J. Hyg., Camb. (1977), **78**, 27
Printed in Great Britain

Decline of maternal antibodies to plague in Norway rats*

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(*Received* 18 *June* 1976)

SUMMARY

The decline of maternal antibodies to the fraction I antigen of *Yersinia pestis* was investigated in newly weaned *Rattus norvegicus* obtained from dams vaccinated with strain EV76(51f) of *Y. pestis*. IHA titre decreased by 50 % each 7·3 days and CF titre declined 50 % each 10·0 days in young rats. An analysis of available data indicated that maternal IHA and CF antibodies could persist to 3 months of age. Therefore, positive serologic reactions in young *R. norvegicus*, detected in the course of serological surveys, could be the result either of active immunization after exposure to the plague bacillus or of transient passive immunization (i.e. maternal antibody).

INTRODUCTION

Maternal antibody to plague has been found in offspring of laboratory Norway rats (Williams *et al.* 1974) and wild-caught gerbils (Levi & Suchkov, 1963), and some evidence indicates that it also occurs in guinea pigs (Jawetz & Meyer, 1944). Apart from any significance it may have in protecting young animals from disease, maternal antibody in young animals should augment the detection of plague foci with serological surveys, because the number of seropositive animals in an area is increased. In counterbalance, the success of flea and rodent control measures, designed to reduce the transmission of plague, could be undervalued should young animals with maternal antibody be misidentified as animals recently infected. The occurrence and persistence of maternal antibody in mammalian reservoirs of plague would seem, therefore, to be significant information for serological surveys and plague control programmes. Herein, we report information on the persistence of maternal antibodies to plague in *Rattus norvegicus*, a cosmopolitan species implicated as an urban reservoir of the disease (Macchiavello, 1954).

MATERIALS AND METHODS

Young albino rats (*Rattus norvegicus* Berkenhout, Wistar strain) were obtained from dams bred after subcutaneous vaccination with the attenuated Madagascar

* In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care', as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences – National Research Council, U.S.A.

Table 1. *IHA titres of female rats and newly weaned offspring*

IHA titre of dam	Geometric mean IHA titre of littermates	Titres of litter-mates (25 days old)
1/32	1/8	1/8, 1/8, 1/8, 1/8
1/64	1/19	1/16, 1/16, 1/16, 1/32
1/128	1/51	1/32, 1/64, 1/64
1/256	1/102	1/64, 1/64, 1/256
1/256	1/203	1/128, 1/256, 1/256
1/1024*	1/813	1/512, 1/512, 1/1024, 1/1024, 1/1024, 1/1024
1/2048	1/861	1/512, 1/1024, 1/1024, 1/1024

* Offspring born 8 months after vaccination of this dam.

strain EV76(51f) of *Yersinia pestis* incubated 24 h at 25° C in brain–heart infusion broth in order to obtain plague bacilli sensitive to phagocytosis (Cavanaugh & Randall, 1959). A single dose of 98,000 *Y. pestis* EV76(51f), as determined by colony counts, was administered to 1 dam that gave birth to a litter 8 months later. Another 6 dams received 3 inoculations with EV76(51f): the first contained 5400 organisms; the second, given at 4 weeks, contained 3800 organisms; and the third, given at 8 weeks, contained a larger booster dose of 25,600 organisms. Litters were born approximately 4 weeks after the third vaccination. All young were separated from their dams when 25 days of age.

Blood samples (0·5 ml/rat) were obtained by cardiac puncture from anesthetized young when 25, 40, 55, 70 and 80 days of age. Sera were tested for antibodies to the fraction I antigen of *Y. pestis* using microtitre adaptations (Cavanaugh *et al.* 1965) of indirect hemagglutination (IHA) and complement fixation (CF) tests. Titre represented the highest initial dilution of serum (i.e. before addition of antigen) showing 4+ haemagglutination or 4+ fixation of complement.

The periods of time in which IHA and CF titres declined by 50 % were calculated from regressions of titre as a function of age. Of various regression lines and curves investigated, the exponential form, $Y = ae^{bX}$ (where Y = titre, X = age, a and b = constants determined by regression analysis) fitted the data best (i.e. gave the highest coefficients of correlation).

RESULTS

IHA titres in offspring were correlated with the IHA titres of dams, and some newly weaned rats possessed a titre equal to their dam's (Table 1). The persistence of IHA antibody in young rats was in direct proportion to the titre at weaning, and the rate of antibody loss was similar in all young (Table 2). Loss-rates were calculated from the ten young rats shown in Tables 1 and 2 which possessed titres of 1/512–1/1024 when 25 days old. IHA titre declined 50 % each 7·9 days in male rats and each 6·9 days in females (Table 3). An analysis of variance showed, however, that this difference was not statistically significant (F = 4·03, D.F. = 1 and 4, $P > 0{\cdot}10$). When young males and females were considered together, IHA antibody titre decreased 50 % each 7·3 days.

https://doi.org/10.1017/S0022172400055893 Published online by Cambridge University Press

Table 2. *IHA titres at various ages in weaned offspring*

IHA titre at 25 days of age	Number studied	Geometric mean IHA titre			
		40 days	55 days	70 days	80 days
1/8	4	1/4	—	—	—
1/16	3	1/5	—	—	—
1/32	2	1/8	—	—	—
1/64	4	1/19	1/3	1/2	—
1/128	1	1/32	1/8	1/4	—
1/256	3	1/32	1/13	1/4	—
1/512	3	1/161	1/32	1/13	1/8
1/1024	7	1/256	1/39	1/14	1/5

Table 3. *Regressions of* $Y = ae^{bX}$ *(where* Y *= titre and* X *= days of age) for maternal antibodies*

Source of data	Data points	Values of constants		Coefficient of correlation	Days for 50% decline in titre
		a	b		
IHA, males	25	5,606	−0·088	−0·94	7·9
IHA, females	25	13,440	−0·101	−0·89	6·9
IHA, both sexes	50	8,680	−0·094	−0·91	7·3
CF, both sexes	12	439	−0·100	−0·97	10·0

Essentially equal loss rates of IHA titre were found in young born 1 and 8 months after the vaccination of dams was completed, as indicated by the very high correlation coefficients for the regressions of titre versus age (Table 3). In no case was a titre observed to increase after weaning in any of the young rats studied.

The persistence of CF antibody was studied in six litter-mates born 8 months after vaccination of the dam. Titres were detected only to 40 days of age. Geometric mean titre for the 6 rats was 1/37 (range = 1/32–1/64) at 25 days of age and 1/8 (range = 1/8) at 40 days of age. These data, although few, suggest a 50% decrease in maternal CF antibody approximately each 10·0 days.

DISCUSSION

In natural foci of plague, female rats probably develop antibodies as a result of sublethal infection with *Y. pestis* via fleabite. In simulating this method of vaccination to obtain data on maternal antibody representative of young *R. norvegicus* encountered in field situations, we employed living attenuated *Y. pestis* in a phagocytosis-sensitive condition to vaccinate dams, rather than a killed vaccine. The patterns subsequently observed for the loss of IHA antibody in offspring complemented data derived from foster nursing experiments in an earlier study (Williams *et al.* 1974), and young born 8 months after the dam's vaccination displayed similar loss-rate of IHA antibody as young born 4 weeks after a third vaccination of dams. We therefore consider it unlikely that any offspring were actively immunized by passage of *Y. pestis* EV76(51f) across placental membranes.

https://doi.org/10.1017/S0022172400055893 Published online by Cambridge University Press

On the average, maternal IHA titre declined 50% each 7·3 days and maternal CF titre declined 50% each 10·0 days in the offspring of vaccinated dams. These rates for loss of titre are possibly slight overestimates, since small amounts of maternal antibodies were removed in the process of bleeding the young rats. Any difference between the sexes in the persistence of antibodies, such as males retaining titres longer, is unlikely to be of consequence in serological surveys.

As this study demonstrated, newly weaned rats occasionally have titres of maternal antibody equal to the titres of their dams. Wild *R. norvegicus* have been found with IHA titres as high as 1/2048 (Cavanaugh *et al.* 1970; Hudson & Quan, 1975) and probably develop CF titres of at least 1/256, similar to wild mice (*Peromyscus maniculatus*; Cavanaugh *et al.* 1965). Therefore, some newly weaned wild rats may inherit IHA titres as high as 1/2048 or CF titres up to 1/256. At the minimum rates of decline measured in this study, maternal IHA or CF antibodies could persist in such young animals at detectable titre up to 3 months of age. Of course, most offspring of feral rats probably do not possess maternal antibodies for this length of time, simply because few feral dams have high titres (Cavanaugh *et al.* 1970; Hudson & Quan, 1975). Nevertheless, positive IHA and CF reactions in young *R. norvegicus* should be interpreted with caution, and active immunization as a result of infection with *Y. pestis* should not be assumed. Unfortunately, a simple and rapid method, adaptable to field surveys, is not available to distinguish plague antibody of maternal origin from antibody produced in response to infection in situations where questions of some urgency arise.

The serological patterns for maternal antibodies described herein for *R. norvegicus* are probably representative of those in other species of *Rattus*, and similar patterns might be expected in mice or other animals which obtain maternal antibody both *in utero* and while nursing. It should be borne in mind, however, that some species of animal obtain maternal antibody only while *in utero*. For this reason alone, the persistence of antibody cannot be assumed to be the same for all species.

The authors gratefully acknowledge the technical assistance of R. Huntley, D. Schaberg and T. Stowells.

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