FULL PAPER *Bacteriology*

Characterization and Identification of *Erysipelothrix rhusiopathiae* Isolated from an Unnatural Host, a Cat, with a Clinical Manifestation of Depression

Jin Ju LEE[1)], Dong Hyeok KIM[1)], Jeong Ju LIM[1)], Dae Geun KIM[1)], Hong Hee CHANG[1)], Hu Jang LEE[1)], Sang Hun KIM[2)], Man Hee RHEE[3)], Mehari ENDALE[3)], Yumiko IMADA[4)], Ok Jin KIM[5)] and Suk KIM[1)]*

[1)]Institute of Agriculture and Life Science, Gyeongsang National University, Jinju 660–701, [2)]College of Veterinary Medicine, Chungnam National University, Daejeon 305–764, [3)]College of Veterinary Medicine, Kyungpook National University, Daegu 702–701, Korea, [4)]National Institute of Animal Health, Tsukuba, Ibaraki 305–0856, Japan and [5)]Center for Animal Resources Development, Wonkwang University, Iksan 570–749, Korea

(Received 8 June 2010/Accepted 25 August 2010/Published online in J-STAGE 8 September 2010)

ABSTRACT. *Erysipelothrix rhusiopathiae* is pathogenic for humans, many domestic animals and wild birds, but infectious cases with clinical symptoms in cats have not been reported. *E. rhusiopathiae* was recovered from a 4-month Russian blue breed cat with a very poor body condition score of 1 (BCS: 1/5). The isolate was typed as serotype 2b. Mice experimentally infected with the clinical isolate of *E. rhusiopathiae* through subcutaneous or intraperitoneal routes survived, and the organism was recovered from the spleen and synovial and pericardial fluids. Cats experimentally inoculated with the isolate either orally or subcutaneously survived but commonly exhibited depression and emaciation together with localized erythemal lesion of the skin accompanied by purulent ocular discharge. On hematological analysis, the number of total white blood cells was high compared with that in normal cats. Histological examination revealed congestion and moderate inflammation with focal necrosis. This observation may provide insight on *E. rhusiopathiae* infection in cats with the possible epidemiological significance and implications as a potential source of infection to other animals and humans.
KEY WORDS: depression, *Erysipelothrix rhusiopathiae,* feline, pathogenicity.

J. Vet. Med. Sci. 73(2): 149–154, 2011

Erysipelothrix rhusiopathiae is a nonmotile, non-sporulating, non-acid-fast and Gram-positive slender rod belonging to the family *Erysipelotrichaceae* that has been isolated from many species of birds and mammals [7, 15]. It has also been reported as an occupational pathogen in humans, is prevalent in those working in association with animals and animal products and has thus far been isolated from many species of birds and mammals, including humans [20]. Human and animal infection can occur from contact with other infected animals, their secretions, wastes, products or organic matter contaminated by any of these [21].

Pigs and birds are believed to be the most important animal reservoirs of *E. rhusiopathiae* [21]. The clinical forms in pigs are acute septicemic, subacute urticarial and chronic associated endocarditis or arthritis [1, 10], while in humans, they are localized cutaneous, generalized cutaneous and septicemic forms associated with endocarditis [1]. An isolated human case report by Callon and Brady [2] indicated that a toothpick perforation of the sigmoid colon was associated with *E. rhusiopathiae* septicemia. Nevertheless, reports on the association of this bacterium with clinical cases in cats are limited.

The organism is shed by diseased animals in feces, urine, saliva and nasal secretions, which can contaminate food, water, soil and bedding, leading to indirect transmission of the organism [21]. Some studies have found both virulent and avirulent serotypes on the tonsils [13], and the feces of apparently healthy animals contained virulent organisms [21]. To our knowledge so far, however, there has been no report on the isolation of the bacterium either from apparently healthy or clinical cases of cats.

Identification of *Erysipelothrix* is based on Gram staining, cultural morphology, motility and hemolytic and biochemical properties, particularly H_2S production [10]. Beside cultural methods, there have been two established PCR methods reported for detecting *E. rhusiopathiae*, the genus-specific and species-specific methods [8, 12]. In addition, the surface protective antigen (Spa) of *E. rhusiopathiae* is reported to be one of the most clearly defined surface proteins such that its detection and identification are important for evaluating the antigenicity and pathogenicity of field *Erysipelothrix* spp. [11]. Shen *et al*. very recently developed *spa* multiplex real-time and conventional PCR assays to detect and differentiate among *spaA*, *spaB* and *spaC* genes within *Erysipelothrix* spp. and suggested that these PCR assays may be useful tools for investigating Spa prevalence among strains isolated from field tissues [11].

The mouse protection test is considered to be the best method for confirming an isolate as *E. rhusiopathiae* [7]. However, it may not be a good confirmatory test for strains that are virulent in other animals such as cats. The recommended method for serologic investigation of *E. rhusiopathiae* has been reported to be the double agar-gel diffusion precipitation test with type-specific rabbit antisera and a heat-stable antigen prepared by hot aqueous extraction [7]. In addition, the *in vitro* antimicrobial susceptibility profiles of the *E. rhusiopathiae* clinical isolates have been reported [13, 16].

* CORRESPONDENCE TO: KIM, S., Institute of Agriculture and Life Science, Gyeongsang National University, 900 Gazwa, Jinju 660–701, Korea.
e-mail: kimsuk@gsnu.ac.kr

Despite the multitude of identification techniques, however, *E. rhusiopathiae* infection is possibly underdiagnosed because of its resemblance to other infections, difficulties in isolation and identification of the causative organism and the rapid response to empiric antimicrobial therapy [4]. Besides, infections of patients with no history of contact with animals or skin lesions have been reported, and in many cases, the source of infection has not been identified [2]. Moreover, potential errors in recognition of this organism isolated from clinical cases due to unusual clinical presentations and the possibility of underdiagnosed infections have been reported [1]. Above all, the pathogenesis and clinical manifestation of depression by *E. rhusiopathiae* is poorly understood in cats, and the organism is barely implicated as an etiological pathogen isolated from feces of clinical cases in cats. This is the first report to isolate, characterize and identify *E. rhusiopathiae* from an immature cat with a clinical manifestation of depression by using culture methods for morphological and biochemical characteristics, PCR assay, serotyping, antimicrobial susceptibility and *in vivo* pathogenicity tests. This study might provide insight on the bacteria as a potential cause of *E. rhusiopathiae* infection in companion animals such as cats with the possible epidemiological significance and implications as a potential source of infection to other animals including humans.

MATERIALS AND METHODS

Sample collection and bacterial culture: A female Russian blue breed cat (4 months of age and weighing 0.85 kg) was presented to a local veterinary hospital (Daejeon, Korea) with a very poor body condition score of 1 (BCS: 1/5). Blood and fecal samples were obtained and cultured on sheep blood agar plates. The cat had received medical treatment with cefazolin at a local clinic for 7 days and recovered. Colonies on the blood agar plates were picked and streaked onto commercially available brain heart infusion agar (pH 7.6, Difco, Detroit, KS, U.S.A.) supplemented with 10% horse serum, 0.1% Tween 80 and 0.03% sodium azide for selective enrichment and incubated at 37°C overnight before further analyses were performed. Both reference strain *E. rhusiopathiae* S-192 (serovar 2), which was obtained from the National Veterinary Research and Quarantine Service, and the clinical isolate were stored at 4°C in brain heart infusion agar supplemented with 10% horse serum until use, suspended in broth medium containing 20% glycerol and kept at –80°C.

Biochemical characteristics of the isolate: Growth and morphological characteristics, Gram staining and hemolytic characteristics were examined. Biochemical characteristics of the isolates were assayed with a commercially available automatic Vitek system. Carbohydrate fermentation and production of hydrogen sulfide in triple sugar iron agar were also tested [10, 19].

PCR assay of the isolate: For extraction of total DNA from the isolate, bacterial suspension from enriched broth culture was pelleted by centrifugation, suspended in 200 μl of TES buffer (50 mM Tris-HCl, 5 mM EDTA, 50 mM NaCl, pH 8.0) containing 10 μl of lysozyme (10 mg/m*l*) and 10 μl (1 mg/m*l*) of N-acetylmuramidase SG (Seikagaku, Tokyo, Japan) and then incubated for 30 min at room temperature or at 37°C before addition of 10 μl of 10% sodium dodecyl sulfate (SDS) and 10 μl of proteinase K (20 mg/m*l*). After further incubation at 55°C for 60 min, the crude DNA preparation was treated with RNase, extracted three times with phenol-chloroform, precipitated with ethanol and dissolved in distilled water. PCR amplification was performed with 25-μl volumes using 100 *n*g of template DNA and 100 pmol of each primer. Primers ER1 (5'-CGATTATATTCTTAGCACGCAACG-3') and ER2 (5'-TGCTTGTGTTGTG ATTTCTTGACG-3') previously designed from the DNA sequence of chromosomal loci that were presumably associated with the virulence of *E. rhusiopathiae* [12] were used to identify this organism. PCR consisting of denaturation at 94°C for 1 min, annealing at 63°C for 30 sec, and extension at 72°C for 1 min was performed for 30 cycles. For *spa* multiplex PCR, the primers were used according to the method described earlier [11]. Primer Ery ABCF (5'-ATGAAAAAGAAAAAACACCTATTTCCG-3') was used as a common forward primer for all three *spa* types, and primers Ery A813R (5'-GCGATTTCTCCGCATAGCA-3'), Ery B1000R (5'-CAATACCCTTTAGAGCCTCAACCA-3') and Ery C1011R (5'-TGCCTCAACTAC GGTTTGATACG-3') were used as reverse primers for *spaA*, *spaB* and *spaC*, respectively. The amplification conditions of the *spa* multiplex PCR were as follows: initial denaturation at 95°C for 5 min; 35 cycles of 40 sec at 95°C, 40 sec at 55°C and 1 min at 72°C; and finally extension at 72°C for 7 min. A total of 10 μl of each of the PCR products was analyzed by 1% agarose gel electrophoresis.

Serotyping of the isolate: Serotyping was carried out using double agar gel precipitation tests with autoclaved extracts of the isolates and rabbit antisera against formalin-killed cells of reference strains [6]. Briefly, rabbits were immunized by five successive intravenous injections of formalin-killed whole-cell suspensions of international reference strains of serotypes 1 to 23. The isolate obtained from the affected cat in the present study was cultivated, and cells were collected by centrifugation at 7,000 × *g* for 5 min, resuspended in 1/30 volume of distilled water and autoclaved at 121°C for 60 min. After centrifugation, the supernatant was reacted with rabbit antisera against serotypes 1a, 1b and 2a in 1% Noble agar with 150 mM sodium chloride-10 mM phosphate buffer (pH 7.2) containing 0.1% sodium azide. Serotype 2b was distinguished from serotype 2a by examining whether the precipitation line between the sample and anti-serotype 2a fused completely with the homologous precipitation line for serotype 2a. If fusion was incomplete, the strain was designated as serotype 2b.

Antimicrobial susceptibility test: Antimicrobial susceptibility tests were determined by the disc diffusion method [16] using discs containing the following antibiotics and contents, which are indicated in parentheses: ampicillin (10 μg), cephalothin (30 μg), chloramphenicol (30 μg), erythro-

mycin (15 μg), norfloxacin (5 μg), penicillin (10 IU), tetracycline (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), vancomycin (30 μg), amikacin (30 μg), amoxicillin/clavulanic acid (20/10 μg), cefazolin (30 μg), colistin (10 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg) and streptomycin (10 μg). *Staphylococcus aureus* ATCC 25923 and *Streptococcus pneumonia* ATCC 49619 were used as quality control. Zone diameter breakpoints [3] were used for interpretation of resistance to those agents.

After incubation for 24 hr at 37°C in 10 m*l* of selective enrichment broth, the isolate was plated onto brain heart infusion agar supplemented with 10% horse serum. The discs were placed using a disc dispenser, and the plates were incubated at 37°C for 24 hr.

Experimental infection of mice with the clinical isolate: Specific-pathogen-free (SPF) female BALB/c mice aged 8 weeks and weighing 25 ± 3 g were used in this study. All mice were kept at 23 ± 1°C with a 12-hr light/dark cycle and had free access to water and diet. The mice were acclimatized for at least 2 weeks before inoculation, after which they were grouped into four treatments with five mice in each group. The first and second groups were subcutaneously (SC) and intraperitoneally (IP) challenged with 100 μ*l* (2×10^4 CFU), respectively [15]. The third and fourth groups were inoculated with 100 μ*l* of the reference strain, *E. rhusiopathiae* S-192 serovar 2, containing 2×10^4 CFU through the same route as the clinical isolate. Infected mice were examined for viability every 24 hr, and the symptoms of the mice were observed for 8 weeks, after which the surviving mice were euthanized and autopsied. The spleen, liver, heart and synovial and pericardial fluids obtained from all the mice were subjected to isolation of *E. rhusiopathiae*, which was confirmed by PCR assay.

Experimental infection of cats with the clinical isolate: Six specific-pathogen-free (SPF) female Korean short-hair breed cats aged 4–5 months were challenged, and the pathogenicity of the isolate was examined. The cats were individually housed, able to visually interact with each other and their caretaker and had free access to feed and water. Before experimental infection, the standard procedure for diagnosis was carried out including a general physical assessment; determination of the number of red blood cells, packed cell volume, hemoglobin concentration and number of white blood cells; and fecal analysis. Three treatment groups of two cats in each group were examined. The first group did not receive any challenge, while the second and third groups were challenged with 1 m*l* of bacterial suspension containing 2×10^4 CFU subcutaneously (SC) and orally (PO), respectively. Fecal samples were collected daily for 4 weeks after inoculation. Daily fecal samples were plated on brain heart infusion agar supplemented with 10% horse serum, 0.1% Tween 80 and 0.03% sodium azide, and cultivated for 48 hr at 37°C. The clinical signs, including appetite, body temperature, defecation and skin condition, were monitored every day for 4 weeks. Blood samples were collected from cats at 14 and 21 days postinfection. The presence of bacteria in the blood was also examined using the combination of blood culture and PCR. After 4 weeks, the cats were euthanized and autopsied. To determine the bacterial presence in organs or body fluids, the synovial fluid (0.3 to 0.5 m*l*), spleen and liver were cultured and subjected to the PCR assay. Histopathological lesions were examined on organs including the spleen, liver and heart in the inoculum challenged and autopsied cats. Tissue biopsies were then fixed in 4% buffered formaldehyde and conventionally stained with hematoxylin-eosin staining techniques. All procedures described were reviewed and approved by the Animal Ethical Committee of Gyeongsang National University (Authorization Number GNU-LA-20).

Statistical analysis: Values are expressed as means ± SD. Statistical analysis was performed using the Student's *t*-test, and a *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Identification and biochemical characteristics of the isolate: The isolates recovered from feces and grown either in agar or in broth were Gram-positive on staining, which decolorized easily, and most of the isolates appeared to be small colonies with a narrow zone of alpha hemolysis on blood agar plates. No bacteria were isolated from blood of the clinical cat. The colonial morphology on agar plates was characterized by little transparency and a smooth convex form (S) that gradually developed into slightly larger and flat rough (R) forms after several subcultures. Microscopically, the bacteria appeared as small, moderately curved and slender rods on the agar plate, but appeared as longer and curved rods in broth subculture. Biochemically, the isolate was negative for catalase, oxidase, methyl red, indole, esculin, nitrate reduction, the Voges-Proskauer reaction and the gelatin liquefaction test. In addition, acid was produced from glucose, fructose and galactose but not from maltose, xylose, mannitol and lactose fermentation. Moreover, the isolate produced hydrogen sulfide on triple sugar iron agar reaction [20]. In the PCR analysis, the expected sizes of the *E. rhusiopathiae*-specific, *spaA*, *spaB* and *spaC* PCR products were 937, 813, 904 and 1,011 bp, respectively. The present study amplified *E. rhusiopathiae* and *spaA*-specific products of 937 and 813 bp, respectively (Fig. 1A and 1B). In the double agar gel precipitation test for serotyping, the precipitation line between our isolate and anti-serotype 2a antibodies was incomplete; therefore, our isolate was designated as serotype 2b.

Antimicrobial susceptibility tests: The clinical isolate was resistant to cephalothin, trimethoprim/sulfamethoxazole, vancomycin, colistin, nalidixic acid, kanamycin and streptomycin and susceptible to amikacin, cefazolin, gentamicin ampicillin, chloramphenicol, erythromycin, norfloxacin, penicillin, tetracycline and amoxicillin/clavulanic acid.

Mice pathogenicity test of the isolate: While all the mice challenged with the reference strain died within 5 days postinfection, those groups of mice inoculated with the clinical isolate survived the infection regardless of the route of

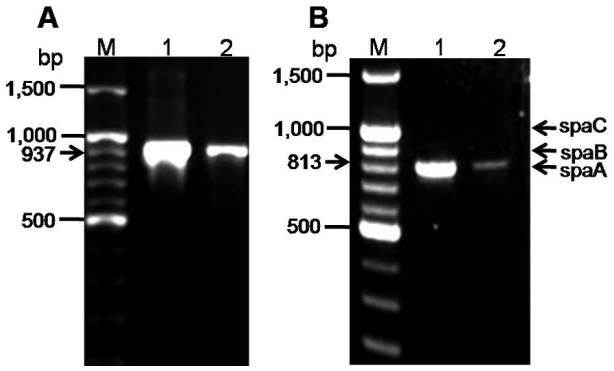

Fig. 1. Detection of DNA fragments amplified from DNA sequences specific to *E. rhusiopathiae* (A) and the *spa* genes (B). Lanes: 1, reference strain of *E. rhusiopathiae* S-192; 2, clinical isolate; M, 100-bp ladder marker.

administration. Both routes (SC or IP) of infection exhibited a weak virulence of the isolate with a clinical manifestation of depression and emaciation except for ruffled hairs observed in 40% of the cases challenged through IP. At 8 weeks after the challenge, the inoculum was recovered by bacterial culture and PCR methods from the spleen and synovial and pericardial fluids in the ratios of 5/5, 4/5 and 5/5 in the SC group and 5/5, 3/5 and 4/5 in those challenged with IP, respectively.

Cat pathogenicity test of the isolate: In the examination of pathogenicity by challenge with the isolate, none of the cats in the inoculated groups died, although clinical signs of varying severity were observed for 4 weeks postinfection. In both routes (SC or PO) of infection, the challenged cats exhibited common symptoms of depression and emaciation. In addition, one of the cats inoculated SC showed a localized erythemal lesion (Fig. 2A) accompanied by unilateral purulent ocular discharge (Fig. 2B). Hematological analyses were performed during the 4-week experimental period. The total white blood cell count was generally lower in inoculated cats, while there was no difference in red blood cell parameters except a slight decrease in mean corpuscular hemoglobin (MCH) levels in the inoculated cats compared with the control group (Table 1). White blood cell values at 14 days postinfection were higher than at 21 days, and in particular, those cats inoculated SC had significantly higher WBC values compared with those inoculated PO. The inoculum was isolated from feces in the PO-challenged groups. However, in the cats challenged SC, the inoculum was not recovered from feces. Our findings revealed that the isolate was found in the liver and spleen, but not in the synovial fluid and heart of both groups at 28 days postinfection (Table 2).

Histological examination of the autopsied spleens, livers and hearts of the inoculated cats revealed infiltration of mononuclear cells with densely aggregated leukocytes in all organs examined and congestion and moderate inflammation with pinpoint focal necrosis in the liver and heart, respectively (Fig. 2C–E).

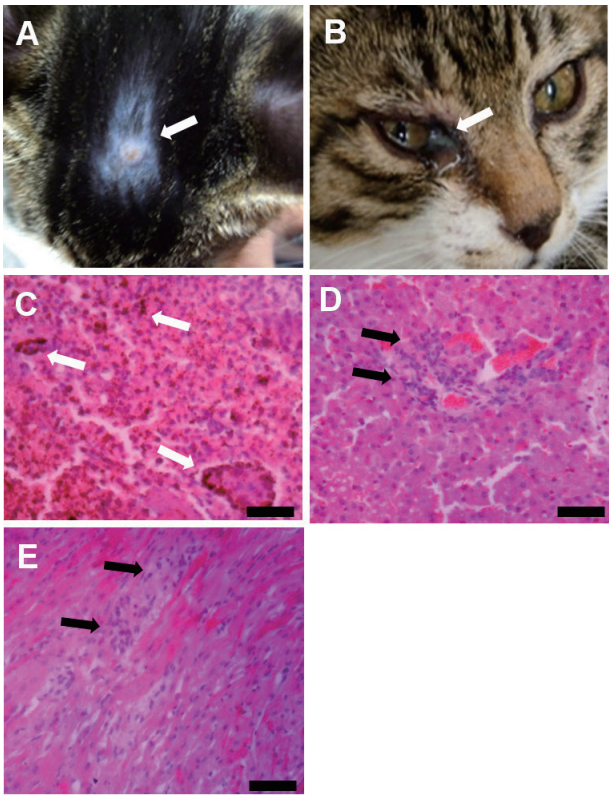

Fig. 2. Gross and histological lesions in cats experimentally infected with the clinical isolate of *E. rhusiopathiae*. Cats were subcutaneously (A and B) and orally (C, D and E) inoculated. A gross local urticarial lesion in skin (A) and purulent unilateral ocular discharge (B) are indicated by arrows. Histological lesions of the spleen (C), liver (D) and heart (E) in the inoculated cats revealed infiltration of mononuclear cells (white arrows) in the spleen with densely aggregated leukocytes and congestion and moderate inflammation (black arrows) with pinpoint focal necrosis in the liver and heart. Magnification, × 400. Bar=50 μm.

DISCUSSION

E. rhusiopathiae is ubiquitous in nature and has been isolated from many species of wild and domestic mammals, birds, reptiles, amphibians and fishes [1]. The organism causes a variety of diseases in many species of birds and mammals, including humans, but it is most important as the causative agent of swine erysipelas [17, 21].

Swine erysipelas may occur as an acute septicemic or chronic disease typically characterized by endocarditis and polyarthritis [1, 21]. It also causes polyarthritis in sheep and lambs and serious death losses in turkeys and chickens. In humans, the organism causes erysipeloid, a local cutaneous lesion, and rarely causes endocarditis or acute septicemic disease [21].

Despite the wide range of the above indicated hosts affected by the organism, reports on the association of this bacterium with infectious cases exhibiting clinical symp-

Table 1. Hematological examination results in cats experimentally infected with the clinical isolate of *E. rhusiopathiae*

	Subcutaneously inoculated		Orally inoculated		Non-infected	
	Day 14 p.i.a)	Day 21 p.i.	Day 14 p.i.	Day 21 p.i.	Day 14 p.i.	Day 21 p.i.
WBC[b] (10^3/mm^3)	50.28 ± 0.47***	32.42 ± 1.18***	28.21 ± 0.82**	23.71 ± 1.38*	13.7 ± 0.17	14.2 ± 0.25
RBC[b] (10^6/mm^3)	5.77 ± 0.13	6.69 ± 0.02	5.46 ± 0.13	6.65 ± 0.11	5.98 ± 0.17	5.9 ± 0.29
PCV[b]	23.75 ± 3.0	27.55 ± 0.10	20.85 ± 3.21	26.3 ± 0.49	33.7 ± 1.20	34.07 ± 1.42
Hb[b] (g/d*l*)	9.6 ± 0.07	9.65 ± 0.03	8.95 ± 0.03	9.05 ± 0.03	11.2 ± 0.99	11.38 ± 0.52

a) Day 14 postinfection. b) Data represent averages ± SD. *Analysis of the various hematologic values in the two groups by the Student's *t*-test revealed statistically significant differences (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$) compared with the non-infected values.

Table 2. Isolation of *E. rhusiopathiae* from cats experimentally infected with the clinical isolate on day 28 postinfection

	Subcutaneously inoculated		Orally inoculated	
	Group 1[a]	Group 2	Group 1	Group 2
Blood	–[b]	–	+	–
Feces	–	–	+	+
Liver	+	+	+	+
Spleen	+	+	+	+
Synovial fluid	–	–	–	–
Heart	–	–	–	–

a) Group of cats challenged with the bacteria. b) Bacteria detected: +. Bacteria not detected: –.

toms in cats and their possible epidemiological implications are limited. Besides, although *E. rhusiopathiae* infection has been reported to be associated with more diverse clinical syndromes [7] and there has been an isolated report of *E. rhusiopathiae* infection in two human cases acquired from two cat bites [14], clinical infection of cats with this organism has not yet been reported.

Norrung and Molin [9] reported that strains of *Erysipelothrix* were determined as belonging to serotypes 1–26 and group N. Most of the isolates from swine are classified into serotype 1 or 2, and serotype 2a is more prevalent in chronic forms of the disease. Imada *et al.* [6] serotyped 800 *Erysipelothrix* strains isolated from pigs with acute and chronic cases of swine erysipelas, and 254 of the strains belonged to serotype 2b, suggesting that serotype 2b may be a crucial pathogen for swine erysipelas. Various virulence factors, such as capsule, hyaluronidase and neuraminidase, have been suggested as being involved in the pathogenicity of this bacterium [18]. Very little, however, is known about the pathogenesis of serotype 2b in other animals such as cats and mice.

In this study, we identified *E. rhusiopathiae* from the feces of a patient cat by using culture methods in combination with a PCR assay. In addition, serotyping of the isolate revealed it to be *E. rhusiopathiae* serotype 2b. The present results on the antimicrobial susceptibilities of the *E. rhusiopathiae* isolate from the affected cat are in general agreement with previous reports on the antimicrobial susceptibility of the clinical isolates of swine [13]. Similar antimicrobial susceptibility observations using antimicrobial agents against various sources of the bacterium were also reported elsewhere [5, 22].

Erysipelothrix spp. have great genetic, serological, biochemical and antigenic variations between strains [11]. In this study, an *spa* multiplex PCR assay was used for molecular identification of the *spa* gene. Our findings detected the *spaA*, but not *spaB* or *spaC*, gene in the clinical isolate.

The virulence of the isolate was less than that of the reference strain in mice, regardless of the route of infection. This is evidenced by the fact that mice inoculated with the isolate survived infection with the same lethal dose given to the reference strain group. In cats, the isolate was not lethal but exhibited common symptoms of depression and emaciation. In addition, challenged cats showed localized urticarial lesions accompanied by purulent ocular discharge, and histological examination of the autopsied spleens, livers and hearts of the inoculated cats revealed congestion and moderate inflammation with pinpoint focal necrosis. Detection of the *spaA* gene in the present study suggests that this surface protective antigen may be a possible virulence factor for the above pathogenic effects of the isolate in cats. *E. rhusiopathiae* strains are known to vary considerably in virulence, and the surface protective antigen (Spa) has been indicated as one of the virulence factors to be involved in the pathogenicity of the disease [11, 16]. To and Nagai further suggested that Spa proteins may be virulence factors specifically involved in the pathogenesis of *E. rhusiopathiae*, and from a practical point of view, the presence or absence of *spa* may be a suitable marker for the differentiation of *E. rhusiopathiae* and *E. tonsillarum* [15].

In conclusion, we report that cases of depression in cats may be associated with *E rhusiopathiae* infection. Our finding may provide insight on a bacterium as a potential cause of *E rhusiopathiae* infection in companion animals such as cats with the possible epidemiological significance and implications as a potential source of infection to other animals and humans.

REFERENCES

1. Brooke, C. J. and Riley, T. V. 1999. *Erysipelothrix rhusiopathiae*: bacteriology, epidemiology and clinical manifestations of an occupational pathogen. *J. Med. Microbiol.* **48**: 789–799.
2. Callon, R. A. Jr. and Brady, P. G. 1990. Toothpick perforation of the sigmoid colon: an unusual case associated with *Erysipelothrix rhusiopathiae* septicemia. *Gastrointest. Endosc.* **36**: 141–143.

3. CLSI. 2007. Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement, CLSI document M100-S17. Vol. 27, Clinical and Laboratory Standards Institute, Wayne.
4. Connor, M. P. and Green, A. D. 1995. Erysipeloid infection in a sheep farmer with coexisting orf. *J. Infect*. **30**: 161–163.
5. Fidalgo, S. G., Longbottom, C. J. and Rjley, T. V. 2002. Susceptibility of *Erysipelothrix rhusiopathiae* to antimicrobial agents and home disinfectants. *Pathology* **34**: 462–465.
6. Imada, Y., Takase, A., Kikuma, R., Iwamaru, Y., Akachi, S. and Hayakawa, Y. 2004. Serotyping of 800 strains of *Erysipelothrix* isolated from pigs affected with erysipelas and discrimination of attenuated live vaccine strain by genotyping. *J. Clin. Microbiol.* **42**: 2121–2126.
7. Jones, D. 1986. Genus *Erysipelothrix* rosenbach. pp. 1245–1249. *In*: Bergey's Manual of Systematic Bacteriology, Vol. 2. (Sneath, P., Mair, N. and Sharpe, M, eds.), Williams and Wilkins, Baltimore.
8. Makino, S., Okada, Y., Maruyama, T., Ishikawa, K., Takahashi, T., Nakamura, M., Ezaki, T. and Morita, H. 1994. Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. *J. Clin. Microbiol.* **32**: 1526–1531.
9. Norrung, V. and Molin, G. 1991. A new serotype of *Erysipelothrix rhusiopathiae* isolated from pig slurry (short communication). *Acta Vet. Hung.* **39**: 137–138.
10. Reboli, A. C. and Farrar, W. E. 1992. The genus *Erysipelothrix*. pp. 1629–1642. *In*: The Prokaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications (Balows, A., Truper, H., Dworkin, M., Harder, W. and Schleifer, K. eds.), Springer–Verlag, New York.
11. Shen, H. G., Bender, J. S. and Opriessnig, T. 2010. Identification of surface protective antigen (*spa*) types in *Erysipelothrix* reference strains and diagnostic samples by *spa* multiplex real-time and conventional PCR assays. *J. Appl. Microbiol.* (in press).
12. Shimoji, Y., Mori, Y., Hyakutake, K., Sekizaki, T. and Yokomizo, Y. 1998. Use of an enrichment broth cultivation-PCR combination assay for rapid diagnosis of swine erysipelas. *J. Clin. Microbiol.* **36**: 86–89.
13. Takahashi, T., Sawada, T., Muramatsu, M., Tamura, Y., Fujisawa, T., Benno, Y. and Mitsuoka, T. 1987. Serotype, antimicrobial susceptibility, and pathogenicity of *Erysipelothrix rhusiopathiae* isolates from tonsils of apparently healthy slaughter pigs. *J. Clin. Microbiol.* **25**: 536–539.
14. Talan, D. A., Citron, D. M., Abrahamian, F. M., Moran, G. J. and Goldstein, E. J. C. 1999. Bacteriologic analysis of infected dog and cat bites. Emergency Medicine Animal Bite Infection Study Group. *N. Engl. J. Med.* **340**: 85–92.
15. To, H. and Nagai, S. 2007. Genetic and antigenic diversity of the surface protective antigen proteins of *Erysipelothrix rhusiopathiae*. *Clin. Vaccine Immunol*. **14**: 813–820.
16. Venditti, M., Gelfusa, V., Tarasi, A., Brandimarte, C. and Serra, P. 1990. Antimicrobial susceptibilities of *Erysipelothrix rhusiopathiae*. *Antimicrob. Agents Chemother.* **34**: 2038–2040.
17. Verbarg, S., Rheims, H., Emus, S., Fruhling, 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. Microbil.* **54**: 221–225.
18. Wang, Q., Chang, B. J., Mee, B. J. and Riley, T. V. 2005. Neuraminidase production by *Erysipelothrix rhusiopathiae*. *Vet. Microbiol.***107**: 265–272.
19. White, C. M., Fan, C., Song, J., Tsikouris J. P. and Chow, M. 2001. An evaluation of the hemostatic effects of hydrophilic, alcohol, and lipophilic extracts of notoginseng. *Pharmacotherapy* **21**: 773–777.
20. Wood, R. L. 2006. *Erysipelas*. pp. 475–486. *In*: Diseases of Swine, 9 ed. (Barbara, E. and Straw, B. eds.), Iowa State University Press, Ames.
21. Wood, R. L. 1992. *Erysipelas*. pp. 475–486. *In*: Diseases of Swine, 7 ed. (Leman, A., Straw, B., Mengeling, W., D'Allaire, S. and Taylor, D. eds.), Iowa State University Press, Ames.
22. Yamamoto, K., Kijima, M., Takahashi, T., Yoshimura, H., Tani, O., Kojyou, 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.