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Title	Serological surveillance for antibodies against Erysipelothrix species in wild boar and deer in Japan
Author(s)	Shimizu, Takae; Okamoto, Chiaki; Aoki, Hiroshi et al.
Citation	Japanese Journal of Veterinary Research, 64(1), 91-94
Issue Date	2016-02
DOI	https://doi.org/10.14943/jjvr.64.1.91
Doc URL	https://hdl.handle.net/2115/61029
Type	departmental bulletin paper
File Information	09_Shimizu_Note.pdf



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

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Received for publication, August 6, 2015; accepted, November 10, 2015

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 bacilli¹⁷⁾; 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 fish²⁾, and is the main causative bacterium for swine erysipelas²⁾, which is a major problem in animal

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doi: 10.14943/jjvr.64.1.91

husbandry¹⁸). 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 soil¹⁷. The organism is widely regarded as an occupational pathogen and is prevalent in humans that work in association with animals and animal products². 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 humans⁵⁻⁷. In order to estimate the status of *Erysipelothrix* infection in Japan, the seroprevalence of antibodies against *Erysipelothrix* spp. has been investigated in pigs¹¹, chickens¹⁴, cattle⁹, and stray dogs¹⁰. 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 yezoensis*) 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 <i>Erysipelothrix</i> 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 Canada³⁾. 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.

This work was supported by Health and Labour Sciences Research Grants from the Ministry of Health, Labour and Welfare.

References

- 1) Boadella, M., Ruiz-Fons, J. F., Vicente, J., Martin, M., Segales, J. and Gortazar, C. 2012. Seroprevalence evolution of selected pathogens in Iberian wild boar. *Transbound Emerg. Dis.*, **59**: 395–404.
- 2) Brooke, C. and Riley, T. V. 1999. *Erysipelothrix rhusiopathiae*: bacteriology, epidemiology and clinical manifestations of an occupational pathogen. *J. Med. Microbiol.*, **48**: 789–799.
- 3) Campbell, G. D., Addison, E. M., Barker, I. K. and Rosendal, S. 1994. *Erysipelothrix rhusiopathiae*, serotype 17, septicemia in moose (*Alces alces*) from Algonquin park, Ontario. *J. Wildl. Dis.*, **30**: 436–438.
- 4) Closa-Sebastià, F., Casas-Díaz, E., Cuenca, R., Lavín, S., Mentaberre, G. and Marco, I. 2011. Antibodies to selected pathogens in wild boar (*Sus scrofa*) from Catalonia (NE Spain). *Eur. J. Wildl. Res.*, **57**: 977–981.
- 5) 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.
- 6) Rebolí, A. C. and Farrar, W. E. 1989. *Erysipelothrix rhusiopathiae*: an occupational pathogen. *Clin. Microbiol. Rev.*, **2**: 354–359.
- 7) Sales, J. and Kotrba, R. 2013. Meat from wild boar (*Sus scrofa* L.): a review. *Meat. Sci.*, **94**: 187–201.

- 8) Sawada, T., Muramatsu, M. and Seto, K. 1979. Response of growth agglutinating antibody and protection of pigs inoculated with swine erysipelas live vaccine. *Jpn. J. Vet. Sci.*, **41**: 593–600.
- 9) Sawada, T., Hassanein, R., Yamamoto, T. and Yoshida, T. 2001. Distribution of antibody against *Erysipelothrix rhusiopathiae* in cattle. *Clin. Diagn. Lab. Immunol.*, **8**: 624–627.
- 10) Shimazaki, Y., Gamoh, K., Imada, Y., Makie, H., Kanzaki, M. and Takahashi, T. 2005. Detection of antibodies to *Erysipelothrix* in stray dogs in Japan. *Acta Vet. Scand.*, **46**: 159–161.
- 11) Takahashi, T., Sawada, T. and Muramatsu, M. 1987. Serotype, antimicrobial susceptibility, and pathogenicity of *Erysipelothrix rhusiopathiae* isolates from tonsils of apparently healthy slaughter pigs. *J. Clin. Microbiol.*, **25**: 536–539.
- 12) Takahashi, T., Fujisawa, T., Tamura, Y., Suzuki, S., Muramatsu, M., Sawada, T., Benno, Y. and Mitsuoka, T. 1992. DNA relatedness among *Erysipelothrix rhusiopathiae* strains representing all twenty-three serovars and *Erysipelothrix tonsillarum*. *Int. J. Syst. Bacteriol.*, **42**: 469–473.
- 13) Takahashi, T., Takagi, M., Sawada, T. and Seto, K. 1984. Cross protection in mice and swine immunized with live erysipelas vaccine to challenge exposure with strains of *Erysipelothrix rhusiopathiae* of serovars and *Erysipelothrix tonsillarum*. *Am. J. Vet. Res.*, **45**: 2115–2118.
- 14) Takahashi, T., Takagi, M., Yamamoto, K. and Nakamura, M. 2000. A serological survey on erysipelas in chickens by growth agglutination test. *J. Vet. Med. B*, **47**: 797–799.
- 15) Verbarg, S., Rheims, H., Emus, S., Frühling, A., Kroppenstedt, R. M., Stackebrandt, E. and Schumann, P. 2004. *Erysipelothrix inopinata* sp. nov., isolated in the course of sterile filtration of vegetable peptone broth, and description of *Erysipelotrichaceae* fam. nov. *Int. J. Syst. Evol. Microbiol.*, **54**: 221–225.
- 16) Vicente, J., Leon-Vizcaino, L., Gortazar, C., Jose Cubero, M., Gonzalez, M., and Martin-Atance, P. 2002. Antibodies to selected viral and bacterial pathogens in European wild boars from southcentral Spain. *J. Wildl. Dis.*, **38**: 649–652.
- 17) Wang, Q., Chang, B. J. and Riley, T. V. 2010. *Erysipelothrix rhusiopathiae*. *Vet. Microbiol.*, **140**: 405–417.
- 18) Yamamoto, K., Kijima, M., Takahashi, T., Yoshimura, H., Tani, O., Kojoyou, T., Yamawaki, Y. and Tanimoto, T. 1999. Serovar, pathogenicity and antimicrobial susceptibility of *Erysipelothrix rhusiopathiae* isolates from farmed wild boars (*Sus scrofa*) affected with septicemic erysipelas in Japan. *Res. Vet. Sci.*, **67**: 301–303.