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Serological and pathogenic characterization of *Erysipelothrix rhusiopathiae* isolates from two human cases of endocarditis in Japan

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SUMMARY

We characterized the serological and pathogenic properties of two *Erysipelothrix rhusiopathiae* isolates from human cases of infective endocarditis in Japan. One isolate was recovered from a fisherman, and was identified as serovar 3, which is known to be prevalent among fish isolates. This strain exhibited high virulence in mice but was avirulent in swine. Another was untypable, and avirulent in both mice and swine. Our results suggest that various serological and athogenical types of *E. rhusiopathiae* can induce human endocarditis. This is the first report to characterize the pathogenicity of *E. rhusiopathiae* isolates from human endocarditis.

KEY WORDS: Endocarditis, *Erysipelothrix rhusiopathiae*, Human infection, Pathogenicity, Serovar

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Erysipelothrix rhusiopathiae is a primary pathogen of swine as well as a cause of sporadic disease outbreaks in humans and other animals. The organism is distributed worldwide and has been isolated from the organs of many wild and domestic mammals, birds, reptiles, amphibians and fish (Wood and Henderson, 2006). Infection in humans is usually a consequence of contact with infected animals, or their products or waste. Thus, it is prevalent in abattoir workers, butchers, farmers, veterinarians, fishermen, fish handlers and housewives (Brooke and Riley, 1999). There are three well-defined clinical syndromes in humans that are caused by *E. rhusiopathiae* infection. The most common is erysipeloid, characterized by swelling and redness of the infected parts of the body, typically the fingers and hands. Less common but more severe is a diffuse cutaneous form. The most serious manifestation of *E. rhusiopathiae* infection is a bacteremia illness, where endocarditis has almost always been linked. Although bacteremia and endocarditis are relatively uncommon, these types of diseases appear to be increasing in incidence (Brooke and Riley, 1999).

Serovars and pathogenicity in test animals, such as mice and pigs, are significant factors in characterizing strains of *Erysipelothrix* strains. Previously, we reported these characteristics of the strains isolated from a wide variety of animals including pigs, cows, chickens, dogs, and various species of fish (Takahashi *et al.*, 2008). For human strains, there are only two publications related to serotyping of the organism (Cross and Claxton, 1979; Kodera *et al.*, 2006). In this

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study, we characterized serovar and pathogenicity in mice and pigs for two *E. rhusiopathiae* isolates from human endocarditis cases in Japan. To our knowledge, this is the first report to characterize the pathogenicity of *E. rhusiopathiae* isolates that cause endocarditis in humans.

Two *E. rhusiopathiae* isolates, strains Nagasaki and Sapporo, from blood samples of two different human endocarditis cases were used. These cases had the chief complaint of systemic edema on both legs, and were found to develop a vegetation on either the tricuspid or the mitral valve based on echocardiogram. In addition, these cases also developed glomerular nephritis. Bacterial isolation and diagnosis were carried out at Sasebo City General Hospital and Teine Keijinkai Hospital. Background, clinical signs and examination results related to the strain Nagasaki have been reported previously (Yamamoto *et al.*, 2008). The strain Sapporo was identified by Gram staining, species-specific polymerase chain reaction (PCR) (Takeshi *et al.*, 1999) and Api Coryne (BioMerieux, Craponne, France).

The serovars of the two strains were determined using an agar gel double-diffusion precipitation system against typing sera representing serovars 1a, 1b, 2–26 and type N of *Erysipelothrix*, as described previously (Takahashi *et al.*, 2008).

For pathogenicity tests, 95 four-week-old female outbred strain ddY mice (Nippon SLC, Hamamatsu, Japan) were used. Five female and five castrated male Yorkshire swine were used when they were 10–11 weeks old. These swine were conventionally farrowed and raised in confinement. The sera of the swine had growth agglutination titers (Sawada *et al.*, 1979) of <1:8. Preparation of inocula and the inoculation of mice and pigs were carried out according to previous protocols (Takahashi *et al.*, 2008). A 0.1 mL volume of 10^0 to 10^{-6} dilutions of each isolate (approximately 10^9 CFU) was inoculated subcutaneously into five mice. The LD_{50} values were determined using the method of Karber (1931), based on mortality rates recorded 10 days after exposure. At the same time, three pigs were inoculated intradermally with 0.1 mL (approximately 10^8 CFU) of the Nagasaki strain and two pigs were inoculated with the Sapporo strain. Their clinical signs were observed every day for 14 days after exposure. All animal studies were conducted in accordance with Japanese law on Welfare and Management of Animals.

The present results were summarized together with a previous case in Japan (Kodera *et al.*, 2006) in Table 1. Presently, the genus *Erysipelothrix* contains three main species, *E. rhusiopathiae*, *E. tonsillarum* (Takahashi *et al.*, 2008), and *E. inopinata* (Verbarg *et al.*, 2004). There have previously been many reports about human cases of *Erysipelothrix* endocarditis, all of them due to in-

TABLE 1 - *Origins, sources, serovars and levels of pathogenicity in mice and swine for Erysipelothrix isolates from all three cases of human endocarditis in Japan.*

	Case 1	*Case 2*	*Case 3*
Year of case report	2006[a]	2008[b]	This study
Name of isolate	None	Nagasaki	Sapporo
Origin (Sex, age, and occupation)	Male, 67 yr, fisherman	Male, 58 yr, fisherman	Male, 40's, unknown
Source	Blood	Blood	Blood
Bacterial identification	*E. rhusiopathiae*	*E. rhusiopathiae*	*E. rhusiopathiae*[d]
Serovar	1[b]	3[d]	Untypable[d]
Pathogenicity for mice (Log LD_{50})	Not tested	0.6[d]	>8.9[d]
Pathogenicity for swine (Clinical sign[c])	Not tested	None[d]	None[d]

[a,b]These cases were reported by Kodera et al. (2006)[a] and Yamamoto et al. (2008)[b]; [c]Clinical signs including pyrexia, claudication, erythema, and death; [d]Results from this study.

fection by *E. rhusiopathiae*, but not other species in the genus (Brooke and Riley, 1999). In Japan, a total of three cases of *Erysipelothrix* endocarditis in humans have been identified (Kodera *et al.*, 2006; Yamamoto *et al.*, 2008), and these isolates were identified as *E. rhusiopathiae*. This particular species is a significant causative organism of human infective endocarditis.

Serovars of *Erysipelothrix* spp. have been extensively investigated to determine the origins of isolates. Most isolates from swine erysipelas belong to serovars 1a, 1b and 2 (Takahashi *et al.*, 1996), while serovar 7 strains are usually associated with endocarditis in dogs (Takahashi *et al.*, 2000). There is little information regarding serovars in human cases of *Erysipelothrix*. In this study, the strain Nagasaki was typed as serovar 3. This serovar was observed among strains of fish origin from our previous study (Takahashi *et al.*, 2008), although serovars 2, 5, and 9 and 1, 2, 5, 6, 8, 9, 10, 11, 14 and type N were also observed among isolates from retail and marine species of fish, respectively (Hashimoto *et al.*, 1974; Stenström *et al.*, 1992).

Yamamoto *et al.* (2008) suggested that the patient was infected due to occupational exposure because he was a fisherman (Brooke and Riley, 1999). The present serological findings support their suggestion. In the other case, Kodera *et al.* (2006) also reported an *Erysipelothrix* endocarditis case in a fisherman, and the isolate was typed as serovar 1b, the prevalence of which in fish was previously reported (Hashimoto *et al.*, 1974). The strain Sapporo was untypable using all antiserum, suggesting that it may be an unknown and/or new serovar that is yet to be classified (Hassanein *et al.*, 2001). Such untypable strains represent a major population of bovine (Hassanein *et al.*, 2001) and poultry isolates (Nakazawa *et al.*, 1998), and a minor population of porcine isolates (Takahashi *et al.*, 1996). However, it was confirmed that the patient had no history of contact with these animals. Likewise, human cases of *Erysipelothrix* infection, without any contact history, have been reported (Schuster *et al.*, 1993). To identify the *E. rhusiopathiae* infection route other than occupational exposure, further epidemiological investigation is required.

Pathogenicity in mice and swine has been investigated in a variety of *Erysipelothrix* strains. Mice are very susceptible to *Erysipelothrix* infection, and thus most of the strains are pathogenic in mice. In contrast, pathogenicity in pigs is observed only in strains belonging to limited serovars, such as 1a, 1b, and 2 (Takahashi *et al.*, 2008). In this study, the strain Nagasaki was highly virulent in mice (log LD_{50} = 0.6 CFU), but avirulent in swine. This result was similar to that in serovar 3 isolates from fish in our previous study (Takahashi *et al.*, 2008), and thus further supports the suggestion that this strain originated from fish. The strain Sapporo was avirulent in both mice (log LD_{50} = >8.9 CFU) and swine. Such avirulence was also occasionally observed in untypable strains (Hassanein *et al.*, 2003) and strains belonging to serovars 1b, 2, 4, 9, 11, 12, 13, 15, 17, 22 and type N (Hassanein *et al.*, 2003; Takahashi *et al.*, 2008). The present results suggest that strains virulent in test animals and avirulent strains can cause human infective endocarditis. For *E. rhusiopathiae*, several virulence factors, such as neuraminidase, hyaluronidase, surface protein, and capsule, have been identified and may be involved in the pathogenesis of disease in animals (Shimoji, 2000). However, the functions of these virulence factors in humans remain to be elucidated.

The present results show that the two *E. rhusiopathiae* isolates of human origin clearly have different characteristics as demonstrated by the results of the serotyping and pathogenicity tests. This implies that a variety of types of *E. rhusiopathiae* can be implicated with human infective endocarditis. Unfortunately, it is likely that infections by *E. rhusiopathiae* in humans are under-diagnosed because of the resemblance it bears to other infections, and the difficulties involved with isolation and identification (Brooke and Riley, 1999). This may result in a decrease in the chance of further investigating *Erysipelothrix* strains. A greater awareness by clinicians of human infection by *Erysipelothrix* is desirable.

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