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Serogroup F, a New Capsule Serogroup of *Pasteurella multocida*

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Four capsule serogroups (A, B, D, and E) have been described by using passive hemagglutination tests. Serogroups A and D predominate in pasteurelloses of avian species. A new capsule serogroup of *Pasteurella multocida* has been isolated from turkeys in Arkansas, California, Indiana, Iowa, Missouri, New Jersey, and Virginia. Strains belonging to the new serogroup were somatic serotype 1, 3, 7, or 12, and they varied in virulence for mice and poults. Antisera made in rabbits passively protected mice against challenge with the same serogroup regardless of somatic serotype.

Pasteurella multocida is one source of systemic and respiratory disease in a variety of domestic and feral animals. Several schemes have been developed for serologic classification and epidemiologic study of the organism (1, 3, 7, 11, 12, 14). These schemes have included specific agglutination, passive hemagglutination (PHA), passive protection of mice, and gel diffusion precipitin (GDP) tests. The PHA test for identification of specific capsule antigen (1) and the GDP test for identification of specific somatic antigen (7) are currently in prominent use. The mouse passive protection test (14), which correlates with the PHA test (4), is sometimes used for identification of specific capsule antigen in areas where hemorrhagic septicemia of cattle and buffalo is endemic, i.e., in Africa and Asia. Four specific capsule serogroups (A, B, D, and E) are recognized by the PHA test, and 16 specific somatic serotypes (1 through 16) are recognized by the GDP test.

It is especially difficult to prepare high-titered antisera against serogroup A- and D-specific antigens. This difficulty may be due in part to the production of inert capsule material, such as hyaluronic acid in serogroup A strains, which interferes with antigen recognition. Hyaluronic acid in serogroup A strains inhibits the reaction between antigen and antisera in the PHA test (2). To overcome the difficulties with the PHA test, nonserologic tests were developed for recognition of serogroups A and D. The tests are based on depolymerization of the hyaluronic acid capsule of serogroup A by *Staphylococcus aureus* (5) and on flocculation of serogroup D with acriflavine (6). Nonserologic tests are not available for recognition of serogroups B and E.

For most purposes, the combined use of the PHA (for serogroups B and E) and nonserologic (for serogroups A and D) tests is adequate for classification of the specific capsule groups of *P. multocida*. However, recognition of a new capsule serogroup with properties similar to those of serogroup A or D could make classification difficult.

In this report, we describe a new capsule serogroup of *P. multocida*, serogroup F, isolated from turkeys in different areas of the United States. Some strains of this new serogroup react similarly to those of serogroup D in the acriflavine test.

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MATERIALS AND METHODS

Bacteria. Nine strains of the new serogroup isolated from turkeys were submitted to our laboratory for somatic antigen typing. The origins of these strains and their somatic types as determined by the GDP test are listed in Table 1. Strains P-1059, M-1404, P-3881, and P-1235 are known strains which represent serogroups A, B, D, and E, respectively, and have been previously described (13).

The Cowan 1 strain of *S. aureus* was used for the hyaluronic acid depolymerization test.

Colony growth. Colonies were described after 18 to 24 h of growth on 5% horse blood agar and glucose starch agar plates. Iridescence was determined by viewing colonies with a stereo dissecting microscope with oblique transmitted light (9).

Biochemical tests. Determinations of production of acid and gas from carbohydrates in phenol red broth, nitrate reduction, urease activity, hydrolysis of gelatin, and the methyl red and Voges-Proskauer reactions were done as described elsewhere (8). Oxidase production was determined by the method of Kovacs (10), and catalase production was determined by the addition of 3% hydrogen peroxide to a colony on glucose starch agar. Determination of capsule hyaluronic acid depolymerization was done as described by Carter and Rundell (5), except that it was done on glucose starch agar. Flocculation with acriflavine was done as described by Carter and Subronto (6).

TABLE 1. Origins and somatic serotypes of capsule serogroup F strains of *P. multocida*

Strain	Origin	Serotype
P-1434	Virginia	3
P-2368	New Jersey	7; SL[a]: 3, 4, 12
P-2369	New Jersey	7; SL: 3, 4, 12
P-2481	Indiana	12
P-2718	Missouri	3
P-3428	Iowa	12
P-3695	Iowa	1
P-4218	California	3
P-4679	Arkansas	1

[a] SL, Serotypes with slight cross-reactions.

TABLE 2. PHA titers against extract-sensitized erythrocytes with antiserum against *P. multocida* capsule serogroups

Sensitizing extract	Titer[a] of antiserum to capsule serogroup:					
	A (P-1059)[b]	B (M-1404)	D (P-3881)	E (P-1235)	F (P-4218)	F (P-4679)
P-1059	2,048	<8	<8	<8	<8	<8
M-1404	<8	1,024	<8	<8	<8	<8
P-3881	<8	<8	256	<8	<8	<8
P-1235	<8	<8	<8	1,024	<8	<8
P-4218	<8	<8	<8	<8	256	256
P-4679	<8	<8	<8	<8	256	256

[a] Values are expressed as the reciprocal of the dilution with complete hemagglutination.
[b] Strain used to make antiserum.

Antiserum and serologic tests. Antisera against capsule serogroups A, B, E, and F were made in pasteurella-free rabbits as described elsewhere (13). Antiserum against capsule serogroup D could not be made in rabbits; therefore, it was made in a sheep by the procedure described elsewhere for rabbits (13). Strains used for antiserum production are listed in Table 2.

For determination of capsule serogroup, turkey erythrocytes were sensitized and PHA tests were done as described previously (13). Antisera were heated at 56°C for 0.5 h to inactivate complement and were adsorbed with normal turkey erythrocytes to remove heterophile antibodies.

For determination of the specific somatic serotype, the GDP test was done as described elsewhere (7).

Passive protection in mice. Groups of five male BALB/c mice (17 to 19 g) were inoculated intraperitoneally (i.p.) with 0.3 ml of antiserum. After 24 h, they were challenged with live organisms of the homologous or a heterologous capsule serogroup in 0.1 ml of tryptose broth (Table 3). The mice were observed for 7 days after challenge.

Virulence of strains. Serogroup F strains with different serotypes were tested for virulence in groups of five male BALB/c mice or 7-day-old turkeys by inoculating the animals i.p. with live organisms in 0.1 ml of tryptose broth. The influence of inoculation route on virulence was determined by inoculating groups of five 7-day-old turkeys intravenously or i.p. with different concentrations of live organisms in 0.1 ml of tryptose broth. After inoculation, the animals were observed for 1 week.

RESULTS

Colony growth and physiologic characteristics. All nine strains of the new serogroup produced colonies on agar medium that were similar to each other in regard to size, shape, and texture. On blood agar, colonies were about 1.5 mm in diameter, glistening, and convex with an entire edge. On glucose starch agar, the colonies had a pearllike iridescence in oblique transmitted light and were about 2.0 mm in diameter.

The physiologic characteristics of the nine strains are shown in Table 4. No strains of the new serogroup produced a hyaluronic acid-containing capsule, as evidenced by the lack of formation of small noniridescent blue colony types when the strains were grown in the presence of hyaluronidase-producing *S. aureus*. Control cultures of strains P-1059 (serogroup A) and P-3881 (serogroup D) produced characteristic reactions. Two of the nine new serogroup strains produced heavy flocculation in acriflavine that could not be distinguished from the reaction produced by the control serogroup D strain (P-3881).

PHA tests. Table 2 shows the PHA test reactions of the different control strains (serogroups A, B, D, and E) and of strains P4218 and P4679 with homologous and heterologous antisera. No reactions occurred between antisera or antigens made from serogroups A, B, D, and E and those made from strains P-4218 and P-4679, indicating that the latter two strains represented a new serogroup. Erythrocytes sensitized with extracts from other strains (Table 1) reacted in PHA tests only with antisera made against strains P-4218 and P-4679; titers were 1:256 against both antisera (data not shown).

Passive protection in mice. Passive protection of mice was evaluated at two levels of challenge with strains representing serogroups A, B, E, and F (Table 3). Strain P-3881 (serogroup D), several other serogroup D strains from our collection, and strain P-4679 were avirulent for nonimmu-

TABLE 3. Challenge of passive immunity of BALB/c mice with virulent capsule serogroups A, B, E, and F of *P. multocida*

Antiserum[a]	No. of dead mice/total tested when challenged by strain:							
	P-1059 (A:3) at:		M-1404 (B:2) at:		P-1235 (E:2) at:		P-4218 (F:3) at:	
	13.8 CFU	138 CFU	43 CFU	430 CFU	33 CFU	330 CFU	300 CFU	3,000 CFU
P-1059 (A:3)	0/5	2/5	ND[b]	ND	ND	ND	1/10	5/5
M-1404 (B:2)	ND	ND	0/5	0/5	ND	ND	10/10	5/5
P-3881 (D:12)	ND	ND	ND	ND	ND	ND	8/10	5/5
P-1235 (E:2)	ND	ND	ND	ND	0/5	0/5	10/10	5/5
P-4679 (F:1)	ND	ND	ND	ND	ND	ND	0/5	0/5
P-4218 (F:3)	ND	ND	ND	ND	ND	ND	0/5	0/5
None	5/5	ND	3/5	ND	5/5	5/5	5/5	ND

[a] In parentheses, the letter indicates the capsule serogroup, and the number indicates the somatic serotype.
[b] ND, Not done.

TABLE 4. Characteristics of serogroup F strains of *P. multocida*

Test	Reaction (no. of positive strains)
Production of:	
Oxidase	+ (9)
Catalase	+ (9)
H_2S (lead acetate strips)	+ (9)
Urease	− (0)
Hemolysis on blood agar	− (0)
Iridescence	+ (9)
Hyaluronic acid	− (0)
Motility	− (0)
NO_3 reduced	+ (9)
Hydrolysis of gelatin	− (0)
Indole	+ (9)
Methyl red reaction	− (0)
Voges-Proskauer reaction	− (0)
Growth on MacConkey agar	− (0)
Flocculates with acriflavine	− (2)
Gas from glucose	− (0)
Acid from:	
Fructose	+ (9)
Glucose	+ (9)
Mannose	+ (9)
Galactose	+ (9)
Rhamnose	− (0)
Xylose	− (2)
Arabinose	− (1)
Raffinose	− (1)
Maltose	− (0)
Sucrose	+ (9)
Lactose	− (1)
Trehalose	− (1)
Sorbitol	+ (9)
Mannitol	+ (9)
Dulcitol	− (0)
Inositol	− (0)
Glycerol	+ (9)
Inulin	− (0)
Dextrin	− (0)
Salicin	− (0)

nized mice when tested at challenge levels of 10^5 organisms or less. Therefore, passive protection could not be completely evaluated with regard to homologous serogroup. From the data, it is evident that none of the antisera representing serogroups A, B, D, and E protected mice against a challenge of 3,000 CFU of strain P-4218, whereas antisera against strains P-4218 and P-4679 did. Antisera against strains P-1059 (serogroup A) and P-3881 (serogroup D) protected slightly against challenge with strain P-4218 at a challenge level of 300 CFU.

Virulence of serogroup F strains. Strains of serogroup F varied in virulence (Table 5). Strains that were highly virulent for turkeys were also highly virulent for mice. No correlation was seen between virulence and somatic serotype. The inoculation route influenced virulence. Of two strains tested (Table 6), both were more virulent when animals were inoculated i.p.

DISCUSSION

A new capsule serogroup of *P. multocida* was found which had biochemical and colony characteristics typical of other serogroups of the species. Two of the nine serogroup F strains produced a floccular reaction with acriflavine that was indistinguishable from that seen with a control serogroup D strain. Therefore, with the exclusive use of nonserologic methods to identify serogroups A and D, serogroup F would probably be classified either as untypable or as a serogroup D strain.

Passive protection tests in mice to determine capsule serogroup are not routinely done; they are more expensive than other tests and require that a strain be virulent. As shown by our data, serogroup F may vary in virulence. Therefore, weakly virulent or avirulent serogroup F strains may not be distinguished by passive protection tests. In addition, depending on the level of challenge organisms, some cross protection can occur when the test is used with unadsorbed antisera. This cross protection is probably due to specific serotypes and common antigens shared among *P. multocida* strains.

Among the serogroup F strains, four somatic serotypes were found. These included serotypes 1 and 3, which are the serotypes most frequently isolated from avian species with fowl cholera. Because of the difficulty in doing the PHA tests, only somatic serotyping is usually done in epidemiologic surveys of avian *P. multocida*. The importance of serogroup F in the epidemiology of fowl cholera remains to be determined. The fact that the new serogroup was isolated

TABLE 5. Virulence of capsule serogroup F strains of *P. multocida* for mice and poults by i.p. inoculation

Somatic serotype	Strain	Virulence for mice		Virulence for poults	
		No. dead/total no. tested	CFU per inoculum	No. dead/total no. tested	CFU per inoculum
1	P-3695	0/5	281	ND[a]	ND
1	P-4679	0/5	270	1/5	210,000
3	P-1434	5/5	333	5/5	7
3	P-2718	0/5	370	1/5	18,000
3	P-4218	5/5	285	5/5	12
7; SL[b]: 3, 4 , 12	P-2369	ND	ND	1/5	200,000
12	P-2481	ND	ND	3/5[c]	200
12	P-3428	ND	ND	0/5	20,000

[a] ND, Not done.
[b] SL, Serotypes with slight cross-reactions.
[c] Five of five poults died when they were given 2,000 CFU.

TABLE 6. Influence of inoculation route on virulence of capsule serogroup F strains of *P. multocida* in poults

Strain (somatic serotype)	Inoculation route	No. of dead poults/no. tested at inoculum CFU of:								
		1.23	12.3	20.7	123	207	1,230	2,070	12,300	20,700
P-2481 (12)	i.p.			0/5		3/5		5/5		5/5
P-2481 (12)	i.v.[a]			ND[b]		1/5		3/5		4/5
P-4218 (3)	i.p.	0/5	5/5		5/5		5/5		5/5	
P-4218 (3)	i.v.	0/5	0/5		1/5		2/5		2/5	

[a] i.v., Intravenous.
[b] ND, Not done.

from turkeys with different geographic origins indicates that it is widespread.

LITERATURE CITED

1. **Carter, G. R.** 1955. Studies on *Pasteurella multocida*. I. A haemagglutination test for the identification of serological types. Am. J. Vet. Res. **16**:481–484.
2. **Carter, G. R.** 1958. Some characteristics of type A strains of *Pasteurella multocida*. Br. Vet. J. **114**:356–357.
3. **Carter, G. R.** 1962. Further observations on typing *Pasteurella multocida* by the indirect hemagglutination test. Can. J. Comp. Med. Vet. Sci. **26**:238–240.
4. **Carter, G. R.** 1964. Correlation between hemagglutinating antibody and mouse passive protection in antipasteurella (*Pasteurella multocida*) sera. Can. J. Microbiol. **10**:753–756.
5. **Carter, G. R., and S. W. Rundell.** 1975. Identification of type A strains of *Pasteurella multocida* using staphylococcal hyaluronidase. Vet. Rec. **96**:343.
6. **Carter, G. R., and P. Subronto.** 1973. Identification of type D strains of *Pasteurella multocida* with acriflavine. Am. J. Vet. Res. **34**:293–294.
7. **Heddleston, K. L., J. E. Gallagher, and P. A. Rebers.** 1972. Fowl cholera: gel diffusion precipitin test for serotyping *Pasteurella multocida* from avian species. Avian Dis. **16**:925–936.
8. **Heddleston, K. L., and G. Wessman.** 1975. Characteristics of *Pasteurella multocida* of human origin. J. Clin. Microbiol. **1**: 377–383.
9. **Henry, B. S.** 1933. Dissociation in the genus Brucella. J. Infect. Dis. **52**:374–402.
10. **Kovacs, N.** 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature (London) **178**:703.
11. **Little, P. A., and B. M. Lyon.** 1943. Demonstration of serological types within the non-hemolytic pasteurellas. Am. J. Vet. Res. **4**:110–112.
12. **Namioka, S.** 1970. Antigenic analysis of *Pasteurella multocida*. Natl. Inst. Anim. Health Q. **10**:97–108.
13. **Rimler, R. B., and K. A. Brogden.** 1986. *Pasteurella multocida* from rabbits and swine: serologic types and toxin production. Am. J. Vet. Res. **47**:730–737.
14. **Roberts, R. S.** 1947. An immunological study of *Pasteurella septica*. J. Comp. Pathol. Ther. **57**:261–278.