ISOLATION OF PATHOGENS OTHER THAN *Yersinia pestis* DURING PLAGUE INVESTIGATIONS

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Abstract: From 1975 to 1978, 37 isolates of *Pasteurella multocida*, 1 of *Salmonella enteriditis*, and 5 of *Francisella tularensis* were recovered from 42 mammalian specimens and 1 flea pool submitted for examination for evidence of infection with *Yersinia pestis*. Most of the specimens were collected during investigations of either a human plague infection or a reported epizootic among rodent populations. All specimens were of species regularly or occasionally involved in plague or tularemia cycles in nature and most were collected in areas of known plague or tularemia activity.

INTRODUCTION

During the 4-year period 1975-1978, 66 persons acquired infections with *Yersinia pestis* as a result of exposure to infected rodents and associated mammals or ectoparasites in the United States.[1] Numerous epizootics of plague among rodent populations also were observed during this period, especially during 1975 and 1976. Epidemiological-epizootiological investigations of the human cases and of some of the reported epizootics were undertaken by state and local health agencies and/or the Plague Branch, Vector-Borne Diseases Division, Center for Disease Control (CDC), to define the extent of the plague hazard to human populations and initiate appropriate control measures.

Many of the mammalian and ectoparasite specimens collected during these investigations were sent to the Plague Branch laboratory and examined specifically for evidence of infection with *Y. pestis*, or with *Francisella tularensis*, since human disease with either agent is clinically and epidemiologically similar. This report concerns the isolation of pathogens other than *Y. pestis* from the specimens processed.

MATERIALS AND METHODS

Mammalian specimens were received as whole carcasses or as excised tissues (spleen, liver, bone). Examinations for lesions were conducted when possible, and portions of spleen and liver (or other tissues) were taken for tests. Impression smears for microscopic examination by conventional staining or fluorescent antibody (FA) staining techniques were prepared, and the remainder of the tissues were triturated with sterile saline and sand. Small volumes (0.1-0.5 ml) of the tissue suspensions were inoculated subcutaneously into laboratory mice. Inoculated mice were held and observed daily for morbidity or mortality over a period of 3 weeks, after which healthy mice were discarded. Necropsies of mice dead or killed when moribund were conducted to obtain tissue specimens for recovery of bacterial agents. Small bits of spleen and liver were inoculated directly onto blood agar and cystine heart-blood agar plates and observed daily for the presence of bacterial colonies. Bacteria recovered were identified by conventional procedures.[2] Serotyping tests were not performed.

Fleas were identified and pooled according to host and location. Pools, con-

taining no more than 25 fleas, were triturated in sterile saline. Suspensions of the pools were then processed in the same manner as tissue suspensions.

RESULTS

Examinations were conducted on 2,097 mammalian specimens during the 4-year period, and cultures of four pathogens were recovered: *Yersinia pestis, Francisella tularensis, Pasteurella multocida,* and *Salmonella enteriditis.* In Table 1 are listed the total numbers of mammalian specimens processed and the numbers found positive for each of the four pathogens. One culture of *P. multocida* was isolated from a pool of fleas (15 *Monopsyllus eumolpi* and *M. ciliatus)* collected from an apparently healthy chipmunk.

Table 2 shows specific host, date and location of collection and data for each isolate of *P. multocida, F. tularensis,* and *S. enteriditis.* Comparable data for isolates of *Y. pestis* are available in Plague Branch Annual Reports, 1975-1978. Also presented in Table 2 are precedent or concurrent epizootics of plague reported in areas of *P. multocida* activity.

DISCUSSION

Although *P. multocida* has been described as a common member of the resident bacterial flora of the respiratory tract of numerous mammalian species and may act as an opportunistic pathogen during periods of physiologic stress,[3] it is noteworthy that this agent's activity and apparent mammalian pathogenicity coincided with, and may have been induced by, activity of the plague organism on several occasions.

To a large extent *P. multocida* was recovered from animals collected from populations concurrently or recently involved with plague, as shown in Table 2. This may simply reflect the fact that the bulk of specimens were collected and submitted in response to suspect plague activity, although many specimens were collected in areas where plague had not been detected in recent years. Only six isolates of *P. multocida* were recovered from these latter specimens. No gross lesions were seen at necropsy of any of the *P. multocida*-infected carcasses, indicating acute onset and rapid death. Usually, laboratory mice also demonstrated a rapid course, with death occurring 24 to 36 h after subcutaneous inoculation with *P. multocida*-infected tissues.

The recovery of *P. multocida* from a pool of fleas may reflect the host's bacteremic (or septicemic) condition and should not be construed as definitive evidence of the vector capacity of fleas for this agent. The usual mode of transmission is considered to be via droplet infection or ingestion (fecal-oral route or by cannibalism).[3]

One specimen, consisting of excised tissues identified only as from a "squirrel," collected in New Mexico, yielded a positive FA test result for *Y. pestis,* but the only bacterial agent which could be recovered from the tissues was

TABLE 1. Mammalian specimens processed for recovery of selected bacterial pathogens, 1975-1978.

	1975	1976	1977	1978	Total
Total Number Processed	395	984	354	364	2907
Number Positive:					
Y. pestis	90	109	22	16	237
P. multocida	4	25	4	3	36
F. tularensis	2	0	1	2	5
S. enteriditis	0	0	1	0	1

TABLE 2. Sources of *Pasteurella multocida, Francisella tularensis,* and *Salmonella enteriditis* isolates, and associated plague status.

Date	Number and Identity of Host	Agent Isolated[a]	Locality	Plague History[b]
ARIZONA				
August 1977	1 *Peromyscus maniculatus*	Pm	Apache Co.	RRP
CALIFORNIA				
June 1975	1 *Sylvilagus bachmani*	Pm	Los Angeles Co.	NPC
June 1975	1 *Spermophilus beecheyi*	Pm	Los Angeles Co.	NPC
July 1975	1 *Spermophilus beecheyi*	Pm	San Diego Co.	NPR
September 1975	1 *Spermophilus beecheyi*	Pm	Plumas Co.	PPC
July 1977	1 flea pool from *Eutamias* sp. (15 *Monopsyllus eumolpi, M. ciliatus)*	Pm	El Dorado Co.	RRP
July 1978	1 *Spermophilus lateralis*	Pm	Nevada Co.	PPC
COLORADO				
April 1976	1 *Spermophilus tridecemlineatus*	Pm	Boulder Co.	RRP
June 1976	1 *Scuirus niger*	Pm	Denver Co.	PPC
July 1976	1 *Spermophilus tridecemlineatus*	Pm	Denver Co.	PPC
July 1976	1 *Neotoma* sp.	Pm	Denver Co.	PPC
June 1976	2 *Spermophilus richardsoni*	Pm	Eagle Co.	PPC
July 1976	1 *Spermophilus tridecemlineatus*	Pm	El Paso Co.	PPC
July 1976	2 *Lepus californicus*	Pm	El Paso Co.	PPC
July 1976	1 *Cynomys ludovicinaus*	Pm	El Paso Co.	PPC
July 1976	2 *Peromyscus maniculatus*	Pm	El Paso Co.	PPC
June 1976	1 *Spermophilus richardsoni*	Pm	Garfield Co.	NPC
June 1976	1 *Spermophilus richardsoni*	Pm	Larimer Co.	PPC
June 1976	4 *Cynomys gunnisoni*	Pm	Montrose Co.	NPC "blasting"
June 1976	2 *Spermophilus richardsoni*	Pm	Pitkin Co.	PPC
June 1976	2 *Eutamias* sp.	Pm	Pitkin Co.	PPC
June 1976	1 *Tamiasciurus hudsonicus*	Pm	Pitkin Co.	PPC
June 1976	1 *Mustela* sp.	Pm	Weld Co.	PPC
June 1977	1 *Spermophilus variegatus*	Pm	Pueblo Co.	PPC
June 1977	1 *Lepus* sp.	Ft	Rio Grande Co.	NPC
August 1977	1 *Spermophilus variegatus*	Pm	Larimer Co.	P in 1976

TABLE 2. (continued)

Date	Number and Identity of Host	Agent Isolated[a]	Locality	Plague History[b]
May 1978	1 *Sylvilagus auduboni*	Ft	Fremont Co.	P in 1976
July 1978	1 *Lepus townsendi*	Ft	Fremont Co.	P in 1976
October 1978	1 *Sylvilagus auduboni*	Pm	Larimer Co.	P in 1976 "human contact"
KANSAS				
December 1975	1 *Sylvilagus* sp. (captive; collected in SE Kansas)	Ft	Riley Co.	NPR
NEVADA				
July 1975	1 *Sylvilagus* sp.	Ft	Washoe Co.	P in adj. CA
June 1978	1 *Sylvilagus auduboni*	Ft	Clark Co.	NPR
NEW MEXICO				
August 1976	1 *Felis domesticus*	Pm	McKinley Co.	RRP
January 1977	1 "squirrel"	Se	?	?
WYOMING				
June 1977	1 *Spermophilus richardsoni*	Pm	Laramie Co.	P in 1976
August 1977	1 *Spermophilus richardsoni*	Pm	Park	NPC

[a]Pm - *Pasteurella multocida*
Ft - *Francisella tularensis*
Se - *Salmonella enteriditis*

[b]RRP - recurrent rodent plague (plague focus)
NPR - no plague ever recorded
PPC - plague present concurrently
NPC - no plague concurrently
P in 1976 - plague in 1976
P in adj. CA - plague present in adjacent areas of California

Salmonella enteriditis. Although the host may have had a dual infection with both *Y. pestis* and *S. enteriditis*, the FA test result may have been falsely positive. With our purified fraction 1 conjugate and appropriate technical controls, however, false positive FA results have been rare. Exact location data were not received with this specimen, but in many areas of northern and central New Mexico ground squirrels have been involved in reported epizootics of plague.

Stress conditions which may have contributed to the induction of oportunistic pathogenicity or secondary invasion by *P. multocida* could include one or more of the following four factors. 1) Concurrent or precedent infection with viral agents or infection with helminth parasites but examinations were not conducted for either class of agent. 2) Other coincidental bacterial disease, e.g., plague. No dual infection with *Y. pestis* and *P. multocida* was demonstrated in any individual animal, but on several occasions, each agent was found in different members of the same populations as indicated in Table 2. 3) Overpopulation. *Spermophilus richardsoni* populations in Eagle and Pitkin Counties, Colorado, were very high in the summer of 1976 (pers. commun., D. Murphy, Vail, Colorado, and T. Dunlop, Aspen, Colorado). 4) Human activity. Ectoparasite and rodent control measures were applied in many areas to reduce the plague hazard. Dynamite blasting at a road construction site in Montrose County, Colorado, may have been the precipitating stress that induced pathogenicity by *P. multocida* among prairie dogs, from four of which the agent was recovered.

No confirmed human infections with *P. multocida* resulted from contact with any of the specimens reported here, although one man was treated empirically with penicillin (effective against *P. multocida)* for a local infection after he punctured his finger with a rabbit bone while extracting the bone from his pet cat. *P. multocida* was recovered at culture from the rabbit bone and a piece of its skin, but the lesion on the patient's finger was not cultured.

Disease agents (e.g., leptospira, rickettsiae, viruses, fungi) other than those recovered *(Y. pestis, F. tularensis, P. multocida, S. enteriditis)* also may have been active and responsible for mortality in the animal populations during this period, since the environmental and/or stress conditions favoring the occurrence and spread of epizootic plague, most probably, also would be conducive to the production of epizootics by other agents. The techniques applied, however, were not designed to demonstrate or isolate other agents.

These results reinforce the concept that agents which may constitute opportunistic pathogens in mammalian populations will be detected when actively sought. Some of these agents *(P. multocida, F. tularensis, S. enteriditis)* are pathogenic for humans when exposure is through the skin or mucous membrane. Their identity therefore, is in situations of human exposure to animals, their ectoparasites, or their feces.

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