Hygiene Service Centers (LHSC), except

Table 1
Seroprevalence of *Neospora caninum* among clinically healthy dairy cattle in 11 surveyed districts with confirmed case reports of bovine neosporosis in Japan

District	Number of sera	Number of positives classified by IFAT titer							Seroprevalence (95% confidence interval)
		200	400	800	1600	3200	6400	Total	
Hokkaido	400		3		3	2	3	11	2.8 (1.1–4.4)
Niigata	100		1		2		1	4	4.0 (0.2–7.8)
Fukushima	100				1		1	2	2.0 (0.0–4.7)
Ibaraki	400	1	2	8	4	5	1	21	5.3 (3.1–7.4)
Chiba	100		1		1	3	2	7	7.0 (2.0–12.0)
Shiga	100		1	3		2	1	7	7.0 (2.0–12.0)
Okayama	100			2	2	1		5	5.0 (0.7–9.3)
Shimane	100	2	3	4	4	1		14	14.0 (7.2–20.8)
Tokushima	100		4	2	2	3	2	13	13.0 (6.4–19.6)
Fukuoka	100	2	4	2		1		9	9.0 (3.4–14.6)
Kumamoto	100		1	2	1			4	4.0 (0.2–7.8)
Total	1700	5	20	23	20	18	11	97	5.7 (4.6–6.8)

Table 2
Seroprevalence of *Neospora caninum* among clinically healthy dairy cattle in seven surveyed districts without confirmed case reports of bovine neosporosis in Japan

District	Number of sera	Number of positives classified by IFAT titer							Seroprevalence (95% confidence interval)
		200	400	800	1600	3200	6400	Total	
Iwate	100		2	1	3	2		8	8.0 (2.7–13.3)
Toyama	100		2	1				3	3.0 (0.0–6.3)
Saitama	100	1		3	2			6	6.0 (1.3–10.7)
Hyogo	100			2	2	2		6	6.0 (1.3–10.7)
Kochi	100			2				2	2.0 (0.0–4.7)
Kagoshima	120	2		3	1	2	1	9	7.5 (2.8–12.2)
Okinawa	100		2	3	3			8	8.0 (2.7–13.3)
Total	720	3	6	15	11	6	1	42	5.8 (4.1–7.5)

in Hokkaido, where only three serum samples per herd were stored. The minimum sample size for estimating seroprevalence of *N. caninum* in each district was set at 100, assuming an expected seroprevalence of 30% and a desired absolute precision of 10% for 95% confidence interval (CI) (Toma et al., 1999). Samples were randomly selected from the stored sera in each district as follows: 20 dairy herds were first selected using a random procedure, then five animals were randomly selected from each herd to fit the minimum number of samplings. The sample size was distributed according to the proportion of cattle allocated to each LHSC in the surveyed districts (Toma et al., 1999). In Kagoshima district, five animals were selected from each of the 20 herds at all six LHSCs; therefore, the total sample size was 120. Hokkaido district is the main dairy farming area and Ibaraki district is the place where *N. caninum* was first isolated in Japan (Yamane et al., 1997); sample size was increased to 400 to obtain more precise results. In Hokkaido district, 100 samples were obtained from all stored sera of the randomly selected herds at each of the four LHSCs with veterinary diagnostic laboratories. A total of 2420 serum samples were obtained from 77 LHSCs in the 18 selected districts. The sera were stored at −20 °C until serological testing.

2.3. Serological testing

An indirect fluorescent antibody test (IFAT) was used to measure the antibodies to *N. caninum*, as described previously (Reichel and Drake, 1996; Yamane et al., 1997). Sera were first screened for the presence of antibodies at a dilution of 1:200 (Dubey and Lindsay, 1996; Reichel and Drake, 1996; Dubey, 1999); positive samples were further titrated in a two-fold dilution to 1:6400. The end-point titer was the last serum dilution showing distinct and whole parasite fluorescence. Positive and negative control sera were diluted 1:200 and used on each slide.

2.4. Data analysis

Statistical differences in proportions were compared using the χ^2-test (Yates corrected) or Fisher's exact test. Statistical analysis was conducted using a statistical software package (StatView, SAS Institute, Cary, IN, USA). Distribution of *N. caninum* seropositive reactions were shown using the Geographical Information System (MapInfo Professional, MapInfo Corporation, Troy, NY, USA).

3. Results

Neospora seropositive cattle were detected in all surveyed districts. Mean seroprevalence of *N. caninum* was 5.7% in the districts with confirmed case reports of bovine neosporosis (Table 1) and 5.8% in those without any reports (Table 2). No significant difference was observed in seroprevalence between the two groups. The overall seroprevalence of 5.7% (139/2420, 95% CI: 4.8–6.7%) was classified by different categories, as shown in Fig. 2. No significant difference was observed in the seroprevalences between the animals' ages. As shown in Fig. 1, a statistically higher seroprevalence ($P = 0.002$) was observed in the southern areas (7.5%, 77/1020, 95%

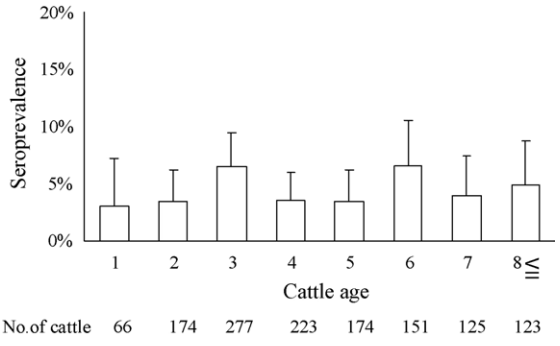

Fig. 2. Seroprevalence of *Neospora caninum* classified by cattle ages with a 95% confidence interval.

CI: 5.9–9.2%) compared to the northern areas (4.4%, 62/1400, 95% CI: 3.4–5.5%) using cut-off titers of 1:200.

4. Discussion

This is the first report to investigate the nationwide seroprevalence of *N. caninum* in cattle in Asia. Seropositive cattle were detected in all surveyed districts despite the evidence of confirmed case reports of bovine neosporosis, showing that *N. caninum* is widely distributed throughout Japan. Furthermore, no significant difference was observed between seroprevalences for the two groups of districts with or without the confirmed case reports. Therefore, the nationwide seroprevalence among clinically healthy dairy cattle in Japan was estimated at 5.7%, which was close to the previous report of 5.0% (1/20) in one region in Japan (Noda and Shirakawa, 1997). To the best of our knowledge, one published report on nationwide seroprevalence in New Zealand showed nearly the same level of the seroprevalence at 8.5% in 1985 and 6.8% in 1995 using 400 sera stored in a serum bank (Reichel, 1998). Considering the large scale and randomized sampling procedure of this survey, the present study may provide a reliable estimate of the nationwide seroprevalence of *N. caninum* among clinically healthy dairy cattle in Japan.

Seroprevalence was not higher among animals in the older age group, which may indicate that vertical transmission may be more common compared to horizontal transmission in Japan. This finding is in accordance with the previous reports, which showed no increase in seroprevalence with animals' ages (Paré et al., 1996; Davison et al., 1999; Mainar-Jaime et al., 1999; Pitel et al., 2001). On the contrary, horizontal transmission could have been hidden if older *N. caninum*-infected cows were culled due to abortion and/or low milk production, as described by Thurmond and Hietala (1996, 1997); therefore, interpretation of this finding should be evaluated with caution.

Considering that cattle with an IFAT titer greater than 1:200 are reported to be 6.1 times more likely to abort in Japan (Koiwai et al., in press), the results of the present study suggest that substantial fetal losses may be induced by *N. caninum* infection in Japan as well. *Neospora* infection may be more widely distributed in the southern part of Japan, although we could not find any explanation for this. Further epidemiological studies, especially focused on the difference in dairy cattle management such as feeding practices and herd replace criteria, may clarify the risk factors influencing seropositive reactions to *N. caninum* in Japan.

In conclusion, devising strategies, such as selective culling of *Neospora* seropositive animals, are needed to reduce the economic impact on the Japanese dairy industry. Epidemiological parameters reported here could assist with the development of a rational control program in Japan.

Acknowledgements

The authors would like to thank the district veterinary officers for their cooperation in submitting serum samples and data. This work was supported in part by a Grant-in-Aid from the National Institute of Animal Health.

References

Davison, H.C., French, N.P., Trees, A.J., 1999. Herd-specific and age-specific seroprevalence of *Neospora caninum* in 14 British dairy herds. Vet. Rec. 144, 547–550.

Dubey, J.P., 1999. Recent advances in *Neospora* and neosporosis. Vet. Parasitol. 84, 349–367.

Dubey, J.P., Lindsay, D.S., 1996. A review of *Neospora caninum* and neosporosis. Vet. Parasitol. 67, 1–59.

Haritani, M., 1997. Bovine neosporosis. J. Anim. Protozooses 10, 1–5 (in Japanese, with English abstract).

Koiwai, M., Hamaoka, T., Haritani, M., Shimizu, S., Kimura, K., Yamane, I., in press. Proportion of abortions due to neosporosis among dairy cattle in Japan. J. Vet. Med. Sci.

Mainar-Jaime, R.C., Thurmond, M.C., Berzal-Herranz, B., Hietala, S.K., 1999. Seroprevalence of *Neospora caninum* and abortion in dairy cows in northern Spain. Vet. Rec. 145, 72–75.

Ministry of Agriculture, Forestry and Fisheries (MAFF), 1997. Annual report of National Livestock Reproductive Disorders Prevention Program in 1996. Ministry of Agriculture, Forestry and Fisheries of Japan, Tokyo, 76-238 (in Japanese).

Ministry of Agriculture, Forestry, Fisheries (MAFF), 1998. Statistics on Animal Hygiene 1997. Ministry of Agriculture, Forestry, Fisheries (MAFF), Nourinkousaikai, Tokyo, 43 pp.

Noda, Y., Shirakawa, H., 1997. Survey of antibody to *Neospora* in aborted dairy cows. J. Jpn. Vet. Med. Assoc. 50, 258–261 (in Japanese, with English abstract).

Ogino, H., Watanabe, E., Watanabe, S., Agawa, H., Narita, M., Haritani, M., Kawashima, K., 1992. Neosporosis in the aborted fetus and newborn calf. J. Comp. Pathol. 107, 231–237.

Paré, J., Thurmond, M.C., Hietala, S.K., 1996. Congenital *Neospora caninum* infection in dairy cattle and associated calfhood mortality. Can. J. Vet. Res. 60, 133–139.

Pitel, P.-H., Pronost, S., Chatagnon, G., Tainturier, D., Fortier, G., Ballet, J.-J., 2001. Neosporosis in bovine dairy herds from the west of France: detection of *Neospora caninum* DNA in aborted fetuses, seroepidemiology of *N. caninum* in cattle and dogs. Vet. Parasitol. 102, 269–277.

Reichel, M.P., 1998. Prevalence of *Neospora* antibodies in New Zealand dairy cattle and dogs. NZ Vet. J. 46, 38.

Reichel, M.P., Drake, J.M., 1996. The diagnosis of *Neospora* abortions in cattle. NZ Vet. J. 44, 151–154.

Smith, R.D. (Ed.), .1995. Veterinary Clinical Epidemiology: A Problem-Oriented Approach,. 2nd ed. CRC Press, Boca Raton, pp. 227–246.

Thurmond, M.C., Hietala, S.K., 1996. Culling associated with *Neospora caninum* infection in dairy cows. Am. J. Vet. Res. 57, 1559–1562.

Thurmond, M.C., Hietala, S.K., 1997. Effect of *Neospora caninum* infection on milk production in first-lactation dairy cows. J. Am. Vet. Med. Assoc. 210, 672–674.

Toma, B., Dufour, B., Sanaa, M., Bénet, J.J., Moutou, F., Louzã, A., Ellis, P. (Eds.), 1999. Applied veterinary epidemiology and the control of disease in populations. AEEMA, Maisons-Alfort, pp. 89–136.

Yamane, I., Kokuho, T., Shimura, K., Eto, M., Shibahara, T., Haritani, M., Ouchi, Y., Sverlow, K., Conrad, P.A., 1997. In vitro isolation and characterisation of a bovine *Neospora* species in Japan. Res. Vet. Sci. 63, 77–80.