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Brief Communication

Detection of Antibodies to *Erysipelothrix* in Stray Dogs in Japan

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Erysipelothrix rhusiopathiae is considered as a pathogenic bacterium for many species of animals. The genus *Erysipelothrix* had been thought to contain a single strain. However, on the basis of genetic homology, the genus *Erysipelothrix* was found to constitute different strains, *E. rhusiopathiae* and a new strain, *E. tonsillarum* (Takahashi et al. 1992). *E. tonsillarum* can be distinguished from *E. rhusiopathiae* by serology, the fermentation of saccharose, genetic homology and the lack of pathogenicity for pigs (*Takahashi et al.* 1992, 1993, 2000a).

The classical host of the bacterium is the pig, but it can also induce a wide variety of disease conditions in other mammals and birds (*Kucsera* 1979, *Takahashi et al.* 1994). The bacterium has been isolated from dogs with endocarditis (*Eriksen et al.* 1987, *Hoenig & Gillette* 1980, *Kucsera* 1979, *Sisson et al.* 1984). In an experimental infection study using an isolate from a dog, it was confirmed that dogs developed endocarditis after intravenous inoculation (*Goudswaard et al.* 1973). The isolates from dogs with endocarditis in Belgium were typed as serovar 7, which is one of the *E. tonsillarum* serovars (*Schrauwen et al.* 1993, *Takahashi et al.* 1993). Furthermore, the isolate was classified into genomic *E. tonsillarum* based on the characteristics and genetic homology (*Takahashi et al.* 2000a). Other strains isolated from several cases of erysipelas in dogs were also typed as serovar 7 (*Hoenig & Gillette* 1980). These reports strongly indicated that *E. tonsillarum* was a canine pathogen.

However, there is no information about serological surveys in dogs to elucidate the epidemiological features of the disease in the field. Furthermore, there are few studies about the mechanism of erysipelas in dogs. As a causative factor of bacterial endocarditis, a pre-existing heart lesion has been suspected, but the relation between them is still obscure (*Goudswaard et al*. 1973, Hoenig & Gillette 1980). It has not been documented, whether erysipelas is actually caused by the mixed infection with *Erysipelothrix* and other organisms in dogs.

In this study, to search for the epidemiological features of erysipelas infection among dogs, we surveyed the levels and the distribution of anti-*Erysipelothrix* antibodies among dogs in the field.

The serum samples used in this study were obtained from 120 stray or homeless dogs in

Tokyo metropolitan animal preservation center, during the period of April 1999 to March 2000. As negative samples, we also used the serum derived from 19 dogs of SPF beagles origin in our laboratory. The growth agglutination (GA) test has been generally applied for the assessment of immunity in the animals to erysipelas (*Wood,* 1993). It is known that *E.rhusiopathiae* antigen in the GA test cross-reacts with *E.tonsillarum* (*Takahashi et al.* 1984, 1994). In the present study, therefore, the GA test was carried out to quantify the antibody responses to *Erysipelothrix* in dog serum. The procedure was carried out by a method of *Sawada et al.* (1979) with some modifications. Two fold dilutions of the serum were prepared with tryptose phosphate broth (pH 7.6, Difco) containing 0.1% Tween 80, 25 μg/ml of gentamicin, and 250 μg/ml of kanamicin in 96 well, V-bottom plates. Overnight broth culture of the Marienfelde strain (serovar 1a of *E.rhusiopathiae,* and international standard strain for the GA test) was used as live antigen. Five μl of the culture was added to 100 μl of each serum dilution. The agglutination was read after incubation at 37 degrees Celsius for 24h, and titres were expressed as the reciprocal of the highest serum dilution causing agglutination. In studies of *Erysipelothrix* infection in pigs and chickens, we previously described that the GA titre rose to 1:16 or higher in the serum experimentally infected with virulent *Erysipelothrix* strains (*Sawada et al.* 1979, *Takahashi et al.* 2000b). Thus, in the present investigation, porcine serum that had GA titre 1:16 to 32 was used as positive control and GA titre of 1:16 or higher was considered to be positive.

The results of serological survey of GA test are shown in Table 1. In total, a GA titre of 1:16 or higher indicating possible *Erysipelothrix* infection was detected in 6 (5.0%) of 120 serum samples derived from dogs in the field. Of these positive sera, four (66.7%) had a GA titre 1:16, one (16.7%) had a GA titre 1:32, and one (16.7%) had a GA titre 1:128. In 19 serum samples derived from laboratory dogs, one sample had a GA titre 1:4, but a sample with GA titer of 1:16 or higher was not detected. As a result of the antibody investigation, we could demonstrate the incidence of dogs having the GA titre 1:16 or higher (suspected *Erysipelothrix* infection) in the field, but there was no statistically significant difference between the population of positive samples in field dogs and that in laboratory dogs (Fisher's exact test).

Erysipelothrix has been isolated from several cases of endocarditis and septicaemia in dogs (*Eriksen et al.* 1987, *Hoenig & Gillette* 1980, *Kucsera* 1979, *Sisson et al.* 1984) and it has been demonstrated that the bacterium could cause endocarditis and arthritis in dogs by the intravenous injection (*Goudswaard et al.* 1973). There are hardly any reports that had examined epidemiological investigation and mechanism of the erysipelas infection in dogs. This is the first report on the existence of dogs having the positive level of antibodies against *Erysipelothrix* with 5% prevalence, even if it is a low proportion, indicating there was a certain risk of *Erysipelothrix* infection among dogs in

Table 1. GA antibody level to *Erysipelothrix* in Japanese dogs.

	No. of sera tested	No. of sera with anti-*Erysipelothrix* antibody at indicated titre							Proportion of positive samples (%) (≥16)
		<4	4	8	16	32	64	128	
Field dogs (%)	120	70	31	13	4	1	0	1	5.0
Laboratory dogs (%)	19	18	1	0	0	0	0	0	0

the field. From only the present data, it is difficult to know whether *Erysipelothrix* is able to cause the endocarditis absolutely or secondary to other organisms. In any case, further investigations are needed to make clear the mechanism of erysipelas infection in dogs.

References

Eriksen K, Fossum K, Gamlem H, Grondalen J, Kucsera G, Ulstein T: Endocarditis in two dogs caused by *Erysipelothrix rhusiopathiae.* J. Small Anim. Pract. 1987, *28,* 117-123.

Goudswaard J, Hartman EG, Janmaat A, Huisman GH: Erysipelothrix rhusiopathiae strain 7, a causative agent of endocarditis and arthritis in the dogs. Tijdschr. Diergeneesk.1973, *98,* 416-423

Hoenig M, Gillette DM: Endocarditis caused by *Erysipelothrix rhusiopathiae* in a dog. J. Am. Vet. Med. Assoc. 1980, *176,* 326-327.

Kucsera G: Serological typing of *Erysipelothrix rhusiopathiae* strains and the epizootiological significance of the typing. Acta Vet. Acad. Sci. Hung. 1979, *27,* 19-28.

Sawada T, Muramatsu M, Seto K: Response of growth agglutinating antibody and protection of pigs inoculated with swine erysipelas live vaccine. Jpn. J. Vet. Sci. 1979, *41,* 593-600.

Schrauwen E, Devriese LA, Hoorens J, Takahashi T: Erysipelothrix tonsillarum endocarditis in a dog. Vlaams Diergeneeskd Tijdschr 1993, *62,* 160-161.

Sisson D, Thomas WP: Endocarditis of the aortic valve in the dog. J. Am. Vet. Med. Assoc. 1984, *184,* 570-577.

Takahashi T, Takagi M, Sawada T, Seto K: 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. 1984, *45,* 2115-2118.

Takahashi T, Fujisawa T, Tamura Y, Suzuki S, Muramatsu M, Sawada S, Bennno Y, Mitsuoka T: DNA relatedness among *Erysipelothrix rhusiopathiae* strains representing all twenty-three serovars and *Erysipelothrix tonsillarum.* Int. J. Syst. Bacteriol. 1992, *42,* 469-473.

Takahashi T, Tamura Y, Yoshimura H, Nagamine N, Kijima M, Nakamura M: Erysipelothrix tonsillarum isolated from dogs with endocarditis in Belgium. Res. Vet. Sci. 1993, *54,* 264-265.

Takahashi T, Takagi M, Yamaoka R, Ohishi K, Norimatsu M, Nakamura M: Comparison of the pathogenicity for chickens of *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum.* Avian Pathol. 1994, *23,* 237-245.

Takahashi T, Fujisawa T, Yamamoto K, Kijima M, Takahashi T: Taxonomic evidence that serovar 7 of *Erysipelothrix* strains isolated from dogs with endocarditis are *Erysipelothrix tonsillarum.* J. Vet. Med. B 2000a, *47,* 311-313.

Takahashi T, Takagi M, Yamamoto K, Nakamura M: A serological survey on Erysipelas in chickens by growth agglutination test. J. Vet. Med. B 2000b, *47,* 797-799.

Wood RL: Swine erysipelas. In: Leman AD, Straw BE, Mengeling, WL, Allarie SD & Taylor DJ (eds.) : Disease of swine, 7th ed., Iowa State University Press, Ames. 1993, 475-486.

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