Prevalence of *Erysipelothrix* Species in Healthy, Slaughtered Cattle

Raafat HASSANEIN¹, Takuo SAWADA¹, Yasushi KATAOKA¹, Mohamed ALI¹,
Kiyomi ITOH² and Yoshikazu SUZUKI²

¹Nippon Veterinary and Animal Science University, Department of Veterinary
Microbiology, Musashino, Tokyo 180-8602, Japan

²Nairiku Meat Inspection Office, Prefecture of Yamagata, 990-0892, Japan

(Received 15 October 2000 / Accepted 10 December 2000)

Summary

Erysipelothrix species were isolated from the tonsils of 79 (6.4%) of 1,236 healthy cattle slaughtered in 4 prefectures, Yamagata, Miyagi, Nagasaki, and Tokyo, in Japan from September, 1998, to August, 1999.

A combination, modified culture medium consisted of brain heart infusion (BHI) broth containing 0.1% Tween 80, 5% horse serum, 50 µg/ml gentamicin, 0.1% sodium azide and 0.001% crystal violet, and BHI agar containing 0.1% Tween 80, 50 µg/ml gentamicin, and 0.1% sodium azide was compared with two traditional media for isolation of *Erysipelothrix* species. The modified media were the most effective since they produced the highest number of *Erysipelothrix* isolates ($p < 0.01$) as well as the greatest degree of inhibition of the growth of contaminated organisms.

The isolation rates in summer (10.3%) and spring (7.1%) was higher than those in autumn (2.6%) or winter (1.3%) ($p < 0.01$).

INTRODUCTION

Erysipelothrix infection has a major economic importance, causing a variety of disease conditions in swine, turkeys, and sheep, and infrequently in dogs, horses, and humans¹⁹. The clinical disease seldom occurs in cattle. However, some disease conditions, especially in young calves, have been recorded. These cases include septicemia in calves showing multiple hepatic and pulmonary abscesses¹², arthritis^{3,8}, encephalomeningitis¹⁶, and polyserositis⁷. Moreover, the organism has been isolated from the tonsils of adult healthy cattle and from endocardial lesions at post-mortem examination^{1,9}, as well as from cattle slurry¹⁰. However the epidemiological significance of *Erysipelothrix* infection in cattle remains unknown.

Our studies of antibody detection using the growth-

agglutination test indicated that Japanese cattle might have been infected with *Erysipelothrix*. Most of the sera collected from 804 cattle in 9 prefectures (ranging from Hokkaido to Okinawa) of Japan between 1988 and 1992 were positive and the antibody titers ranged from below 4 to above 128. Seventy-seven percent of the sera showed titers of 32 or above (T. Sawada, R. Hassanein, T. Yamamoto, and T. Yoshida, submitted for publication).

Erysipelothrix is easily isolated from the blood of infected animals with septicemia or other acute forms of erysipelas, but a small number of bacteria with low viability may occur in chronic cases, so enrichment cultures are necessary to isolate the microorganisms from heavily contaminated materials such as arthritic joints, tonsils, or feces¹⁹. Numerous techniques for isolation of *Erysipelothrix* species have been attempted using different culture media with various inhibitory substances for organisms other than *Erysipelothrix*^{13,14,18}. Direct inoculation of mice can also be used⁵.

The objectives of this study were to investigate the

Corresponding author: Nippon Veterinary and Animal Science University, Department of Veterinary Microbiology, Musashino, Tokyo 180-8602, Japan
Tel : 0422-31-4151 Fax : 0422-31-4560
e-mail : t-sawada@mub. biglobe.ne.jp.

epidemiological significance of *Erysipelothrix* infection in cattle as well as the prevalence of *Erysipelothrix* species with characterization of the isolates, and to evaluate modified culture media in comparison with other traditional culture media for isolation of *Erysipelothrix*.

MATERIALS AND METHODS

Bacterial strains: Type strains ATCC 19414 (serovar 2) of *Erysipelothrix rhusiopathiae* and ATCC 43339 (serovar 7) of *Erysipelothrix tonsillarum* were used as reference strains of *Erysipelothrix* species. Representative strains of several species isolated frequently in the present study for the culture of *Erysipelothrix* species were used as associated microorganisms on evaluation of selective broth and agar media. These were *Proteus* spp., *Lactobacillus* spp., *Streptococcus pneumoniae*, and *Candida albicans*.

Specimens: Tonsils were obtained from 1,236 healthy cattle showing no clinical symptoms at slaughterhouses in Yamagata, Miyagi, Tokyo, and Nagasaki prefectures of Japan between September, 1998, and August, 1999. Tonsils from the cattle were placed in a sterile specimen bag, and transported at frozen status to our laboratory in Tokyo. All the samples were subjected to bacteriological examination.

Culture media: Medium I was prepared as described by Packer¹¹⁾, containing the following: Brain heart infusion (BHI) broth (pH 7.6, Difco) or BHI agar (pH 7.6, Difco) containing 0.1% Tween 80,

0.1% sodium azide (NaN_3) and 0.001% crystal violet (CV). Medium II was prepared as described by Takahashi et al.¹⁴⁾, containing the following: BHI broth (pH 7.6, Difco) or BHI agar (pH 7.6, Difco) containing 0.1% Tween 80, 50 $\mu\text{g}/\text{ml}$ gentamicin (GM) and 500 $\mu\text{g}/\text{ml}$ kanamycin (KM).

Culture procedures: Approximately 1 g of tonsil tissue was chopped into pieces and homogenized after addition of 1 ml sterilized physiological saline. Each homogenate (0.1 ml) was inoculated into 10 ml of two broth media. The inoculated broth was incubated at 37°C for 24 h, and then a loopful of each broth culture was streaked onto the agar plates of the same type. The agar plates were incubated at 37°C for 48 h and then examined for the presence of typical colonies of *Erysipelothrix* species^{11,14)}. The isolation rate of *Erysipelothrix* species from both media was statistically analyzed using Student's t-test employing 6 replicates.

Evaluation of selective broth and agar media for cultivation of *Erysipelothrix* species and inhibition of associated organisms: Four types of BHI broth (Table 2) containing 0.1% Tween 80 and: A) 50 $\mu\text{g}/\text{ml}$ GM, 500 $\mu\text{g}/\text{ml}$ KM, and 0.0015% CV; B) 50 $\mu\text{g}/\text{ml}$ GM, 0.001% CV, 0.1% NaN_3 , and 5% horse serum (HS); C) 50 $\mu\text{g}/\text{ml}$ GM, 500 $\mu\text{g}/\text{ml}$ KM, and 0.15% NaN_3 ; and D) 50 $\mu\text{g}/\text{ml}$ GM and 0.1% NaN_3 were used for enrichment culture. Five types of BHI agar (Table 2) containing 0.1% Tween 80 and: a) 50 $\mu\text{g}/\text{ml}$ GM, 500 $\mu\text{g}/\text{ml}$ KM, and 0.0015% CV; b) 50 $\mu\text{g}/\text{ml}$ GM, 0.001% CV, 0.1% NaN_3 , and 5% HS; c) 50 $\mu\text{g}/\text{ml}$ GM,

Table 1. Comparison between two traditional culture media for isolation of *Erysipelothrix* species and growth inhibition of associated organisms

Culture media	<i>Erysipelothrix</i> species	No. of isolates / No. of samples (% $\pm$ SE)			
		Associated organisms			
		<i>Proteus</i> species	<i>Lactobacillus</i> species	<i>Streptococcus pneumoniae</i>	<i>Candida albicans</i>
Medium I	5/300 (1.7 $\pm$ 0.6)	7/300 (2.3)	0/300 (0)	12/300 (4)	6/300 (2)
Medium II	16/300 (5.3 $\pm$ 1.2) ^a	84/300 (28)	18/300 (6)	0/300 (0)	39/300 (13)

Medium I: BHI broth and agar containing Tween 80, sodium azide, and crystal violet.

Medium II: BHI broth and agar containing Tween 80, gentamicin and kanamycin.

^a Statistically significant ($p < 0.05$).

Table 2. Evaluation of various combinations of different media for cultivation of *Erysipelothrix* strains and growth inhibition of associated organisms

Organisms	Broth and agar culture media ^a														
	BHI, GM, KM, and CV					BHI, HS, GM, CV, and NaN ₃					BHI, GM, KM, and NaN ₃				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
<i>E. rhusiopathiae</i> ATCC 19414	- ^b	-	-	-	+ ^c	-	-	-	+	+	-	-	+	-	+
<i>E. tonsillarum</i> ATCC 43339	-	-	-	-	+	+	-	-	+	+	+	+	-	+	+
<i>E. rhusiopathiae</i> (Isolate 1) ^d	-	-	-	-	+	+	-	-	+	+	-	-	+	+	+
<i>E. rhusiopathiae</i> (Isolate 2) ^e	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>Proteus</i> spp.	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+
<i>Lactobacillus</i> spp.	-	-	+	-	+	-	-	+	-	+	-	-	+	-	+
<i>Streptococcus pneumoniae</i>	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
<i>Candida albicans</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+

^a BHI: brain heart infusion, GM: gentamicin, KM: kanamycin, CV: crystal violet, HS: horse serum, and NaN₃: sodium azide

A: BHI agar, GM, KM, and CV

B: BHI agar, horse serum, GM, NaN₃, and CVC: BHI agar, GM, KM, and NaN₃D: BHI agar, GM, and NaN₃

E: BHI agar

^b No bacterial growth^c Bacterial growth^d Field isolate (Miyagi), *Erysipelothrix* spp.^e Field isolate (Nagasaki), *Erysipelothrix* spp.

Table 3. Comparison between modified and traditional media for isolation of *Erysipelothrix* species and growth inhibition of associated organisms

Culture media	<i>Erysipelothrix</i> species	No. of isolates / No. of samples (%±SE)			
		Associated organisms			
		<i>Proteus</i> species	<i>Lactobacillus</i> species	<i>Streptococcus pneumoniae</i>	<i>Candida albicans</i>
Medium II	9/300 (3±0.9)	84/300 (28)	18/300 (6)	0/300 (0)	39/300 (13)
Medium III	36/300 (12±2.5) ^a	0/300 (0)	0/300 (0)	0/300 (0)	0/300 (0)

Medium II : BHI broth and agar containing Tween 80, Gentamycin(GM) and Kanamycin.

Medium III: BHI broth containing Tween 80, horse serum, GM, sodium azide, crystal violet and BHI agar containing Tween 80, GM, and sodium azide

^a Statistically significant ($p<0.01$).

500µg/ml KM, and 0.15% NaN₃; d) 50µg/ml GM and 0.1% NaN₃; e) BHI agar containing 0.1% Tween 80 were used for isolation culture.

Comparison of the modified and traditional medium: The medium (II), described by Takahashi et al.¹⁴⁾ was compared with the modified medium (III) for isolation of *Erysipelothrix* species from 300 tonsils collected from healthy slaughtered cattle (Table 3). The isolation rate of *Erysipelothrix* species from both media was statistically analyzed using Student's t-test employing 6 replicates.

Morphological and biochemical identification of isolates: Isolates were identified as *Erysipelothrix* species on the basis of cellular morphology, biochemical and biological properties during growth in triple sugar iron agar slants and gelatin medium, and the reactions for catalase, oxidase, and urease tests. Carbohydrate fermentation tests were carried out using 20 sugars as listed in Table 4 in a fermentation base with Andrade's indicator and supplemented with 10% HS according to the methods described by Wood¹⁹⁾. Other associated organisms were identified morphologically and biochemically according to the methods described by Holt et al.⁶⁾

RESULTS

Bacterial isolation: Out of 300 samples obtained from the tonsils of healthy, slaughtered cattle in different prefectures in Japan, 5 (1.7%) isolates of *Erysipelothrix* species were recovered and identified

using medium I, while 16 (5.3%) were recovered and identified using medium II. Commonly associated microorganisms were isolated and identified as *Proteus* spp., *Lactobacillus* spp., *Streptococcus pneumoniae*, and *Candida albicans* (Table 1).

Based on trials for the evaluation of selective broth and agar media for the cultivation of *Erysipelothrix* species and inhibition of associated organisms, the most efficient broth medium that allowed the rapid and heavy growth of all reference strains and field isolates of *Erysipelothrix* species as well as the inhibition of all associated organisms was BHI broth containing 0.1% Tween 80, 5% HS, 50µg/ml GM, 0.001% CV, and 0.1% NaN₃. Concerning the agar media, the best type was BHI agar containing 0.1% Tween 80, 50µg/ml GM, and 0.1% NaN₃ (Table 2).

Of the other 300 tonsil samples used in comparison between modified medium (III) and traditional medium (II), nine (3%) isolates of *Erysipelothrix* species were recovered by the traditional medium (II) while 36 (12%) by the modified medium (III) (Table 3).

Finally, out of 1,236 samples obtained from healthy, slaughtered cattle in different prefectures in Japan, 79 (6.4%) isolates of *Erysipelothrix* species were recovered and identified. The isolation rate during either the summer (10.3%) or spring (7.1%) season was higher than during the autumn (2.6%) or winter (1.3%) season (Table 5) (Analysis of variance test, $n=1,236$, 4 replicates, $p<0.01$).

Table 4. Biochemical characteristics of 79 *Erysipelothrix* isolates

Characteristics	Result		
	79 isolates	ATCC 19414 ^a	ATCC 43339 ^b
Motility	—	—	—
Haemolysis	Non-haemolytic	Non-haemolytic	Non-haemolytic
H ₂ S production on triple sugar iron agar	+	+	+
"Test-tube brush" growth on gelatin	+	+	+
Catalase	—	—	—
Urease	—	—	—
Oxidase	—	—	—
Acid from:			
Glucose	+	+	+
Lactose	+	+	+
Galactose	+	+	+
Dextrin	+	+	+
Maltose	+	+	+
Fructose	+	+	+
Saccharose	V ^c (+ ^e 28) ^d	+ ^e	+
Levulose	+ ^e	+	+
Mannose	V(+31)	+ ^e	—
Salicin	V(+35)	—	—
Trehalose	V(+35)	—	—
Mannitol	—	—	—
Sorbitol	—	—	—
Rhamnose	—	—	—
Dulcitol	—	—	—
Inositol	—	—	—
Arabinose	—	—	—
Raffinose	—	—	—
Inuline	—	—	—
Adonitol	—	—	—

^a The type strain of *E. rhusiopathiae*.^b The type strain of *E. tonsillarum*.^c Variable.^d Number between parenthesis is the number of positive isolates / total number of isolates (79).^e Acid was produced slowly (5 to 10 days).

The prevalence of *Erysipelothrix* species in 4- to 6-year-old cattle was higher than in 2- to 3-year-old cattle (Table 6). The Japanese black cattle showed a higher rate of isolation (6.8%) than did other breeds (Table 7).

Morphological and biochemical identification: All the isolates had identical morphology and biochemical properties of genus *Erysipelothrix*. The organisms from smooth colonies were rod-shaped, while those from rough colonies formed into long, non-branching filaments. As shown in Table 4, all the isolates showed

typical reactions of *Erysipelothrix* in triple sugar iron agar slants, "test-tube brush" growth in gelatin medium, and negative production of catalase, oxidase and urease. The carbohydrate fermentation patterns using 20 sugars were variable among isolates. All isolates produced acid from glucose, dextrin, galactose, fructose, maltose, lactose, and levulose, but not from mannitol, sorbitol, rhamnose, dulcitol, inositol, arabinose, raffinose, inuline, and adonitol. Variable reactions were recorded for the fermentation of mannose, salicin, saccharose, and trehalose.

Table 5. Isolation of *Erysipelothrix* species from tonsils of apparently healthy, slaughtered cattle in Japan

Sampling period	No. of samples examined	No. (%±SE) of isolates
Autumn (Sept.~ Nov.)	229	6 (2.6±0.3)
Winter (Dec.~ Feb.)	232	3 (1.3±0.5)
Spring (Mar.~ May)	309	22 (7.1±0.5) ^a
Summer (Jun.~ Aug.)	466	48 (10.3±0.04) ^a
Total	1,236	79 (6.4)

^a Statistically significant ($p < 0.01$) against Autumn and Winter.

Table 6. Prevalence of *Erysipelothrix* species in different ages of apparently healthy, slaughtered cattle in Japan

Age (yr)	No. of examined samples	No. of positive samples (%±SE)
2	24	0 (0±0)
3	50	1 (2.0±0.9)
4	835	55 (6.6±1.8)
5	289	21 (7.3±1.3)
6	38	2 (5.3±3.1)
Total	1,236	79 (6.4)

Table 7. Prevalence of *Erysipelothrix* species among various breeds in Japan

Breed	No. of examined samples	No. of positive samples (%±SE)
Japanese black	1037	71 (6.8±2.2)
Japanese brown	8	1 (12.5±3.1)
Holstein	115	2 (1.7±1.7)
Frisian	76	5 (6.6±2)
Total	1,236	79 (6.4)

DISCUSSION

Until now, clinical cases of erysipelas in cattle have not been recorded in Japan in spite of one investigation in 1959 in which *E. rhusiopathiae* was isolated from the tonsils of healthy cattle⁹⁾. The present investigation illustrated that *Erysipelothrix* species could be isolated from the tonsils of 79 (6.4%) out of 1,236 healthy, slaughtered cattle, with the highest rate of isolation during summer and spring seasons. Similarly, a tendency was reportedly shown in which the isolation rate of *Erysipelothrix* from pigs was higher during spring and summer than during autumn and

winter^{2,14)}. In addition, adult cattle showed a higher incidence of *Erysipelothrix* than did younger cattle in the present study.

These results proved that healthy cattle act as carriers for the organism and are considered to be a potential source for infection to other animals and humans, supporting the incidence of antibodies in healthy cattle demonstrated in our previous study (T. Sawada, R. Hassanein, T. Yamamoto, and T. Yoshida, submitted for publication). Moreover, the exposure of carrier cattle to stress factors or reduction in local resistance may result in the spread of the organisms into the regional lymphatic system and

blood leading to potentially fatal septicemia¹²⁾.

The morphological and biochemical properties of *Erysipelothrix* isolates were identical to those of the type strains of *Erysipelothrix* species, and correlated with those reported^{14,19)}.

Carbohydrate fermentation patterns of *Erysipelothrix* species varied according to medium, indicator, and method of measuring acid production. Andrad's base with 10% serum was the most dependable medium because it allowed the least variations in patterns¹⁷⁾. *Erysipelothrix* sometimes produced a slight amount of acid from saccharose^{15,19)}. Our present result on saccharose utilization of *Erysipelothrix* isolates, strains ATCC 19414 and ATCC 43339, was the same as that described previously^{15,17,19)}, while it having been reported that the isolates of *E. tonsillarum* are positive and those of *E. rhusiopathiae* are negative for saccharose¹³⁾. We consider that it must be positive even if the reaction is weak or slow. Further studies are needed to establish the standard for carbohydrate fermentation by *Erysipelothrix* species.

Because the tonsils may harbor many associated organisms accompanying *Erysipelothrix* species, it is difficult to isolate *Erysipelothrix* in a pure culture. This necessitates the use of culture media containing inhibitory substances for organisms other than *Erysipelothrix*. In our present study, modified media were evaluated and compared with two traditional media^{11,14)}. The combination of BHI broth containing Tween 80, HS, GM, NaN₃, and CV and BHI agar containing the same constituents except CV and HS was the best for providing a highest rate of isolation of *Erysipelothrix* as well as inhibition of the growth of other organisms. In contrast, the selective media, which were modified by Takahashi et al.¹⁴⁾, containing GM and KM allowed the growth of *Proteus* species, *Lactobacillus* species and *Candida albicans* at rates of 28%, 6%, and 13%, respectively, from examined samples, while Packer's media allowed the growth of *Proteus* species, *Streptococcus pneumoniae*, and *Candida albicans* at rates of 2.3%, 4%, and 2%, respectively. Further, associated organisms should be attended because these organisms may complicate or may be complicated with *Erysipelothrix* organisms especially when the animal is exposed to stress factors. In a previous study, Kluge and Perl⁷⁾ isolated *E.*

rhusiopathiae and *Streptococcus* species from cases of septicemia, polyserositis, and encephalitis, confirming the role of the combination of both organisms in developing these cases. Additionally, *Candida* species occurs as a normal inhabitant of the digestive tract, oral cavity, and vagina of cattle, and with stress factors or in combination with *Erysipelothrix* may cause a disease condition⁴⁾.

It could be concluded that 6.4% of healthy cattle in Japan harbor the *Erysipelothrix* organisms and may act as a potential source of infection for other animal species or humans. The addition of GM, NaN₃, and CV in combination with the culture media is effective in inhibiting of organisms other than *Erysipelothrix*. Lastly, associated organisms should be regarded as complicating pathogens in order to avoid diagnostic confusion in such cases.

ACKNOWLEDGMENTS

We wish to thank Dr. M. Katsumi of The Sendai Meat Inspection Center, City of Sendai, Dr. T. Fukuda of The Kenpoku Livestock Hygiene Service Station, Prefecture of Nagasaki, and Drs. T. Suzuki and H. Takagi of The Hachioji Branch Station, Shibaura Meat Inspectors, Station, Tokyo Metropolitan Government, for kindly supplying the tonsil samples.

REFERENCES

- 1) Arsov, R.: Carriers of the swine erysipelas organism among healthy cattle, sheep, and fowls. Nauchni. Trud. Vissh. Vet. Med. Inst. Prof. G. Pavlov 12, 361-366, 1964.
- 2) Connell, R. and Langford, E. V.: Studies of swine erysipelas. V. Presence of *Erysipelothrix rhusiopathiae* in apparently healthy pigs. Can. J. Comp. Med. 17, 448-453, 1953.
- 3) Dreyfuss, D. J. and Stephens, P. R.: *Erysipelothrix rhusiopathiae*-induced septic arthritis in a calf. J. Am. Vet. Med. Assoc. 197, 1361-1362, 1990.
- 4) Foley, G. L. and Schlafer, D. H.: Candida abortion in cattle. Vet. Pathol. 24, 532-536, 1987.
- 5) Geissler, S.: Untersuchungen über das Vorkommen von Rotlaufbakterien in den Tonsillen gesunder Schlachtschweine. Zentralbl. Bakteriologie (Origin A), 159, 335-338, 1953.
- 6) Holt, J. G. et al.: Bergey's manual of determina-

- tive bacteriology, 9th ed. The Williams & Wilkins Co., Baltimore, Md. 1994.
- 7) Kluge, J. P. and Perl, S.: *Erysipelothrix rhusiopathiae* septicemia-polyserositis and streptococcal encephalitis in a calf. J. Vet. Diagn. Invest. 4, 196-197, 1992.
- 8) Moulton, J. E., Rhode, E. R. and Wheat, D. J.: Erysipelas arthritis in calves. J. Am. Vet. Med. Assoc. 123, 335-340, 1953.
- 9) Murase, N., Suzuki, K. and Nakahara, T.: Studies on the typing of *Erysipelothrix rhusiopathiae* II. Serological behaviours of the strains isolated from fowls including those from cattle and humans. Jpn. J. Vet. Sci. 21, 177-181, 1959.
- 10) Nørrung, V., Munch, B. and Errebo Larsen, H.: Occurrence, isolation and serotyping of *Erysipelothrix rhusiopathiae* in cattle and pig slurry. Acta. Vet. Scand. 28, 9-14, 1987.
- 11) Packer, R. A.: The use of sodium azide (NaN_3) and crystal violet in a selective medium for streptococci and *Erysipelothrix rhusiopathiae*. J. Bacteriol. 46, 343-349, 1943.
- 12) Rebhun, W. C.: *Erysipelothrix insidiosa* septicemia in neonatal calves. Vet. Med. Small Anim. Clin. 71, 684-686, 1976.
- 13) Takahashi, T. et al.: DNA relatedness among *Erysipelothrix rhusiopathiae* strains representing all twenty-three serovars and *Erysipelothrix tonsillarum*. Int. J. Syst. Bacteriol. 42, 469-473, 1992.
- 14) Takahashi, T. et al.: Serotype, antimicrobial susceptibility, and pathogenicity of *Erysipelothrix rhusiopathiae* isolates from tonsils of apparently healthy slaughter pigs. J. Clin. Microbiol. 25, 536-539, 1987.
- 15) Watarai, M. et al.: Comparison of etiological and immunological characteristics of two attenuated *Erysipelothrix rhusiopathiae* strains of serotypes 1 a and 2. J. Vet. Med. Sci. 55, 595-600, 1993.
- 16) Whaley, A. E., Robinson, V. B., Newberre, J. W. and Sipple, W. L.: Bovine encephalomyelitis. Vet. Med. 53, 475-476, 1958.
- 17) White, T. G. and Shuman, R. D.: Fermentation reactions of *Erysipelothrix rhusiopathiae*. J. Bacteriol. 82, 595-599, 1961.
- 18) Wood, R. L.: A selective liquid medium utilizing antibiotics for isolation of *Erysipelothrix insidiosa*. Am. J. Vet. Res. 26, 1303-1308, 1965.
- 19) Wood, R. L.: Swine erysipelas. In: Leman, A. D., Straw, B. E., Mengeling, W. L., Allarie, S. D. and Taylor, D. J., ed., Diseases of swine, 475-486, Iowa State University Press, Ames, IA, 1992.

原 著

和文要約

健康牛における *Erysipelothrix* 属菌の保菌状況

Raafat HASSANEIN¹, 澤田拓士¹, 片岡 康¹, Mohamed ALI¹, 伊藤喜代美², 鈴木吉一²(¹日本獣医畜産大学獣医微生物学教室, ²山形県内陸食肉衛生検査所)

牛における豚丹毒菌 (*Erysipelothrix* 属菌) の保菌状況を明らかにする目的で, 1998年9月から1999年8月にかけて山形, 宮城, 長崎および東京の4都県のと畜場で採取した合計1,236頭の健康牛の扁桃から本菌の分離を試みたところ, 合計79株 (6.4%) の豚丹毒菌が分離された。培地として, これまで伝統的に用いられてきた50 μ g/ml ゲンタマイシン (GM), 500 μ g/ml カナマイシン (KM) あるいは0.001%クリスタル紫 (CV) と NaN₃を選択剤として加えた0.1% Tween80加ブレインハートインフュージョン (BHI) 培地で増菌および分離培養を行ったところ, GM・KM 加 BHI 培地で最高の分離率が得

られた。また, 分離株および基準株を用いて添加剤の比較検討を行ったところ, 0.1% Tween80, 5%馬血清, 50 μ g/ml GM, 0.001% CV, 0.1% NaN₃カロブロスによる増菌培養と, 0.1% Tween80, 50 μ g/ml GM, 0.1% NaN₃加 BHI 寒天培地による分離培養の組合せが豚丹毒菌の増殖性と雑菌の増殖阻止の程度から最良である ($p < 0.01$) ことが判明した。分離株はすべてが H₂S を産生し, ゼラチン培地で試験管ブラシ状発育を示した。以上より, 健康牛の扁桃における豚丹毒菌保菌状況と, 菌分離により適した培地が明らかとなった。