

lose about one-third of the initial body weight during a 5 day fast. The liver weight decreases logarithmically. In the first 2 days of fasting a marked increase was observed

in total liver lipid of young, male mice. Thereafter a decrease to less than normal values followed on the third to fifth fasting days.

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Studies on Q Fever: Complement-Fixing Antibodies in Meat Packers at Fort Worth, Texas.*

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Previous studies^{1,2} have suggested that risk of infection with Q fever is greater among stockyard and packinghouse workers than among the general population. The recovery of *Rickettsia burneti* from ticks in Liberty County, Texas,³ and the more recent demonstration of the same organism as the causative agent of a natural outbreak of Q fever among stock handlers and slaughterhouse workers at Amarillo, Texas,⁴ stimulated interest in the possibility of unrecognized human infections in this area. Accordingly, a serologic survey was undertaken to determine the frequency of complement-fixing antibodies to *R. burneti* in a group of packing plant employees at Fort Worth, Texas.

Serum specimens were obtained from the laboratory of the Fort Worth Health Department. The blood samples were collected during May and June, 1947, for the performance of routine serological tests for syphilis, from employees of meat packing plants. The sera used in this study were obtained only from workers who handled meat or meat products in raw or prepared states. Of the 1,433 specimens examined, approximately $\frac{3}{4}$ were secured from employees of one large packing house, the remainder being obtained from workers at a number of smaller establishments. When the routine tests for syphilis were completed, the sera were transported to this laboratory and stored in the deep freeze cabinet until tested for antibodies against *R. burneti*.

The Q fever antigen[†] was prepared from yolk sacs of embryonated eggs infected with a strain of *R. burneti* (American Nine Mile) isolated in Montana in 1935.⁵ The antigen consisted of a washed rickettsial suspension prepared by a combination of ether extractions to remove the fats and repeated centrifugation cycles to free the rickettsial bodies from extraneous proteins and fats. All tests

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¹ a. Derrick, E. H., *Med. J. Australia*, 1937, **2**, 281; b. Freeman, M., Derrick, E. H., Brown, H. E., Smith, D. J. W., and Johnson, D. W., *Australian J. Exp. Biol. and Med. Sc.*, 1940, **18**, 193; c. Derrick, E. H., *J. Hyg.*, 1944, **43**, 357.

² Shepard, C. C., *Am. J. Hyg.*, 1947, **46**, 185.

³ Parker, R. R., and Kohls, G. M., *Pub. Health Rep.*, 1943, **58**, 1510.

⁴ a. Topping, N. H., Shepard, C. C., and Irons, J. V., *J. A. M. A.*, 1947, **133**, 813; b. Irons, J. V., and Hooper, J. M., *J. A. M. A.*, 1947, **133**, 815; c. Irons, J. V., Murphy, J. N., and Wolfe, D. M., *J. A. M. A.*, 1947, **133**, 819; d. Cox, H. R., Tesar, W. C., and Irons, J. V., *J. A. M. A.*, 1947, **133**, 820.

[†] The authors are indebted to Dr. Herald R. Cox, Virus and Rickettsial Section, Lederle Laboratories Division, American Cyanamid Company, for generously supplying the yolk sac antigen (lot No. Q 9M-5-16) used in these studies.

⁵ Davis, G. E., and Cox, H. R., *Pub. Health Rep.*, 1938, **53**, 2259.

were performed with a single lot of antigen. Complement-fixation tests were carried out according to the method described by Bengtson.⁶ The antigen was diluted 1:32 (2 units). Complement was titrated in the presence of antigen; 2 units of complement were used in the tests.

Serial 2-fold dilutions of inactivated sera were made, using a single pipette for each serum specimen. The hemolytic system consisted of equal volumes of 2% sheep erythrocytes and amboceptor made up to 2 units. In performing the tests, 0.2 cc of diluted antigen, serum, and complement were added to each tube, mixed thoroughly, and stored at ice-box temperature for 18 hours. Sensitized sheep erythrocytes (0.4 cc) were then added, and the tubes held at 37°C in a water bath for one hour or until control tubes showed complete hemolysis. Appropriate serum and antigen controls, as well as known positive and negative human and guinea pig sera, were included in each series of tests. The end point was read as the highest serum dilution showing at least 3+ fixation.

All sera were examined in a "screen" test using 2 dilutions of serum: 1:8 and 1:16. Sera having titers of 1:16 in the "screen" test were retested in 2-fold serial dilutions from 1:8 to 1:512 or greater.

Complement-fixing antibody titers against the *R. burneti* antigen are recorded in the accompanying table. Of the 1,433 specimens examined, 81 were anti-complementary and

1,238 showed less than 3+ fixation in a serum dilution of 1:8. One hundred and fourteen of the sera (8.0%) showed titers of 8 or greater, while 80 (5.6%) had a titer of 16 or more and 32 (2.3%), 32 or more. Seventeen serum specimens (1.2%) showed titers of 64 or more; among these, 10 had a complement-fixing antibody titer of 64, 2 a titer of 128, 3 a titer of 256, and 2 a titer of 512.

The individuals with antibodies for Q fever worked in various departments of the plants and there was no clear evidence that the positive reactors were engaged in any particular job. Some suggestive evidence was found that the higher titers occurred in individuals dealing with cattle rather than in those engaged in work with hogs and sheep. Epidemiological significance cannot be attached to these findings since the total number of individuals employed or tested in each department at the time of the study is unknown.

Among all the specimens examined, serological tests for syphilis† (Kahn, Kolmer) were positive in 39 instances (2.7%) and doubtful in 19 (1.3%). Among the 114 sera showing complement-fixing antibody titers of 8 or more against *R. burneti*, 4 (3.5%) had positive serological tests for syphilis and 2 (1.8%) had doubtful tests. The complement-fixation titers for *R. burneti* among these 6 individuals with positive or doubtful serological tests for syphilis were 8 in 2 instances, 16 in 3, and 512 in 1. The findings suggest only a coincidental relationship between serum reactivity with antigens for syphilis and Q fever.

Discussion. Considerable evidence exists to indicate that the complement-fixation test with yolk-sac antigens of *R. burneti* is reliable and specific for the serologic diagnosis of Q fever. In proved infections in which *R. burneti* was recovered from the blood stream, a rise in titer of complement-fixing antibodies has been demonstrated during convalescence.^{4,7} Serological cross-reactions with other rickettsial diseases do not occur,^{4,6,7} but it should be noted that there is

TABLE I.
Complement-Fixation Titers Against *R. burneti* in Sera from Meat Packers at Fort Worth, Texas.

Titer*	No. of serum specimens	%	Total % positive
AC	81	5.7	
<8	1238	85.7	
8	34	2.4	
16	48	3.3	
32	15	1.1	8.0
64 or >	17	1.2	
Total	1433	99.5	

* Highest serum dilution yielding 3+ or greater fixation.

AC Serum anticomplementary.

⁶ Bengtson, I. A., PROC. SOC. EXP. BIOL. AND MED., 1941, 46, 665.

† These tests were performed by Mrs. Edith Mazurek in the laboratory of the Fort Worth Health Department.

little available information with regard to possible cross-reactions with other non-rickettsial diseases, with the exception of primary atypical pneumonia.^{7,8} However, agglutination tests with *R. burneti* have been negative in a variety of other diseases.⁹

It is not yet certain whether non-specific complement-fixation may occur with egg yolk sac antigens of *R. burneti*. In this laboratory a number of sera which reacted with the egg yolk sac antigen of *R. burneti* were also tested with an antigen prepared from uninoculated normal egg yolk sacs, but in no instance was complement fixation accomplished. Non-specific complement-fixation with syphilitic sera has been reported with a variety of other egg yolk sac antigens.^{10,11} Further studies with regard to these problems are in progress in this laboratory.

The significance that can be attached to the results of a complement-fixation test for Q fever on a single serum sample is uncertain, particularly when the serum titer is low (8 or 16) and when a clinical history of illness is unavailable. Early in convalescence considerably higher titers are to be expected^{2,4,7} but at longer intervals after infection lower titers might be anticipated. Elsewhere, however, it has been demonstrated that elevated serum antibody titers may persist for 1½ years after natural infection with Q fever.¹² Illness due to Q fever may vary in severity

from a trivial attack of "grippe"¹³ to fatal pneumonia,⁴ and subclinical infection may result in elevated antibody titers.^{2,4} It is apparent, therefore, that a history of illness may have little value in the interpretation of antibody titers obtained by serological survey. In the present study, the plant physicians were unaware of any unusual prevalence of undiagnosed febrile illness.

The results of the studies herein reported are believed to indicate the existence of unrecognized exposure to *R. burneti*. Similar data have not been reported in this country from other geographic areas or in other occupational groups. At present no conclusion can be drawn as to whether this frequency of occurrence of antibodies for Q fever is unusual. The occupation of these individuals may be immaterial and it is possible that a similar prevalence of antibodies for Q fever may be found in other occupational groups or in the general population in this area or elsewhere. Studies designed to elucidate some of these problems are in progress. Recent studies have indicated the occurrence of Q fever among persons exposed to dairy cattle in the Los Angeles area. *R. burneti* has also been isolated from the milk of such cattle.¹⁴

Summary. Complement-fixation tests employing an egg-yolk sac antigen of *R. burneti* (American Nine Mile strain) were performed on sera from 1,433 packinghouse workers at Fort Worth, Texas. Evidences of previous infection with *R. burneti* were obtained. Antibody titers of 8 or more were found in 8.0%, 16 or more in 5.6%, 32 or more in 2.2%, and 64 or more in 1.2%.

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¹³ Commission on Acute Respiratory Diseases, *Am. J. Hyg.*, 1946, **44**, 123.

¹⁴ Huebner, R. J., *et al.*, *Public Health Rep.*, 1948, **63**, 214.

⁷ a. Robbins, F. C., and Ragan, C. A., *Am. J. Hyg.*, 1946, **44**, 6; b. Robbins, F. C., Gould, R. L., and Warner, F. B., *Am. J. Hyg.*, 1946, **44**, 23; c. Robbins, F. C., Rustigian, R., Snyder, M. J., and Smadel, J. E., *Am. J. Hyg.*, 1946, **44**, 51; d. Robbins, F. C., and Rustigian, R., *Am. J. Hyg.*, 1946, **44**, 64.

⁸ Dyer, R. E., *Am. J. Hyg.*, 1944, **39**, 308.

⁹ Commission on Acute Respiratory Diseases, *Am. J. Hyg.*, 1944, **44**, 110.

¹⁰ Wertman, K., *J. Lab. and Clin. Med.*, 1945, **30**, 112.

¹¹ Van der Scheer, J., Bohnel, E., and Cox, H. R., *J. Immunol.*, 1947, **56**, 365.

¹² Sulkin, S. E., and Strauss, E., *Proc. Soc. Exp. Biol. and Med.*, 1948, **67**, 142.