JOURNAL OF CLINICAL MICROBIOLOGY, June 1980, p. 646-649
0095-1137/80/06-0646/04$02.00/0

Vol. 11, No. 6

Physiological and Serological Characteristics of 48 *Pasteurella multocida* Cultures from Rabbits

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Forty-eight *Pasteurella multocida* cultures collected from rabbits over a 56-year period were examined to determine their physiological characteristics and to determine their serological types in the gel diffusion precipitin test. Generally, the physiological characteristics from 30 tests were typical for *P. multocida*. There were a few atypical variations in the fermentation of lactose and maltose and variations in trehalose, dulcitol, xylose, sorbitol, and glycerol. Seven cultures did not produce indole, and four cultures did not produce detectable amounts of hydrogen sulfide. In obliquely transmitted light, 26 cultures formed large, slightly iridescent, mucoid colonies, 17 cultures had iridescent colonies, and 5 cultures had blue colonies. Heat-stable antigens from the 48 cultures reacted with antisera prepared from *P. multocida* type cultures representing serotypes 1, 3, 4, 12, and 15. Antigens from 15 cultures reacted slightly with antisera from more than one serotype. Overall, gel precipitin reactions involving serotype 3 (25%) and serotype 12 (66.7%) were the most prevalent.

Pasteurella multocida may infect the respiratory tract and the inner ears of rabbits, producing snuffles, pneumonia, or septicemia (8, 9). The mortality rate in these infections may range from 25 to 50% (1, 18).

The morphological and physiological characteristics of *P. multocida* from rabbits were first described as early as 1921 (7). Since then most studies have centered on reporting the pathology of the infection (9), serology (10), or the antibiotic sensitivities of the isolates (15). Some descriptions of rabbit cultures have been included in physiological studies of *P. multocida* isolated from a variety of hosts, but they have only included a few rabbit cultures (11). Other descriptions have been limited to enzootics in individual breeding or holding colonies.

Various methods have been used to serotype *P. multocida* cultures. Carter (5) and Carter and Bain (6) reported capsular types A and D among rabbit cultures with an indirect hemagglutination test. In contrast, types B and C were reported by Hagen based on a slide agglutination test using antisera to Carter's capsular types (10). By another method, *P. multocida* from a single holding colony of rabbits was typed as Namioka's capsular-somatic serotype 1:A (17).

The purpose of this study was to examine the physiological characteristics of 48 rabbit cultures of *P. multocida* collected from a variety of geographic sources over a 56-year period and to determine the serological types by using the gel diffusion precipitin test.

MATERIALS AND METHODS

The 48 *P. multocida* cultures of rabbit origin are listed in Table 1. These cultures have a variety of geographic origins, with no more than 10 cultures from any one source. The cultures were collected from 1922 to 1978, and, except for four that were lost before complete characterization, are maintained in a lyophilized state at 4°C. The 10 cultures from the National Animal Disease Center were collected over a 6-year period.

Lyophilized cultures were reconstituted in tryptose broth (Difco Laboratories, Detroit, Mich.) and streaked on dextrose starch agar (Difco). Their colonial morphology on dextrose starch agar was determined using obliquely transmitted light, and colonies were described as being iridescent, blue, or watery-mucoid.

The cellular morphology of the organisms was determined by Gram stain, and the presence of a capsule was determined by suspending a colony of organisms on a slide in a drop of saline and adding a drop of India ink. The suspension was mixed, covered with a cover slip, and examined. Capsules appeared as clear halos around the cells.

Physiological tests were performed as described (11, 14). Carbohydrate fermentation results were recorded after cultures were incubated for 24 and 48 h at 37°C and 7 and 14 days at 23°C. Indole production was tested for in a solution of 2.0% tryptose in 0.85% NaCl. Negative indole-producing cultures were retested daily up to 5 days. Hydrogen sulfide production was detected as described (13). Other physiological tests included changes in litmus milk, gelatin liquefaction, motility in semisoft agar, nitrate reduction, methyl red test, urease production, and growth on MacConkey agar.

TABLE 1. *P. multocida isolates of rabbit origin*

Source	Year	No. of cultures
N.Y. State Health Dept., N.Y.	1922	1
Not recorded[a]	1956	2
Animal Disease Station, Beltsville, Md.	1958–60	4
National Animal Disease Center, Ames, Iowa	1964–70	10
C. I. Boyer, Baltimore, Md.	1966	1
L. Leibovitz, Cornell Univ., N.Y.	1971	1
W. T. Derieux, Clemson Univ., S.C.	1972	1
Veterinary Diagnostic Laboratory, Springfield, Mo.	1972	1
R. E. Flatt, Iowa State Univ.	1973	4
G. R. Bubash, Pennsylvania State Univ.	1972	1
P. C. Kradel, Pennsylvania State Univ.	1975	1
D. L. Brooks, Univ. of California, Davis	1975	8
Inst. Bio. de São Paulo, Brazil	1975	10
R. B. Rimler, Univ. of Georgia	1977	1
National Institutes of Health, Bethesda, Md.	1978	2

[a] Origin of rabbit isolates is not known.

The gel diffusion precipitin test as described by Heddleston et al. (12) was used to serotype the rabbit cultures. This test has been used successfully to serotype cultures of *P. multocida* regardless of the host species from which they were isolated (2, 14). The agar gel consisted of 0.9% Noble agar and 8.5% NaCl in distilled water. Five milliliters of molten agar was pipetted onto standard microscope slides (25 by 75 mm). Heat-stable antigens from the rabbit cultures were prepared by suspending the overnight growth from a heavily inoculated dextrose starch agar plate in 1.0 ml of a solution containing 0.02 M phosphate buffer, 8.5% NaCl, and 0.3% Formalin (pH 7.0). The suspension was heated in a boiling-water bath for 1 h and centrifuged to sediment the cells. The supernatant fluid was used in the gel diffusion precipitin test. The major component responsible for the type specificity of the heat-stable antigen is thought to be a lipopolysaccharide (3). Standard antisera to each of the 16 *P. multocida* serotypes were prepared as previously reported (4).

RESULTS

When cultivated on blood agar, 26 of the rabbit cultures had large (2 to 2.5-mm), circular, convex, entire, viscous colonies after 24 h at 37°C. These cultures were slightly iridescent on dextrose starch agar when viewed with oblique transmitted light. Sixteen cultures had large (1- to 2-mm), iridescent, circular, convex, entire, smooth colonies on dextrose starch agar, and five cultures had small (0.5- to 1-mm), blue, circular, convex, entire, rough colonies. In addition, one culture had tiny, punctiform, iridescent colonies.

Gram-stained preparations from all cultures varied from uniform arrangements of gram-negative short coccobacillary-shaped rods occurring singly, in pairs, and occasionally in short chains, to preparations of gram-negative rods of varying lengths. Large distinct capsules were observed on organisms from watery-mucoid colonies, smaller capsules were observed on organisms from iridescent colonies, and no capsules were observed on organisms from blue colonies.

Glucose, sucrose, mannose, mannitol, galactose, and levulose were fermented by all cultures of *P. multocida*, but arabinose, raffinose, rhamnose, dextrin, inulin, salicin, and inositol were not fermented by any culture. A few cultures fermented lactose, maltose, trehalose, dulcitol, xylose, sorbitol, and glycerol (Table 2). Carbohydrate fermentation generally occurred within 18 to 24 h. However, some cultures slowly fermented the carbohydrates, requiring the reactions to be read after 3 days of incubation.

Forty-one of the 48 cultures produced indole. Seven cultures did not, even when retested daily for up to 5 days. Forty of 44 cultures produced detectable amounts of hydrogen sulfide within 14 days.

No liquefaction of gelatin, production of urease, or change in litmus milk was noted with any culture. None of the cultures grew on MacConkey agar or exhibited motility in semisolid media. All isolates reduced nitrates.

Heat-stable antigens from the 48 rabbit cultures reacted with antisera prepared from *P. multocida* type culture representing serotypes 1, 3, 4, 12, and 15 in the gel diffusion precipitin test. The distribution of rabbit cultures into these serotypes is presented in Table 3. Antigens from 15 cultures reacted slightly with antisera from more than one serotype. Overall, precipitin reactions involving serotypes 3 and 12 were the most prevalent, occurring 25 and 66.7%, respectively.

TABLE 2. *Fermentation patterns not common to all rabbit isolates of P. multocida*

Carbohydrate	No. of isolates	
	Positive	Negative
Lactose	2	46
Maltose	1	47
Trehalose	2	42
Dulcitol	1	47
Xylose	46	2
Sorbitol	38	6
Glycerol	40	4

TABLE 3. *Distribution of P. multocida serotypes of rabbit origin*

Serotype	Frequency
1	2
3	6
3 crossing with 2	1
3 crossing with 4, 12	5
4 crossing with 7	1
12	23
12 crossing with 2, 5	8
12 crossing with 14	1
15	1

Serotypes 3 and 12 were observed in rabbits regardless of where and when the cultures were isolated. Precipitin reactions involving serotypes 3, 12, and 15 were observed among the 10 cultures from Brazil, serotypes 3 and 12 were found among the 8 cultures from California, and reactions involving serotypes 3, 4, and 12 were seen among the 10 cultures from the National Animal Disease Center in Iowa. The two type 1 cultures were isolated from a domestic and a wild rabbit.

DISCUSSION

The physiological characteristics of the 48 cultures were typical for *P. multocida*. Even the few atypical variations that are listed in Table 2 have been reported (11). Trehalose, dulcitol, xylose, sorbitol, and glycerol fermentation have been observed to vary among cultures, but the fermentation of lactose and maltose are characteristics rarely observed (11). Several isolates were found not to produce detectable amounts of indole and hydrogen sulfide. Variations in definitive characteristics such as indole and H_2S production, as well as rare variations of lactose and maltose fermentation, make the few atypical isolates of *P. multocida* from rabbits difficult to identify, often necessitating serological confirmation.

The serological results in Table 3 show the prevalent serotypes of *P. multocida* from rabbits in this study to be types 3 and 12. Antigens from some of the cultures that reacted with antisera from either serotypes 3 or 12 also reacted slightly with antisera from other serotypes. The cross-reacting precipitin lines were generally weaker than the type-specific line. The significance of these cross-reactions in the gel diffusion precipitin test is not yet known.

Serotypes 3 and 12 have been isolated from a variety of hosts. Serotype 3 has been isolated from cattle, poultry, waterfowl, feral birds, and humans (2, 14). Serotype 12 has been isolated from poultry, cattle, cats, and humans (2, 14). Like many of the rabbit isolates, P-1573 (type strain of serotype 12 from humans) has large, slightly iridescent, viscous, watery-mucoid colonies.

A few of Heddleston's serotypes have been reported to be related to immunological antigens in those that have been examined. The total number of immunotypes within the 16 serotypes has not yet been determined (12).

If serotypes 3 and 12 are similar immunotypes, a monovalent bacterin of either type might provide protection. However, if they are distinct immunotypes, a cross-protecting bacterin, similar to that prepared by Rimler et al. (16), could be examined.

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