Collective Outbreaks of Bovine Actinobacillosis

Muneo NAKAZAWA and Ryoza AZUMA

National Institute of Animal Health, Kodaira-shi, Tokyo 187

Tatsuo YAMASHITA

Goto Branch, Livestock Hygiene Service Center, Prefecture of Nagasaki,
Fukue-shi, Nagasaki 853

Toshihiko IWAO

Ishikari Livestock Hygiene Service Center, Sapporo-shi, Hokkaido 061-01

Masuo UCHIMURA

Miyazaki Livestock Hygiene Service Center, Sadohara-cho, Miyazaki 880-03

(Received for publication March 1, 1977)

Abstract. Three collective outbreaks of bovine actinobacillosis were observed in Japan during the past ten years. The results of observation are summarized as follows. (1) In Tottori Prefecture, 50 affected cows were reported during a period from 1962 to 1965. Then 35 cows in Hokkaido and 123 in Miyazaki Prefecture were affected during a period from 1975 to 1976. Morbidity was 8.8, 7.1 and 13.8 per cent, respectively. The majority of the affected animals were females of the Japanese Black breed 2 to 10 years old. The disease occurred most frequently in autumn to winter. (2) In the affected cows, marked swelling and abscess developed in the submaxillary, pharyngo-laryngeal, buccal and/or prescapular areas. Discharge of pus from the lesion was observed in some cows. Most of the affected cows recovered after antibiotic or surgical treatment. (3) Six strains of *Actinobacillus lignieresii* were isolated in pure culture from 6 out of 14 nodular lesions examined. They belonged to serotype 4 or 5. (4) The affected cows had a significantly higher agglutinin titer in serum than healthy ones ($P < 0.001$). (5) Histologically, all the nodular lesions contained characteristic eosinophilic sulfur granules.

Actinobacillosis due to *Actinobacillus lignieresii* in domestic animals has been found in cattle [3, 5, 6, 14-16], sheep [17], horse [4] and ducks [2]. In cattle digestive organs, particularly the oral cavity and associated lymph nodes, are frequently involved, showing abscess formation and the subsequent development of granulomatous lesion [7]. The outbreak is usually sporadic. Occasionally, a collective outbreak has been reported in England [6] and the United

States [3]. In Japan, the first collective outbreak of actinobacillosis in cattle was found in Tottori Prefecture during a period from 1962 to 1965. The second and third outbreaks were recognized in Hokkaido and Miyazaki Prefecture, respectively, during a period from 1975 to 1976.

The present paper deals with the three collective outbreaks mentioned above with special reference to their epizootiology, bacteriology, serology and pathology.

牛の actinobacillosis の集団発生病例：中沢宗生・東 量三（農林省家畜衛生試験場），山下達夫（長崎県家畜保健衛生所五島支所），岩尾俊彦（石狩家畜保健衛生所），内村益雄（宮崎家畜保健衛生所）

Materials and Methods

Cattle examined: The majority of the affected cattle were females of the Japanese Black breed 2 to 10 years old. In case 1 (Tottori) and case 2 (Hokkaido), affected cows were of the same herd. They were housed in a stall from November to April and pastured from May to October. In case 3 (Miyazaki), 1,665 cows were involved. They had been raised on 546 farms and housed in stalls for all the seasons.

Bacteriological examination: Subcutaneous nodules of the affected cows were sampled by surgical excision. Each sample was emulsified in 2 volumes of physiological saline with a glass-mortar. One-tenth ml of the resulting emulsion was inoculated onto 5% sheep blood agar plates and into modified VL medium. The plates were incubated aerobically at 37°C for 2 days, and the modified VL medium was anaerobically by the CO₂ jet method at 37°C for 4 days [1]. The isolates were examined for bacteriological characteristics by the procedures described by Phillips [9], Till and Palmer [18] and Mráz [8], in comparison with the reference strain, *Actinobacillus lignieresii* NCTC 10563.

Serotyping of isolates: Serotyping was performed by the method reported by Phillips [13]. Each reference strain was cultured on trypticase soy agar (BBL) at 37°C for 24 hours. Growing organisms were harvested and suspended in physiological saline. They were washed once with and resuspended in the same solution. The suspension was boiled for 2 hours. The concentration of organisms in it was adjusted to an optical density of 1.5 by a spectrophotometer (Klett-Summerson) with a green filter (500 to 560 nm in wavelength). Rabbits received seven intravenous injections of boiled antigen (1.0 ml) at intervals of 4 days. Preliminary bleeding was made 10 days or more after the last injection. Then complete bleeding was made by cardiac puncture, if the agglutinin titer was satisfactory. Serotyping was performed by the agglutination test with antisera and boiled antigens prepared from the reference strains and isolates.

Measurement of agglutinin titers of sera from affected and healthy cows: Each strain of the isolates was cultured on brain heart infusion agar (Difco) at 37°C for 24 hours. Organisms were suspended in, washed once with, and resuspended in physiological saline. Formalin was added to the suspension to provide a final concentration of 0.5%. The concentration of each antigen suspension was adjusted to McFarland opacity tube No. 3. Test sera were collected from affected and healthy cows and

stored at -20°C until use. Serial twofold serum dilutions (0.5 ml) were mixed with an equal volume of formalinized antigen. The mixtures were kept at 37°C for 18 to 20 hours. The titer was expressed as the reciprocal of the highest dilution of test serum showing complete agglutination.

Pathological examination: The subcutaneous nodules were fixed in 10% neutral formalin, dehydrated in ethanol, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Results

Epizootiological findings: As summarized in Table 1, the outbreak of case 1 was detected in cows in Tottori Prefecture during a period from 1962 to 1965. Every year, of 122 to 144 cows raised in a herd, 8 to 15 were affected. Cases 2 and 3 occurred over a period from 1975 to 1976. In case 2 found in Hokkaido, 35 of 437 cattle were affected in a herd. In case 3, 123 of 1,665 cows housed on farms in Miyazaki Prefecture were affected. The mean morbidity was 8.8%, 7.1% and 13.8% in cases 1, 2 and 3, respectively. Each affected cow had 1 to 8 subcutaneous nodules 2 to 7 cm in diameter. Marked swelling and abscess formation were seen in such nodules in the submaxillary, pharyngo-laryngeal, buccal and/or prescapular areas (Fig. 1). Other clinical signs, such as anorexia and pyrexia, were lacking. Some had a purulent discharge from ulcerative nodules. Oxytetracycline (100 to 200 mg per day) and potassium iodide (3 g per day) were usually given intramuscularly and per os for 3 to 5 consecutive days. Additionally, penicillin (3,000,000 units) and streptomycin (1 to 2 g) were given intramuscularly and surgical operation was performed in some cows. The therapy was apparently effective in 13 of 50, 28 of 35 and 111 of 123 in cases 1, 2 and 3, respectively.

Bacteriological examination: *A. lignieresii* was isolated in pure culture from 1 of 3, 1 of 5 and 4 of 6 samples examined in

Table 1. Epizootiological findings of three cases of collective outbreaks of bovine actinobacillosis

Prefecture Period	Case of collective outbreaks					
	1				2	3
	Tottori				Hokkaido	Miyazaki
	1962	1963	1964	1965	Oct. 1975 to May 1976	Apr. 1975 to July 1976
Raised	130	122	144	141	437	1665
Affected	8	15	12	15	35	123
Morbidity (%)	6.1	12.2	8.4	10.6	7.1	13.8
Termination						
Recovered	13				28	111
Relapsed	37				7	12

Remarks.

These affected cattle showed subcutaneous swelling and abscess formation in submaxillary, pharyngo-laryngeal and prescapular areas. They received treatment with penicillin, streptomycin, oxytetracycline, potassium iodide and surgical operation. For details see the text.

Table 2. Bacteriological examination

Case	No. of samples examined*	Isolate	Bacterial count**
1 (Tottori)	1	<i>Actinobacillus lignieresii</i>	10 ⁴
	2	<i>Actinomyces bovis</i> , <i>Propionibacterium acnes</i> , <i>Selenomonas</i> spp. and Gram positive cocci	10 ⁸
	3	<i>Actinomyces naeslundii</i> , <i>Leptotrichia</i> spp. and <i>Propionibacterium acnes</i>	10 ⁸
2 (Hokkaido)	1	<i>Propionibacterium acnes</i> , <i>Staphylococcus</i> spp. and <i>Streptococcus</i> spp.	10 ⁸
	2	<i>Propionibacterium acnes</i> , <i>Staphylococcus</i> spp. and <i>Streptococcus</i> spp.	10 ⁴
	3	<i>Propionibacterium acnes</i> and <i>Streptococcus</i> spp.	10 ¹
	4	<i>Propionibacterium acnes</i>	10 ¹
	5	<i>Actinobacillus lignieresii</i>	10 ²
3 (Miyazaki)	1	(—)	•
	2	<i>Actinobacillus lignieresii</i>	•
	3	(—)	•
	4	<i>Actinobacillus lignieresii</i>	•
	5	<i>Actinobacillus lignieresii</i>	•
	6	<i>Actinobacillus lignieresii</i>	•

Remarks.

*: Samples were of granulomatous lesions characterized histopathologically by the presence of eosinophilic sulfur granules.

** : Estimated by the role tube method in cases 1 and 2 and by the aerobic method in case 3.

• : Not estimated.

cases 1, 2 and 3, respectively. From 6 other samples were isolated *Actinomyces bovis*, *Actinomyces naeslundii*, *Leptotrichia* spp., *Propionibacterium acnes*, *Selenomonas* spp., *Staphylococcus* and *Streptococcus*. No bacteria were isolated from the remaining 2 samples (Table 2). In cases 1 and 2, bacterial colonies were counted in each sample by the role tube method. They were isolated from each sample in the order of 10¹ to 10⁸ per gram (Table 2). Colonies grown on blood agar were 1.5 to 3.0 mm in diameter, smooth, round, bluish-gray, translucent, adherent and non hemolytic, and those on MacConkey's agar 0.5 to 1.0 mm in diameter, smooth, round and adherent. The isolates were nonmotile and Gram-negative and composed of coccal or bacillary forms mixed with some filamentous ones. They had the same biological properties as the reference strain (NCTC 10563), except that in the VP reaction they produced acid but no gas from dextrin, fructose, galactose, maltose, mannose and xylose within 4 days. Adonitol, arabinose, lactose, raffinose, rhamnose, salicin, sorbitol and trehalose were not fermented. Catalase

Table 3. Serotyping of isolated and reference strains by agglutination test

	Antigen									
	Reference strain						Isolated strain			
	NCTC 10563	NCTC 10565	NCTC 10566	NCTC 10567	NCTC 10568	NCTC 10569	T-4	I-1	M-1	M-2
Antiserum										
Serotype 1	2560*	<10	80	<10	20	20	<10	20	20	40
Serotype 2	<10	2560	80	10	10	10	20	20	10	40
Serotype 3	<10	160	2560	40	10	<10	160	20	<10	20
Serotype 4	<10	10	40	1280	<10	40	1280	1280	10	10
Serotype 5	<10	<10	80	20	2560	20	320	40	2560	2560
Serotype 6	<10	10	80	40	20	1280	80	20	20	20

Remarks.

*: Reciprocal of agglutinin titer.

Table 4. Agglutinin titers of sera from affected and healthy cows

Group	Case No.	Reciprocal of agglutinin titer								
		<20	40	80	160	320	640	1280	2560	5120
Affected cows	2	3*	3	2	3	6	2	1	0	0
	3	0	1	0	2	0	4	2	7	1
Healthy cows	2	12	7	1	0	0	0	0	0	0
	3	1	2	4	3	0	0	0	0	0

Remarks.

* Number of cattle having the corresponding titer.

In case 2, antigen prepared from strain I-1 (serotype 4) was used.

In case 3, antigen prepared from strain M-1 (serotype 5) was used.

weakly positive, oxidase and urease positive, gelatine liquefaction and sodium hippurate hydrolysis negative, nitrate reduced to nitrite, hydrogen sulfide produced, weakly positive for the methylene blue test, and VP, MR and indole reaction negative.

Serotyping of isolates: The results obtained are shown in Table 3. It was evident that at least two serotypes of *A. lignieresii* were concerned with bovine actinobacillosis in Japan. Strains T-4 and I-1 isolated from an affected cow in Tottori and in Hokkaido, respectively, belonged to serotype 4. On the other hand, strains M-1 and M-2 isolated from affected cows in Miyazaki belonged to serotype 5.

Serum agglutinin titers in affected and healthy cows: The sera of affected and

healthy cows involved in cases 2 and 3 were examined for agglutinin titer. Since boiled antigens were low in sensitivity, formalinized antigens were used in this examination. In case 2, antigen was prepared from strain I-1 (serotype 4) isolated from an affected cow in the same herd. The titers of 12 of 20 sera from affected cows ranged from 1:160 to 1:1,280, while those of 20 sera from healthy cows raised in the same or neighboring herds were 1:80 or lower.

In case 3 antigen prepared from strain M-1 (serotype 5), an isolate from an affected cow in the same area, was used. The titers of 14 of 17 sera from affected cows ranged from 1:320 to 1:5,120. The serum titers of healthy cows raised in this area were 1:160 or lower (Table 4). When these data were

analyzed by the t-test, there was a significant difference ($P < 0.001$) between affected and healthy cows. Most of the sera examined showed similar titers against formalinized antigens prepared from strains belonging to serotype 2 (NCTC 10565) and serotype 3 (NCTC 10566).

On the other hand, serum samples were collected from 13 cattle involved in the outbreak of case 3 at given intervals during 4 months after therapy and examined for agglutinin. Their titers ranged from 1:320 to 1:5,120.

Pathological findings: Histopathologically, subcutaneous nodules were typical granulomatous lesions of actinobacillosis. They were characterized by a purulent center surrounded by fibrous connective tissue. Eosinophilic sulfur granules were present within the pus surrounded by a number of neutrophils, mononuclear cells, and a few giant cells (Fig. 2).

Discussion

This paper is the first to report the collective outbreaks of bovine actinobacillosis found in Japan during the last decade. As for actinobacillosis, it is said that a sporadic outbreak is "typical" [3] and a collective or epizootic outbreak, such as reported by Hebeler et al. [6], "atypical". Outbreaks of the latter form have been most frequent during autumn and winter in England [6] and the United States [3]. The present three cases also showed the same trend as these.

Phillips [10, 11] described that *A. lignieresii* was isolated usually from the oral cavity and the contents of the rumen of healthy cattle. From this, the organism is considered to invade the tissue injured by some hard feedstuffs or any other foreign body, producing a chronic local inflammatory reaction leading to granuloma forma-

tion [7]. In the present outbreak of case 3, dried stalks of sweet potato were detected in the diet given, probably playing some role in the pathogenesis of the disease.

A. lignieresii was isolated in pure culture from 6 of 14 samples collected from subcutaneous nodules containing characteristic eosinophilic sulfur granules. Some other kinds of bacteria were isolated from other 6 samples. *Actinomyces bovis* and *Actinomyces naeslundii* were isolated from 2 of 3 samples in case 1 and presumed to be causative agents. Theoretically, most of the infections of soft tissue, including lymph nodes, tongue and buccal mucous membrane, are caused by *A. lignieresii*, whereas actinomycosis due to *Actinomyces bovis* has frequently developed in the mandibula [7]. It is said that actinobacillosis occasionally takes an epizootic form, but that actinomycosis does not. Therefore, it was considered that the main causative agent might be *A. lignieresii* in the outbreaks of cases 1, 2 and 3. At any rate, *A. lignieresii* was isolated from only about a half of a total of 14 samples examined. This may be attributed to the treatment with antibiotics before sampling. Till and Palmer [18] described that this organism was sensitive to penicillin, streptomycin, tetracyclin and chloramphenicol.

Phillips [13] reported that the strains isolated from bovine and ovine lesions were divided into 6 serotypes, and that the majority of bovine strains belonged to serotypes 1, 5 and 6, and most ovine strains to serotypes 2, 3 and 4. In the present studies, it was revealed that at least two serotypes of the organism were prevalent in Japan; that is, 2 strains isolated in Tottori and Hokkaido belonged to serotype 4 and other 2 strains in Miyazaki to serotype 5. It is of interest to note that the strains belonging to serotype 4 were detected from cattle in

Japan, because this type had been isolated only from sheep since Phillips [13]. It is not clear whether other serotypes are also related to bovine actinobacillosis in Japan, because the present investigation was limited. Further studies are needed to clarify the epizootiology of the disease.

Campbell et al. [3] indicated that the agglutination test was not so useful for the diagnosis of actinobacillosis. In contrast, Phillips [12] reported that affected cattle usually showed higher agglutinin titers than healthy ones, although there may be some difficulty in setting a border between the two. In cases 2 and 3, the affected cows had significantly higher agglutinin titers than healthy ones ($P < 0.001$). Most of the sera examined showed similar titers against formalinized antigens prepared from strains of serotypes 2 (NCTC 10565) and 3 (NCTC 10566). It is indicated that these antigens may be useful for detecting natural infections of *A. lignieresii*.

Acknowledgments: The authors wish to thank Dr. J. E. Phillips, of the Department of Veterinary Pathology, Royal School of Veterinary Studies, Edinburgh, Scotland, for supply of the type strains of *A. lignieresii* and valuable suggestions. Thanks are also due to the veterinary staffs of the Ishikari and Miyazaki Livestock Hygiene Service Centers and the Tottori Breeding Station for detailed information on epizootiology.

References

- [1] Azuma, R. (1968). CO₂ jet method. In *Anaerobes in Clinical Medicine*, Kosakai, M., and Suzuki, S., editors, Igaku Shoin, Tokyo, 36-40 (in Japanese).
- [2] Bisgaard, M. (1975). Characterization of atypical *Actinobacillus lignieresii* isolated from ducks with salpingitis and peritonitis. *Nord. vet. Med.*, **27**, 378-383.
- [3] Campbell, S. G., Whitlock, R. H., Timoney, J. F., and Underwood, A. M. (1975). An unusual epizootic of actinobacillosis in dairy heifers. *J. Amer. vet. med. Ass.*, **166**, 604-606.
- [4] Chladek, D. W., and Ruth, G. R. (1976). Isolation of *Actinobacillus lignieresii* from an epidural abscess in a horse with progressive paralysis. *J. Amer. vet. med. Ass.*, **168**, 64-66.
- [5] Franco, D. A. (1970). Generalized actinobacillosis in a Holstein cow (post-mortem lesions). *Vet. Med. Small Anim. Clin.*, **65**, 562.
- [6] Hebel, H. F., Linton, A. H., and Osborne, A. D. (1961). Atypical actinobacillosis in a dairy herd. *Vet. Rec.*, **73**, 517-521.
- [7] Jansen, R., and Mackey, D. R. (1971). Actinobacillosis. In *Diseases of Feedlot Cattle*, 2nd ed., Lea and Febiger, Philadelphia, 91-94.
- [8] Mráz, O. (1969). Vergleichende Studie der Arten *Actinobacillus lignieresii* und *Pasteurella haemolytica*. I. *Actinobacillus lignieresii* Brumpt, 1910; emend. *Zbl. Bakt., I. Abt. Orig.*, **209**, 212-232.
- [9] Phillips, J. E. (1960). The characterization of *Actinobacillus lignieresii*. *J. Path. Bact.*, **79**, 331-336.
- [10] Phillips, J. E. (1961). The commensal role of *Actinobacillus lignieresii*. *J. Path. Bact.*, **82**, 205-208.
- [11] Phillips, J. E. (1964). Commensal actinobacilli from the bovine tongue. *J. Path. Bact.*, **87**, 442-444.
- [12] Phillips, J. E. (1965). The incidence of agglutinating antibodies to *Actinobacillus lignieresii* in the sera of normal and infected cattle. *J. Path. Bact.*, **90**, 557-566.
- [13] Phillips, J. E. (1967). Antigenic structure and serological typing of *Actinobacillus lignieresii*. *J. Path. Bact.*, **93**, 463-475.
- [14] Ristic, M., Herzberg, M., Sanders, D. A., and Williams, J. W. (1956). Actinobacillosis. I. An evaluation of cultural characteristics of selected strains of *Actinobacillus lignieresii*. *Amer. J. vet. Res.*, **17**, 555-562.
- [15] Sanders, D. A., and Ristic, M. (1956). Actinobacillosis of cattle. *J. Amer. vet. med. Ass.*, **129**, 478-481.
- [16] Swarbrick, O. (1967). Atypical actinobacillosis in three cows. *Brit. vet. J.*, **123**, 70-75.
- [17] Taylor, A. W. (1944). Actinobacillosis in sheep. *J. comp. Path.*, **54**, 228-237.
- [18] Till, D. H., and Palmer, F. P. (1960). A review of actinobacillosis with a study of the causal organism. *Vet. Rec.*, **72**, 527-533.

Explanation of Figures

Fig. 1. Abscess formation in the pharyngo-laryngeal area of an affected cow in case 2.

Fig. 2. Characteristic eosinophilic sulfur granules within granulomatous lesion of an affected cow in case 2. H-E staining. $\times 100$.

①

②