

**ACKNOWLEDGMENTS**

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## Atypical Plague Bacilli Isolated from Rodents, Fleas, and Man

JAMES E. WILLIAMS, PHD, DANIEL N. HARRISON, PHD, THOMAS J. QUAN, PHD,  
JANET L. MULLINS, ALLAN M. BARNES, PHD, AND DAN C. CAVANAUGH, PHD

*Introduction*

Although most strains of the plague bacillus (*Yersinia pestis*) isolated from man, animals or fleas have been typical in terms of virulence determinants and susceptibility to antibiotic drugs, unusual forms have been encountered on occasion. In Vietnam, a virulent streptomycin-resistant strain of *Y. pestis* was isolated from a Norway rat (*Rattus norvegicus*) collected in Saigon in 1965.<sup>1</sup> During 1965 and 1966, two streptomycin-resistant and nine tetracycline-resistant strains were isolated from Vietnamese patients.<sup>2</sup> Obviously, drug resistant *Y. pestis* present a potential threat. In addition, plague bacteria lacking virulence determinants have been recovered from patients, including a strain deficient in the fraction 1 capsular antigen from a fatal case in the United States in 1957,<sup>3</sup> a nonpesticinogenic strain from a case in Arizona in 1967,<sup>4</sup> and nonpigmented *Y. pestis* from a clinically mild case in South Africa in 1971.<sup>5</sup> Such variants have been detected infrequently and fortuitously. The laboratory methods usually employed for the isolation of plague bacilli from clinical specimens favor the recovery of typical *Y. pestis*, so the natural occurrence of unusual forms might be greater than pres-

ent data suggest. Results reported herein represent deliberate attempts to assess the problems presented by aberrant *Y. pestis*.

*Methods*

In an effort to detect variant forms, plague strains isolated during 1975 and 1976 in the United States, 1974 in Vietnam, and from 1972 to 1974 in Central Java were screened for antibiotic susceptibilities and determinants of virulence. Clinical materials examined included bubo aspirates in Cary-Blair transport medium<sup>6</sup> from Vietnamese patients and tissues from rock squirrels (*Spermophilus variegatus*) collected in New Mexico. Original clinical specimens were not available for other isolates, therefore cultures established on agar slopes following animal passage were tested.

Typical plague bacilli are characterized by fraction 1 (F1) capsular antigen, pigmentation on certain culture media, a VW complex of antigens, and a PCF complex (pesticin I, coagulase, and fibrinolytic factor). Plague strains lacking any of these usually exhibit reduced virulence in laboratory animals.<sup>7</sup> Tests used to detect F1 antigen included antiserum-agar and Ouchterlony gel diffusion agar-plate techniques.<sup>8</sup> Tests for VW antigens, pigmentation and the PCF complex employed magnesium oxalate, congo-red and pesticin I agars, respectively.<sup>9</sup>

Antibiotic susceptibility tests employed discs (Difco, Detroit, Michigan) with 10 µg streptomycin or 30 µg tetracycline at 35°C on Mueller-Hinton agar.<sup>10</sup> Plates were swabbed with broth cultures photometrically standardized at 610 nm to give half the density of a McFarland no. 1 standard. Broth cultures of *Y. pestis* were prepared at 25°C, near optimal growth temperature, while broth cultures of *Escherichia coli*

Address reprint requests to Dr. James E. Williams. Drs. Williams, Harrison, and Cavanaugh (Chief) are in the Department of Hazardous Microorganisms, Division of Communicable Disease and Immunology, Walter Reed Army Institute of Research, Washington, DC, 20012; Dr. Barnes is Chief and Dr. Quan is Assistant Chief of the Plague Branch, Vector-borne Diseases Division, Bureau of Laboratories, Center for Disease Control, Fort Collins, Colorado; Ms. Mullins is Chief, General Microbiology Section, Health and Social Services Department, State of New Mexico Scientific Laboratory System, Albuquerque, NM. This paper, submitted to the Journal June 24, 1977, was revised and accepted for publication August 12, 1977.

TABLE 1—Observations of Atypical Plague Bacilli

Source	Number of <i>Y. pestis</i> Strains Tested	Observations of Atypical <i>Y. pestis</i>
United States, 1976:		
Plague patients	13	None*
Rock squirrels	4	One strain exhibited several pigment negative colonies.
United States, 1975:		
Plague patients	20	None*
Rock squirrels	6	None*
Vietnam, 1974:		
Plague patients	12	One strain was completely negative for pigment; 3 strains exhibited many pigment negative colonies.
Central Java, Indonesia, 1972-1974:		
Rats	2	None*
Rodent fleas	6	All strains exhibited a mix of VW positive and VW negative colonies.
Total	63	11 strains (17%) observed with some atypical bacilli.

\* Strains demonstrated FI and VW antigens, pigmentation, pesticin I, and susceptibility to discs of 10  $\mu$ g streptomycin (clear zone of inhibition of  $\geq$  15 mm) and 30  $\mu$ g tetracycline (clear zone  $\geq$  19 mm).

(ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), used as controls, were prepared at 35°C.

## Results

All plague strains tested, including isolates from patients in the 1975 and 1976 outbreaks of plague in the United States, were completely susceptible to streptomycin and tetracycline. The *in vitro* data indicate that these drugs remain effective.

While most of the plague strains examined possessed the virulence factors attributed to classical *Y. pestis* (Table 1), one strain from a 2 cm femoral bubo of a Vietnamese was entirely nonpigmented when isolated from Cary-Blair holding media and, after subsequent passage, from the spleens of laboratory rats. The patient, a 29 year-old woman, had been moderately ill with a temperature of 39°C and recovered in 2 days. Pigment-negative *Y. pestis*, mixed with pigmented *Y. pestis*, were found in several other holding media containing bubo aspirates from Vietnamese cases. Nonpigmented *Y. pestis* also accounted for 1-2 per cent of the plague bacilli isolated from triturated spleen and liver tissues of a rock squirrel collected during June 1976, in New Mexico.

Tests of Javanese plague strains indicated that the two isolates from rats were almost pure populations of VW-positive *Y. pestis*, whereas the six isolates from fleas were all mixed populations containing large numbers of plague bacilli lacking VW.

## Discussion

The results suggest that atypical plague bacilli may be quite common in nature, although pure populations of such organisms may not be the rule. Populations of *Y. pestis* infecting man, rodents, or fleas may include variants mixed with classical plague bacilli.

Nonpigmented *Y. pestis* appear to occur frequently. By employing special methods, nonpigmented strains have been isolated from fleas and rabbits collected in New Mexico,<sup>11</sup> and two human cases associated with pigment-deficient plague bacilli have been documented, one in South Africa,<sup>5</sup> and now one in Vietnam. Both were mild cases in young women. The strains recovered from these cases resembled the *Y. pestis* strains used as living-avirulent plague vaccines in some countries.<sup>12</sup> Such vaccines are not presently employed in the United States or South Africa but have been used recently in Vietnam.

The large numbers of *Y. pestis* lacking VW antigens in isolates from fleas collected in Central Java suggest that conditions in the flea may favor the selection or growth of such bacteria. Plague bacilli without VW presumably would be phagocytized and destroyed in a mammalian host. Exposure could, however, result in natural immunization, as encapsulated plague strains lacking pigmentation and the VW antigens function as excellent vaccines.<sup>13</sup>

Many questions concerning the natural occurrence and consequence of atypical plague bacilli remain. Experimental infections of laboratory animals with *Y. pestis* of varying

phenotype result in chronic and cryptic infections that present many problems for diagnosis, vaccination, and surveillance.<sup>14</sup> The experienced clinician in the midst of an epidemic expects to see patients exhibiting varying degrees of illness, and the possibility exists that infections with *Y. pestis* lacking certain virulence determinants could be responsible for many of the mild cases of plague (*Pestis Minor*) that have been observed throughout the history of this disease. The potential for human infection with variant plague bacteria may be better assessed by including a surveillance for atypical *Y. pestis* in surveys of rodents and fleas in enzootic plague foci in the United States or elsewhere.

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## New Mexico Health Day: A University-Community Partnership in Recruiting Health Professionals

BENSON R. DAITZ, MD, AND MAX D. BENNETT, PHD

Medical housestaff, nursing, and pharmacy students are not generally given any formal guidance in finding jobs at the conclusion of their training programs.

It has been assumed that the health care establishment is replete with opportunities for health professionals, and yet, rural and semi-rural community experience in recruiting health professionals has been fraught with difficulty. Communities are frequently unaware of their medical manpower needs. Questions of economic base, needs for specialists or

generalists, and organization of referral patterns are largely an unknown quantity to community lay persons interested in recruiting health professionals. Although some communities have been successful, the efforts of the Sears-AMA program and the Vermont Regional Medical Care Project, among others, have been terminated because of discouraging results.<sup>1, 2</sup>

The medical manpower distribution and recruitment problems in New Mexico are certainly similar and transferable to many areas in North America. New Mexico, as a predominantly rural state, has struggled with the problems of recruiting health professionals. The cities of Albuquerque, Santa Fe, and Los Alamos account for approximately 40 per cent of the state's 1,122,500 people. Sixty-seven per cent of New Mexico's physicians practice in these metropolitan areas. Twenty-four of New Mexico's 32 counties are classified as medically underserved according to the Federal Index of Medical Underservice.<sup>3</sup>

Address reprint requests to Dr. Benson R. Daitz, Assistant Professor, Department of Family, Community and Emergency Medicine, University of New Mexico School of Medicine, Albuquerque, NM 87131. Dr. Bennett is Assistant to the Vice-President for Health Sciences, UNM School of Medicine. This paper, submitted to the *Journal* March 14, 1977, was revised and accepted for publication July 29, 1977.