Isolation of *Fusobacterium necrophorum* from Bovine Ruminal Lesions

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Abstract. A survey was conducted on the occurrences of rumen parakeratosis, rumenitis, traumatic reticulitis, and reticular and hepatic abscesses among 492 slaughtered cattle in Yamaguchi and Fukuoka Prefectures, Japan. These diseases occurred to 59 animals (12.0%), 83 (16.9%), 26 (5.3%) and 25 (5.1%), respectively. Hepatic abscesses were detected from 18 (12.0%) of 149 animals with rumen parakeratosis and/or rumenitis, 3 (11.5%) of 26 with traumatic reticulitis, 1 (12.5%) of 8 with reticular abscesses, and 3 (1.0%) of 309 with no disorders. *Fusobacterium necrophorum* was detected from 9 of 20 cases of rumen parakeratosis, 12 of 20 cases of rumenitis, 1 of 10 cases of unaffected ruminal tissue, and all of the cases of reticular and hepatic abscesses. The viable count of *F. necrophorum* was 10^4 to 10^6 per gram in the cases of rumen parakeratosis, 10^3 to 10^7 in those of rumenitis, and 10^5 in that of unaffected ruminal tissue.

Relatively frequent occurrence of hepatic abscess has hitherto been described in barley beef and feedlot beef cattle by several workers [4, 6, 10]. In Japan, a similar finding has recently been obtained from fattened dairy steers [1, 12, 15]. In these animals, attention was paid to a correlation between the incidence of hepatic abscess and that of ruminal lesions, such as rumen parakeratosis and rumenitis. Consequently, the terms rumenitis-liver abscess complex [4] and rumen parakeratosis-rumenitis-liver abscess complex [15] were proposed. Those previous reports, however, gave few detailed bacteriological observations, especially on *Fusobacterium necrophorum*.

In their previous papers, the authors reported the bacteriology of bovine hepatic abscess and the distribution of *F. necrophorum* in the alimentary tract of cattle [7,

8]. The present paper deals with a survey on the incidence of bovine ruminal lesions and the isolation of *F. necrophorum*, which is considered as one of the most important agents of hepatic abscess formation.

Materials and Methods

Reticulo-ruminal lesions

A total of 492 cattle slaughtered at the Ube Abattoir in Ube-shi, Yamaguchi Prefecture, and the Yahata Meat Center in Kitakyushu-shi, Fukuoka Prefecture, Japan, over a period from September, 1973 to July, 1974 were examined. By referring to the literature [5, 15], the reticulo-ruminal lesions grossly observed in them were recorded as follows.

Rumen parakeratosis: Lesions were grossly characterized by spotted adhesion of feed and hairs to the mucosal epithelium of the rumen, and clumping, enlargement and black pigmentation of villi.

Rumenitis: Foci of lesions grossly exhibited necrosis, swelling, edema and hemorrhage in the mucosa. Some areas of the mucosa manifested devillation, often depigmentation, ulcers, nodules of

granulation tissue, and scars.

Traumatic reticulitis and reticular abscess: At slaughter, foreign bodies, such as ingested metal fragments and similar rigid materials, were grossly found in the lumen of the reticulum and in reticular abscesses. For convenience sake, lesions without these bodies were regarded as reticular abscesses.

Isolation and enumeration of *F. necrophorum*

Twenty samples of rumen parakeratosis, 20 of rumenitis, 25 of hepatic abscesses, and 8 reticular abscesses were collected in a chilled container for laboratory examination. A total of 101 samples of rumen fluids were obtained from animals with these lesions and 10 samples from those with apparently unaffected ruminal mucous membrane. All the samples were brought to the laboratory in the same manner.

Homogenized tissues with and without lesions, rumen fluids, and pura were used for the isolation and enumeration of *F. necrophorum*. Instead of FM medium (Nissui Seiyaku Co., Ltd., Tokyo), 5% rabbit blood FM medium (BFM) was routinely employed. The cultivation, identification, enumeration and differentiation of the isolates were made by referring to previous reports [2, 3, 7, 8, 10].

Results

Incidence of bovine reticulo-ruminal lesions

Of the 492 cattle examined, 149 (30.3%) were affected with varying degrees of rumen parakeratosis and/or rumenitis (Figs. 1 and 2). As shown in Table 1, the former disease was detected from 59 (12.0%) animals and the latter from 83 (16.9%). Both diseases were observed in 7 (1.4%) animals. Reticular abscess was found in 8 (1.6%) ani-

mals and traumatic reticulitis in 26 (5.3%). The breeds and sexes of these animals are listed in Table 2. In Holstein steers, reticulo-ruminal lesions were detected from 74 (54.0%) of 137 animals of varying age. Table 3 indicates the occurrence of hepatic abscess. This abscess was found in 18 (12.0%) of 149 animals with ruminal lesions, 3 (11.5%) of 26 with traumatic reticulitis, 1 (12.5%) of 8 with reticular abscess, and 3 (1.0%) of 309 with no diseases. Isolation and colony count of *F. necrophorum*

Mucous membrane: As shown in Table 4, *F. necrophorum* was found in 9 of 20 specimens of rumen parakeratosis, 12 of 20 specimens of rumenitis, and 1 of 10 unaffected tissue specimens. The colony count of the organism was 10^4 to 10^6 , 10^3 to 10^7 and 10^5 per gram, respectively.

Rumen fluid: *F. necrophorum* was detected from 59 of 101 specimens affected with rumen parakeratosis and/or rumenitis. The bacterial count ranged from 10^2 to 10^6 .

Abscess: The organism was detected from all the specimens of hepatic and reticular abscesses. The viable bacterial count of *F. necrophorum* ranged from 10^4 to 10^8 per gram in 22 specimens of hepatic abscess. It was not examined in the remaining specimens.

Table 1. Occurrence of bovine reticulo-ruminal lesions

Lesion \ Abattoir	Ube	Yahata	Total (%)
Rumen parakeratosis	21*	38	59 (12.0%)
Rumenitis	57	26	83 (16.9%)
Rumen parakeratosis and rumenitis	2	5	7 (1.4%)
Traumatic reticulitis	11	15	26 (5.3%)
Reticular abscess	6	2	8 (1.6%)
Unaffected mucous membrane	157	152	309 (62.8%)
Total	254	238	492 (100%)

Remarks.

* : No. of animals.

Table 2. Breed and sex of cattle with reticulo-ruminal lesions

Breed	Sex	No. of animals inspected	No. of animals with lesions
Holstein	Male	25	6
	Female	88	26
	Steer	137	74
Japanese Black	Male	2	0
	Female	23	9
	Steer	106	29
Japanese Brown	Male	7	1
	Female	16	7
	Steer	88	31
Total		492	183 (37.2%)

Table 3. Relationship between hepatic abscess and reticulo-ruminal lesions

	Hepatic abscess		Total
	+	-	
Rumen parakeratosis	13*	46	59
Rumenitis	3	80	83
Rumen parakeratosis and rumenitis	2	5	7
Traumatic reticulitis	3	23	26
Reticular abscess	1	7	8
Unaffected mucous membrane	3	306	309
Total	25	467	492

Remarks.

*: No. of animals.

Table 4. Isolation and colony count of *F. necrophorum*

Sample	Isolation rate	Colony count (log ₁₀)							
		2	3	4	5	6	7	8	
Mucous membrane	Rumen parakeratosis	9/20*	0	0	5	3	1	0	0
	Rumenitis	12/20	0	2	2	2	5	1	0
	Unaffected	1/10	0	0	0	1	0	0	0
Rumen fluid	Rumen parakeratosis	20/32	0	3	10	6	1	0	0
	Rumenitis	37/64	1	9	14	11	2	0	0
	Rumen parakeratosis and rumenitis	2/5	0	0	0	1	1	0	0
Hepatic abscess		22/22	0	0	2	3	7	8	2
Reticular abscess		8/8	.*	.*	.*	.*	.*	.*	.*

Remarks.

*: No. of positive samples/No. of samples examined.

**.: Not tested.

Distribution of phases of *F. necrophorum*

Based on biological properties, the isolates were differentiated into two phases, A and B. The organism belonging to phase A was detected from 19 specimens of ruminal mucous membrane and fluid, 22 specimens of hepatic abscess, and 3 specimens of reticular lesion, and that belonging to phase B from 64, 3 and 5 specimens, respectively.

Discussion

Relatively high incidences of rumen parakeratosis and rumenitis in beef cattle and steers have been reported in U.S.A. and the northern district of Japan in the recent years. In the present survey, this finding was observed in Holstein steers. In order to clarify the predisposing factors of these diseases, several workers investigated the feeds and fattening process of these animals in detail [1, 5, 15]. No attempts succeeded in elucidating any definite cause for this finding.

Hepatic abscess occurred quite frequently to cattle with ruminal lesions and traumatic reticulitis. Jensen [4] reported a significant correlation between bovine rumenitis and hepatic abscess, and proposed the term "rumenitis-liver abscess complex". Later, Tamate [15] used the term "rumen parakeratosis-rumenitis-liver abscess complex". On the other hand, it is generally considered that both traumatic reticulitis and reticular abscess are predisposing conditions for hepatic abscess formation. It seems necessary to pay attention to the incidence of these lesions together with those of rumen parakeratosis and rumenitis. It is a future subject of study to elucidate the correlation between hepatic abscess and these reticulo-ruminal lesions.

F. necrophorum was detected from lesions of the ruminal walls and from reticular

abscesses. No specimens of traumatic reticulitis were collected. In some specimens of rumenitis, the viable count of the organism was relatively high. On the other hand, the bacterium was not frequently detected from the apparently unaffected ruminal mucous membrane. These findings may indicate the participation of *F. necrophorum* in causing an injury of the ruminal mucous membrane, and the presence of a significant correlation between reticulo-ruminal lesions and hepatic abscess. Jensen [5] described the inability of the organism to penetrate into the unaffected, intact mucous membrane. Recently, the bacterium was detected from the sound hepatic tissue around the abscess [1, 13]. It seems necessary to investigate the problem how *F. necrophorum* in the ruminal content penetrates into veins in the ruminal wall and reaches the hepatic tissue. It is also needed to clarify the virulence factors of the bacterium.

Reports [2, 3, 7] have hitherto been made on several biotypes of strains of *F. necrophorum* divided by the difference in biological properties. The present isolates were tentatively differentiated into two phases by referring to Bergey's Manual [2]. On the other hand, it is well known that the organism is distributed in wide ranges of domestic and wild animals [9, 14]. Details in the biological, serological and pathogenic properties of the bacterium from these sources remain to be elucidated. Attempts should be made in near future to compare several properties between the two phases by obtaining isolates from more different sources. The results of comparison will contribute to a more efficient differentiation of the bacterium and to the establishment of an adequate infrasubspecific designation, the term which has appeared in the recommendation by Lapage et al.

[10].

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Explanation of Figures

Fig. 1. Rumen parakeratosis of ventral sac of rumen. Clumps of villi with feed and hair are evident.

Fig. 2. Rumenitis of ventral sac of rumen. Mucosa is hemorrhagic, devillated and erosive.