NOTE

Isolation of *Mycoplasma bovis* from Calf Pneumonia in Japan

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Mycoplasma bovis was isolated from an outbreak of bovine mastitis for the first time in the United States [4]. Pathological roles of this species in mastitis [4, 5], arthritis [15] and calf pneumonia [3, 17] have been confirmed. Occurrence of pneumonia or pneumo-arthritis syndrome in calves associated with *M. bovis* has been reported in the United States [6], Italy [9], England [17], Canada [7], Hungary [10] and West Germany [8]. In Japan, the same organisms have been isolated from mastitic milk samples [13] and calf arthritis [12], but there is no information about the detection of *M. bovis* from pneumonic calves.

In June, 1978, pneumonic lungs of three calves died of chronic pneumonia and nasal discharges from 33 calves affected with respiratory syndrome in four farms in Miyazaki were subjected to the bacterial examination. A modified Hayflick medium used for the classical mycoplasmas composed of 70 ml of PPLO agar medium (Difco), 20 ml of heat-inactivated horse serum, 10 ml of 25% fresh yeast extract, 1 ml of 0.2% DNA (calf thymus extract, Sigma), 0.5 ml of 5% thallium acetate and 250 mcg/ml of ampicillin with the pH adjusted to 7.6. A modified GS agar medium [2] in which Hartley's broth was replaced by brain heart infusion broth (Difco) was used for the isolation of *M. dispar*. For the

primary isolation of ureaplasmas, a liquid medium of Taylor-Robinson et al. [16] excluding thallium acetate and penicillin and containing 250 mcg/ml of ampicillin was employed. An agar medium consisting of 70 ml of PPLO agar medium (Difco), 20 ml of horse serum, 10 ml of fresh yeast extract, 1 ml of 10% urea solution, 0.2 ml of 1% phenol red, 2.5 ml of a 7.3 mixture of 1.5 M KH_2PO_4 and 1.5 M Na_2HPO_4 solutions [18] and 250 mcg/ml of ampicillin was used for the confirmation of growth of dark-brown colonies of ureaplasmas. Heart infusion agar plates supplemented with 5% sheep blood and 5% fresh yeast extract were employed for the common bacteria. Three pieces of samples were taken from different lobes of pneumonic lungs and triturated in PPLO broth base to make 10% (w/v) suspensions. Ten-fold dilutions of the homogenates were made in the same broth. Plates inoculated with diluted samples were incubated at 37°C for ten days in an atmosphere of 10% CO_2 in air. Serological identification of the isolates was performed by the growth inhibition test [1] and indirect epi-immunofluorescence test [11] with antisera against bovine *Mycoplasma* and *Acholeplasma* species prepared and used in a previous study [13]. In addition, reference sera against *M. bovis* Donetta, *M. canadense* 257C and *M. dispar*

Table 1. Mycoplasmal and bacterial isolations from pneumonic lungs of 3 calves

Calf No.	Pneumonic lobe No.	Isolation of			Identified species of classical mycoplasma
		Classical mycoplasma (CFU/g)	Ureaplasma (CCU/g)	Bacteria (CFU/g)	
1	1	6.2×10^8	10^7	$2.0 \times 10^5^*$	<i>M. bovis</i> $\gg$ <i>M. bovirhinis</i>
	2	2.4×10^8	10^7	$3.8 \times 10^5^*$	"
	3	1.1×10^8	10^6	1.3×10^6	"
2	1	3.4×10^7	NG	$4.4 \times 10^4^*$	<i>M. bovis</i> $\gg$ <i>M. bovirhinis</i>
	2	1.0×10^7	NG	$2.0 \times 10^6^*$	"
	3	8.6×10^6	NG	NG	"
3	1	4.8×10^8	10^7	$9.1 \times 10^6^{**}$	<i>M. bovis</i> $\gg$ <i>M. bovirhinis</i>
	2	6.5×10^8	10^6	$6.5 \times 10^6^{**}$	"
	3	6.2×10^8	10^6	$2.0 \times 10^6^{**}$	"

* *Pasteurella haemolytica*: ** *P. haemolytica* and *Corynebacterium pyogenes*.

NG, negative for isolation.

Table 2. Incidence of mycoplasmal and bacterial isolates from nasal discharges of pneumonic calves¹

Farm	A	B	C	D	Total (%)
No. of calves tested	10	6	10	6	33
<i>Mycoplasma bovis</i>	5*	5	3	0	13 (39)
<i>M. bovirhinis</i>	11	5	8	6	30 (91)
<i>Achole. laidlawii</i>	2	3	2	2	9 (27)
<i>Ureaplasma</i> sp.	6	2	9	4	21 (64)
Streptococci	9	4	6	6	25 (76)
Staphylococci	5	1	3	4	13 (39)
<i>Pasteurella</i> sp.	3	0	2	0	5 (15)
<i>Haemophilus</i> sp.	0	0	2	0	2 (6)
<i>Pseudomonas</i> sp.	4	2	0	1	7 (21)
Others	6	1	4	2	13 (39)

* No. of positive samples.

462/2 were obtained from Dr. H. Enr of the University of Aarhus, Aarhus C, Denmark and used for the confirmation of serological reactions.

Many mycoplasma colonies, ranging from 10^6 to 10^8 CFU per gram of tissue, were isolated from all of the pneumonic lungs tested, as are shown in Table 1. In all cases colonies grown on the plates inoculated with the aliquots of the highest dilutions were identified as *M. bovis*, while *M. bovirhinis* was detected from the plates inoculated with lower dilutions of the lung

homogenates. No other species of *Mycoplasma* or *Acholeplasma* was detected by the immunofluorescence test. Ureaplasmas were isolated from two of the three pneumonic lungs. Bacterial species isolated in significant number were *Pasteurella haemolytica* and *Corynebacterium pyogenes* (Table 1).

M. bovis was isolated from 13 of 33 nasal discharges originating from three farms (Table 2). It was not easy to discriminate the colonies of *M. bovis* from those of *M. bovirhinis* which always grew

dominantly on plates inoculated with nasal discharges. Once isolated, cloned and grown massively on a plate, all the isolates of *M. bovis* produced film and spots, in contrast to the type strain Donetta. No strain of *M. bovis*, including Donetta, split glucose, arginine or urea. Weak color change of phenol red contained in the liquid medium with or without glucose was produced by all strains of this species.

Although results of our serodiagnostic study [14] indicated the association of *M. bovis* in calf pneumonia observed on many farms, this is the first report on the isolation of this species from clinically apparent calf pneumonia in Japan. Previous isolation of *M. bovis* from bovine mastitis [13] suggests that the invasion of this organism into Japan would be in 1977 or earlier. One of the reason why this species had not detected previously from prevalent calf pneumonia in this country is probably due to the presence of predominating colonies of other mycoplasma species which prevented previous workers from detecting the colonies of *M. bovis*, especially when nasal discharge was used for cultivation. It seems to be necessary to devise a selective medium for the reliable isolation of *M. bovis* from the materials concomitant with other mycoplasma species.

REFERENCES

- [1] Black, F. T. (1973). *Appl. Microbiol.* **25**, 528-533.
- [2] Gourlay, R. N., and Leach, R. H. (1970). *J. Med. Microbiol.* **3**, 111-123.
- [3] Gourlay, R. N., Thomas, L. H., and Howard, C. J. (1976). *Vet. Rec.* **98**, 506-507.
- [4] Hale, H. H., Helmboldt, C. F., Plastringe, W. N., and Stula, E. F. (1962). *Cornell Vet.* **52**, 582-591.
- [5] Jasper, D. E., Jain, N. C., and Brazil, L. H. (1966). *J. Am. Vet. Med. Assoc.* **148**, 1017-1029.
- [6] Kehoe, J. M., Norcross, N. L., and Carmichael, L. E. (1967). *Ann. New York Acad. Sci.* **143**, 337-344.
- [7] Langford, E. V. (1977). *Can. J. Comp. Med. Vet. Sci.* **41**, 89-94.
- [8] Pfützner, H., Bocklisch, H., und Zepezauer, V. (1980). *Muench. Vet. Med.* **35**, 499-561.
- [9] Pignattelli, P., and Galassi, D. (1971). *Atti Soc. It. Buiatria* **3**, 544-554.
- [10] Romváry, J., Rózsa, J., Stipkovits, L., and Mészáros, J. (1979). *Acta Vet. Acad. Sci. Hungaricae* **27**, 29-37.
- [11] Rosendal, S., and Black, F. T. (1973). *Acta Pathol. Microbiol. Scand., B* **80**, 615-622.
- [12] Shibata, E., Koyama, M., Yasui, T., Yabuki, Y., Igarashi, Y., Tatebu, T., Ishikura, K., and Hiramata, T. (1980). *J. Hokkaido Vet. Assoc.* **24**, 212-217 (in Japanese).
- [13] Shimizu, T., and Nagatomo, H. (1977). *Jpn. J. Vet. Sci.* **39**, 581-585.
- [14] Shimizu, T., Nagatomo, H., and Hataya, M. (1981). *Jpn. J. Vet. Sci.* **43**, 191-199.
- [15] Stalheim, O. H. V., and Page, L. A. (1975). *J. Clin. Microbiol.* **2**, 165-168.
- [16] Taylor-Robinson, D., Haig, D. A., and Williams, M. H. (1967). *Ann. New York Acad. Sci.* **143**, 517-518.
- [17] Thomas, L. H., Howard, C. J., and Gourlay, R. N. (1975). *Vet. Rec.* **97**, 55-56.
- [18] Windsor, G. D., Edward, D. G. N., and Trigwell, J. A. (1975). *J. Med. Microbiol.* **8**, 183-187.

要 約

子牛肺炎からの *Mycoplasma bovis* の分離 (短報): 清水高正 (宮崎大学農学部家畜衛生学講座) —— 慢性肺炎により死亡した子牛 3 頭の肺, および肺炎発生飼育場 4 場の罹患牛 33 頭の鼻汁のうち, 3 場由来の 13 例から *M. bovis* が分離された. 臨床診断の目的で鼻汁を培養に供する場合, *M. bovirhinis* が優勢に発育し, *M. bovis* の集落の検出を困難にする例が多かった.