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RECOVERY AND IDENTIFICATION OF *PASTEURELLA MULTOCIDA* FROM MAMMALS AND FLEAS COLLECTED DURING PLAGUE INVESTIGATIONS

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ABSTRACT: During the 12-yr period, 1973–1984, 243 isolates of *Pasteurella multocida* were recovered or identified from specimens submitted for plague tests. Of the isolates, 79% were from rodents, 10% from lagomorphs, and 7% from carnivores; eight isolates were recovered from pools of rodent or carnivore fleas, and two were recovered from cat-bite wounds of human patients. No correlations of host or geographic sources, season, or biotypic or serotypic characteristics were found. Of the rodent strains serotyped, most were found to be serotypes 1A or 3A, which suggests a possible epizootiologic role for rodents in outbreaks of avian cholera that commonly involve these serotypes.

INTRODUCTION

Pasteurella multocida is an opportunistically or facultatively pathogenic bacterium with an extensive mammalian and avian host range. For many animals, it is considered as a normal oral, or respiratory tract resident which, following some predisposing stress, may flare to produce pathologic lesions and, often, fulminating septicemia and death. It has been isolated from a variety of pathologic processes in human patients also, commonly following animal bites. For many non-bite associated cases, it is unclear whether or not *P. multocida* was resident prior to the lesion, but the organism does not appear to be "normal" flora among the general human population. The wide variety of conditions and lesions associated with it is indicative of its opportunistic nature.

Our recovery of 243 isolates from various sources during the 12-yr period 1 January 1973 to 31 December 1984 (71% or 173 during the 5-yr period 1980–1984) aroused our curiosity about the effect of *P. multocida* on rodent populations and also about the role of rodents in the ecology and epizootiology of pasteurellosis. Neither of these aspects has been widely studied.

The recovery of these *P. multocida* isolates was a fortuitous, but natural, outcome of the examination of mammalian and flea specimens submitted for plague tests. Recovery was accomplished easily because of our reliance on mouse inoculation rather than on in vitro culture methods using inhibitory media.

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MATERIALS AND METHODS

Specimens: With the two exceptions noted below, all mammalian specimens were submitted either as whole carcasses of animals found dead, or as excised tissues (spleen/liver) in Cary Blair or other transport media. Two specimens were received as pure cultures isolated from swabs of cat-bite wounds of human patients. Two specimens were received as pure cultures of: 1) abscess material and 2) sputum from different dogs. Another specimen consisted of spleen and liver tissues excised from a striped skunk (*Mephitis mephitis*) found sick and later euthanized. Carcasses were examined for gross lesions at necropsy. Portions of spleen and liver were excised, triturated and suspended in sterile saline. Fleas were identified and pooled (up to 25/pool) by species, host species, and geographic location. Flea pools were triturated and suspended in sterile saline. Laboratory mice (National Institutes of Health general purpose strain, Division of Vector-Borne Viral Diseases, Centers for Disease Control specific pathogen free colony) were inoculated subcutaneously

TABLE 1. Host distribution of 243 isolates of *Pasteurella multocida*, United States, 1973–1984.

Host	No. isolates
Lagomorphs	
1. *Sylvilagus bachmani*	2
2. *Sylvilagus auduboni*	10
3. *Sylvilagus* species and "rabbits"	10
4. domestic rabbit	1
Rodents	
5. *Eutamias minimus*	2
6. *Eutamias amoenus*	9
7. *Eutamias townsendi*	2
8. *Eutamias quadrivittatus*	1
9. *Eutamias quadrimaculatus*	4
10. *Eutamias speciosus*	2
11. *Eutamias* species	10
12. *Marmota flaviventris*	2
13. *Spermophilus richardsoni*	12
14. *Spermophilus beldingi*	1
15. *Spermophilus tridecemlineatus*	8
16. *Spermophilus variegatus*	5
17. *Spermophilus beecheyi*	49
18. *Spermophilus lateralis*	16
19. *Cynomys ludovicianus*	2
20. *Cynomys gunnisoni*	5
21. *Sciurus niger*	28
22. *Sciurus griseus*	1
23. *Sciurus aberti*	1
24. *Sciurus* species	1
25. *Tamiasciurus hudsonicus*	1
26. *Tamiasciurus douglasii*	7
27. *Glaucomys sabrinus*	2
28. *Thomomys bottae*	1
29. *Peromyscus maniculatus*	4
30. *Peromyscus* species	1
Rodents (cont.)	
31. *Neotoma lepida*	2
32. *Neotoma fuscipes*	3
33. *Neotoma* species	2
34. *Microtus montanus*	2
35. *Ondatra zibethicus*	1
36. *Rattus rattus*	3
37. *Rattus norvegicus*	1
38. *Mus musculus*	1
Carnivores	
39. *Canis domesticus*	2
40. *Mustela* species	1
41. *Mephitis mephitis*	1
42. *Felis catus*	10
43. (*Homo sapiens* after bite of *F. catus*)	2
44. *Felis rufus*	4
Flea pools (Siphonaptera, Insects)	
45. Rabbit fleas (*Cediopsylla inaequalis*) 1 from *Sylvilagus auduboni* 1 from *Lynx rufus*	2 pools
46. *Spermophilus variegatus* fleas (*Diamanus montanus*)	3 pools
47. *Cynomys gunnisoni* fleas (*Hoplopsyllus anomalus*)	1 pool
48. *Spermophilus beecheyi* fleas (*Diamanus montanus*)	1 pool
49. *Canis latrans* fleas (*Pulex simulans*)	1 pool

with 0.5 ml of individual tissue or flea suspensions. Inoculated mice were housed with adequate food and water and observed daily for 21 days. Spleens and livers were excised at necropsy of mice dying after inoculation with test materials, and were cultured on blood agar plates at 28 C.

Resultant bacterial colonies were identified by standard methods such as those outlined by Cowan and Steel (1965) or by using the API 20E® (Analytab Products, Inc., Carle Place, New York, New York 11514, USA) enteric bacterial identification kit. *Pasteurella multocida* was suspected for colonies showing appropriate growth, colonial morphology, odor, positive oxidase, and microscopic morphology and staining characteristics. Further biochemical characterization tests are discussed later.

RESULTS AND DISCUSSION

Specimens infected with *P. multocida* caused death of mice usually within 24 hr and always within 48 hr following subcutaneous inoculation. The agent was usually recovered in pure culture from blood agar plates inoculated with spleen and liver tissues excised at necropsy of the affected mice.

Data on host distribution (Table 1) re-

flected more those animals thought to be involved in plague cycles than they did a true distribution of *P. multocida* in mammals, which distribution is probably ubiquitous. Hosts comprised six species of carnivores, including the two human isolates which may be properly thought of as cat strains, at least two species of lagomorphs, and at least 30 species of rodents.

The presence of *P. multocida* in fleas is interesting, but probably only signifies that one or more flea in each pool had fed recently on a septicemic host. Three of the pools were taken from a single rodent from which the agent was also isolated. We doubt that fleas play a significant role as vector for *P. multocida*, although we have not tested this hypothesis.

Data on temporal distribution, shown in Table 2, likewise probably reflected dates of suspected plague activity and not a true representation of *P. multocida* activity.

Data on geographic distribution indicated that the sources of specimens were limited primarily to western states, not that *P. multocida* was so distributed. Few specimens collected in eastern U.S. locations are processed at this laboratory. It is interesting, however, that only three isolates originated in Arizona, a state that sends us a fair percentage of our specimens. Specimens collected in California yielded 82 isolates; Colorado, 79; Nevada, 51; New Mexico, 4; Oregon, 6; Texas, 2; Washington, 3; and Wyoming, 13. The numbers of isolates from these states are about proportional to the total numbers of specimens submitted from them.

All of the isolates from rodents were recovered from tissues of animals found dead. Except in two cases, *P. multocida* was the only pathogen, or opportunistic pathogen, recovered and, thus, the presumed cause of death. The excepted two rodents also yielded evidence of active plague infection, as did three carnivores. Most of the carcasses were found singly and in separate areas indicating sporadic disease rather than active epizootics. This is consistent with the widely held belief that *P. multocida* is resident, but not pathogenic, in the respiratory tract of most mammals, until some predisposing stress condition(s) allows the infection to proceed to fulminant, perhaps fatal, disease. The recovery of *P. multocida* from at least 32 species of rodent is consistent with the wide host range speculated for this agent.

TABLE 2. Temporal distributions of 243 isolates of *Pasteurella multocida*, United States, 1973–1984.

	Year	No.	Year	No.
Annual:	1973	10	1979	20
	1974	1	1980	16
	1975	4	1981	36
	1976	28	1982	28
	1977	5	1983	36
	1978	2	1984	56
	Overall annual average: 20 isolates/year. Annual average, last 5 years: 34			
	Month	No.	Month	No.
Monthly:	Jan.	5	Jul.	65
	Feb.	3	Aug.	49
	Mar.	2	Sep.	17
	Apr.	6	Oct.	5
	May	7	Nov.	2
	June	81	Dec.	1
	Season	No.	Season	No.
Seasonal:	Winter	9	Summer	195
	Spring	15	Fall	24

Many of the isolates have been sent to Dr. Jessie Price of the National Animal Health Laboratory in Madison, Wisconsin, for serotyping and further study. Serotyping information is available currently for 31 isolates: all are capsule serogroup A; 12 (39%) are serotype 1 or 1 with cross reactivity; 16 (42%) are serotype 3 or 3 with cross-reactivity, and 6 (19%) were nontypeable (Table 3). Serotyping results, so far, don't seem to correlate with host species or geographic origin.

Of the four capsular antigenic varieties of *P. multocida*, types B and E, which are

TABLE 3. Serotypic distribution of 31 isolates of *Pasteurella multocida*, United States, 1973–1983.

Somatic serotype	No. isolates	Source of isolates	
		Host species[a]	State[b]
1	6	11, 11, 17, 18, 29, 32	OR, CA
1, 5	6	15, 17, 17, 24, 33, 39	CA, CO
3	6	6, 10, 18, 22, 32, 41	CA, CO, NV
3, 4	3	17, 42, 44	CA, NV, NM
3, 4, 5	1	42	CO
3, 5	3	6, 17, 39	CA, NM
Non-reactive	6	15, 17, 17, 21, 21, 25	CA, CO, TX

[a] Host species: number corresponds to that given in Table 1 for each species.
[b] CA = California, CO = Colorado, NV = Nevada, NM = New Mexico, OR = Oregon, TX = Texas.

associated with the hemorrhagic septicemias sensu stricto of cattle, buffalo, and bison, are not common in North America. Type A strains of various somatic serotypes are the most common cause of fowl cholera and sometimes cause respiratory infections of mammals. Type D strains are the common causes of respiratory infections of swine and are isolated occasionally from other mammals (Carter, 1967).

Among isolates from migratory waterfowl, those from areas in the Central, Mississippi, and Pacific flyways were principally serotype 1A, and those from Atlantic flyway sites were serotype 3A or 4A. Isolates from non-waterfowl birds were more varied, but serotypes 1A and 3A accounted for 74% of the 55 isolates examined. A small number of mammalian isolates examined in this study were serotypes 1A, 2A, or 3A, but relationships between species, serotypes, or geographic locations were not evident (Brogden and Rhoades, 1983).

Only 31 of our isolates have been serotyped, but 81% fall into serotypes 1A or 3A, none were of capsule group D. The predominance of serotypes 1A and 3A suggests a possible role of small mammals, particularly rodents, in the epizootiological cycle of avian cholera, perhaps as reservoir or maintenance hosts.

None of the hosts of the isolates examined in this study are known to be from specific sites where large-scale avian cholera epornitics have occurred although many came from areas along the Pacific and Rocky Mountain flyways of migratory waterfowl. Further study is needed to define the precise role, if any, of rodents in the epizootiology of *Pasteurella* infections. Results of such studies may indicate, for example, that management of rodent populations in areas at high risk of avian cholera or pasteurellosis (e.g., wintering grounds for migratory waterfowl, zoos, other impoundments) would be helpful in reducing, if not eliminating, epornitics and/or epizootics.

Biochemical characterizations were initially tested with the API 20E system. We found 35 seven-digit profiles for the 131 isolates tested. Only 14 profiles are given for *P. multocida* in the API Data Registry. The most common profile we found (representing 16 isolates) is not indexed, nor were 27 other profiles found for one to eight isolates each. These aberrant API results were verified by repeat tests at least once and for four isolates twice. The profiles found were probably truly aberrant especially in light of conventional biochemical test results discussed later, but were indicative of the inadequacy of the system for use with Pasteurellae. A real deficiency found with the API system, however, was the negative oxidase test for many strains that yielded positive oxidase

TABLE 4. Results of conventional biochemical tests for isolates of *Pasteurella multocida* from small mammals, United States, 1973–1984.

Test	No. of strains tested	Result	Percent positive	Test	No. of strains tested	Result	Percent positive
Hemolysis	147	–	0	Acid from:			
Growth:				Adonitol	147	–	0
MacConkey agar	147	–	0	Alpha CH_3 glucoside	50	–	0
Citrate agar	147	–	0	Amygdalin	147	–	0
Urease	147	–	0	Cellobiose	147	–	0
DNase	147	–	0	Dulcitol	147	–	0
Catalase	147	+	100	Fructose	147	+	100
Oxidase	147	+	100	Galactose	147	+	100
ONPG	45	–	0	Glucose	147	+	100
NO_3	105	+	93	Glycerol	147	–	2
Esculin hydrolysis	105	–	0	Inositol	147	–	0
Gelatin hydrolysis	105	–	0	Lactose	137	–	0
Arginine dihydrolase	101	–	0	Maltose	147	–	0
Arginine decarboxylase	101	–	0	Mannitol	147	v	92
Lysine decarboxylase	101	–	0	Mannose	141	+	100
Ornithine decarboxylase	147	v	76	Melibiose	143	–	0
Motility 25 C	105	–	0	Melizitose	143	–	0
Malonate	105	–	0	Raffinose	143	–	1
Indol	147	v	60	Rhamnose	147	–	0
Voges Proskauer	105	–	0	Salicin	147	–	1
				Sorbitol	143	v	68
				Starch	44	–	0
				Sucrose	147	+	99
				Trehalose	147	v	84
				Xylose	147	v	93

tests by conventional methods, a finding in general agreement with that reported by Weaver and Collins (1982).

We feel we've had better success identifying *P. multocida* by the initial criteria outlined earlier (colonial and microscopic morphology, odor, positive oxidase, and lack of growth on MacConkey agar) than we've had relying on API results. We do not condemn API 20E; it was designed for use with and works well for enteric bacteria. The *P. multocida* included in API data bank were mostly human isolates that probably reflect only a small percentage of biotypes available. API profiles do not appear to correlate to other parameters—serotype, host species, or geographic origin.

In conventional biochemical test reactions (using agar formulations with phenol red indicator for carbohydrate fermentation rather than fluid media), the isolates of *P. multocida* which were tested were remarkably consistent—especially if compared to API results. Variable results were found for only six of 43 (14%) tests included in the battery. Our results (Table 4) generally agree with those reported by Heddleston (1976) for 1,268 isolates of *P. multocida* of diverse origin. Differences in the physiologic characteristics reported from these 1,268 strains, of which less than 6% were from small mammals, were not sufficient to allow reliable identification of host sources.

Another biotyping scheme—also based on human clinical isolates—was presented by Oberhofer (1981). His scheme, based

TABLE 5. *Pasteurella multocida* Oberhofer biotype patterns (1981).

	Biotypes (%)[a]										
Substrate	A (12)	B (25)	C (2)	D (12)	E (13)	F (8)	G (2)	H (6)	I (4)	J (10)	K (8)
Maltose	+	−	+	−	−	−	+	+	−	−	−
Mannitol	+	+	+	+	+	−	−	−	+	−	−
Xylose	+	+	+	+	+	−	+	−	−	−	−
Sorbitol	+	+	+	−	+	−	−	−	+	−	−
Trehalose	+	+	−	+	−	+	+	+	−	−	+W[b]
135 mammal strains studied:											Untypeable:
No.	15	60	4	17	5	—	—	—	—	—	33
%	11	44	3	13	4						24

[a] Percentage of human isolates found for biotypes.
[b] W = weak.

on fermentation patterns of five carbohydrates for 52 isolates, allows for 11 biotypes. As shown in Table 5, data from 135 of our isolates from mammals or fleas allow us to place most (44%) in biotype B, 13% in biotype D, 11% in biotype A, 4% in biotype E, and 24% were nontypeable. The Oberhofer biotypic patterns apparently don't correlate with the other parameters either (serotype, host species, geographic origin, or API profile).

Biotyping by schemata presented by Fredriksen (1973) or Carter (1976) was not attempted.

In summary, our isolates have diverse mammalian origins and diverse characteristics. Those of rodent origin seem to be distinct, but may be related to those isolates of fowl cholera of serotype 1:A or 3:A but not to isolates associated with hemorrhagic septicemias of wild or domestic livestock, which are largely of capsule type D.

A line listing of the strains studied is available on request from the authors, as are those strains that remain viable.

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