BRIEF NOTE

AN ENZOOTIC OF CALF PNEUMONIA ASSOCIATED WITH *MYCOPLASMA BOVIRHINIS*

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Various etiologies have been incriminated as a cause of respiratory disease of calves [12]. Among them, mycoplasma was isolated firstly from nasal swabs of cattle with shipping fever [1]. Thereafter, reports concerning calf pneumonia associated with mycoplasmas appeared [3, 4, 7, 8], in which *M. bovirhinis* [9], *M. dispar* [6], *M. alkalescens* [10] and T-mycoplasmas [5, 11, 13] have hitherto been detected from pneumonic lungs and respiratory tracts of cattle.

This brief note contains the results of investigation for an enzootic pneumonia of calves associated with mycoplasma which was found for the first time in Japan.

Outbreak of the disease: The farm premises had been situated in the suburbs of Miyakonojo city, Miyazaki. Five hundred to 650 Holstein calves, both male and female, had been reared from their age of 2 to 3 weeks up to 6 months old with accidental losses of 10 to at most 29 calves annually for these years. Since an enzootic of respiratory disease occurred in the beginning of 1973, as many as 83 calves were dead or discarded during January to August; 8 to 17 calves every month regardless of the season. Treatment with spiramycin was ineffective to prevent deaths. On post-mortem examination, 46 of them were affected with suppurative or croupous pneumonia, 14 with gastroenteritis,

17 with both pneumonia and enteritis, and the remainder 6 with other etiologies.

Results and discussion: Pathological and microbiological tests were performed with 14 dead or discarded calves. Medium used for isolating mycoplasmas had the following ingredients: heart infusion broth base (Eiken), 2.5%; phytone (BBL), 1%; yeast extract (Difco), 1%; dextrose, 0.5%; heat-inactivated swine serum, 15%; thallium acetate, 0.025%; penicillin, 1,000 I.U. per ml of medium. Both liquid and solid media were used for the primary isolation. Incubations were made in candle jars placed at 37°C for 7 days. A modified medium of Taylor-Robinson [14] was used for T-mycoplasmas. Besides, heart infusion agar plate supplemented with 10% swine serum, selenite broth, DHL agar plate and corn meal agar plate were employed for common bacteria and fungi. Results are summarized in Table 1.

Mycoplasmas were isolated abundantly from 5 out of 6 pneumonic lungs without heavy contamination by common bacteria. They were also isolated from 3 tracheas, 2 spleens and a kidney, as well as 9 nasal swabs.

Antisera were prepared by immunizing rabbits with 3 of our isolates, namely strain BP 7 and BP 19 which were isolated from lungs of calf No. 7 and No. 13, and strain 351 which was isolated from nasal cavity of an apparently healthy calf. Antisera to both lung strains similarly inhibited growth of two substrains of *M. bovirhinis* strain PG

短報: 清水高正, 野坂 大, 中村憲雄 (宮崎大学農学部, 宮崎市船塚町) *M. bovirhinis* の関与する子牛の肺炎について

Table 1. Pathological Diagnosis and Results of Detection of Mycoplasmas and Antibodies from 14 Calves

Calf No.	Pathological diagnosis	Detection of mycoplasma					Antibody titer	
		Lungs	Trachea	Spleen	Kidneys	Nose	Aggl.**	M.I.
1	Pneumoenteritis	NT	NT	NT	NT	NT	NT	NT
2	Pneumoenteritis	(—)	NT	—	—	+	NT	NT
3	Pneumoenteritis	(—)	NT	—	—	+	16	40
4	Pneumoenteritis	(—)	NT	—	—	+	8	40
5	Pneumoenteritis	—	NT	—	—	+	64	80
6	Pneumonia	+	—	+	—	(—)	64	320
7	Pneumonia	+	—	+	+	—	16	40
8	Enteritis	—	—	—	—	+	8	<10
9	Abomasumitis	—	+	—	—	+	8	<10
10	Pneumonia	(—)	(—)	—	—	(—)	16	20
11	Pneumonia	+	(—)	—	—	+	NT	NT
12	Pneumonia	(—)	(—)	—	—	+	16	20
13	Pneumoenteritis	+	+	—	—	(—)	64	320
14	Pneumonia	+	+	—	—	+	128	320

(—): Not detected due to heavy contamination with common bacteria.

* All the isolates in this table were serologically identified as *M. bovirhinis*.

** Geometric mean agglutinin titer was 23.3.

NT: Not tested.

Table 2. Isolation and Serological Identification of Mycoplasmas from Nasal Discharges

Farm	Herd	Outbreak of pneumonia	Isolation of mycoplasmas	Identification of isolates		
				Antiserum**		Unidentified
				BP 7	351	
A	1	+	63/117* (54%)	55	4	4
	2	—	3/ 14 (21)	3	0	0
B		—	3/ 25 (12)	3	0	0
Various		—	0/ 43 (0)	—	—	—

* Number positive for mycoplasma per number of calves examined.

** Rabbit antiserum to strain BP7 isolated from pneumonic lung of calf No. 7 shown in Table 1 and identified as *M. bovirhinis*, and to strain 351 which was a nasal isolate and belongs to *Acholeplasma* sp. biologically.

Table 3. Agglutinin Titers in Sera of Affected and Non-Affected Calves

Herd	Respiratory symptom	No. of serum	Agglutinin titer							GMT
			≤2	4	8	16	32	64	128	
A1	+	14			3	5	3	2	1	22.6
A2	—	11	6		3	2				3.5
Various	—	35	31	3	1					<2

43 obtained through the courtesy of Prof. M. Ogata, University of Tokyo, and Dr. R. H. Leach, Mycoplasma Reference Laboratory, London, by the method of Clyde [2]. Other reference strains of bovine mycoplasmas excluding *M. mycoides* subsp. *mycoides* reported [10] and furnished by Dr. Leach were inhibited by none of three antisera tested. Strain 351 was proved to belong to *Acholeplasma* sp. from the biological properties

All the isolates from pneumonic lungs and related materials were identified as *M. bovirhinis* by the growth inhibition test. Both agglutinin and metabolic inhibition (MI) titers of the sera from 11 out of 14 dead calves against strain BP 7 are shown in Table 1.

Except for mycoplasmas, *Salmonella typhimurium* was isolated from lungs and/or other organs of calves No. 1 to No. 5. Agglutinin to live and heat-inactivated antigens of this organism was not detected in the sera of dead calves. Pasteurella organism was never isolated, but coli-groups and Proteus organism were the frequent contaminants in the present materials.

Isolation of mycoplasmas from nasal discharges of calves with or without respiratory symptoms were performed. As shown in Table 2, mycoplasmas were isolated from 63 out of 117 calves kept in the very herd where pneumonia had been prevalent.

Sera were taken from affected calves in this herd and from calves in disease-free herd simultaneously, and their agglutinin titers were estimated. As shown in Table 3, a higher geometric mean agglutinin titer (GMT) was proved for the affected calves as compared with those of the disease-free samples.

M. bovirhinis has been considered as a secondary invader in pneumonic lungs of cattle associated with other agents such as parainfluenza type 3 virus [4, 8]. In the present

study, only two pneumonic lungs were examined and considered negative for the presence of virus with BK cell culture; therefore, the primary pathogene, if such had existed in this enzootic of calf pneumonia, was not apparent. Although isolation of mycoplasmas from pneumonic lungs were not always successful, infection of all dead calves excluding Nos. 8 and 9 with *M. bovirhinis* was proved from the results of serological investigation. It is probable from these results that infection or superinfection with mycoplasmas such as *M. bovirhinis* would result in severe pneumonia, which was frequently lethal for calves.

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