Table 3

"XENOPSYLLA CHEOPIS" INFESTATION OF COMMENSAL RATS BY COUNTY AND OPERATIONAL YEAR
May 1946 - November 1949

Operational year	Grady	Thomas	Brooks
	Percent infested	Percent infested	Percent infested
May 1946 - April 1947	60.7	28.4	13.2
May 1947 - April 1948	41.4	5.2	1.8
May 1948 - April 1949	42.1	11.2	3.6
May 1949 - November 1949	59.0	19.1	11.6

REFERENCES

1. Hill, Elmer L. and Morlan, Harvey B.: Evaluation of county-wide DDT dusting operations in murine typhus control. Pub. Health Rep. 63:1635-1653 (1948).
2. Dent, Jack E., Morlan, Harvey B., and Hill, Elmer L.: Effects of DDT dusting on domestic rats under colony and field conditions. Pub. Health Rep. 64:666-671 (1949).
3. Morlan, Harvey B., Hill, Elmer L., and Schubert, Joseph H.: Serological survey for murine typhus infection in southwest Georgia animals. Pub. Health Rep. 65:57-63 (1950).

SEROLOGICAL DIAGNOSIS OF TYPHUS

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Infection with any of the rickettsial diseases results in the production of antibodies in man or rodents. The diagnosis of typhus fever is established serologically by demonstrating the presence of specific antibodies in the blood serum. Two methods commonly used are known as the Weil-Felix agglutination test and the complement fixation test.

The use of *Proteus* OX19 in an agglutination test for typhus stems back to 1915 when Weil and Felix observed that serum from typhus fever patients would agglutinate in certain strain (OX19) of *Proteus* bacilli. Since that time other *Proteus* antigens, OX2 and OXK, have been found useful for the agglutination test in rickettsial diseases. The OX2 strain was reported as specific for Rocky Mountain spotted fever, and OXK for scrub typhus (tsutsugamushi) of the Orient. The OXK antigen, however, has little use in this country.

The Weil-Felix test has numerous disadvantages. The antigen is nonspecific in the sense not only that it is nonrickettsial, but that it does not differentiate clearly between certain rickettsial diseases. For example, it was found that 70 percent of the sera from cases of Rocky Mountain spotted fever agglutinated the OX19 antigen which is used primarily for typhus, although usually not in very high dilutions. The OXK antigen, useful for diagnosing scrub typhus, agglutinates to high titers in serum from patients with louse-borne relapsing fever. Q fever and rickettsialpox infections fail to develop agglutinins in significant amounts to

agglutinate *Proteus* antigens. Patients having infections with *Proteus* organisms may give falsely positive reactions for typhus with the Weil-Felix test. These disadvantages have stimulated study of other tests and more specific antigens for the diagnosis of rickettsial infections, including typhus fever.

Modifications of the Weil-Felix agglutination test have been made by using suspensions of specific rickettsiae in place of *Proteus* antigens. Comparative studies made with the rickettsial antigens and *Proteus* antigens have shown little advantage of one over the other. The greater expense and difficulty in preparing rickettsial suspensions has made them impractical for use in routine testing.

The complement fixation test first was applied to the diagnosis of typhus in 1936, by Castaneda in Mexico. The antigen used by him was prepared from peritoneal washings of X-rayed, infected rats. Upon the introduction of the embryonated egg yolk sac culture technique of Cox, it became possible to grow large numbers of rickettsial organisms for antigen preparations. The typhus antigen used in the CDC serology laboratory at present is of this type and is purchased from a commercial producer.

The complement fixation test has a wider application as a serological test for typhus than does the agglutination test, because it is more specific, especially the improved antigens which are now in use. The course of the patient's disease under medical treatment for typhus can be followed by the complement fixation test because the complement fixation antibodies tend to persist after the antibodies detectable by the Weil-Felix test have disappeared. In addition, surveys can be made of human and rodent (rat) populations of various areas in order to determine the normal antibody level in the blood. If a new epizootic develops in the rodent population, an increase in the antibody level of the blood of rats would be demonstrable, and would indicate activities to suppress rats and their fleas. The fleas transmit the infection among rats and from rats to man.

As a rule, the complement fixation test does not become positive in man until about the second week of infection. The Weil-Felix test is generally positive in the first week of infection. In some 322 cases studied by Bengtson in 1941, about 24 percent gave positive results with the complement fixation test whereas the Weil-Felix test was negative.

Specimens of serum are often sent to the laboratory with the request that a diagnosis be made. Since certain low levels of antibodies are often found in normal sera, it is not possible to report any single titer as diagnostic. A RISING TITER, however, is strong evidence of active rickettsial infection. Such information could only be obtained from successive specimens of serum taken 5 to 10 days apart in the early phase of the disease. It is strongly recommended that, wherever possible, such pairs of serum ("acute" and "convalescent") be sent in for diagnosis.

The persistences of antibodies resulting from typhus infection have been studied in great detail by many workers. Antibodies can act as indicators of such infection for periods up to 3 or 4 years following illness. Average positive titers taken at yearly intervals show no pronounced loss of titer with passage of time, according to some recent work.

Routine complement fixation testing for typhus fever is now made with a soluble antigen. This antigen is prepared from yolk sac culture of rickettsial organisms in chick embryos. After the yolk sacs are harvested, they are extracted with ether and benzene and precipitated with sodium sulfate. This material then is titered and standardized to a constant level of antigenicity.

Considerable care must be practiced when taking specimens for serologic studies. At least 5 milliliters of blood are drawn aseptically from the patient's vein with a sterile, dry syringe and needle. Best results are obtained if the blood is centrifuged soon after clotting, and the sterile serum is shipped promptly to the laboratory. Specimens of whole blood shipped during the high temperature of the summer months tend to become hemolyzed. Hemolyzed specimens are tested only with difficulty or cannot be tested at all. Sera from rats and other rodents must be collected with great care to avoid contamination. Sera which become contaminated will generally be anticomplementary when they are tested in the laboratory. Specimens should be shipped at the beginning of a week, to prevent lay-over in post offices during week ends.

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