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NOTE

Serological surveillance for antibodies against *Erysipelothrix* species in wild boar and deer in Japan

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Abstract

We investigated the seroprevalence of antibodies against *Erysipelothrix* in wild animals in Japan. Serum samples were collected from 48 wild boar, 26 Yezo deer and 26 Japanese deer in Japan. Growth agglutination (GA) test was performed to estimate antibody titers. As a result, positive results were obtained from 32 (66.7%), 1 (3.6%) and 6 (23.1%) samples from wild boar, Yezo deer and Japanese deer, respectively. Our findings suggest that wild animals may be an important reservoir of *Erysipelothrix*.

Key Words: Antibody, *Erysipelothrix* spp., Wild animals

Members of the genus *Erysipelothrix* are facultative, non-spore-forming, non-acid-fast, and small Gram-positive bacilli[17)]; the three main species in the genus are *E. rhusiopathiae*, *E. tonsillarum*[12)], and *E. inopinata*[15)]. *E. rhusiopathiae* is prevalent among a variety of mammals, birds, and fish[2)], and is the main causative bacterium for swine erysipelas[2)], which is a major problem in animal

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husbandry[18]. In humans, this species causes erysipeloid, which manifests as skin lesions, endocarditis and sepsis and is contracted by direct contact with contaminated animals, their products and wastes, or contaminated soil[17]. The organism is widely regarded as an occupational pathogen and is prevalent in humans that work in association with animals and animal products[2]. In Japan, *Erysipelothrix* spp. are regarded as a food safety risk, and any edible meat from livestock infected with *Erysipelothrix* is discarded.

Wild animal populations are known reservoirs of *E. rhusiopathiae*[17], and species such as boar and deer, which are frequently hunted and processed for consumption by humans, are considered to be a potential risk of infection in humans[5–7]. In order to estimate the status of *Erysipelothrix* infection in Japan, the seroprevalence of antibodies against *Erysipelothrix* spp. has been investigated in pigs[11], chickens[14], cattle[9], and stray dogs[10]. However, the status of *Erysipelothrix* infection in wild animals has not yet been comprehensively examined. We therefore investigated the seroprevalence of antibodies against *Erysipelothrix* in wild boar and deer in Japan.

Serum samples were collected from 48 wild boar (*Sus scrofa leucomystax*), 26 Yezo deer (*Cervus nippon yesoensis*) and 26 Japanese deer (*Cervus nippon centralis*), which were harvested by hunting or trapping in Japan between 2011 and 2012. Wild boar and Japanese deer were harvested in Kyushu district, whereas Yezo deer were harvested in Hokkaido prefecture. The growth agglutination (GA) test was performed as described previously[8,10]. The GA test has been generally applied for the assessment of immunity in the animals to erysipelas[8–10,14]. It is known that *E. rhusiopathiae* antigen in the GA test cross-reacts with *E. tonsillarum*[13]. In the present study, therefore, the GA test was carried out to quantify the antibody responses to *Erysipelothrix* spp. Briefly, the serum samples were inactivated by heating at 56°C for 30 min. Two-fold dilutions (4–128-fold) of the serum samples were prepared with tryptose phosphate broth (pH 7.6; Difco Laboratories Inc., MI) containing 0.1% Tween 80, 25 μg/ml of gentamicin, and 250 μg/ml of kanamycin in 96-well plates. Overnight broth culture of the Marienfelde strain (serovar 1a of *E. rhusiopathiae*) was used as a live antigen. Five microliters of the culture was added to 100 μl of each serum dilution and agglutination was evaluated after incubation at 37°C for 24 h. The GA titer was expressed as the reciprocal of the highest serum dilution causing agglutination. In studies of *Erysipelothrix* infection in pigs and chickens[8,14], the GA titers rose to 1 : 16 or higher in the serum experimentally infected with virulent *Erysipelothrix* strains. Thus, GA titers of 16 or higher are considered positive in this study. Fisher's exact test was used to compare positive rates and among animal species. Significance was set at $P < 0.05$.

In wild boar, a GA titer of 16 or higher was detected in 32 of 48 (66.7%) serum samples (Table 1). Few reports have been published on the global prevalence of antibodies to *Erysipelothrix* among wild boar, except in Spain, where several research groups reported that 5–15% of wild boars were positive for antibodies to these organisms[1,4,16]. The results of the present study imply that, compared with Spain, *Erysipelothrix* spp. are more prevalent among wild boar populations in Japan. However, a direct comparison of the obtained results is complicated by the fact that different methods have been employed to monitor the seroprevalence of *Erysipelothrix* antibodies; for example, different antibody detection techniques and cut-off values have been employed by different researchers. It is considered that standardizing monitoring methods among areas and countries would facilitate a more comprehensive and accurate understanding of the seroprevalence of antibodies to *Erysipelothrix*.

Unlike wild boars, the prevalence of antibodies to *Erysipelothrix* in wild deer has not yet been reported elsewhere. Indeed, this is the first study, to our knowledge, to investigate the seroprevalence of *Erysipelothrix* antibodies in wild

Table 1. Growth agglutination titer of antibody against *Erysipelothrix* species in wild boar and deer in Japan

Animal species	No. of sera tested	No. of sera with antibody to *Erysipelothrix* species at[a)]:							No. of positive samples (%)
		<4	4	8	16	32	64	128	
Wild boar	48	3	3	10	13	13	5	1	32 (66.7)
Yezo deer	26	0	1	24	1	0	0	0	1 (3.8)
Japanese deer	26	2	10	8	6	0	0	0	6 (23.1)
Total	100	5	14	42	20	13	5	1	39 (39)

a) Vertical lines indicate cut-off values.

deer. The results showed that a GA titer of 16 was detected in 1 (3.6%) and 6 (23.1%) Yezo deer and Japanese deer, respectively, and that there was no significant difference in seroprevalence of the *Erysipelothrix* antibodies between the Yezo and Japanese deer samples analyzed (Table 1, $P > 0.05$). Although infection by pathogenic *Erysipelothrix* spp. has not been comprehensively examined in deer, a case of septicemia attributable to *E. rhusiopathiae* was reported in moose in Canada[3)]. Our findings suggest that wild deer and wild boar may act as reservoirs for *Erysipelothrix*, even though the observed seroprevalence of *Erysipelothrix* antibodies was relatively low.

When the seroprevalence of *Erysipelothrix* antibodies was compared among the three species examined in this study, positive rates of infection were significantly higher in the wild boar samples (66.7%) than in the wild deer samples (13.5%, $P < 0.05$). In addition, a GA titer of 32 or higher was only observed in wild boar. Therefore, wild boars should be regarded as an important reservoir for *Erysipelothrix* spp.

In conclusion, we investigated the seroprevalence of antibodies against *Erysipelothrix* spp. in wild boar and deer. Our results showed that *Erysipelothrix* antibodies were detected in both wild boar and deer at high and low rates, respectively. In addition, the present findings emphasize the need for a standardized risk management strategy for mitigating the transmission of *Erysipelothrix* spp. from wild animals, especially wild boar, to humans involved in activities such as hunting, dressing carcasses, and selling and cooking meat products for consumption.

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