Serovars and Biovars of *Pasteurella haemolytica* Isolated from Calves

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Abstract. This study was carried out to clarify the serovar and biovar distribution of *P. haemolytica* isolated from calves in this country. A total of 113 isolates of *P. haemolytica* were examined. Eighty-five isolates from the lung lesions of 36 pneumonic calves were determined as serovar 1; one isolate from the apparently normal lung of a healthy calf was serovar 2. The remaining 27 isolates belonged to Frank's new serovars 13 to 15 except five untypable strains; one isolate belonged to serovar 13, four to serovar 14 and 17 to serovar 15. Serovars 13 (one isolate), 14 (4) and 15 (1) were all from pneumonic tissues. Isolates of serovar 15 (16) were from eye swabs of calves. All 113 isolates were considered to correspond to biovar A from such biochemical characteristics as production of catalase and β -galactosidase, and the fermentation pattern of D-trehalose, D-xylose and D-mannose. The different biochemical characteristics between established serovars 1 and 2 and new serovars 13, 14 and 15 were also discussed.

Although *Pasteurella haemolytica* is often isolated from the lesions of calf pneumonia, the etiological role of this organism in pneumonia is controversial. Ishino et al. [10] observed 194 cases of calf pneumonia in mass rearing facilities in the northern part of Japan. They pointed out that calf pneumonia was a complex disease resulting from interaction between a bacterial agent and stress due to environmental factors. These authors and Jericho [11] emphasized the pathogenic role of *P. haemolytica* in respiratory disease of young cattle.

P. haemolytica from sheep and cattle were divided into 12 serovars by indirect hemagglutination (IHA) [3, 5]. Recently, Frank [7] reported that isolates from the nasal passages of cattle were untypable by IHA and grouped into three serovars by rapid plate agglutination. With respect to

biotyping, Smith [16] divided the organism into biovars A and T on the bases of biochemical characteristics and antibiotic susceptibility.

Little has been reported on serotyping and biotyping of *P. haemolytica* in Japan. We tried to clarify the serovar and biovar distribution of *P. haemolytica* isolated from calves during epizootiological survey in this country.

Materials and Methods

Strains: A total of 113 isolates of *P. haemolytica* were examined. Of them, 96 and 1 isolates were from the lung lesions of 42 pneumonic calves and from the apparently normal lung of a healthy one submitted for bacteriological diagnosis, and 13 and three isolates were from eye swabs taken from seven healthy calves and one with pneumonia, respectively. All male calves of the Holstein breed examined were less than 2 months of age except one of 6 months of age.

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Serotyping: Rapid plate agglutination tests were performed by the method of Frank and Wessman [8] with 15 kinds of antisera kindly supplied by Dr. G. H. Frank, the National Animal Disease Center, Ames, Iowa, USA. Of these antisera, 12 were prepared from established serovar 1 to 12 [8]; the others were from three untypable strains, 282-76 194-76 and 148-78 [7], by IHA. In this report, the latter strains are designated as new serovars 13, 14 and 15.

Biotyping: Identification of species and biotyping were performed by the method reported by Wessman and Hilker [17] and Biberstein [1]. Strains 129 of biovar A and JF2 of biovar T were used as control. Biberstein and Gills's medium [2] with various sugars at 1% was used to test for carbohydrate fermentation; Smith's medium [16] for arabinose fermentation.

Penicillin susceptibility and fuchsin sensitivity tests were performed by the method reported by Olmos and Biberstein [13].

Results

Serotyping: First, the 113 isolates were tested with antisera to established serovars 1 to 12; 85 belonged to serovar 1 and another to serovar 2. The remaining 27 were not typable; one of them autoagglutinated. The untypable 26 isolates were then retested with antisera to new serovars 13 to 15. Of them, one belonged to serovar 13, four to serovar 14, 17 to serovar 15, and four were untypable. All the 85 isolates belonging to serovar 1 originated from 36 of 42 pneumonic calves. Serovar 2 (one isolate) was

isolated from the apparently normal lung. Serovars 13 (one isolate), 14 (four isolates) and untypable five isolates including the autoagglutinating strain were from pneumonic tissues. Among 17 isolates belonging to serovar 15, 13 were from eye swabs of 7 healthy calves, other three from eye swab of a pneumonic calf and the other one from pneumonic tissue of a calf (Table 1).

Biochemical characterization and biotyping: All 113 isolates were identified as *P. haemolytica*. The common features were as follow: they were aerobic, nonmotile, β -haemolytic on sheep blood agar and gram-negative rods. They grew on MacConkey agar, reduced nitrate to nitrite, failed to hydrolyze urea and to produce indole, and exhibited catalase and oxidase activities.

Biochemical characteristics of biovar A (strain I29) and biovar T (strain JF2) are shown in Table 2. All isolates produced catalase and β -galactosidase and fermented D-xylose as did strain I29. D-trehalose or D-mannose was not fermented by any strain except strain JF2. All isolates belonging to serovar 13 to 15, untypable and the autoagglutinating strain fermented L-arabinose. However, all isolates of serovars 1 and 2 and reference strain I29 did not ferment L-arabinose, in either Biberstein and Gills's or Smith's medium. All isolates of serovar

Table 1. Sources and serovars of 113 strains of *P. haemolytica* isolated from calves

Source	Number of strains in serovars															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	UT
Pneumonic tissue	85* (36)												1 (1)	4 (1)	1 (1)	5** (3)
Apparently normal lung		1 (1)														
Eye swab from healthy calf															13 (7)	
Eye swab from calf with pneumonia															3 (1)	

*: Number of strains. **: One strain is autoagglutinating.

UT: Untypable strain. (): Number of calves.

Table 2. Biochemical characteristics of 113 strains of *P. haemolytica* isolated from calves

Differential characteristics	Reference strain		Serovar					Untypable strain (4)	Autoagglutinating strain (1)
	129 Biovar A	JF2 Biovar T	1 (85)	2 (1)	13 (1)	14 (4)	15 (17)		
Catalase	+	—	85*	1	1	4	17	4	1
β -galactosidase	+	—	85	1	1	4	17	4	1
Fermentation of									
D-trehalose	—	+							
D-xylose	+	—	85	1	1	4	17	4	1
L-arabinos	—	—			1	4	17	4	1
salicin	—	+				4			1
D-cellobiose	—	+				4			1
D-mannose	—	+							
lactose	+	—	85					2	
esculin	—	+				4			1
Penicillin (5 IU) susceptibility	+	—	22	1	1	4	3		
Fuchsin (2.0 μ g) sensitivity	+	—	85	1	1		4	4	

+: Positive reaction. —: Negative reaction. (): Number of strains tested.

*: Number of positive strains.

14 and the autoagglutinating strain fermented salicin and D-cellobiose and hydrolyzed esculin. Lactose was fermented by all isolates of serovar 1 and two untypable isolates.

Twenty-two isolates of serovar 1, one of serovar 2, one of serovar 13, four of serovar 14, and three of serovar 15 were susceptible to penicillin at a concentration of 5 IU/disk, but 63 of serovar 1, 14 of serovar 15 and four untypable strains were resistant. All isolates of serovars 1, 2 and 13, four of serovar 15, and four untypable strains were sensitive to fuchsin at a concentration of 2 μ g/ml.

Discussion

Various bacteria and mycoplasmas have been isolated from pneumonic cases of calves by many investigators [10, 12, 14]. As bacteria, *P. haemolytica* and/or *P. multocida* have mainly been isolated. Schiefer et al. [14] reported that the lesion of fibrinous pneumonia was undoubtedly associated with *P. haemolytica*. In the present survey, *P.*

haemolytica was isolated from the lung lesions of 42 of 51 pneumonic calves. Therefore, this organism may be regarded as one of the important pathogenic organisms in calf pneumonia. We consider that more attention should be paid to *P. haemolytica* as the causative agent of calf pneumonia in this country.

Frank and Wessman [8] proved that the rapid plate agglutination procedure is rapid and specific for serotyping of *P. haemolytica*. By this procedure, we clarified serovar distribution of *P. haemolytica* isolated from calves in this country. The predominant serovar in pneumonic tissues was 1; new serovars 13, 14 and 15, and untypable strains were also found. According to Frank and Wessman [8] and Wessman and Hilker [17], serovar 1 was predominant in USA, and serovar 2 was infrequently isolated from calves. The serovar distribution in Japan seems to be similar to that in USA. It is not clear whether new serovars are pathogenic for calves, since the number of strains of these serovars isolated was limited and

particularly most serovar 15 strains were from eye swabs of healthy calves.

Smith [16] divided the organisms into two biovar A and T from the fermentation pattern of arabinose and trehalose; biovar A strains ferment arabinose but not trehalose, and biovar T strains indicate the reverse pattern. Shreeve et al. [15], however, reported that some strains of biovar A failed to ferment arabinose. They stated that the arabinose and trehalose fermentation tests were more reliable for T than for A strains. Frederiksen [9] also reported that strains of biovar A were negative in arabinose fermentation. In this study, including I29 as a reference for biovar A, 85 strains of serovar 1 and one strain of serovar 2 failed to ferment arabinose. These isolates, however, are considered to belong to biovar A because of production of catalase and β -galactosidase, and the fermentation pattern of D-trehalose, D-xylose, salicin, D-cellobiose, D-mannose, lactose and esculin (Table 2). The results well correspond to the description by Frederiksen [9]. Therefore, we consider that fermentation of D-xylose instead of L-arabinose is a useful criterion to distinguish biovar A from biovar T.

Susceptibility to penicillin has also been used as a criterion for biotyping [4, 16]; biovar A is more susceptible than biovar T. In this study, however, 63 of the 85 isolates of biovar A of serovar 1 were resistant to penicillin, which disagreed with the findings by other workers [4, 13]. Penicillin resistance of this bacteria may have resulted from frequent use of this antibiotic against calf pneumonia in this country. Chang and Carter [6] stated that 78 of 127 isolates of *P. haemolytica* from bovine were resistant to penicillin. We considered from such a situation that the dye-sensitivity test reported by Olmos and Biberstein [13] is more

useful for differentiation between biovars A and T than the penicillin-susceptibility test, since all the strains of biovar A of serovars 1 and 2 were more sensitive to basic fuchsin than strain JF2 of biovar T.

Frank [7] identified his new serovars as biovar A from the fermentation of arabinose and production of catalase. In the present study, all isolates of serovars 13, 14, and 15 and untypable strains were positive in catalase and β -galactosidase production, arabinose and xylose fermentation, and negative in trehalose and mannose fermentation. These isolates were identified as so-called biovar A from the above-mentioned characteristics. These findings agreed with those by Frank [7]. Unlike biovar A of serovars 1 and 2, however, new serovars 13, 14, and 15 and the untypable isolates did ferment arabinose. It is uncertain whether these isolates should be regarded as the same to biovar A.

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References

- [1] Biberstein, E. L. (1978). Biotyping and serotyping of *Pasteurella haemolytica*. In *Methods in Microbiology*, vol. 10, Bergan, T., and Norris, J. R., editors, Academic Press Inc., New York, 253-269.
- [2] Biberstein, E. L., and Gills, M. G. (1962). The relation of the antigenic types to the A and T types of *Pasteurella haemolytica*. *J. Comp. Pathol.* **72**, 316-320.
- [3] Biberstein, E. L., Gills, M. G., and Knight, H. (1960). Serological types of *Pasteurella haemolytica*. *Cornell Vet.* **50**, 283-300.
- [4] Biberstein, E. L., and Kirkham, C. (1979). Antimicrobial susceptibility patterns of the A

- and T types of *Pasteurella haemolytica*. *Res. Vet. Sci.* **26**, 324-328.
- [5] Biberstein, E. L., and Thompson, D. A. (1966). Epidemiological studies on *Pasteurella haemolytica* in sheep. *J. Comp. Pathol.* **76**, 83-94.
- [6] Chang, W. H., and Carter, G. R. (1976). Multiple drug resistance in *Pasteurella multocida* and *Pasteurella haemolytica* from cattle and swine. *J. Amer. Vet. Med. Assoc.* **169**, 710-712.
- [7] Frank, G. H. (1980). Serological groups among untypable bovine isolates of *Pasteurella haemolytica*. *J. Clin. Microbiol.* **12**, 579-582.
- [8] Frank, G. H., and Wessman, G. E. (1978). Rapid plate agglutination procedure for serotyping *Pasteurella haemolytica*. *J. Clin. Microbiol.* **7**, 142-145.
- [9] Frederiksen, W. (1973). *Pasteurella* taxonomy and nomenclature. In *Contributions to Microbiology and Immunology*, vol. 2, Winblad, S., editor, S. Karger Publishing Co., Basel, 170-176.
- [10] Ishino, S., Oka, M., Terui, S., and Ikeda, S. (1979). Pathological and microbiological studies on calf pneumonia occurring in mass rearing facilities. *Natl. Inst. Anim. Health, Q. (Jpn.)* **19**, 91-103.
- [11] Jericho, K. W. F. (1979). Update on *Pasteurella* rellosis in young cattle. *Can. Vet. J.* **20**, 333-335.
- [12] Kuniyasu, C., Yoshida, Y., Ueda, H., Sugawara, H., and Ito, Y. (1977). Isolation of *Mycoplasma dispar* from pneumonic calf lungs in Japan. *Natl. Inst. Anim. Health, Q. (Jpn.)* **17**, 75-76.
- [13] Olmos, A., and Biberstein, E. L. (1979). Differentiation of *Pasteurella haemolytica* biotypes A and T with growth inhibitors. *J. Clin. Microbiol.* **10**, 231-234.
- [14] Schiefer, B., Ward, G. E., and Moffatt, R. E. (1978). Correlation of microbiological and histological findings in bovine fibrinous pneumonia. *Vet. Pathol.* **15**, 313-321.
- [15] Shreeve, B. J., Ivanov, I. N., and Thompson, D. A. (1970). Biochemical reactions of different serotypes of *Pasteurella haemolytica*. *J. Med. Microbiol.* **3**, 356-358.
- [16] Smith, G. R. (1961). The characteristics of two types of *Pasteurella haemolytica* associated with different pathological conditions in sheep. *J. Pathol. Bacteriol.* **81**, 431-440.
- [17] Wessman, G. E., and Hilker, G. (1968). Characterization of *Pasteurella haemolytica* isolated from the respiratory tract of cattle. *Can. J. Comp. Med.* **32**, 498-504.

要 約

子牛由来 *Pasteurella haemolytica* の血清型と生物型：中沢宗生・石野清之（家畜衛生試験場）——子牛から分離された *P. haemolytica* 113 株の血清型と生物型を調べた。肺炎子牛36頭の肺病変部由来85株は血清型 1，また，健康子牛の外見上正常な肺より分離された 1 株は血清型 2 であった。残りの 27 株は 5 株の型別不能株を除いて Frank の新しい血清型 13，14 および 15 に属していた。すなわち，1 株が血清型 13，4 株が血清型 14，また，17 株が血清型 15 であった。血清型 13（1 株），14（4 株）および 15（1 株）は血清型 1 が分離された肺炎子牛とは別の子牛の肺病変部からそれぞれ分離された。また，血清型 15 の 16 株は子牛の眼のスワブより分離された。一方，113 株の生物型はカタラーゼと β -ガラクトシダーゼの産生や数種類の糖の発酵パターンから生物型 A に該当すると考えられた。また，血清型 1 および 2 と新しい血清型 13，14 および 15 の生化学的性状の相違についても検討した。