

Capsular and Somatic Serotypes of *Pasteurella multocida* Isolates Recovered from Healthy and Diseased Rabbits in Texas

YUE-SHOUNG LU,^{1*} STEVEN P. PAKES,¹ AND CONSTANTINE STEFANU²

Division of Comparative Medicine, Department of Pathology,¹ and Department of Family Practice and Community Medicine,² University of Texas Health Science Center at Dallas, Dallas, Texas 75235

Received 7 March 1983/Accepted 29 April 1983

A total of 111 *Pasteurella multocida* isolates recovered from healthy and diseased rabbits were typed for capsular and somatic antigens by the typing systems of Carter and Heddeleston, respectively. The major serotypes of the 48 *P. multocida* isolates recovered from nasal cavities of healthy rabbits were serotypes 12:A (33%), nontypable:A (50%), and nontypable:D (10%). Similarly, the major serotypes of the 63 *P. multocida* isolates obtained from rabbits with rhinitis, pneumonia, conjunctivitis, tympanitis, or cutaneous abscesses were serotypes 12:A (32%), nontypable:A (30%), and 3:A (16%). Serotype 12:A was predominant, regardless of whether the isolates were recovered from healthy or diseased rabbits.

It is well recognized that *Pasteurella multocida* infection in rabbits is common and widespread (11). Pasteurellosis can cause high morbidity and mortality but is most often characterized by subclinical infections of the respiratory, genital, and sensory systems. The effect of this disease on research rabbits is so profound that study of the pathogenesis, serotypes, and development of effective control and preventive methods is of the utmost importance.

In our efforts to develop live vaccines and test their efficacies in preventing rabbit pasteurellosis, it is necessary to obtain epizootiological data, such as serological identification of *P. multocida* isolates from clinically healthy and diseased rabbits and correlation of serotypes of *P. multocida* isolates to health states and disease patterns. These data are especially useful in selecting isolates for potential vaccine candidates. Studies in several species suggest that protective immunity elicited by *P. multocida* vaccines is probably associated with capsular (1, 10, 15) or somatic (12, 14) antigen or both, although there is not agreement as to whether one or both of the antigens are most important in stimulating immunity, which may vary with animal species (12, 14). Serotyping studies of rabbit isolates are limited (2, 3, 18), and the information is incomplete in that only one of these studies identifies both capsular and somatic antigens (9). Furthermore, the study (9) did not indicate whether the isolates were recovered from clinically healthy or diseased rabbits. We feel it is important to type rabbit *P. multocida* for both capsular and somatic antigens and to

correlate serotypes with health states in order to provide more precise information for selecting vaccine candidates. A rational approach for selecting potential candidates is to identify the most commonly distributed, antigenically characterized isolates associated with rabbit pasteurellosis. The purposes of this study were to determine the prevalence of capsular and somatic types of *P. multocida* isolates recovered from rabbits in Texas and to correlate the serotypes with health status and specific disease patterns.

MATERIALS AND METHODS

Bacterial isolates. A total of 111 *P. multocida* isolates were recovered from weanling and adult male and female rabbits supplied by two Texas rabbitries from 1977 to 1981. Forty-eight isolates were recovered from the nasal cavities of clinically healthy rabbits. Sixty-three isolates were recovered from diseased rabbits, of which 20 isolates were from nasal cavities of rabbits with rhinitis, 25 were from rabbit lungs with gross or microscopic (or both) lesions of pneumonia, and 18 were from rabbits with conjunctivitis, tympanitis, or skin abscesses. Each isolate was identified as *P. multocida* by standard procedures (4) and stored in stock culture agars (Difco Laboratories, Detroit, Mich.).

Capsular typing. Isolates were tested only for A or D capsular antigen, since capsular types B and E *P. multocida* isolates are not usually found in animal populations in North America (5). A staphylococcal hyaluronidase inhibition test was used to identify capsular type A *P. multocida*. This non-serological procedure is specific for the identification of capsular type A *P. multocida* (6). Type A *P. multocida* organisms manifest a diminution in colony size in the area surrounding staphylococcal streaks and loss of capsu-

lar hyaluronic acid, whereas types B, D, and E *P. multocida* organisms are not affected. An acriflavine flocculation test (7) was used to identify capsular type D *P. multocida* because these organisms are the only ones that flocculate and precipitate in an 0.1% aqueous solution of neutral acriflavine. Known capsular type A (strain PM 1062), type B (strain 656), type D (strain KOBE 6), and type E (strain Bunia II) *P. multocida* isolates were included as controls.

Somatic typing. (i) **Antigen extraction.** Organisms grown overnight in a heavily inoculated glucose starch agar plate (100 by 15 mm) were harvested in 0.7 ml of 0.01 M phosphate-buffered solution containing 8.5% NaCl and 0.3% Formalin, boiled in a 100°C water bath for 60 min, and centrifuged at $27,000 \times g$ for 10 min. The supernatant was saved and used for the antigen in the agar gel diffusion precipitin test (13).

(ii) **Antiserum preparation.** Ammonium sulfate-precipitated rabbit antibodies to the known 16 *P. multocida* somatic antigens were used. Specific antisera were prepared in pasteurized rabbits by repeated intramuscular injections of Formalin-killed *P. multocida* organisms of a known somatic serotype mixed with incomplete Freund adjuvant. The 16 somatic types of *P. multocida* were obtained from the National Animal Disease Laboratory, Ames, Iowa. The type and strain identifications of the 16 isolates are as follows: X-73, type 1; M-1404, type 2; P-1059, type 3; P-1662, type 4; P-1702, type 5; P-2192, type 6; P1997, type 7; P-1581, type 8; P-2095, type 9; P-2100, type 10; P-903, type 11; P-1573, type 12; P-1591, type 13; P-2225, type 14; P-2237, type 15; and P-2723, type 16. Antibody specificity is confirmed when the antibody reacts only with the specific somatic antigen in the agar gel diffusion precipitin test.

(iii) **Agar gel diffusion precipitin test.** A slightly modified procedure of a method described previously (13) was used. The agar gel was prepared by dissolving 0.9 g of Special Noble agar in 100 ml of 0.01 M phosphate-buffered saline (pH 7.2) containing 0.05% sodium azide. Five milliliters of the melted agar was placed on a slide (25 by 75 mm). Nine wells (3 mm in diameter, 8 mm from center to center) were cut and arranged in a circular pattern, with one well in the center and eight wells in the periphery. Usually, antiserum was placed in the central well and testing antigens were placed in the outer wells. The results were recorded after 72 h of incubation of the slide at room temperature in a humidified chamber. Known positive and negative sera and known antigens were included as controls.

Statistical analysis. Frequency tabulations and appropriate percentage values were calculated for each of the isolate categories. Basic statistics of proportions were also applied for comparative purposes. In addition, tests were conducted to determine whether the distribution of *P. multocida* isolates between healthy and diseased rabbits differed from a predetermined theoretical distribution computed by the use of the nonparametric chi-square statistic.

RESULTS

Capsular and somatic typing of *P. multocida* isolates. The 111 isolates recovered from healthy and diseased rabbits were typed for capsular and somatic antigens. All 111 isolates could be typed

for capsular antigens, with 92% of the isolates identified as type A and 8% as type D. In contrast, only 57% of the 111 isolates could be typed for somatic antigens, 43% being nontypable for the presently known 16 somatic antigens. The major somatic types of the 111 isolates were type 12 (45 of 111; 40%) and type 3 (13 of 111; 12%). Other somatic types were 1, 2, 6, 7, 10, 13, and 16 (20 of 111; 18%). Nine of the 111 isolates (8%) reacted with 2 or 4 of the 16 typing sera. When isolates were identified by both capsular and somatic antigens, the major serotypes were serotypes 12:A (32%), 3:A (9%), nontypable:A (37%), and nontypable:D (6%). *P. multocida* isolates which did not react with the 16 somatic typing sera were designated as nontypable serotypes; however, these isolates may have a somatic antigen comparable to the 16 established serotypes and possibly representing several somatic groups.

Correlation of *P. multocida* serotypes and health status of rabbits. Of the 111 *P. multocida* isolates, 48 were recovered from clinically healthy rabbits and 63 from diseased rabbits. The distribution of serotypes from healthy and diseased rabbits is shown in Table 1. The major serotypes from healthy animals were serotypes 12:A (33%), nontypable:A (50%), and nontypable:D (10%). Similarly, the major serotypes from diseased rabbits were serotypes 12:A (32%), 3:A (16%), and nontypable:A (30%). Statistical analyses of the nontypable and typable serotypes indicated that nontypable serotypes were isolated more frequently ($P < 0.01$) from healthy animals. Serotype 3:A was isolated only from diseased rabbits. When compared with the other typable serotypes, serotype 12:A was recovered more frequently ($P < 0.01$) from clinically healthy rabbits than from diseased rabbits and was the predominant isolate recovered from all rabbits.

TABLE 1. *P. multocida* serotypes and health status of Texas rabbits

Serotype	No. of isolates in:	
	Healthy rabbits	Diseased rabbits
Typable		
3:A	0	10
12:A	16	20
Miscellaneous	3 ^a	14 ^b
Nontypable		
Nontypable:A	24	17
Nontypable:D	5	2

^a 2:A, 16:A, and 10,12:A.

^b 1:A, 6:A, 16:A, 2,12:A, 10,12:A, 3,6,7,10:A, 3,6,12,13:A, 3,7,10,12:A, and 12:D.

TABLE 2. Serotype distribution of *P. multocida* isolates from rabbits with respiratory and nonrespiratory diseases

Serotype	No. of isolates in rabbits with:		
	Respiratory diseases		Nonrespiratory diseases ^a
	Rhinitis	Pneumonia	
Typable			
3:A	1	3	6
12:A	9	7	4
Micellaneous	4 ^b	7 ^c	3 ^d
Nontypable			
Nontypable:A	6	7	4
Nontypable:D	0	1	1

^a Conjunctivitis, tympanitis, or cutaneous abscesses.

^b 1:A, 6A:, 10,12:A, and 3,6,12,13:A.

^c 16:A, 2,12:A, 3,6,7,10:A, 3,7,10,12:A, and 12:D.

^d 6:A and 2,12:A.

Correlation of *P. multocida* and rabbit disease patterns. The 63 isolates (Table 2) from diseased rabbits were further divided into respiratory system and non-respiratory system isolates to determine whether a pattern existed between serotypes and lesions. Isolates recovered from rabbits with rhinitis (snuffles) or pneumonia were classified as respiratory isolates, and isolates from rabbits with conjunctivitis, tympanitis, or cutaneous abscesses were classified as nonrespiratory isolates, although it is recognized that infection of these sites may extend from a primary respiratory infection. Statistical analysis revealed that, compared with the other typable serotypes, 3:A was isolated much more frequently from rabbits with non-respiratory lesions than from rabbits with respiratory lesions ($P < 0.05$). Additional analysis of respiratory disease isolates did not reveal any statistical association between any one serotype and respiratory lesions.

DISCUSSION

Combined capsular and somatic typing data indicate that serotype 12:A is the major serotype associated with healthy and diseased rabbits in Texas. Capsular typing showed that all 111 isolates could be typed as A or D, with A greatly predominating (92%). *P. multocida* serotype A was also the predominant isolate (67%) from Michigan rabbits, whether diseased or healthy (18). Since *P. multocida* serotype A is apparently widespread in many animal species (3), it is not surprising that it is probably the major capsular type in rabbits. *P. multocida* somatic serotypes 12 and 3 were the most commonly identifiable somatic types in the present study,

with type 12 predominating (40%). These findings are in agreement with those of a recent somatic typing study of 48 *P. multocida* isolates from American and Brazilian rabbits (2), in which it was reported that somatic serotypes 12 and 3 were the two major types, with type 12 greatly predominating (75%). On the basis of our findings and the report (9) that 12:A was the major serotype among the 79 *P. multocida* isolates recovered from rabbits with unknown health status, we feel that *P. multocida* serotype 12:A is probably the major serotype associated with healthy and diseased rabbits in the United States. However, a large-scale study of rabbit populations from various geographic locations is required to make a definitive conclusion.

When we correlated serotypes with health and disease states of rabbits, we noted that *P. multocida* serotype 3:A was isolated only from diseased rabbits, whereas *P. multocida* serotype 12:A was isolated from healthy and diseased rabbits. Rabbits with respiratory lesions from which *P. multocida* serotype 3:A was cultured also had a higher frequency of conjunctivitis and tympanitis than diseased rabbits from which *P. multocida* serotype 12:A was cultured. This finding suggests that serotype 3:A may be more virulent than serotype 12:A. Studies from our laboratory (Y. S. Lu, S. P. Pakes, and J. E. Rehg, Abstr. Annu. Meet. Am. Assoc. Lab. Anim. Sci. 1982, 116, p. 65) have indeed shown that rabbits experimentally infected with *P. multocida* serotype 3:A have a higher mortality and more extensive lesions than rabbits infected with *P. multocida* serotype 12:A even though lower doses of serotype 3:A were used.

Since a high percentage of clinically healthy rabbits were found to carry serotype 12:A in their nasal cavities, we suspect that this serotype most often causes disease after a stressful event, such as sudden changes in environments or various experimental manipulations. Indeed, *P. multocida* serotype 12:A was the most prevalent isolate from diseased rabbits (Table 1). In addition, recent experiments (17) from our laboratory have shown that rabbits pretreated with hydrocortisone and experimentally infected with *P. multocida* serotype 12:A develop significantly higher prevalence and more severe lesions than rabbits not pretreated with hydrocortisone and infected with *P. multocida* serotype 12:A.

It was surprising that 43% of the 111 isolates were somatically untypable with the 16 known somatic antisera. Although the nontypable serotypes were isolated much more frequently from healthy rabbits, nontypable isolates were also recovered from diseased rabbits. A recent study (19) in Israel reported that a significant number (48 of 51; 94%) of *P. multocida* isolates from rabbits were somatically unidentifiable as one of

the 16 known somatic antigens. Further studies are needed to determine the somatic antigenic components of the nontypable *P. multocida* isolates and their significance in the pathogenesis of rabbit pasteurellosis.

Recent studies in our laboratory and another laboratory (8, 16) showed that live mutant vaccines produced from serotypes 12:A and 3:A *P. multocida* isolates significantly reduced or prevented disease in rabbits challenged with homologous virulent *P. multocida*. The observation in this study that serotypes 12:A and 3:A combined accounted for only about 50% of the *P. multocida* isolates has highlighted the consideration of using multivalent vaccines to control rabbit pasteurellosis on a broader basis. Also, the efficacy of cross-protection of monovalent vaccines in controlling rabbit pasteurellosis must also be evaluated to determine the need for mono- or multivalent vaccines.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grants RR01311 and RR00890 from the National Institutes of Health and by a grant from the Samuel Roberts Noble Foundation, Ardmore, Okla.

LITERATURE CITED

1. Bain, R. V. S. 1955. A preliminary examination of the antigens of *Pasteurella multocida* type 1. *Br. Vet. J.* 111:492-498.
2. Broden, K. A. 1980. Physiological and serological characteristics of 48 *Pasteurella multocida* cultures from rabbits. *J. Clin. Microbiol.* 11:646-649.
3. Carter, G. R. 1967. Pasteurellosis: *Pasteurella multocida* and *Pasteurella hemolytica*. *Adv. Vet. Sci.* 11:321-379.
4. Carter, G. R. 1979. Diagnostic procedures in veterinary bacteriology and mycology, p. 99-107. Charles C Thomas, Publisher, Springfield, Ill.
5. Carter, G. R. 1982. Whatever happened to hemorrhagic septicemia? *J. Am. Vet. Med. Assoc.* 180:1176-1177.
6. Carter, G. R., and S. W. Rundell. 1975. Identification of type A strains of *P. multocida* using staphylococcal hyaluronidase. *Vet. Rec.* 96:343.
7. Carter, G. R., and P. Subronto. 1973. Identification of type D strains of *Pasteurella multocida* with acriflavine. *Am. J. Vet. Res.* 34:293-294.
8. Chengappa, M. M., R. C. Meyers, and G. R. Carter. 1980. A streptomycin-dependent live *Pasteurella multocida* vaccine for the prevention of rabbit pasteurellosis. *Lab. Anim. Sci.* 30:515-518.
9. Chengappa, M. M., R. C. Meyers, and G. R. Carter. 1982. Capsular and somatic types of *Pasteurella multocida* from rabbits. *Can. J. Comp. Med.* 46:437-439.
10. Duo, S. K., and C. C. Panduranga Rao. 1978. Serological tests as indicators of immunity against *Pasteurella multocida* infection in sheep. *Can. J. Comp. Med.* 4:489-495.
11. Flatt, R. E. 1974. Bacterial diseases, p. 194-205. In S. H. Weisbroth, R. E. Flatt, and A. L. Kraus (ed.), *The biology of the laboratory rabbit*. Academic Press, Inc., New York.
12. Garfield, J., P. A. Rebers, and K. L. Heddleston. 1976. Immunogenic and toxic properties of a purified lipopolysaccharide-protein complex from *Pasteurella multocida*. *Infect. Immun.* 14:990-999.
13. 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.
14. Heddleston, K. L., and P. A. Rebers. 1972. Fowl cholera: cross-immunity induced in turkeys with formalin-killed in vivo-propagated *Pasteurella multocida*. *Avian Dis.* 16:578-586.
15. Kodama, H., M. Matsumoto, and L. M. Snow. 1982. Immunogenicity of capsular antigens of *Pasteurella multocida* in turkeys. *Am. J. Vet. Res.* 42:1838-1841.
16. Lu, Y. S., and S. P. Pakes. 1981. Protection of rabbits against experimental pasteurellosis by a streptomycin-dependent *Pasteurella multocida* serotype 3:A live mutant vaccine. *Infect. Immun.* 34:1018-1024.
17. Lu, Y. S., S. P. Pakes, J. E. Rehg, and D. H. Ringler. 1982. Pathogenesis of a serotype 12:A *Pasteurella multocida* in hydrocortisone-treated and non-treated rabbits. *Lab. Anim. Sci.* 32:258-262.
18. Lu, Y. S., D. H. Ringler, and J. S. Park. 1978. Characterization of *Pasteurella multocida* isolates from the nares of healthy rabbits and rabbits with pneumonia. *Lab. Anim. Sci.* 28:691-697.
19. Mushim, R., and M. Schoenbam. 1980. A strain of *Pasteurella multocida* associated with infection in rabbit colonies. *Lab. Anim.* 14:353-356.