Two Cases of *Erysipelothrix rhusiopathiae* Serotype 2 Infection in Bottlenose Dolphins

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豚丹毒菌血清型 2 型感染のバンドウイルカ 2 例

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ABSTRACT. 20-year-old male and a 6-year-old male bottlenose dolphin (*Tursiops truncatus*), both born in captivity, were suspected of *Erysipelothrix rhusiopathiae* infection in August 1999. The older dolphin died and *E. rhusiopathiae* serotype 2 was isolated from his blood samples. The younger dolphin recovered from subacute erysipelas by administering sultamicillin tosilate (15 mg/kg, BID) and doxycycline (8 mg/kg, BID) although skin lesions were showing. Antibody against *E. rhusiopathiae* serotype 2 in the sera from these dolphins was detected.

Key Words : bottlenose dolphin, *Erysipelothrix rhusiopathiae* serotype 2, septicemia

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Erysipelothrix rhusiopathiae is the cause of erysipelas in swine [1], and turkeys [2] but is seen less frequently in other animals and humans. In cetaceans, this disease has been reported worldwide [3-9]. *E. rhusiopathiae* is classified into 23 serotypes and type N based on peptidoglycan antigens of the cell wall [1, 10]. Among the serotypes of *E. rhusiopathiae*, serotypes 1 and 2 are the most important [1, 11-14]. In Japan, there has been only one case reporting serotyping in cetaceans [15]. In this paper we describe the first two cases of *E. rhusiopathiae* ser-

otype 2 infection in bottlenose dolphins (*Tursiops truncatus*) in the world.

Case 1 : On August 16 1999, a 20-year-old male bottlenose dolphin's behavior and appetite began to deteriorate. He had stopped eating fish on August 23 and the next day he began to float head first in an abnormal vertical pattern. On August 24, bacterial septicemia was suspected from the WBC value (Table 1). It has been suggested that elevations in AST, LDH and T-BIL values are associated with necrosis in the liver, and that elevations in FFA

Table 1 Blood data in two bottlenose dolphins

Test (Unit)	20-year-old		6-year-old		Asper et al., 1990 ^{*1}	Bossart and Dierauf 1990
	Aug. 16	Aug. 24	Aug. 31	Sep. 16		
WBC($\times 10^2/\mu\text{l}$)	90	19	128	49	56–73 ^{*2}	4.1–10.8
Bas(%)	0.0	0.0	0.0	1.0		0–3
Eos(%)	13.0	0.0	12.0	11.0	9–16	7–37
Sta(%)	2.0	1.0	3.0	1.0		0–5
Seg(%)	60.0	60.0	65.0	66.0	62–72 ^{*2,3}	49–77
Lym(%)	21.0	25.0	16.0	19.0	13–22 ^{*2}	15–28
Mon(%)	4.0	14.0	4.0	2.0	1–4	0–8
RBC($\times 10^4/\mu\text{l}$)	404	451	333	358	343–412 ^{*4}	2.9–5.4
FIB(mg/dl)	234	342	472	241		
TP(g/dl)	7.2	8.6	6.6	6.4	6.5–7.2	6.2–8.3
A/G	1.57	1.31	1.66	1.88		
AST(IU/l)	176	601	171	157	102–133 ^{*5}	48–250
LDH(IU/l)	870	1851	840	777	177–216 ^{*5}	159–1393
ALP(IU/l)	434	178	631	803	196–341 ^{*5}	123–1050
CK(IU/l)	62	93	44	62	47–74 ^{*5}	14–486
BUN(mg/dl)	50.2	66.6	54.2	54.3	44–56	31–73
CRE(mg/dl)	1.63	3.22	1.49	1.63	1.2–1.6	1.1–2.5
FFA(mEq/l)	0.60	2.37	0.61	0.53		
T-BIL(mg/dl)	0.27	1.16	0.26	0.18	0.19–0.31	0.0–0.1
Na(mEq/l)	154	147	152	156		141–168
Cl(mEq/l)	114	106	113	117	105–114	100–128
Fe($\mu\text{g/dl}$)	192	31	85	46	110–175 ^{*6}	110–175 ^{*6}

^{*1}Broad range(5–95%), ^{*2}Adults, ^{*3}Neutrophils, ^{*4}Intermediate hematological ecotype, ^{*5}Adults and unit (U/liter),

^{*6}Males.

and T-BIL are affected by his losing appetite [16]. The values of TP, CRE and Fe were also out of the range of the previous studies [17, 18]. He was treated by the administration of gentamicin sulfate (2 mg/kg, every 12 hr) and cefazolin (20 mg/kg, every 12 hr) through the dorsal fin intravenously. The animal died on August 25. A necropsy was performed 2 hr postmortem. The dolphin was 290.0 cm long and weighed 267.0 kg. The organs and gross lesions for histological examinations were fixed in 10% neutral buffered formalin, and sections were cut and stained with hematoxylin and eosin, and the Gram's method. In the macroscopical findings, the lungs were severely congested and edematous. A large quantity of slightly reddish, foamy and viscous fluid was seen in the trachea and bronchi. Histopathologic examination revealed hemorrhagic pneu-

monia, hemorrhagic necrosis in the adrenal cortex and multiple necrosis in the liver. Large numbers of small gram-positive bacilli were present within macrophages and in the blood vessels of the lung, liver and adrenals, and also in some other organs including the kidney with no other apparent abnormalities.

The blood was sampled on August 24, and 10 ml of the sample was placed in culture bottles (BACTEC PLUS Aerobic 96 F and Anaerobic 97 F, BBL). The bottles were incubated using the automatic blood culture system (BACTEC® 9240) for 48 hr, and then subcultured using a TAS II 5% sheep blood agar plate (BBL) aerobically and a Brucella agar plate (Kyokuto Seiyaku) anaerobically at 37°C for 24 hr. The isolates were identified by standard microbiological procedures [19]. The bacteria showed

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α -hemolytic colonies and were 1.5 to 2.0 mm in diameter. They were identified as *E. rhusiopathiae* on the basis of cellular morphology, a typical reaction in triple sugar iron agar, test-tube brush growth in gelatin semi-agar, positive reactions for glucose, lactose and arginine, and negative reactions for catalase, oxidase and urease. In addition, H₂S was produced. Serotyping of *E. rhusiopathiae* was carried out by the agar gel diffusion test [10] using 23 anti-*E. rhusiopathiae* rabbit sera. As a result of serotyping, this isolate was determined to be serotype 2. An antimicrobial susceptibility test was performed using disk diffusion methods. This isolate was susceptible to ampicillin, ciprofloxacin, imipenem and cephalothin, and resistant to vancomycin and gentamicin (Eiken KB disk). The antibody titer against *E. rhusiopathiae* serotype 2 in dolphins was determined by the agar gel diffusion test using the autoclave-extracted antigen of *E. rhusiopathiae* ATCC 19414-type strain. The antibody titer against serotype 2 in his serum at the necropsy was $\geq 1:32$.

In this case, acute *E. rhusiopathiae* serotype 2 septicemia was diagnosed from the results of clinical signs, histopathological, bacteriological and serological examinations.

Case 2 : A 6-year-old male dolphin had been slightly febrile (37.2°C) since August 28. However, he showed no other clinical signs. The dolphin was estimated to be 270.0 cm in length and weigh 240.0 kg. On August 29, cutaneous lesions characterized by grayish, raised and rhomboid shaped plaques that varied 5 to 10 cm in diameter, appeared on the abdominal and the dorsal regions (Fig. 1). From these clinical signs we suspected an erysipelas infection, and then treated him with an oral administration of sultamicillin tosilate (SBTPC; 15 mg/kg, BID) and doxycycline (DOXY; 8 mg/kg, BID). On August 31, the blood data showed marked leukocytosis, moderate erythrocytopenia, marked

Fig. 1 Plaques in the dorsal region

hyperfibrinogenemia and a decrease in Fe (Table 1). After antibiotic therapy, the younger dolphin gradually recovered. On September 16, the values of the WBC and FIB had decreased. The antibody titer against *E. rhusiopathiae* serotype 2 of his serum on October 1 was 1:16. Therefore, we also diagnosed this case to be an *E. rhusiopathiae* serotype 2 infection.

In swine and small cetaceans, rhomboid plaques of the skin develop as the disease progresses. Marked leukocytosis associated with left shift has also been observed [20]. In the younger dolphin, cutaneous lesions were seen and blood analysis showed marked leukocytosis. It has been reported that *E. rhusiopathiae* was isolated from blood sampled at 2 days prior to the onset of cutaneous plaques [7]. In the present study, a blood culture was not performed. No clinical signs were observed and the body temperature was within normal ranges at 2 days prior to the onset of cutaneous lesions.

In the older dolphin, specific clinical signs were not observed and the diagnosis was made postmortem as described previously [3, 6, 9]. This is similar to acute erysipelas in swine [1]. In the juvenile Pacific white-sided dolphin [9], gross examination revealed only moderately enlarged mesenteric lymph nodes. In this case, the lungs were severely con-

gested and edematous. The cause of disease in both cases was not determined by the results of clinical findings and gross examination. The small gram-positive bacilli presenting within macrophages were similar to those seen in other dolphins [3, 4, 9]. *E. rhusiopathiae* was isolated from the internal organs [4, 9]. However, we did not perform a bacterial isolation from them, and *E. rhusiopathiae* was isolated from blood.

Little information is known regarding the serotyping of *E. rhusiopathiae* in small cetaceans. In Enoshima Aquarium, the isolate from the endocarditis in the bottlenose dolphin was identified as serotype 6 [8, 15]. Serotypes 1 and 2 account for over 90% of all isolates from pigs, and serotype 2 has been isolated from samples of septicemia, arthritis, endocarditis and tonsils [12]. In our cases, serotype 2 was isolated from septicemia. Furthermore, antibody against serotype 2 in the sera from these two dolphins was detected. As a negative control, sera from one healthy adult female bottlenose dolphin and one healthy adult male Pacific white-sided dolphin were used. Specific antibody against serotype 2 was not detected in either, and was below 1:1. The sample from the female bottlenose dolphin was taken at Enoshima Aquarium in 1996 and the other sample from the Pacific white-sided dolphin was taken at a different aquarium. Further research will be necessary in order to determine specific serotypes for erysipelas in dolphins.

The epizootiologic features of erysipelas in dolphins have not been established. Much speculation exists as to the role of food fish in the transmission of the disease [6]. It has been reported that the disease is probably contracted through ingestion of contaminated fish [9]. Other possible sources of contamination include: trainer's hands, flies, wild pigeons and carrier states in dolphins [7]. *E. rhusiopathiae* had not been isolated from food fish, pool water or the samples from blowhole and feces

in the older dolphin over the last ten years (unpublished data). Free chlorine, maintained at levels from 0.3 up to 3.0 ppm at pH 7.6 and above, is safe and controls most bacteria [21]. In our pool, levels of free chlorine range from 0.05 to 2.0 ppm. From August to September in 1999, two Risso's dolphins (*Grampus griseus*), two false killer whales (*Pseudorca crassidens*), two Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) and another 16 bottlenose dolphins were kept in the same pool with these two dolphins. The antibody titer against *E. rhusiopathiae* serotype 2 in these dolphins was not measured and they were administered DOXY (6~8 mg/kg, BID) orally to prevent erysipelas. The original source of infection could not be determined.

In Enoshima Aquarium, two bottlenose dolphins showed severe leukopenia (less than 10×10^2 WBC / μ l) and died of septicemia within a week. On August 24 1999, antibiotic therapy was started in the older dolphin since septicemia was suspected from severely lowered WBC counts. From June 22 to July 6, the older dolphin was administered SBTPC (12 mg/kg, BID) orally due to blood data and his behavior. The prolongation of penicillin therapy for 14 days has been advised in cases of erysipelas [7]. In the present study, the younger dolphin recovered from subacute erysipelas by administering SBTPC and DOXY for 13 days. The dosage of DOXY was less than half of that given in the previous study [22] in which two different effective antibiotic drugs for erysipelas were used. We have only applied antibiotic therapy for the treatment of dolphins with erysipelas. It has been reported that the commercial bacterin of *E. rhusiopathiae* for pigs is effective for the prevention of erysipelas in captive dolphins [6], and currently an *E. rhusiopathiae* vaccine, and the survey of the antibody titers and the serotyping are being studied [23].

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要 約

1999年8月、共に飼育下生まれのバンドウイルカ雄、20歳と6歳が豚丹毒菌に感染した。前者は死亡し、血液から豚丹毒菌血清型2型が分離された。後者は、皮膚に菱形疹を認めたものの、トシル酸スルタミシリン(15 mg/kg, 2回/日)と塩酸ドキシサイクリン(8 mg/kg, 2回/日)との投与により治癒した。両者の血清中には、豚丹毒菌血清型2型に対する抗体が認められた。

キーワード：バンドウイルカ、豚丹毒菌血清型2型、敗血症

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