日本で分離されたPasteurella multocida血清型A:3株を用いた牛感染試験

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NOTE *Bacteriology*

Experimental Infection of Calves with *Pasteurella multocida* Serovar A: 3 Isolated in Japan

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ABSTRACT. *Pasteurella multocida*, serovar A: 3, selected by pathogenicity in mice from among 10 strains isolated from pneumonic lesions of calves, was adjusted to 10^7, 10^8 and 10^{10} colony-forming units (CFU), and inoculated intratracheally into four calves. All calves showed pyrexia and had lungs with congestion and hepatization. Inoculation with 10^{10} CFU of bacteria produced respiratory symptoms and abscesses in lungs. This information will aid elucidation of the pathogenicity of *P. multocida* and the development of vaccines.
KEY WORDS: bovine, experimental infection, *Pasteurella multocida*.

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Bovine respiratory disease (BRD) is associated with significant economic losses in cattle production [5,16], but efficient measures to prevent this disease have yet to be established. BRD is primarily caused by infection with viruses such as parainfluenza virus-3 (PI3V), bovine respiratory syncytial virus (RSV), bovine herpes virus-1 (BH1V) and bovine adenoviruses (BAV). The disease may worsen with secondary bacterial and mycoplasmal infections when calves are placed under stressful conditions, for example during transport or following a change of environment [5, 16]. In Japan, vaccines against these viruses have been used to prevent BRD, but it still occurs endemically. To control this disease, the prevention not only of viral but also of bacterial and mycoplasmal infections may be essential.

Pasteurella multocida is one of the bacteria associated with BRD. It has been frequently isolated from pneumonic [1, 8, 18] and healthy [16, 17] calves. By itself, this bacterium does not usually cause serious disease, but it can be a significant pathogen if associated with infections with other bacteria [7], viruses [15], or *Mycoplasma* [14, 17] when calves are stressed [5]. It has been isolated frequently from the nasal cavity or pneumonic lesions of calves with BRD [8, 9, 13]. Most such isolates from calves are of capsular type A [5] and somatic type 3 [12]. The pathogenicity of capsular type A has not been clearly defined. There are several reports on experimental infections of calves with a strain of serovar A: 3 [4, 6]. However, these required large doses to reproduce pneumonic lesions. In this study, we aimed to reproduce pneumonia in calves by experimental inoculation with lower amounts of bacteria than the previous reports.

Ten strains of *P. multocida* were obtained from the National Institute of Animal Health (NIAH), Tsukuba, Japan. These were isolated from pneumonic lesions and identified as serovar A: 3 at NIAH. For comparison of growth capacity, 5×10^4 colony-forming units (CFU) of bacteria of each strain were inoculated into 100 m*l* of brain heart infusion (BHI) broth (Difco Laboratories, Tucker, GA, U.S.A.) and cultured at 37°C for 18 hr. The number of live bacteria per 1 m*l* was calculated in triplicate. All bacterial strains showed good growth with counts of $1.3–2.4 \times 10^9$ CFU/m*l* with no significant difference between strains (Table 1).

For comparison of pathogenicity between strains, 10 eight-week-old female ddY mice (Japan SLC Inc., Hamamatsu, Japan) were inoculated intraperitoneally with $1–2 \times 10^2$ CFU/m*l* of bacterial dilution for each strain. The dose of the inoculum was determined from previous reports [3, 10, 19]. Deaths were recorded daily for 7 days after the inoculation. Pathogenicity differed between strains, with strain BP165 showing the highest lethality (Table 1). This was therefore selected as the experimental strain.

Four male Holstein calves, approximately three months

Table 1. Growth capacity in medium and pathogenicity in mice of 10 strains of *P. multocida* isolated from diseased calves

Strains	Growth capacity	Pathogenicity in mice
	Bacterial yield[a)] (Mean ± S.D. × 10^9 CFU/m*l*)	Lethality[b)] (%)
BP151	1.8 ± 0.22	10
BP154	1.8 ± 0.053	10
BP156	1.6 ± 0.19	50
BP160	2.4 ± 0.13	80
BP163	1.3 ± 0.16	20
BP165	1.8 ± 0.19	100
BP174	2.0 ± 0.45	90
BP175	1.8 ± 0.078	70
BP177	1.7 ± 0.19	70
BP181	1.7 ± 0.29	40

a) Bacterial yield is shown as bacterial count after cultivation in BHI broth at 37°C for 18 hr.
b) Lethality was calculated at 7 days after inoculation into 10 mice.

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Table 2. Clinical signs, lung lesions and bacterial recovery from lung tissue of calves inoculated with *P. multocida* strain BP165

Calf	Dose (CFU/head)	Volume (m*l*)	Clinical signs				Lung lesion			Recovered bacteria[c]
			Attitude[a]	Appetite	Pyrexia[b]	Respiratory symptom	Congestion	Hepatization	Abscess	
0441	2.7×10^7	5	–	+	+	–	+	+	–	*P. multocida*
1639	7.4×10^8	5	–	+	+	–	+	+	–	*P. multocida*
0801	3.3×10^{10}	5	+	–	+	+(Cough)	+	+	+	*P. multocida*
8902	6.7×10^{10}	10	+	–	+	+(Cough)	+	+	+	*P. multocida*

a) –: Normal, +: depressed.
b) –: <40°C, +: 40–41°C.
c) Samples were collected from the lung lesions at one week (calves 0441 and 1632) or 6 weeks (calves 0801 and 8902) after inoculation.

of age and weighing ~100 kg were used for experimental infection. Serum samples were assayed for possible prior exposure to *P. multocida* by indirect enzyme-linked immunosorbent assay (ELISA) against whole bacteria, and for exposure to BH1V, PI3V, RSV, BAV and bovine viral diarrhea virus (BVDV) by neutralization tests. These four samples showed an optical density (OD) at 492 nm of < 0.3 when *P. multocida* positive samples showed an OD of 0.4–0.6. No calf had neutralizing antibodies for the five viruses.

All the calves were pyretic after the inoculation. Calf 0441, inoculated with 2.7×10^7 CFU of bacteria, had a rectal temperature above 40°C for 1 day. The other three had fevers for 2 day. Fevers of 39.5–39.9°C lasted for about 1 week and produced poor conditions in all the calves. Calves 0801 and 8902, inoculated with high doses of bacteria (10^{10} CFU) also showed deep coughing (Table 2). Coughing started 6 (calf 8902) and 10 (calf 0801) days after the inoculation, then worsened until the day of autopsy. In these 2 calves, depression and anorexia appeared the day after the inoculation, lasted for about 1 week, and then disappeared. Calves 0801 and 8902 were maintained for 6 weeks. IgG antibody titers against *P. multocida* had risen at the day of autopsy in these 2 animals.

At 1 week (calves 0441 and 1632) or 6 weeks (calves 0801 and 8902) after the inoculation, russet lung lesions with congestion and hepatization were present, mainly in the cranial lobes on the inoculated side (Figs. 1, 2), with pleural adhesions prominent. Divisions between the lesions were clear. The cranial lobes had hypertrophied and the insides were fibrosed. In calves 0801 and 8902, inoculated with high doses of bacteria, abscesses were observed in the same region (Fig. 2).

Bacteria were recovered from 4 lung lesions at levels of more than 10^4 CFU/0.5 g and identified as *P. multocida* by morphological and biochemical examinations (Table 2). No bacteria were detected from the unaffected regions of the lungs.

The prevention of *P. multocida* pneumonia is significant in the control of BRD. Experimental infections with *P. multocida* serovar A: 3 have been reported by several authors, but the methods and results differed considerably. We therefore aimed to establish experimental pneumonia using *P. multocida* serovar A: 3 in calves to find the most important immunogens and develop an effective vaccine.

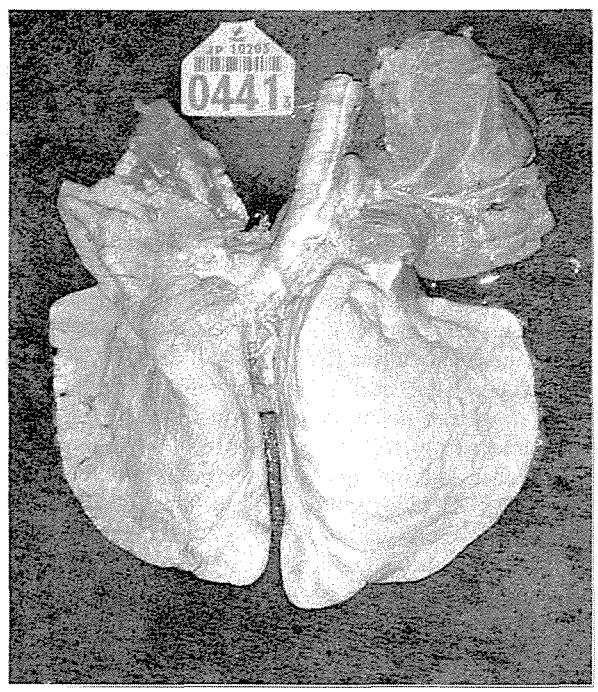

Fig. 1. A whole lung from a calf given 10^7 CFU of *P. multocida* strain BP165 (calf 0441). The cranial lobes show congestion and hepatization.

In previous experimental infections, only one strain of *P. multocida* was used [2, 4, 6, 11], therefore any differences among strains remain unknown. A high inoculation dose of 10^{10} CFU/m*l* and high inoculation volumes of 50–300 m*l* were required to produce serious pneumonic lesions [4, 6, 11]. Lower doses and volumes resulted in only mild disease [2]. In the present study, strain BP165 isolated from pneumonic lesions of a calf in Japan caused serious clinical signs and pneumonic lesions in calves at lower doses and volumes than previously reported. Furthermore, the calves inoculated with higher doses showed more severe clinical signs and pneumonic. We confirmed that the symptoms and lung lesions were caused by *P. multocida*, because the bacteria were recovered from the lesions. Reproduction of the disease in calves was successful because we used calves lacking initial antibodies against this bacterium and employed

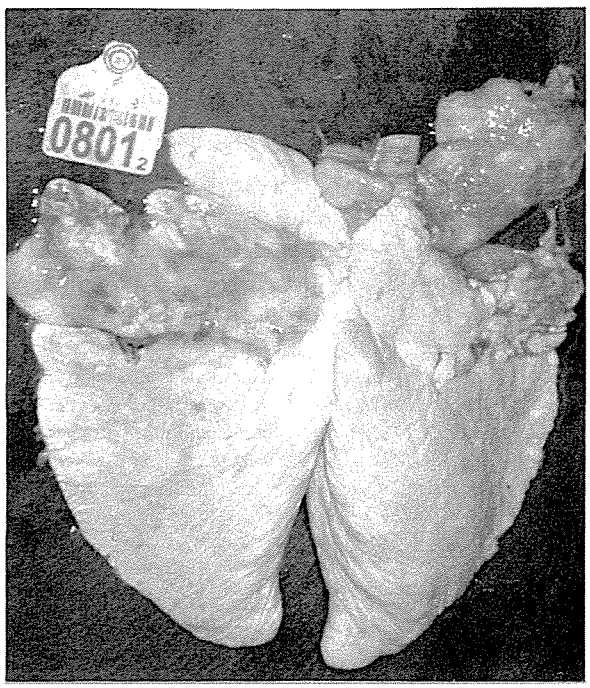

Fig. 2. A whole lung from a calf given 10^{10} CFU of *P. multocida* strain BP165 (calf 0801). The cranial lobes show congestion, hepatization and an abscess.

intratracheal inoculation to disperse the bacteria on the mucosal surface of the upper trachea. We also used a strain selected based on its growth capacity and pathogenicity against mice, whether theses properties relate to pathogenicity in cattle remains to be studied. Thus, respiratory disease and pneumonic lesions can be reproduced in calves infected with serovar A: 3 strain of *P. multocid*a. The model deserves further examination to elucidate the exact pathogenicity of this bacterium and to develop a vaccine.

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