Isolation of *Pasteurella ureae* from Reproductive Tracts of Congenic Mice

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Infertility noted in two congenic strains of mice was associated with abortion, metritis, and stillbirths. *Pasteurella pneumotropica* was recovered from affected uterine tracts of ATL mice. Tetracycline treatment for 6 weeks was instituted in an attempt to minimize *Pasteurella*-associated infertility. Nine ATL mice and 12 ATH mice were examined 4 and 12 months after cessation of tetracycline therapy, respectively. Histopathologically, all animals appeared normal. *P. pneumotropica* was recovered from six of nine prepuces of both ATH and ATL mice; similarly, two of three vaginal cultures of the ATL mice yielded this bacterium. *Pasteurella ureae* was isolated from three of eight reproductive tracts of the female ATH mice. Negative indole and ornithine decarboxylase reactions and positive acid production for mannitol differentiated *P. ureae* from *P. pneumotropica*. This report represents the first confirmed isolation of *P. ureae* from animals. Biochemical profiles of atypical strains of *P. pneumotropica* isolated from animals are discussed and compared with those of *P. ureae*. The need for careful biochemical characterization of all *Pasteurella* isolates from rodents is emphasized.

Pasteurella infections in mice have been limited primarily to those caused by *Pasteurella pneumotropica*. The organism frequently produces a latent infection and is usually considered a secondary invader and opportunistic pathogen. Since its first isolation from mice (14, 15), *P. pneumotropica* has been recovered from abscesses of the skin, panophthalmitis, orbital abscesses (27), dacryoadenitis and conjunctivitis (23), metritis, cystitis, pleural abscesses, peritoneal abscesses and dermatitis (4), pneumonia (4, 8, 27), and suppurative or necrotizing metritis associated with abortion (25). Humans have also been infected with this organism (6, 19, 20), as have rats, hamsters, guinea pigs, dogs, cotton rats, kangaroo rats, and cats (4, 14, 22).

Another *Pasteurella*-like organism was first isolated from the human respiratory tract in 1960 by Henriksen and Jyssum, who described it as a variant of *Pasteurella haemolytica* (10, 11). This same organism was also isolated from sputum in 17 patients with chronic bronchitis and bronchiectasis and was named *Pasteurella ureae* based on distinct cultural and serological characteristics (16, 17). It has subsequently been isolated from humans suffering from pneumonia (21), meningoencephalitis (18), meningitis, and septicemia (2, 24). However, to our knowledge, *P. ureae* has never been isolated from animals. The purpose of this report, therefore, is to de-

scribe the isolation of *P. ureae* from the reproductive tracts of female mice of congenic origin.

CASE REPORT

In 1977, a small breeding nucleus of two congenic strains of mice, ATL and ATH, was obtained from Stanford Medical School and maintained at Brandeis University Foster Biomedical Animal Laboratory. The two strains were maintained as a breeding colony for approximately 18 months without incident other than occasional sporadic neonatal mortality. However, the animal research technician in charge of the breeding colony began noting infertility in the colony. Clinical examination of the near-term pregnant female mice revealed a greater than 20% incidence of abortion, stillbirths, and uterine infections. Histopathological evaluation of five affected uteri revealed acute suppurative metritis and in utero dead fetuses. *P. pneumotropica* was recovered from four (80%) of the five affected uteri. Both congenic strains of mice were placed on tetracycline in the water for 6 weeks to minimize infertility due to the apparent bacterial metritis and subsequent abortion. In an effort to determine the prevalence of *Pasteurella* infection remaining in the two strains of mice, 9 ATL mice (3 females, 6 males) were sacrificed 4 months after cessation of tetracycline therapy, and 11 ATH mice (8 females and 3 males) were sacrificed 12 months after tetracycline therapy was terminated. Neither strain was producing offspring 4 months before being sacrificed.

MATERIALS AND METHODS

Routine necropsies were performed on all mice submitted. Samples of all major organs and tissues were

placed in 10% buffered Formalin. All tissues were processed by conventional methods, sectioned, and stained with hematoxylin-eosin.

At the time of necropsy, specimens of uterus and vagina from all female mice, specimens of prepuce of all male mice, and the accessory sex glands and testes from the male ATH mice were collected aseptically and submitted for microbiological examination. All tissues were minced in sterile saline and plated onto blood-MacConkey split plates and chocolate agar and into thioglycolate and brain heart infusion broths. All media were incubated at 37°C in a 5% CO₂ atmosphere, and any broths showing growth were subcultured after 24 or 48 h.

Minced tissues also were plated onto a heart infusion-yeast extract-horse serum mycoplasma agar plate and into a mycoplasma broth tube. All inoculated mycoplasma media were incubated at 37°C in a candle jar, and mycoplasma broths were subcultured onto a second mycoplasma plate after 48 to 72 h.

Biochemical characterization of bacterial isolates was performed using heavy inocula from a chocolate agar plate and incubating at 37°C in a 5% CO₂ atmosphere for 48 to 72 h. With the exception of xylose, all carbohydrate media were commercial phenol red broth bases with 0.5% added carbohydrate. Xylose reactions were tested in bromcresol purple broth base. Christensen urea agar was used to test urease activity and SIM medium was used to test indole production; motility was tested using SIM medium and wet mounts; Moeller decarboxylase broth was the basal medium used to test ornithine and lysine decarboxylase reactions. Nitrate reduction and triple sugar iron reactions were determined by using conventional tubed media. All plate and tubed media were obtained from Scott Laboratories and GIBCO Diagnostics. The oxidase reaction was measured with PathoTec CO strips (General Diagnostics).

Tests of antimicrobial susceptibility to ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), kanamycin (30 µg), nitrofurantoin (300 µg), penicillin G (10 units), streptomycin (10 µg), and tetracycline (30 µg) were performed by the disk method of Bauer et al. (1) for isolates of *P. ureae*. Antibiotic sensitivities for isolates of *P. pneumotropica* were determined by a modification of the above method using Mueller-Hinton agar with 5% sheep blood (Scott). All antimicrobial disks were obtained from Difco.

RESULTS

Gross and microscopic examination of all tissues revealed no pathological abnormalities. All mycoplasma cultures were negative after 14 days of incubation at 37°C in candle jars.

Data on the isolation of *P. ureae* and *P. pneumotropica* from various reproductive tissues are presented in Table 1. *P. pneumotropica* was recovered from 67% of both male and female ATL mice and from 67% of male ATH mice. This organism was not recovered from female ATH mice. However, a second species of *Pasteurella*, identified as *P. ureae*, was recovered from 38% of female ATH mice. On blood and

chocolate agars at 24 h, colonies were 1 to 2 mm in diameter and were circular, convex, and smooth in appearance. No hemolysis of sheep blood was noted, although a slight green discoloration of the medium was produced. On Gram stain, highly pleomorphic gram-negative rods were observed. Cells often contained unstained vacuoles, and cell sizes ranged from short rods (1 to 2 µm) to short filaments (10 µm). Since the tentative identification indicated an organism not previously reported in animals, we sent the isolate to the U.S. Department of Agriculture, National Veterinary Laboratory Division in Ames, Iowa. The bacterium was confirmed as *P. ureae*.

The biochemical profiles of the *P. ureae* mouse isolates along with three profiles of human strains are presented in Table 2. The strains obtained from mice show complete biochemical agreement with the original strains of *P. ureae* isolated from the human respiratory tract by Jones (16). Biochemically, our isolate is in complete agreement with the criteria compiled by Weaver and Hollis for identification of *P. ureae* (26). Although it is reported that some strains of *P. ureae* may require added serum for enhanced growth, these strains from mice grew readily on unsupplemented media. The use of heavy inocula facilitated interpretation of carbohydrate fermentation reactions within 48 to 72 h. Indole reactions for *P. ureae* were negative at both 48 and 96 h.

Positive reactions for indole, ornithine and lysine decarboxylase, and lactose fermentation differentiated *P. pneumotropica* from *P. ureae*. Also, *P. pneumotropica* did not ferment mannitol. Morphologically, as compared to *P. ureae*, *P. pneumotropica* cells were less pleomorphic, did not form filaments, and did not appear to be vacuolated.

All *P. pneumotropica* isolates were sensitive to all antimicrobial agents tested. All *P. ureae* isolates were sensitive to all antimicrobial agents, with the exception of streptomycin, to which the bacterium was intermediately sensitive.

DISCUSSION

Three of the species of *Pasteurella*, i.e., *P. multocida*, *P. pneumotropica*, and *P. ureae*, are related biochemically, and *P. multocida* and *P. pneumotropica* often infect similar animal hosts. *P. pneumotropica* has been repeatedly cultured from various sites in both humans and mice. First described as a variant of *P. haemolytica* in 1960 (10), *P. ureae* was classified in 1962 as a separate species, based on distinct cultural and serological characteristics (16). Since that time,

TABLE 1. Incidence of *P. pneumotropica* and *P. ureae* in two congenic mouse strains

Strain	No. and sex	No. of sites positive/no. cultured					
		<i>P. pneumotropica</i>			<i>P. ureae</i>		
		Uterus	Vagina	Prepuce	Uterus	Vagina	Prepuce
ATL	3 F	0/3	2/3		0/3	0/3	
	6 M			4/6			0/6
ATH	8 F	0/8	0/8		2/8	3/8	
	3 M ^a			2/3			0/3

^a Accessory sex glands and testes all negative for *Pasteurella* spp.

TABLE 2. Biochemical characteristics of *P. ureae*

Tests for:	Results ^a			
	1	2	3	4
Hemolysis	— ^b	—	— ^b	— ^b
Motility	—	—	—	—
Gas from glucose	—	—	—	—
Acid from:				
Glucose	+	+	+	+
Xylose	—	—	—	—
Mannitol	+	+	+	+
Lactose	—	—	—	—
Maltose	+	+	+	+
Catalase	+	V	+	—
Oxidase	+	+	+	+
Growth on MacConkey agar	—	—	—	—
Urease	+	+	+	+
Indole	—	—	—	—
TSI ^c slant, acid	+	+	—	+
TSI butt, acid	+	+	—	+
Lysine decarboxylase	—	—	—	—
Ornithine decarboxylase	—	—	—	—
Nitrate reduction without gas	+	+	—	+

^a Isolates (reference): 1, mouse (this study); 2, human (26); 3, human (16); 4, human (2). — Negative; +, positive; V, variable.

^b No hemolysis, but green discoloration of medium noted.

^c TSI, Triple sugar iron.

it has been isolated from different body fluids and tissues of humans. The fact that *P. ureae* was not identified earlier in mice may in part be due to the variability of the biochemical reactions encountered when identifying isolates as *P. pneumotropica* or other *Pasteurella* spp. This was noted in an early investigation by Henriksen and Jyssum, describing *Pasteurella* strains from the human respiratory tract (11). In this study, two *Pasteurella* strains were classified as special biotypes of *P. multocida* because of their inability to ferment mannitol (11). A later paper by Henriksen corrected this classification on the basis of the fact that *P. pneumotropica* strains were invariably mannitol negative (9). This difficulty in differentiating the species was due to inability to correctly interpret fermentation reactions in conventional media. A review of the literature comparing biochemical characteristics of *P. pneumotropica* from mice

attests to the variability in biochemical reactions (Table 3). It is recognized that differences in media and incubation times among different investigators may account for some of this variability. It is interesting, however, that the inability to ferment mannitol, first noted by Henriksen as a consistent biochemical reaction for *P. pneumotropica*, has been verified in almost all subsequent reports surveyed, with the exception of three (12, 13, 25). In two of these reports, the *P. pneumotropica* strains with variable mannitol fermentation reactions were isolated from mouse uteri. Two of the studies which reported mannitol-variable *P. pneumotropica* strains also reported isolates that were indole negative. In one report, 5 of 9 isolates were indole negative (25), whereas 14 of 28 additional *P. pneumotropica* strains were indole negative (13). In another report, over half of the *P. pneumotropica* strains isolated from the uteri of rats and mice were indole negative and mannitol negative (5). In still another study, a strain of *P. pneumotropica* recovered from a contaminated specific-pathogen-free, barrier-maintained rodent colony was indole and ornithine decarboxylase negative (3). All other reports specify that isolates of *P. pneumotropica* are indole positive and mannitol negative.

The *P. ureae* isolates in this report and those *P. ureae* strains isolated by others are indole negative and mannitol positive. Clearly, some organisms isolated from mice and classified as *P. pneumotropica* in the literature, based on interpretations of only indole and mannitol reactions, could have been classified as *P. ureae*. It is interesting that all articles dealing with *P. pneumotropica* isolated from mice, with the exception of that by Ward et al. (25), fail to mention *P. ureae* as a possibility when noting variation in biochemical reactions. Ward et al. based their classification of indole-negative organisms as *P. pneumotropica* and not *P. ureae* on the isolates' ability to ferment trehalose and produce H₂S as detected by lead acetate strips (25). Significantly, data obtained by the Centers for Disease

TABLE 3. Biochemical profiles of *P. pneumotropica* from rodents

Biochemical characteristics	Results ^a								
	1	2	3	4	5	6	7	8 ^b	9
Indole	+	+	+	+	V	V	V	—	+
H ₂ S ^c	V ³	+ ² , — ⁴	— ¹ , + ²	+ ³	— ¹ , + ²				— ¹ , + ²
Growth on MacConkey agar	—	—		—	—		+	+	—
Lactose	+	+	+	+	V	V		—	+
Mannitol		V	—	—	V	V	—	—	—
Trehalose		+			+	V		+	

^a Isolates (reference): 1, mice (14); 2, mice (12); 3, mice, rats, and hamsters (4); 4, mice (27); 5, mice (25); 6, rats and mice (13); 7, mice and rats (5); 8, mice and rats (3); 9, mice (23). —, Negative; +, positive; V, variable.

^b Isolate reported as ornithine decarboxylase negative (3).

^c Methods of hydrogen sulfide gas detection: 1, Kligler iron or triple sugar iron agar; 2, lead acetate strips; 3, method not stated; 4, iron salts incorporated into medium.

Control on 97 strains of *P. ureae* indicate that 91% are positive for H₂S as detected by lead acetate strips (Robert Weaver, personal communication).

According to recent data (26) comparing *P. multocida*, *P. urea*, and *P. pneumotropica* (Table 4), urea and indole reactions will differentiate the three species, except for indole-negative strains of *P. pneumotropica*. These indole-negative *P. pneumotropica* strains are differentiated from *P. ureae* strains on the basis of negative acid production for mannitol and a positive reaction for ornithine decarboxylase. One hundred percent of *P. pneumotropica* strains are ornithine decarboxylase positive, compared to none of *P. ureae* isolates. Those *P. pneumotropica* strains which ferment lactose or grow on MacConkey agar can also be differentiated from *P. ureae*.

In this study, *P. ureae* was isolated from only one strain of female mice (ATL), and *P. pneumotropica* was isolated from the males of both congenic strains. The findings of *P. ureae* in normal-appearing uterine tracts of ATL mice is consistent with asymptomatic *P. pneumotropica* uterine infections seen in the ATH mice, as well

as the high incidence of asymptomatic *P. pneumotropica* uterine infections reported in the literature (4, 5, 7, 12). The significance of *P. ureae* in the reproductive tract and *P. pneumotropica* in the prepuce and vagina of infertile mice reported herein is not known. *P. pneumotropica* has also been found in the uteri of other non-productive breeding mice (4, 12).

Our earlier findings of abortion and metritis associated with *P. pneumotropica* agree with the recent findings of Ward et al. (25). Even though both strains had previously experienced abortions, none of the remaining mice examined several months later had demonstrable lesions. Administration of tetracycline during this period before sacrifice appeared to minimize the incidence of abortion and metritis within the mouse colony. Since all *Pasteurella* isolates were sensitive to tetracycline, this antibiotic regimen may account for the lack of pathology noted in the mice subsequently examined in this study. The recovery of tetracycline-sensitive strains of *Pasteurella* at necropsy was not unexpected, since tetracycline therapy was terminated 4 and 12 months before the sacrifice of the ATL and ATH mice, respectively.

It is not known whether *P. ureae* can be a cause of various pathological abnormalities similar to those previously reported in mice with *P. pneumotropica* infections. However, careful biochemical profiling of *Pasteurella* isolates from mice in future studies may delineate whether indole-negative, mannitol-positive isolates are indeed *P. ureae* and not atypical strains of *P. pneumotropica*.

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TABLE 4. Differentiation of three *Pasteurella* species

Biochemical characteristics	% Positive reactions ^a		
	<i>P. multocida</i>	<i>P. pneumotropica</i>	<i>P. ureae</i>
Urea	0	95	100
Indole	99	90	0
Ornithine decarboxylase	94	100	0
Growth on MacConkey agar	2	36	0
Acid from:			
Mannitol	78	2	99
Lactose	8	14	0
Xylose	2	36	0

^a From reference 26.

LITERATURE CITED

1. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 45: 493-496.
2. Bia, F., R. Marier, W. F. Collins, Jr., and A. von Graevenitz. 1978. Meningitis and bacteremia caused by *Pasteurella ureae*. Report of a case following intracranial surgery. *Scand. J. Infect. Dis.* 10:251-253.
3. Blackmore, D. K. 1972. Accidental contamination of a specific-pathogen-free unit. *Lab. Anim.* 3:257-271.
4. Brennan, P. C., T. E. Fritz, and R. J. Flynn. 1965. *Pasteurella pneumotropica*: cultural and biochemical characteristics and its association with diseases in laboratory animals. *Lab. Anim. Care* 15:307-312.
5. Casillo, S., and D. K. Blackmore. 1972. Uterine infections caused by bacteria and mycoplasma in mice and rats. *J. Comp. Pathol.* 82:477-482.
6. Copper, A., R. Martin, and J. A. R. Tibbles. 1973. *Pasteurella* meningitis. *Neurology* 23:1097-1100.
7. Flynn, R. F., P. C. Brennan, and T. E. Fritz. 1965. Pathogen status of commercially produced laboratory mice. *Lab. Anim. Care* 15:440-447.
8. Grey, D. F., and A. L. Campbell. 1953. The use of chloramphenicol and foster mothers in the control of natural pasteurellosis in experimental mice. *Austr. J. Exp. Biol. Med. Sci.* 31:161-166.
9. Henriksen, S. D. 1962. Some *Pasteurella* strains from the human respiratory tract. *Acta Pathol. Microbiol. Scand.* 55:355-356.
10. Henriksen, S. D., and K. Jyssum. 1960. A new variety of *Pasteurella haemolytica* from the human respiratory tract. *Acta Pathol. Microbiol. Scand.* 50:443.
11. Henriksen, S. D., and K. Jyssum. 1961. A study of some *Pasteurella* strains from the human respiratory tract. *Acta Pathol. Microbiol. Scand.* 51:354-367.
12. Hoag, W. G., P. W. Wetmore, J. Rogers, and H. Meir. 1962. A study of latent *Pasteurella* infection in a mouse colony. *J. Infect. Dis.* 111:135-140.
13. Hooper, A., and A. Sebesteny. 1974. Variation of *Pasteurella pneumotropica*. *J. Med. Microbiol.* 7:137-140.
14. Jawetz, E. 1950. A pneumotropic *Pasteurella* of laboratory animals. I. Bacteriological and serological characteristics of the organism. *J. Infect. Dis.* 86:172-183.
15. Jawetz, E., and W. H. Baker. 1950. A pneumotropic *Pasteurella* of laboratory animals. II. Pathological and immunological studies with the organism. *J. Infect. Dis.* 86:184-196.
16. Jones, D. M. 1962. A *Pasteurella*-like organism from the human respiratory tract. *J. Pathol. Bacteriol.* 83:143-151.
17. Jones, D. M., and P. M. O'Connor. 1962. *Pasteurella haemolytica* var. *ureae* from human sputum. *J. Clin. Pathol.* 15:247-248.
18. Kolyvas, E., S. Sorger, M. I. Marks, and C. H. Pai. 1978. *Pasteurella ureae* meningoencephalitis. *J. Pediatr.* 92:81-82.
19. Miller, J. K. 1966. Human pasteurellosis in New York State. *N.Y. St. J. Med.* 66:2527-2531.
20. Rogers, B. T., J. C. Anderson, C. A. Palmer, and W. G. Henderson. 1973. Septicemia due to *Pasteurella pneumotropica*. *J. Clin. Pathol.* 26:396-398.
21. Starkebaum, G. A., and J. J. Florde. 1977. *Pasteurella* pneumonia: report of a case and review of the literature. *J. Clin. Microbiol.* 5:332-335.
22. Van Dorssen, C. A., A. C. DeSmidt, and J. W. E. Stam. 1964. Over het voorkomen van *Pasteurella pneumotropica* bij katten. (The occurrence of *Pasteurella pneumotropica* in cats.) *Tijdschr. Diergeneesk.* 89: 674-682.
23. Wagner, J. E., R. G. Garrison, D. R. Johnson, and T. J. McGuire. 1969. Spontaneous conjunctivitis and dacryoadenitis of mice. *J. Am. Vet. Med. Assoc.* 155:1211-1217.
24. Wang, W. L. L., and G. Haiby. 1966. Meningitis caused by *Pasteurella ureae*. *Am. J. Clin. Pathol.* 45:562-565.
25. Ward, G. E., R. Moffatt, and E. Olfert. 1978. Abortion in mice associated with *Pasteurella pneumotropica*. *J. Clin. Microbiol.* 8:177-180.
26. Weaver, R. E., and D. G. Hollis. 1980. Gram negative fermentative bacteria, p. 242-262. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), *Manual of clinical microbiology*, 3rd ed. American Society for Microbiology, Washington, D.C.
27. Weisbroth, S. H., S. Scher, and I. Boman. 1969. *Pasteurella pneumotropica* abscess syndrome in a mouse colony. *J. Am. Vet. Med. Assoc.* 155:1206-1210.