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Prevalence of anti-*Toxoplasma gondii* antibody in domestic horses in Japan

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ABSTRACT

The present study is the first report that investigated the seroprevalence of *Toxoplasma gondii* infection in domestic horses in various prefectures of Japan and analyzed risk factors for seropositivity. We performed a latex agglutination test for riding/racing horses from 11 prefectures in Japan (783 samples) and 4 groups of Japanese native horses (254 samples). The total seroprevalence of anti-*T. gondii* antibody in horses examined in this study was 4.24% (44/1037). As for riding/racing horses, we did not find a statistically different *T. gondii* seroprevalence between sampling prefectures. In contrast, seroprevalence of *T. gondii* in older horses (>21 years) was significantly higher than that in younger horses (<5 years and 11–15 years). There was no significant difference in *T. gondii* seroprevalence between riding/racing horses and Japanese native horses. Logistical regression analysis revealed that age, but not sex and usage, is a significant risk factor of *T. gondii* infection for domestic horses in Japan. These findings suggest that domesticated horses in Japan can be horizontally infected with *T. gondii* by ingestion of food or water contaminated with oocysts.

1. Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite belonging to the phylum Apicomplexa. Infection with this parasite, namely, toxoplasmosis, is globally prevalent in most warm-blooded intermediate hosts including humans [1]. The parasite has a complex lifecycle, and multiple routes of infection are possible. Felids are the definitive hosts of this parasite and are able to produce as well as shed oocysts in their feces. Consumption of infected meat containing cysts and ingestion of food/water contaminated with oocysts are the two major modes of transmission of *T. gondii* to hosts [2]. *T. gondii* forms cysts in host tissues, and infection is considered to be lifelong. Although most infections are asymptomatic in healthy individuals, the parasite can cause severe complications in immunocompromised individuals, such as patients with AIDS or those receiving immunosuppressive therapy [3]. Moreover, *T. gondii* may cause abortion, stillbirth, and congenital abnormalities if infection occurs during pregnancy. Thus, *T. gondii* infection not only results in reproductive and economic loss in livestock but also has implications for public health because the consumption of contaminated meat can result in zoonotic infection [4,5].

Horses interact with humans in a wide variety of working activities such as recreation, agriculture, carriage, and sports. Toxoplasmosis in horses is generally asymptomatic [4]. However, fever, ataxia, retinal degeneration, and encephalitis and abortion or stillbirth in pregnant equids may occasionally occur [6]. In contrast, horse meat consumption is popular in some countries, and some cases of severe toxoplasmosis in humans, due to the consumption of horse meat, have been reported [7]. In Japan, horses are mainly reared for riding and racing. In addition, there are eight Japanese indigenous horses [Hokkaido, Kiso, Misaki, Tsushima (also known as Taishu horse), Noma, Tokara, Miyako, and Yonaguni horses], and these native species are in danger of extinction [8]. Although surveillance of horse toxoplasmosis is important to maintain the health of horses, there is little information relating to toxoplasmosis of horses in Japan. Moreover, raw horse meat consumption is popular in some prefectures in Japan; therefore, it is also important to understand the prevalence of *T. gondii* in horses to prevent zoonotic infection from infected meat. In this study, to elucidate the seroprevalence of *T. gondii* in horses raised in Japan, we performed serological tests for domestic horses from various prefectures and some groups of indigenous Japanese horses.

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2. Materials and methods

2.1. Blood samples

A total of 783 serum samples from riding or racing horses were obtained across 11 prefectures (Miyagi, Ibaraki, Chiba, Saitama, Kanagawa, Gifu, Mie, Nara, Okayama, Hyogo, and Oita) of Japan between April and November 2014 (Fig. 1 and Table 1). These sera had been previously collected for routine health management and were made available for this investigation. Sera from Japanese native horses (Kiso, Ttsushima, Miyako, and Yonaguni horses) were also collected (Fig. 1 and Table 2). None of the horses used in this study exhibited clinical abnormalities. All serum samples were stored at −80 °C until use.

2.2. Latex agglutination test (LAT)

Antibodies to *T. gondii* were examined using a commercially available LAT kit (Toxocheck-MT; Eiken-Kagaku, Tokyo, Japan) according to the manufacturer's instruction. Serum was tested at 1:8, 1:16, 1:32, 1:64, and 1:128 dilutions. An antibody titer of ≥1:64 was considered positive [9,10].

2.3. Parasites

T. gondii RH strain tachyzoites (kindly provided by Dr. Kami Kim, Albert Einstein College of Medicine, NY, USA) were maintained in human foreskin fibroblast cells cultured in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NE, USA) supplemented with 10% heat-inactivated fetal calf serum, L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml). For the purification of *T. gondii* tachyzoites, infected cells were washed with cold phosphate buffered saline (PBS) and detached using a cell scraper. Cell pellets were re-suspended in PBS and passed through a 27-gauge needle and then through a 5.0 μm pore filter (Millipore, Bedford, MA, USA).

2.4. Indirect fluorescence antibody test (IFAT)

The IFAT slides were prepared with whole tachyzoites of *T. gondii* according to Gupta *et al.* [11]. In brief, purified *T. gondii* RH strain tachyzoites were dispensed in each well of 10-well heavy Teflon-coated antigen slides (Matsunami Glass, Osaka, Japan). Slides were air-dried at room temperature and fixed in cold acetone for 5 min. The slides were then rinsed in PBS and air-dried. *T. gondii*-positive riding/racing horse sera (40 samples) and randomly selected

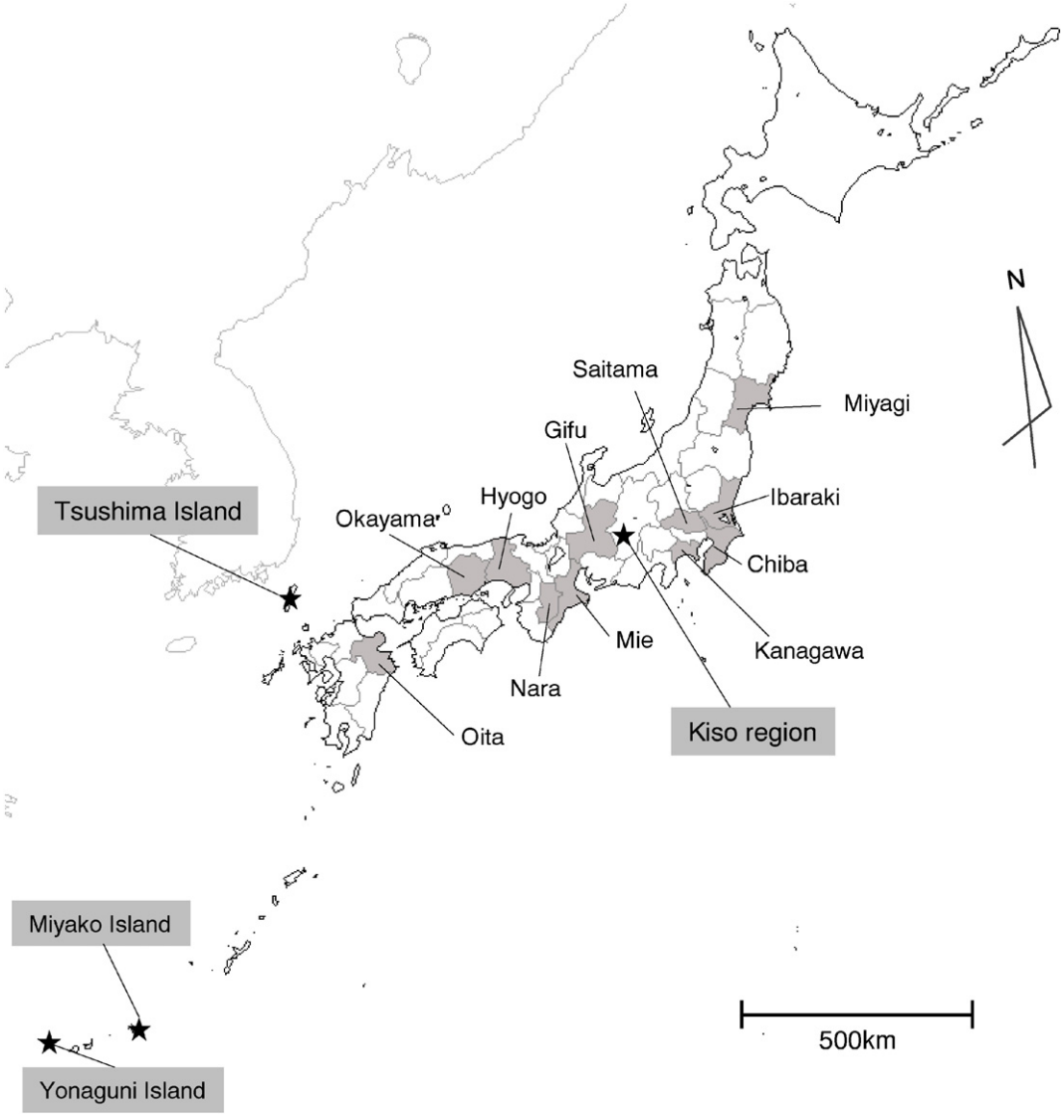

Fig. 1. *Map of Japan where samples were collected.* Map of Japan showing the sampling regions in this study. Prefectures of sampled sera from riding horses are shaded. Stars indicate the location sampled for Japanese native horses.