# Serological Survey of Toxoplasma gondii in Wild Sika Deer in Eastern Hokkaido, Japan

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#### ABSTRACT

*Toxoplasma gondii* is one of the most common protozoan parasites globally and requires both a definitive and an intermediate host to complete its life cycle. The population of wild sika deer (*Cervus nippon yesoensis*) in eastern Hokkaido, Japan, has recently increased and is considered a potential intermediate host of *T. gondii*. In this study, the seroprevalence of *T. gondii* infection in 201 wild sika deer from 10 geographical regions in eastern Hokkaido in 2010 and 2011 was analyzed using the latex agglutination test. Antibodies to *T. gondii* were found in three cases (1.5% of samples), suggesting that deer have started to function as intermediate hosts. This is the first report of seropositivity against *T. gondii* in wild sika deer in eastern Hokkaido.

Keywords: Estern Hokkaido, Seroprevalence, Toxoplasma gondii, Wild sika deer

*Toxoplasma gondii* is an obligate intracellular protozoan parasite and a common parasitic zoonosis in many countries (Kopecna *et al.*, 2006). Cats and other members of the feline family represent the definitive hosts and main reservoirs for *T. gondii*, but many other warm-blooded animals can also act as intermediate hosts in parasite transmission (Dubey and Beatt, 1989, Hill and Dubey, 2002, Murata, 1989). In the past, the major transmission route of *T. gondii* to humans has been the ingestion of raw or undercooked meat from pigs and sheep. Thus, inhibiting *T. gondii* infection in intermediate hosts is therefore considered important for the control of this disease in humans, and recent studies have shown that the prevalence of the parasite in domestic animals could be reduced considerably with intensive hygiene management (Tenter, 2009, Tenter

*et al.*, 2000). However, the prevalence of *T. gondii* infection in wildlife representing potential intermediate hosts is still unclear.

There are currently ~640,000 wild sika deer (*Cervus nippon yesoensis*) on the island of Hokkaido, Japan, and numbers have been increasing in recent years (Yamamura *et al.*, 2008). These deer populations may harbor zoonotic diseases thus raising potential public health risks (Asakura *et al.*, 1998, Sato *et al.*, 2000). The first seropositive case of *T. gondii* infection in wild deer was reported from Gunma Prefecture, Japan, during the 2004–2007 period (Masuko *et al.*, 2011). However, information on the current prevalence of *T. gondii* infection in wild sika deer in Hokkaido is limited. In the present study, we therefore determined the seroprevalence of *T. gondii* infection in wild sika deer from ten regions of eastern Hokkaido in 2010 and 2011 using the latex agglutination test (LAT).

Eighty-five wild sika deer plasma samples from nine geographical regions of eastern Hokkaido obtained in 2010 were analyzed (Fig. 1). Blood was collected from 44 male and 41 female animals, aged 1–7 years. The plasma was separated from whole blood by centrifugation at 2,400 x g for 15 minutes and stored at -20 °C until use. Plasmas were tested for *T. gondii* antibodies using a commercial LAT kit (Toxo Check, Eiken Chemical, Tokyo, Japan) following Omata et al., 2005. Antibody titers of 1:64 or above were regarded as positive (Matsumoto *et al.*, 2011, Omata *et al.*, 2005). Seropositivity was found in 1 of 15 serum samples (6.7%) collected from Urakawa and in 1 of 18 samples (5.6%) from Toyokoro, for a total of 2 in 85 deer (2.4%) (Table 1).

Prompted by the detection of seropositivity in the 2010 survey, a further 116 wild sika deer from five regions of eastern Hokkaido were sampled in 2011, with a focus on Toyokoro and the neighboring regions (Fig. 1). Blood samples were collected from 43 male and 73 female animals, aged 1–7 years. One of 116 serum samples (0.8%) tested positive for *T. gondii* antibodies (LAT titer 1:128), originating from a region (Toyokoro) that had already been implicated in the 2010 survey (Table 1). Seroprevalences were thus of a comparable magnitude between surveys.

Although it had previously been reported that no *T. gondii* antibodies were detected in wild sika deer in eastern Hokkaido in 2003 (Omata *et al.*, 2005), we detected an overall *T. gondii* infection seroprevalence of 1.5% in 2010–2011. This is the first report of *T. gondii* antibody detection in wild deer in Hokkaido. Our results have direct implications for toxoplasmosis control in this area and indicate the importance of investigating *T. gondii* transmission routes in wild sika deer. Although transmission routes are still unclear, the recent increase in deer density is likely to improve the chances of contact between deer and definitive hosts. Previous studies have discussed the importance of waterborne transmission of *T. gondii* (Dubey, 2004), and *T. gondii* infections in wild waterfowl in Hokkaido have indeed been reported (Murao *et al.*, 2008). In addition, the consumption of wildlife is part of traditional local food culture in Hokkaido, and the consumption of deer in particular, has steadily increased. We therefore suggest that periodic monitoring

and further investigation of *T. gondii* infections in both domestic and wildlife species are necessary to improve toxoplasmosis prevention.

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Table 1. Seroprevalence of *T. gondii* infection in wild sika deer from 10 regions of eastern Hokkaido, Japan, in 2010 and 2011.

	2010			2011			2010-2011	
Regi	М	Fe	No. of	М	Fe	No. of	No. of	No. of
on	a 1	m əl	positive	a 1	m əl	positive	samples	positive
	e	e	(%)	e	e	(%)	(70)	(%)
Frim	7	0	0	0	0	0	7	0
0	7	0	0	0	0	0	7	0
Hida	1	1	0	0	0	0	2	0
ka								
Hiro	6	1	0	3	4	0	14	0
0								
Obihi	0	2	0	1	3	0	6	0
ro	•	•	0	0	0	0		0
Onbe	2	2	0	0	0	0	4	0
tu Sama	3	0	0	0	0	0	2	0
ni	5	0	0	0	0	0	5	0
Taiki	0	0	0	0	1	0	1	0
Toyo	8	10	1 (5.6)	31	45	1 (1.3)	94	2 (2.1)
koro								
Urah	9	18	0	8	20	0	55	0
oro								
Urak	8	7	1 (6.7)	0	0	0	15	1 (6.7)
awa		4.1		10	70	1 (0 0)	201	0 (1 5)
Total (%)	44	41	2 (2.4)	43	13	1 (0.8)	201	3 (1.5)
(70)								



Fig. 1. Geographical locations of the 10 sampling regions in Hokkaido, Japan. Geographical coordinates are as follows: ON (Onbetu) 42°53'N, 143°55'E; UH (Urahoro) 42°48'N, 143°39'E; TY (Toyokoro) 42°47'N, 143°30'E; HK (Hidaka) 42°52'N, 142°26'E; OH (Obihiro) 42°55'N, 143°11'E; TK (Taiki) 42°29'N, 143°16'E; HO (Hiroo) 42°25'N, 143°12'E; UK (Urakawa) 42°10'N, 142°45'E, SN (Samani) 42°07'N, 142°55'E; EM (Erimo) 42°01'N, 143°08'E.

### REFERENCES

Asakura, H., Makino, S., Shirahata, T., Tsukamoto, T., Kurazono, H., Ikeda, T. and Takeshi, K., 1998. Detection and genetical characterization of Shiga toxin-producing *Escherichia coli* from wild deer. Microbiol. Immunol. 42, 815-822.

Dubey, J. P., 2004. Toxoplasmosis - a waterborne zoonosis. Vet. Parasitol. 126, 57-72.

- Hill, D. and Dubey, J. P., 2002. *Toxoplasma gondii*: transmission, diagnosis and prevention. Clin. Microbiol. Infect. 8, 634-640.
- Kopecna, J., Jirku, M., Obornik, M., Tokarev, Y. S., Lukes, J. and Modry, D., 2006. Phylogenetic analysis of coccidian parasites from invertebrates: search for missing links. Protist 157, 173-183.
- Masuko, T., Souma, K., Kudo, H., Takasaki, Y., Fukui, E., Kitazawa, R., Nishida, R., Niida, T., Suzuki, T. and Nibe, A., 2011. Effects of the feeding of wild Yeso sika deer (*Cervus nippon yesoensis*) on the prevention of damage due to bark stripping and the use of feeding sites. Anim. Sci. J. 82, 580-586.

- Matsumoto, J., Kako, Y., Morita, Y., Kabeya, H., Sakano, C., Nagai, A., Maruyama, S. and Nogami, S., 2011. Seroprevalence of *Toxoplasma gondii* in wild boars (*Sus scrofa leucomystax*) and wild sika deer (*Cervus nippon*) in Gunma Prefecture, Japan. Parasitol. int. 60, 331-332.
- Murao, T., Omata, Y., Kano, R., Murata, S., Okada, T., Konnai, S., Asakawa, M., Ohashi, K. and Onuma, M., 2008. Serological survey of *Toxoplasma gondii* in wild waterfowl in Chukotka, Kamchatka, Russia and Hokkaido, Japan. J. Parasitol. 94, 830-833.
- Murata, K., 1989. A serological survey of *Toxoplasma gondii* infection in zoo animals and other animals. J. Vet. Med. Sci. 51, 935-940.
- Omata, Y., Ishiguro, N., Kano, R., Masukata, Y., Kudo, A., Kamiya, H., Fukui, H., Igarashi, M., Maeda, R., Nishimura, M. and Saito, A., 2005. Prevalence of *Toxoplasma gondii* and *Neospora caninum* in sika deer from eastern Hokkaido, Japan. J. Wildl. Dis. 41, 454-458.
- Sato, Y., Kobayash, C., Ichikawa, K., Kuwamoto, R., Matsuura, S. and Koyama, T., 2000. An occurrence of *Salmonella typhimurium* infection in sika deer (*Cervus nippon*). J. Vet. Med. Sci. 62, 313-315.
- Tenter, A. M., 2009. *Toxoplasma gondii* in animals used for human consumption. Memorias Do Instituto Oswaldo Cruz 104, 364-369.
- Tenter, A. M., Heckeroth, A. R. and Weiss, L. M., 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. 30, 1217-1258.
- Yamamura, K., Matsuda, H., Yokomizo, H., Kaji, K., Uno, H., Tamada, K., Kurumada, T., Saitoh, T. and Hirakawa, H., 2008. Harvest-based Bayesian estimation of sika deer populations using state-space models. Popul. Ecol. 50, 131-144.