Chronic infections in laboratory rodents from inoculation of nonencapsulated plague bacilli *(Yersinia pestis)*

J. E. Williams and D. C. Cavanaugh

Department of Hazardous Microorganisms, Walter Reed Army Institute of Research, Washington, DC 20012 (USA), July 7, 1982

Summary. Infections with nonencapsulated *Y. pestis* persisted for 9 weeks in mice and 56 weeks in rats after experimental inoculation. Mice succumbed with bacteremia. Rats developed chronic lesions containing nonencapsulated plague bacilli.

Nonencapsulated (Fl^-) *Yersinia pestis* are plague bacilli that lack the ability to elaborate fraction 1 antigen (Fl) on their surface. Such organisms are potential causative agents of undiagnosed or misdiagnosed plague infection in man[1-3]. Problems with diagnosis arise because serological tests employed to detect plague infections rely on Fl. The frequency with which Fl^- *Y. pestis* occur in nature remains unknown, but Fl^- *Y. pestis* have been implicated in 1 human death[4]. Infections with Fl^- plague bacilli can develop in rodents following infection with encapsulated (Fl^+) *Y. pestis*[5,6]. This report describes the virulence, immunogenicity and persistence of Fl^- *Y. pestis* in rodents.

Materials and methods. White mice (Walter Reed ICR strain) and albino rats (Wistar strain)[7] having high susceptibility to lethal infection with virulent Fl^+ *Y. pestis* (LD_{50} = 1-10 bacilli) were inoculated with the Fl^- *Y. pestis* strain CPS-1, which originally came from the abdominal bubo of a vaccinated rat that died 424 days after challenge with the virulent Fl^+ *Y. pestis* strain 195/P[6]. Two titrations with Fl^- *Y. pestis* CPS-1 were done in mice. In each titration, 70 mice were inoculated (10 per decimal dilution). Rats were inoculated with Fl^- *Y. pestis* CPS-1 and later challenged with Fl^+ *Y. pestis* strain 195/P. Animals were inoculated and challenged by s.c. injection. Plague infection was confirmed by isolation of *Y. pestis* from tissues, bacteremia was demonstrated in blood films with Wayson's stain, and sera were tested for Fl antibody by passive microhemagglutination test[8].

Results. Deaths from plague occurred over 9 weeks in mice. The effect on mouse titrations was a progressive decrease in LD_{50}-values (table 1). None of 60 normal mice placed in cages with inoculated mice, to test for contact transmission, died of plague. Bacteremia was present at time of death in 97% (29/30) of mice that died of plague, and some mice died after inoculation of small numbers of bacilli (eg, 50 organisms). Titrations with Fl^- *Y. pestis* cultured at 25 and 37 °C gave similar results. Mice surviving the titrations were seronegative for Fl antibody.

Only 1 of 30 rats inoculated with Fl^- *Y. pestis* died of plague (table 1). Death occurred without bacteremia at 6 days post inoculation. Fl antibody was not present in sera of the other rats a month after inoculation. 25 rats were challenged with Fl^+ *Y. pestis*, and 9 (36%) survived (table 2). Survival was related to dose of Fl^- *Y. pestis* received before challenge. Most surviving rats remained seronegative after challenge, indicating that immunity from

Table 1. Virulence of Fl^- *Y. pestis* strain CPS-1 for mice and rats

Inoculum	Mouse LD_{50} 2 weeks	4 weeks	6 weeks	9 weeks	No. rats died/ No. inoculated
Y. pestis CPS-1 cultured at 25 °C	> 502,000	71,000	62,000	23,000	
550					0/8
850					0/3
50,000					0/4
600,000					0/4
Y. pestis CPS-1 cultured at 37 °C	> 121,000	121,000	55,000	43,000	
250					0/3
1,200					0/4
120,000					1/4

Table 2. Chronic infections with $F1^-$ *Y. pestis* in rats that survived inoculation of $F1^-$ *Y. pestis* and challenge with $F1^+$ *Y. pestis*

Challenge	No. $F1^-$ *Y. pestis* CPS-1 inoculated prior to challenge	No. survived/ No. challenged	Code letters for rats developing chronic lesions
1450 $F1^+$ *Y. pestis* 195/P	0	0/6	
75 days after inoculation of $F1^-$ *Y. pestis* CPS-1	250-850	2/6	A, B
13,800 $F1^+$ *Y. pestis* 195/P	0	0/6	
50 days after inoculation of $F1^-$ *Y. pestis* CPS-1	550-1200	1/11 (9%)	C
	50,000-600,000	6/8 (75%)	D, E, F, G

Rat	Titer of Fl antibody post challenge	Day of death post CPS-1	Lesions at time of death	Phenotype of *Y. pestis* isolated
A	< 1:4	150	Large abscess in spleen	$F1^-$
B	< 1:4	319	Large abscess in spleen	$F1^-$
C	< 1:4	110	Pleural bubo (left lung) and abscess in liver	$F1^-$
D	< 1:4	391	Pleural bubo (4 cm diameter) and two abdominal buboes (2-3 cm diameter)	$F1^-$
E	1:32	379	Abdominal bubo (3-4 cm diameter)	$F1^-$
F	< 1:4	269	Abdominal buboes (one 3 cm diameter)	$F1^-$
G	< 1:4	391	Abdominal buboes (one 3 cm diameter)	$F1^-$

inoculations of Fl^- bacilli frequently prevented active infections with Fl^+ *Y. pestis*. 7 rats developed remarkable lesions filled with purulent material and Fl^- *Y. pestis*. Buboes were first detected 2–3 months after inoculation of Fl^- *Y. pestis* and increased in size with time. Infections with Fl^- *Y. pestis* persisted over a year in rats.

Discussion. Laboratory mice are more susceptible than rats to fatal disease from Fl^- *Y. pestis*, and similar differences in susceptibility may exist among species of wild rodents, which differ in their susceptibility to Fl^+ *Y. pestis*[9–12]. Experimental data illustrate how Fl^- *Y. pestis* might arise[5,6] in wild rodents, persist via chronic infections, and immunize animals against acute plague, thereby reducing the potential for epizootic outbreaks in plague foci.

1 Williams, J.E., Harrison, D.N., Quan, T.J., Mullins, J.L., Barnes, A.M., and Cavanaugh, D.C., Am. J. Public Health *68* (1978) 262.
2 Poland, J., and Barnes, A., in: CRC handbook series in zoonoses, vol. 1, section A, p. 515. Ed S.F. Steele. CRC Press, Boca Raton 1979.
3 Cavanaugh, D.C., and Williams, J.E., in: Fleas, Proc. int. Conf. fleas, p. 245. Aston Wold, Peterborough, UK, 21–25 June 1977, Balkema, Rotterdam 1980.
4 Winter, C.C., Cherry, W.B., and Moody, M.D., Bull. Wld Hlth Org. *23* (1960) 408.
5 Burrows, T.W., Nature *179* (1957) 1246.
6 Williams, J.E., Harrison, D.N., and Cavanaugh, D.C., Trans. R. Soc. trop. Med. Hyg. *69* (1975) 171.
7 In conducting the research described in this report, the investigators adhere to the 'Guide for the Care and Use of Laboratory Animals'; as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council.
8 Bahmanyar, M., and Cavanaugh, D.C., in: Plague manual, p. 16, 17 and 26. World Health Organization, Geneva 1976.
9 Pirie, J.H.H., Publs S. Afr. Inst. med. Res. *3* (1927) 119.
10 Kartman, L., Prince, F.M., Quan, S.F., and Stark, H.E., Ann. N.Y. Acad. Sci. *70* (1958) 668.
11 Baltazard, M., WHO Chronicle *14* (1960) 419.
12 Isaäcson, M., Arntzen, L., and Taylor, P., J. Infect. Dis. *144* (1981) 80.

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Size and shape in *Poecilia reticulata*

G. Rocchetta, M.-L. Vanelli and C. Pancaldi

Institute of Genetics, University of Bologna, Via F. Selmi 1, I-40126 Bologna (Italy), May 25, 1982

Summary. In this paper some aspects of size and shape variation in 3 inbred lines of *Poecilia reticulata* reared under different environmental conditions are described. The results obtained suggest that size variation is principally due to environmental and/or developmental modifications; differences in shape, on the other hand, seem to be mainly correlated with a different genetic constitution of the inbred lines considered.

The difficulty of separating genetic from environmental variation of polygenic traits led to a situation in which studies which measure the degree of genetic variation within and between populations became rather rare[1]. However, since modern theories on evolutionary change stress the unity of the genotype[2], many researchers have again begun to carry out quantitative studies on morphometric variations of size and shape.

Spielman[3], in his study of anthropometric resemblance between men and women from the same and different villages, has shown that differences in shape and size can be correlated to genetic and environmental differences respectively. Festing[4] has shown that the shape of mandible in mice is a highly heritable trait, and that it can be used to determine single individuals as members of different inbred strains. The shape of the head has been also used by Templeton[5] to distinguish between 2 sympatric species of *Drosophila* which were otherwise very similar by electrophoretic and cytogenetic criteria. Finally, Atchley[6] in his study on the genetic components of size and shape in different lines of rats has pointed out that the allometric relationships estimated by him were highly heritable and would thus respond to selection.

The aim of this paper is to show the results of a preliminary investigation on size and shape estimates made in inbred strains of *Poecilia reticulata* reared under different environmental conditions and measured at different times of their post-embryonic development.

Materials and methods. The experiment was performed with 3 lines of *Poecilia reticulata* derived from 3 subsequent full-sib matings. All these lines, called A, B and C, were started with a single inseminated female (W) from a free breeding population. The complete scheme of breeding is described in a previous paper by Vanelli et al.[7].

Fish from each line were reared individually under 3 different environmental conditions: environment 1 = pots with 2000 ccm of water, environment 2 = pots with 250 ccm, environment 3 = pots with 100 ccm. The water was composed of $^2/_3$ tap water and $^1/_3$ distilled water. The environmental temperature was maintained at 25 ± 1 °C, with a photoperiod of 12 h. The fish were fed with standard dry food.

Body length and height were measured for each fish after anesthesia; the 1st measurement was from the distal opercular edge to the end of the spine, excluding the caudal pin; the 2nd was from the fore portion of the dorsal pin to the fore portion of the anal pin. These measurements were made in units of a micrometer (1 unit corresponds to 1.56 mm) and were collected fro fish aged 30, 40, 50, 60 and 70 days without sexual identification.

The values for size and shape were obtained from a system of Cartesian coordinates constituted by the X-variable = body length and by the Y-variable = body height. Each single individual can be identified in this Cartesian plane by a point P_i characterized by the pair of measures $x_i y_i$. The length of the vector connecting the measurement point P_i to the origin defines the size of the pth individual and the angle in radiants between the vector and the X-axis defines the shape of the same individual.