The Complement-Fixation and Weil-Felix Reactions in a Study of Typhus Among Wild Rats[1]

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SINCE the announcement about five years ago[2] of the existence of murine typhus in Puerto Rico, over five hundred cases of the disease have been reported from San Juan and Santurce.[3] The number of reported cases has been increasing yearly, but it is not yet clear whether this figure represents an actual increase in incidence, or whether it is due to a growing awareness of the disease. We therefore thought it pertinent to carry out serological studies, from the point of view of murine typhus, of the wild rats trapped in both sections of the city.

A total of 443 rats was caught in San Juan and Santurce from December 7, 1944, to June 22, 1945; serums from these animals were tested by the complement-fixation (C.F.) and Weil-Felix (W.F.) reactions. The technique utilized for the C.F. test was that described by Bengtson,[4] but as endemic antigen was not available, epidemic typhus antigen was substituted.[5] For the W.F. reaction, an alcohol-treated proteus OX19 suspension, prepared from proteus OX19 strain No. 504 obtained from the National Institute of Health, Washington, D. C., was used.

RESULTS

Complement-Fixation Reaction. Thirty (30) serums were anti-complementary; of the remaining 413, 223 (54 percent) were positive and 190 (46 percent), negative. Two hundred and thirty-seven (237)

1. Received for publication October 17, 1945.

2. J. A. Pons, Is there Brill's disease in Puerto Rico? Bol.Asoc.Méd.de Puerto Rico, 32:196, 1940; There is endemic murine typhus in Puerto Rico. Puerto Rico Health Bulletin, 5:383–386, 1941.

3. San Juan is the capital of Puerto Rico (latitude 18° 30′ N.) and is made up of San Juan proper, the old town where the commercial and shopping sections are located, and Santurce, which is mainly residential. Their combined population is around 200,000.

4. I. A. Bengtson, Complement fixation in the rickettsial diseases. Technique of the test. Pub. Health Rep. 59, March 24, 1944.

5. Epidemic typhus antigen for the complement-fixation test, lot 285H618A. Lederle Laboratories, Pearl River, New York.

of the rats were classified as to size into small (25–50 g.), medium (50–100 g.), and large (100–200 g., or more). The results obtained are shown in the following table.

TABLE 1

Complement-Fixation Reaction in Wild Rats of Different Sizes

Size	*Small*	*Medium*	*Large*
Number Examined	24	72	141
Number Positive	10	40	98
Percentage Positive	42	55	70

A common practice in surveys of this type has been to capture large rats mostly, because of the relative ease with which these larger animals can be bled. However, such a procedure, as shown by Table 1, gives a higher proportion of positives than can be expected from a representative sample of the murine population of the area under investigation. It is probable that the larger (older) the animal, the longer the exposure, and the greater the probabilities of infection.

Three hundred and seventy-eight (378) animals were classified according to species. The results of the C.F. test are given in Table 2.

TABLE 2

Complement-Fixation Reaction in Wild Rats of Different Species

Classification	*Norvegicus*	*Rattus*	*Alexandrinus*
Number Examined	175	93	110
Number Positive	94	48	63
Percentage Positive	53	52	57

No significant differences were observed in the proportion of positive reactors among rats of different species (*norvegicus*, *rattus*, and *alexandrinus*).

One hundred and eighty-nine (189) animals were classified as to sex. Table 3 gives the results obtained.

TABLE 3

Complement-Fixation Reaction in Wild Rats of Different Sexes

Sex	*Male*	*Female*
Number Tested	66	123
Number Positive	40	70
Percentage Positive	60	57

Again no significant difference was noted in the proportion of male and female animals giving a positive complement-fixation reaction.

It is known that murine typhus, as reported from San Juan and Santurce, shows a definite seasonal variation in incidence. Table 4 illustrates this seasonal incidence.

TABLE 4

Cases of Murine Typhus Reported in San Juan, Puerto Rico, from July 1, 1944, to June 30, 1945

1944			1945		
July	*Aug.*	*Sept.*	*Jan.*	*Feb.*	*March*
34	8	6	7	8	11
Oct.	*Nov.*	*Dec.*	*Apr.*	*May*	*June*
6	3	4	17	21	33
Total					158

In order to determine whether there existed a corresponding variation in the proportion of rats giving a positive C.F. reaction, a number of animals trapped in the same places, but caught at various times of the year, were studied. One hundred and thirty-one (131) rats were taken from widely separated spots in San Juan and Santurce during the interval of January 1, 1945 to March 30, 1945. From April 1, 1945 to June 22, 1945, 88 more rats were trapped in these same places. The results obtained with these two groups of animals are shown in Table 5. For purposes of comparison, the number of cases of typhus fever reported during these periods of time is also given.

TABLE 5

Complement-Fixation Reaction in Wild Rats Captured in the Same Places from January 1 to March 30, 1945, and from April 1 to June 22, 1945, Together with the Number of Cases of Typhus Fever Reported in San Juan During These Same Months

Time of Year	*Jan. 1–March 30, 1945*	*April 1–Jan. 22, 1945*
Number of Animals Examined	131	88
Number of Positive Reactors	68	47
Percentage Positive	52	53
Number of Typhus Cases (1945)	26	71
Percentage of Total Number of Cases (1945)	26.8	73.2

Table 5 demonstrates that no significant difference existed between the proportion of positive reactors found among the rats captured from January 1 to March 30 and those trapped from April 1 to June 22. However, in strong contrast was the existing fluctuation in the number of typhus cases reported for these same periods of time. Fluctuation was also noted in the proportion of positive reactors among the animals captured in different locations.

Table 6 shows that the morbidity rate of murine typhus for San Juan is considerably higher than that for Santurce. Table 7 reveals a slightly higher percentage of positive reactors among the rats trapped in San Juan.

TABLE 6

Typhus Fever Morbidity Rates in San Juan and Santurce During the Period of July 1, 1944 to June 30, 1945

Section of City	*Population*	*Cases Reported*	*Rate per 100,000 Population*
Santurce	170,678	116	67.91
San Juan	37,360	34	91.27
Total	208,038	150	72.10

TABLE 7

Complement-Fixation Reaction Among Wild Rats Trapped in San Juan and Santurce

Section of City	*Number Examined*	*Number Positive*	*Percentage Positive*
Santurce	175	89	50.9
San Juan	238	134	56.3
Total	413	223	54.0

The Weil-Felix Reaction. Two hundred and fifty-one (251) rat serums were tested for both the C.F. and W.F. reactions; 61 (24 percent) were positive W.F. reactors; 139 serums were positive for complement-fixing antibodies. Of these last, 97 gave a completely negative W.F., and 42 (30 percent) were positive as follows: 1:25 (14 serums), 1:50 (9 serums), 1:100 (7 serums), 1:200 (8 serums), 1:800 (2 serums), and 1:1,600 (2 serums). One hundred and twelve serums gave a negative complement-fixation reaction. Of these, 93 gave a completely negative W.F., and 19 (17 percent) gave positive reactions as follows: 1:25 (8 serums), 1:50 (4 serums), 1:100 (4 serums), and 1:200 (3 serums). In reading the W.F. test, a one plus reaction was considered the end point (see table 8).

TABLE 8

The Weil-Felix Reaction in Wild Rats with a Positive or Negative Complement-Fixation Reaction

Number of Serums Tested	*Number and Percentage of Serums Giving a Positive W.F. Reaction in the Indicated Dilutions*							
	1:25	*1:50*	*1:100*	*1:200*	*1:400*	*1:800*	*1:1600*	*Totals*
Positive C.F.—139	14	9	7	8	0	2	2	42 (30%)
Negative C.F.—112	8	4	4	3	0	0	0	19 (17%)
Total Positive and Negative C.F.—251	22	13	11	11	0	2	2	61 (24%)

Of the 3,400 rat serums tested by Brigham and Bengtson,[6] 64 (1.9 percent) were positive with the W.F. test; out of 651 serums with a positive C.F. (46.7 percent of the total number of serums tested), 51 (3.6 percent) were positive with the W.F. test. Brigham and Bengtson used formalin-treated suspensions of proteus OX19; suspensions employed in this study were alcohol-treated. It must be noted that the highest titer obtained was 1:1,600 (2 serums). These low titers are also being obtained in a separate investigation on the experimental infection of white and wild *norvegicus* rats with murine rickettsiae, and contrast strongly with the high titers frequently encountered in human typhus infections.

Brigham and Bengtson also give a concise and complete summary of the results obtained by different investigators with the W.F. reaction in wild rats. The results range from 51 percent of positive reactors among 93 rats, found by Leccisotti in Taranto, Italy, to negative results in all the rats examined, as reported by several workers. Such a variation in the results obtained may be explained, in addition to probable differences in infection rates, by the differences in susceptibility to agglutination of the OX19 strains used, by differences in the nature of the suspension (alcohol-treated, formalin-treated, living), and by differences in the methods of reading and recording the results.

Among the rats examined, there were 2 small ones that must have been less than two months old and gave a positive complement-fixation and a negative W.F. This would suggest that the W.F. of wild rats, infected under natural conditions, is also fleeting, as has been shown to be the case in experimentally infected white rats.[7]

6. G. D. Brigham and I. A. Bengtson, A study of the complement fixation and Weil-Felix reactions in wild rats related to isolation of the virus of endemic typhus. Pub. Health Rep. 60, January 12, 1945.

7. *Ibid.*

SUMMARY AND CONCLUSIONS

Four hundred and forty-three (443) rats were trapped in the City of San Juan, where murine typhus is endemic. Thirty (30) of their serums were anticomplementary; of the remaining 413, 223 (54 percent) were positive to the complement-fixation reaction. Although this percentage may not indicate that such a proportion of the murine population is infective, it definitely shows that typhus was very common among the rats studied.

Two hundred and fifty-one (251) serums were tested with the Weil-Felix reaction and 61 (24 percent) found positive. One hundred and thirty-nine (139) of these gave a positive complement-fixation of which 42 (30 percent) were positive to the W.F. test. Among the 112 serums with a negative complement-fixation, there were 19 (17 percent) positive for the W.F.

Significant differences were observed in the proportion of positive reactors to the complement-fixation test among the animals trapped in different sections of the city and during different months. However, when the rats were captured in the same places but during different periods of time, such differences were not apparent. This would suggest that the rate of infection, as determined by complement-fixation among rats, may depend on the locality in which they are trapped.

The lack of correlation between the seasons and the number of positive reactors to the complement-fixation test stands out in strong contrast to the definite seasonal variation established in the incidence of human murine typhus in Puerto Rico.[8] The effect that environmental changes may have on the transmission of the disease by the flea vector may explain this lack of correlation. However, it must be remembered that a positive complement-fixation may not always mean the actual presence of a transmissible virus.

As regards sex or species, no notable difference was observed in the proportion of rats with a positive complement-fixation.

On the other hand, there was noted a difference in the proportion of animals of various sizes (ages) with a positive complement-fixation. This is an important point, since there is a tendency in studies of this nature to select large animals, as they are easier to bleed. The results obtained, when such a procedure is employed, are not therefore representative of the whole murine population. This fact must be taken into consideration because the selection of

8. G. Arbona, Atabrine in the treatment of endemic typhus fever. Bol.Asoc.Méd.de Puerto Rico, 37:208–210, 1945.

the larger (older) animals will tend to give a higher proportion of positive reactors.

There was a marked lack of correlation between the complement-fixation and the Weil-Felix reactions among wild rats. Brigham and Bengtson[9] have demonstrated the fleeting nature of this last reaction in experimentally infected rats. Two small *norvegicus* rats (probably less than two months old) gave a positive complement-fixation and a negative Weil-Felix, suggesting that the latter reaction in wild rats, infected under natural conditions, may also be evanescent in character.

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9. G. D. Brigham and I. A. Bengtson, *op. cit.*