Q Fever

Complement-Fixing Antibodies with *C. burnetii* Antigens in Various Geographic Areas and Occupational Groups in the United States *†

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Q FEVER is world-wide in its distribution. Within a decade since its recognition in Australia [1] the disease has been identified in the United States, the Mediterranean area,[2] Switzerland,[3] Germany,[4] and Panama.[5] Although a few sporadic cases had been recognized previously,[6, 7] the first epidemic occurring under natural conditions in this country was reported from Amarillo, Tex., in March, 1946, and involved persons employed in a stockyard and meat packing plant.[8] In August, 1946, a second natural epidemic, also involving packinghouse workers, occurred in Chicago.[9] In the fall of the same year an endemic focus of Q fever was discovered in Los Angeles County, and in the course of the subsequent year approximately 150 cases were identified.[10, 11]

The mode of transmission of Q fever to human beings has not been defined satisfactorily nor has its natural reservoir or vector been clearly identified. In Australia [12] and in the United States [13, 14] ticks infected with *Coxiella burnetii* have been found in nature and in sporadic instances have been responsible for the human disease.[7, 15] Investigations of the epidemics in this country and in the Mediterranean area, however, have not incriminated ticks or other arthropods as the vectors of Q fever. Two of the 3 American epidemics have involved packinghouse and stockyard workers but the manner in which infection took place was not established. In the Los Angeles County outbreak the investigators pointed to a relationship with dairy cows, and indeed milk from such animals was found to be infected with *C. burnetii*.[10] However, it was not established that infection took place through the ingestion of milk. At present, no single explanation seems adequate to satisfy the varying circumstances under which the disease has been recognized.

A complement-fixation test has been devised for the detection of antibodies against a *C. burnetii* antigen prepared from the infected yolk sacs of embryonated hen's eggs.[16] Such antibodies have been found to persist in sera following naturally acquired infections for as long as 17 months.[17]

In view of these facts, it appeared desirable to undertake a serological survey to determine the frequency with which antibodies against a *C. burnetii* antigen occurred in different geographic areas and among different occupational groups. Sera from packinghouse workers and dairy workers were selected because of the previous occurrence of Q

* Presented at a Joint Session of the Epidemiology and Laboratory Sections of the American Public Health Association at the Seventy-sixth Annual Meeting in Boston, Mass., November 12, 1948.

† This study was aided by grants from the Lederle Laboratories Division of the American Cyanamid Company, Pearl River, N. Y., and the Rose Lampert Graff Foundation, Los Angeles, Calif.

fever among such workers. In addition, samples of sera from the general population in widely separated areas of the country were secured. A preliminary report of the occurrence of antibodies against *C. burnetii* in packinghouse workers in Fort Worth, Tex., has already been presented.[18] In this report the results of complement-fixation tests on 5,470 sera are summarized as follows: packinghouse workers at Fort Worth, 1,433 sera; packinghouse workers at Austin, Minn., 150 sera; routine serological laboratory specimens from Amarillo, Tex., 175 sera; routine serological laboratory specimens from Dallas, Tex., 1,033 sera; routine serological laboratory specimens from Boston, Mass., 965 sera; routine specimens from prospective blood donors from Dallas, 798 sera; specimens from dairy workers in the Dallas milk shed, 350 sera; and, finally, routine serological laboratory specimens from Portland, Ore., 566 sera (the latter collected and tested for antibodies by Dr. A. W. Frisch and the results included in this report with his kind permission). The results have been analyzed with regard to the specificity of the serologic tests employed, and for the information they yield with regard to the frequency of occurrence of antibodies for *C. burnetii* in the United States.

METHODS

Serum specimens were transported to this laboratory within 2 days after collection if obtained locally, or by air express from distant points, and were stored in a deep freeze cabinet until tested.

The Q fever antigen was prepared from the yolk sacs of embryonated eggs infected with a strain of *C. burnetii* (American Nine Mile) isolated in Montana in 1935.[13] The antigen was a washed rickettsial suspension prepared by a combination of ether extraction to remove fats and repeated centrifugation cycles to remove extraneous proteins and fats.[19] Two lots of antigens were used (Q9M-5-16 and Q9M-4-26). Almost all sera reacting with *C. burnetii* antigens were also tested with a yolk sac antigen prepared from uninfected embryonated eggs.*

Complement-fixation tests were performed according to the method of Bengtson.[16] The antigen was diluted 1:32 (2 units). Complement was titrated in the presence of antigen, and 2 units of complement were used in the tests.

Serial twofold dilutions of inactivated sera were made, using a single pipette for each serum specimen. The hemolytic system consisted of equal volumes of 2 per cent sheep erythrocytes and amboceptor made up to 2 units. In performing the tests, 0.2 ml. of diluted antigen, serum, and complement were added to each tube, mixed thoroughly, and stored at icebox temperature for 18 hours. Sensitized sheep erythrocytes (0.4 ml.) were then added, and the tubes held at 37° C. in a water bath for 1 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 endpoint 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 twofold serial dilutions from 1:8 to 1:512 or greater.

Serological Tests for Syphilis—A variety of serological tests for syphilis were performed. Approximately 40 per cent of the sera were tested in one Dallas laboratory (Parkland Hospital) by routine Kahn and Kline tests, Kolmer type

* The *C. burnetii* and normal yolk-sac antigens were prepared in the laboratories of Dr. Herald R. Cox, Lederle Laboratories Division, American Cyanamid Company; generous supplies were made available by Dr. Cox.

TABLE 1

Complement-Fixation Titers against C. burnetii and Results of Serological Tests for Syphilis among 5,470 Sera from Various Geographic Areas and Occupational Groups in the United States

Series	Date of Collection	Results of STS				Results of C-F Tests against C. burnetii								
		No. + or ±	No. Neg.	No. Unknown	Per cent + or ±	No. Sera Tested	No. Positive C. burnetii and + or ± STS	Serum Titers *						Per cent Positive 8 or >
								AC	< 8	8	16	32	64 or >	
Fort Worth meat packers	May-June 1947	58	1,375	0	4.0	1,433	6	81	1,238	34	48	15	17	8.0
Minnesota meat packers	Nov. 1947 Jan. 1948	1	142	7	0.7	150	0	5	144	1	0	0	0	0.7
Amarillo serology lab.	Aug. 1947	10	165	0	5.7	175	2	19	142	6	6	0	2	7.9
Dallas serology lab.	July 1947	397	636	0	38.4	1,033	63	78	850	39	55	7	4	10.2
Boston serology lab.	Oct.-Nov. 1947	102	963	3	9.6	965	1	45	909	1	8	1	1	1.1
Dallas blood donors	Dec. 1947 Mar. 1948	24	774	0	3.0	798	1	10	771	9	8	0	0	2.1
Dallas dairy workers	Mar.-Apr. 1948	4	311	35	1.1	350	1	6	337	3	2	2	0	2.0
Portland, Ore. serology lab.	1948	1	470	95	0.2	566	0	2	554	6	4	0	0	1.8

+ = positive STS
± = doubtful STS
AC = anticomplementary serum
* = highest serum dilution yielding 3+ or greater fixation

complement-fixation tests, and by the use of cardiolipin antigens. Another 20 per cent were tested by similar techniques, including the use of cardiolipin antigens in the laboratory of the Fort Worth Health Department. The sera from Boston were tested by the Hinton test in the laboratory of Dr. William A. Hinton. The remainder were tested by the Amarillo Health Department and by Dr. A. W. Frisch by the usual Kline and Kahn techniques.

RESULTS

The results of the studies are presented in 2 parts: first a description of the origin and composition of the various groups from which serum specimens were obtained and the antibody titers found; and second, data bearing on the specificity of the test.

Fort Worth: packinghouse workers—Serum specimens obtained from 1,433 persons employed in the actual handling and processing of meat and meat products in one large and several small packinghouses at Fort Worth, Tex., were tested for complement-fixing antibodies against *C. burnetii* antigen. A preliminary report of this study has been published.[18] The data will be summarized here for comparison with the results of other studies. The specimens were collected during May and June, 1947, by the Fort Worth Health Department for the performance of routine serological tests for syphilis required of food handlers. The majority of persons tested were adult white males but complete data on age, sex, and racial composition of the total group were not available.

Among the entire group of 1,433 sera, 8 per cent exhibited complement-fixing antibodies in a titer of 8 or more. The titers are recorded in Table 1. Seventeen serum specimens (1.2 per cent) had titers of 64 or more; among these, 10 had a titer of 64, 2 a titer of 128, 3 a titer of 256, and 2 a titer of 512.

It was not possible to interview the individuals with high titers of antibodies against *C. burnetii* or to obtain information with regard to illness. The plant physicians were unaware of the occurrence of an epidemic or of an unusual number of cases of unexplained illness. Analyses of the specific work performed by those with elevated antibody titers did not suggest a correlation with any particular job or department in the plants.

Minnesota: packinghouse workers—From November, 1947, to January, 1948, 150 serum specimens were received from a large meat packing plant in Austin, Minn. The sera were obtained from employees who came to the plant dispensary for first aid and for various medical complaints. Specimens were collected only from those employees who actually handled meat and meat products within the plant. All but 6 were white males between 20 and 61 years of age. One-third of the group had been employed at the plant for more than 15 years each, and 90 per cent of the group had been employed there for more than 5 years. In contrast with the results among packinghouse workers in Fort Worth, only one serum (0.7 per cent) in the Minnesota series exhibited complement-fixing antibodies with *C. burnetii* antigen, and then only in a titer of 8 (Table 1).

Amarillo: Serological laboratory—In August, 1947, 175 serum specimens were obtained from the Amarillo (Texas) City Health Department after routine serological tests for syphilis had been performed. Most of the sera were obtained from adult white males but the specific age, sex, and racial composition of the group was not available.

Fourteen sera (7.9 per cent) exhibited titers of 8 or more (Table 1). Both of the individuals with high titers (64 and 1,024) were subsequently found to have been ill with Q fever during the stockyard epidemic of March, 1946.[8] Histories of illness were not available from

the other 12 individuals with titers of 8 or more.

Dallas: serological laboratory—During July, 1947, 1,033 consecutive serum specimens were obtained from the serological laboratory at Parkland Hospital, Dallas. All sera were originally submitted for serological tests for syphilis. Approximately 60 per cent of the sera were obtained from the venereal disease clinic and the remainder from inpatients, prospective blood donors, and patients attending other clinics at the hospital. Almost all persons were adults, 43 per cent were male, 57 per cent female, 33 per cent were white, and 67 per cent were Negroes.

One hundred and five sera (10.2 per cent) were found to have complement-fixing antibody titers of 8 or more against *C. burnetii* (Table 1). However, only 11 sera had titers of 32 or more and only 4 had titers of 64; none had titers in excess of 64. The sex and racial distribution of those with titers of 8 or more was the same as for the series as a whole. The clinic or hospital records of these patients were reviewed. Several gave a history of pneumonia. No clear evidence of an illness which might have been Q fever was obtained, but the records were considered inadequate for the purpose.

Boston: serological laboratory—During October and November, 1947, 965 sera were received from the Wassermann Laboratory of the Massachusetts State Department of Health and were tested for complement-fixing antibodies against *C. burnetii.* These blood samples had been submitted from various sources for routine serological tests for syphilis. Almost all were from adults, 64 per cent were from females, and 98 per cent were from white persons.

Eleven of the 965 sera (1.1 per cent) contained antibodies for *C. burnetii* in a titer of 8 or more; the titer was 8 in one instance, 16 in 8, 32 in 1, and 64 in 1 (Table 1). The sex and racial distribution of these 11 persons conformed to the distribution in the series as a whole. A questionnaire inquiring into occupation, history of "virus" pneumonia, and service in the Mediterranean area during World War II was answered by 7 of the 11 positive reactors. One whose serum had a titer of 32 had "virus" pneumonia in 1943; another with a serum titer of 16 had pneumonia in the winter of 1947. The individual whose serum showed a titer of 64 denied any illness in the past 2 years.

Dallas: blood donors—Because of the high incidence of positive reactors with both *C. burnetii* and syphilitic antigens in the first Dallas series, another series of different composition was obtained. From December, 1947, to March, 1948, consecutive samples of blood were obtained from prospective blood donors, the samples being submitted for routine serological tests for syphilis. (The latter were performed in the same laboratory as the first Dallas series.) A total of 798 blood samples were obtained. Two-thirds of the donors were white, and one-third colored; 80 per cent were males and 20 per cent females.

Seventeen (2.1 per cent) of this group had complement-fixing antibodies; 9 had a titer of 8, and 8 a titer of 16 (Table 1). The racial and sex distribution for these persons was the same as for the whole group. A history of occupation or of illness was not available.

Dallas: dairy workers—A total of 350 blood samples was obtained during March and April, 1948, from dairy and dairy plant workers in the Dallas area. One hundred and one of these actually lived and worked on dairy farms in close association with dairy cows. All but one were white adults, from 16 to 69 years of age; 87 were males and 14 females. All but 14 per cent had been so employed for more than 1 year; 60 per cent for more than 5 years; and 40

per cent for more than 10 years. In this group, only one individual's serum fixed complement with *C. burnetii* antigen in a dilution of 8 or more; in this person, the titer was 32.

In addition to the farm workers, 249 blood samples were secured from employees of dairy plants, the majority of whom were white males. Approximately two-thirds of the total were indoor plant workers engaged in various phases of the pasteurization and packaging of milk and milk products; the remainder were mostly route salesmen. Of this group of 249, 6 (2.4 per cent) reacted positively with *C. burnetii* antigen. Three had a serum titer of 8, 2 a titer of 16, and one a titer of 32. Combining both groups of dairy workers, 7 of 350 (2.0 per cent) reacted positively; the highest titer being 32 in 2 persons (Table 1).

Oregon: serological laboratory — Through the courtesy of Dr. A. W. Frisch it was possible to include the results on 566 specimens submitted to several serological laboratories in Portland, Ore., for routine serological tests for syphilis. Dr. Frisch's technique in testing for antibodies to *C. burnetii* was similar to that used here with 2 exceptions: (1) the serum dilutions were 10, 20, etc., instead of 8, 16, etc., and (2) the Henzerling instead of American (Dyer) strain of *C. burnetii* was used as antigen. The results are recorded in Table 1. Ten of 566 samples (1.8 per cent) reacted positively in a titer of 10 or more. Six had a titer of 10, and 4 a titer of 20. These results are recorded in Table 1, under the columns for titers of 8 and 16 respectively.

SPECIFICITY OF THE SEROLOGICAL TEST

Relation between STS and serum reactivity with C. burnetii—The unexpectedly high (10.2 per cent) frequency of serum reactors to *C. burnetii* antigen among the 1,033 specimens in the original Dallas series prompted further analysis of the data. The results clearly revealed a significant correlation between a positive or doubtful STS and reactivity in the complement-fixation test with *C. burnetii*.

Of the 1,033 sera tested, 38.4 per cent yielded positive (27.1 per cent) or doubtful (11.3 per cent) tests for syphilis. However, in the group of 105 sera yielding positive complement-fixation tests with *C. burnetii* antigen, 60 per cent also had positive or doubtful tests for syphilis. This relationship, expressed according to the titers of rickettsial antibodies, is shown in Table 2. The whole series was then divided into 2 groups according to whether the STS was negative (636 sera) or positive or doubtful (397 sera), and the number and percentage of reactors with the rickettsial antigen calculated (Tables 3 and 4). A fourfold table relating positive and negative reactivity with *C.*

TABLE 2

Dallas Serological Laboratory Series
Number and Per cent of Sera with Positive or Doubtful STS among 105 Positive Reactors with C. burnetii

Serum Titer for C. burnetii	*Number of Sera*	*Positive or Doubtful STS*	
		Number	*Per cent*
8	39	19	48.7
16	55	37	67.3
32	7	4	57.1
64	4	3	75.0
Total	105	63	60.0

TABLE 3

Dallas Serological Laboratory Series
Complement-Fixation Titers against C. burnetii among 636 Sera with Negative STS

Titer	*No. of Sera*	*Per cent*	*Total Per cent Positive*
AC	38	6.0	
<8	556	87.4	
8	20	3.1	
16	18	2.8	6.6
32	3	0.5	
64	1	0.2	
Total	636	100.0	

TABLE 4

Dallas Serological Laboratory Series Complement-Fixation Titers against C. burnetii among 397 Sera with Positive or Doubtful STS

Titer	No. of Sera	Per cent	Total Per cent Positive
AC	40	10.1	
<8	294	74.1	
8	19	4.8	
16	37	9.3	15.9
32	4	1.0	
64	3	0.8	
Total	397	100.1	

burnetii with the results of the tests for syphilis, in the whole series, yielded a chi square value of 23 and the highly significant P value of less than 0.01.

In the Fort Worth series 4.0 per cent had positive or doubtful STS; among the 114 sera in this series which reacted positively with *C. burnetii,* 6 (5.4 per cent) also had positive or doubtful STS. The rickettsial complement-fixation titers among these 6 individuals with positive or doubtful STS were: 8 in 2 instances, 16 in 3, and 512 in 1. The relationship between the 2 serologic tests could be fortuitous and the P value was not statistically significant.

In the Amarillo series, 5.7 per cent of the sera yielded positive or doubtful results in the STS. Among the 14 sera which fixed complement in the presence of the rickettsial antigen, 2 (14.3 per cent) also had positive STS. The titers for *C. burnetii* in both of these sera were 16. The series is too small for statistical analysis although the results suggest more than a coincidental relationship between the two serologic tests.

Among the 101 specimens from dairy farmers in the Dallas milk shed, the only serum which reacted positively with *C. burnetii* (titer 32) also yielded a doubtful reaction in the serologic tests for syphilis. In all of the other series studied, the relationship between reactivity with syphilitic antigens and the rickettsial antigen could be explained by chance association. Likewise, statistical analysis of all series combined (excluding the original Dallas series) yielded a P value which was not significant.

Reactions with normal yolk-sac antigen—The possibility was considered that some of the sera which reacted in the presence of *C. burnetii* yolk-sac antigen might also fix complement in the presence of normal, uninfected yolk sacs. Accordingly an antigen was obtained from Dr. Herald R. Cox consisting of the yolk sacs of normal embryonated hen's eggs prepared by the same technique as the rickettsial yolk-sac antigens. Almost all sera which reacted in a titer of 8 or more with rickettsial antigen in the "screen" test were again tested with normal yolk-sac antigen and with *C. burnetii* antigen. In no instance was a positive reaction encountered with normal yolk-sac antigen.

Effect of temperature of inactivation—In further attempts to determine the specificity, or lack of specificity, of serological reactions with *C. burnetii,* a number of tests were performed at various temperatures of inactivation of the sera. Inactivation was carried out at 56° C. for 10 minutes and at 65° C. for 15 minutes on sera which in the usual tests reacted with both syphilitic and rickettsial antigens. There were no significant differences in the rickettsial complement-fixation titers at different temperatures of inactivation, nor was complement-fixation demonstrated with the normal yolk-sac antigen under these circumstances.

Reproducibility of results—Sera reacting positively with *C. burnetii* antigen were retested at least once and in many instances several times. Titers tended to fall off, and the sera to become anti-complementary, with repeated thawing and freezing incidental to storage and retesting. The results of repeated tests, however, conformed to the usual reproducibility of most quan-

titative complement-fixation tests. Unusually discrepant results were not encountered. Two different lots of antigen were employed and many of the positive sera were tested with both antigens, with similar results. A group of positive and negative sera were also tested in the laboratory of Dr. Cox with results corresponding well with those obtained in this laboratory.

Persistence of antibodies — Complement-fixing antibodies persist for many months following proved natural infection with Q fever.[17] It was, therefore, of interest to determine the antibody titers on serum specimens obtained several months after the original samples. Nonspecific reactivity might be expected to fluctuate or disappear while true antibody might be expected to persist or decline in titer slowly. Second blood samples were obtained in 42 instances from Fort Worth meat packers 4 months after the original bleedings; in all cases a titer of 8 or more was demonstrated. A comparison of the titers of original and second specimens is shown in Table 5. A distinction is made between a titer of 0, indicating a complete absence of complement-fixation in a final serum dilution of 8, and a titer of <8, indicating some fixation of complement but less than a 3+ reading. With a few exceptions the titers declined in the 4 months interval. However, the falls in titer were moderate and uniform at all initial levels. Of 42 persons, with titers of 8 or more, 23 still had titers of 8 or more 4 months later.

In the series from the Boston laboratory, second samples of blood were obtained from 3 individuals, 1 month, 2 months, and 6 months after the original specimens. Two with original titers of 16 still had titers of 16, 2 and 6 months later; while a third with an original titer of 64 had a titer of 32, 1 month later. One of the positive sera was originally also positive in the serologic test for syphilis. The second specimen from this subject, while exhibiting the same titer with *C. burnetii* antigen, was negative in the test for syphilis.

Hospital patients with suspected Q fever and miscellaneous diseases—Acute and convalescent phase (3–4 weeks after onset) blood specimens were obtained from 22 patients suspected of having Q fever. Seven patients had pulmonary infiltrations without evidence of bacterial pneumonia. The remainder were sporadic cases of acute febrile illnesses in which the usual studies did not establish a specific diagnosis. In addition, acute and convalescent blood was obtained from 2 cases of infectious mononucleosis. Single blood samples were also obtained from 22 cases of lymphopathia venereum. Complement-fixation tests with *C. burnetii* antigen were negative in all instances, with one exception:

A 20 year old male white college student with previous Army service in Korea was hospitalized with fever and pulmonary infiltration. Malaria was proved by examination of blood smears. On appropriate antimalarial chemotherapy, fever and the pulmonary lesion disap-

TABLE 5

Complement-Fixing Antibodies against C. burnetii in Fort Worth Meat Packers
Comparison of Titers in May, 1947, and September, 1947

Titers in September, 1947	Titers in May, 1947							Total
	8	16	32	64	128	256	512	
0	1	3	..	..	..	..	..	4
<8	7	6	1	1	..	..	..	15
8	..	1	..	1	..	..	..	2
16	1	3	2	2	..	..	..	8
32	..	3	1	2	..	..	..	6
64	..	..	..	2	1	1	1	5
128	..	..	..	..	..	1	1	2
Total	9	16	4	8	1	2	2	42

peared within a week. Blood serum taken 5 days after onset was anticomplementary; the STS at this time was negative. Blood serum taken 12 days after onset fixed complement with *C. burnetii* in a titer of 16, but another sample taken 6 weeks after onset had a titer of less than 8. It is difficult to evaluate the significance of these serological reactions. The relatively low titer and its rapid fall suggested a nonspecific reaction, possibly related to malaria.

In the original Dallas series were included the results of complement-fixation tests on sera of 129 hospitalized patients. The single blood samples available were obtained, in general, within a few days after admission to the hospital. These patients, representing consecutive admissions to all services of a general hospital, suffered from a wide variety of illnesses. Approximately one-third had acute or chronic infections and many were febrile at the time blood was drawn. No evident correlation was found between the diagnosis established during hospitalization and the complement-fixation titer against *C. burnetii*, with the exception, already mentioned in this series, of the association between positive STS and reactivity with the rickettsial antigen.

DISCUSSION

A considerable body of evidence attests the diagnostic significance of a rising titer of complement-fixing antibodies for *C. burnetii* following an illness. Such antibody responses have been demonstrated following illness proved to be Q fever by recovery of the rickettsiae from the bloodstream.[2, 8] It is also established that such antibodies persist for prolonged periods of time.[16, 17]

On the other hand, there are few data by which can be assessed the specificity and diagnostic significance of complement-fixation with *C. burnetii* antigen of single serum specimens obtained without knowledge of the clinical history, from presumably well persons. To our knowledge, no large serological surveys employing this test have been recorded. Cross-reactions with other rickettsial antibodies do not occur in Q fever,[16] but systematic studies of possible nonspecific reactions or cross-reactions with other diseases have not come to our attention. Moreover, the data are not available which would permit one to regard any particular serum titer as being significant of a previous attack of Q fever.

In the original Dallas series the incidence of complement-fixing antibodies to *C. burnetii* was 15.9 per cent among persons with positive or doubtful serologic tests for syphilis and 6.6 per cent among those with negative tests for syphilis. Since this difference was not likely to arise by chance, some other explanation must be sought. Possibilities to be considered include: (1) false positive serologic tests for syphilis occurred in the presence of antibodies for *C. burnetii;* (2) some factor of selection in the series, such that *C. burnetii* antibodies were more common in persons who also had syphilis; and (3) nonspecific complement-fixation occurring with *C. burnetii* yolk-sac antigen in the presence of syphilis.

The occurrence of false positive serologic tests for syphilis in persons with complement-fixing antibodies for *C. burnetii* seems unlikely. Three-fourths of the 105 persons with rickettsial antibodies were attending a venereal disease clinic. In almost all instances the diagnosis of syphilis was well established. Most patients had been seen originally with primary or secondary syphilis; several had neurosyphilis; and in 9 instances Kahn titers of 64 or more units were present at the time of this study. Available evidence indicates, therefore, that most of these subjects had syphilis and not false positive serologic tests for syphilis.

That the observed relationship between serologic tests for syphilis and

complement-fixation with *C. burnetii* was due to some factor of selection in the group studied, cannot be supported or denied with assurance. The race and sex distribution of the *C. burnetii* reactors was the same as for the group as a whole. Most of them were urban dwellers. The females worked most often as domestics, the males as unskilled laborers. Considering the clientele of the clinic it seems reasonable to believe that positive and negative reactors to *C. burnetii* antigen as well as those who had positive or negative serologic tests for syphilis all came from the same general environment and engaged in the same occupations. Specific data to substantiate these assumptions are not available.

The possibility of nonspecific complement-fixation with *C. burnetii* antigen in sera with positive or doubtful serologic tests for syphilis should receive serious consideration. Previous studies have indicated that other yolk-sac antigens may also fix complement with syphilitic sera.[20, 21] The present results cannot be explained satisfactorily, but they raise a question with regard to the specificity of this test which can only be settled by further investigation.

If it is assumed that the presence of complement-fixing antibodies for *C. burnetii* indicates previous infection with this organism one must conclude that this survey disclosed a considerable number of otherwise unrecognized cases of Q fever. This assumption seems particularly strong in connection with the results obtained in the packinghouse workers at Fort Worth. Not only was the total incidence of positive serological reactions high (8 per cent), but the incidence of high titers was also considerable (2.3 per cent had titers of 32 or more). The persistence of the antibodies for a period of 4 months further supports the belief that they indeed represented infections with Q fever. The fact that an epidemic was not recognized may be explained on the assumption that the illnesses were sporadic, that they masqueraded under more banal clinical labels, or that they were subclinical. All of these possibilities are known to occur with Q fever.

The serological results obtained among meat packers at Fort Worth thus represent the third instance in this country in which Q fever has been identified in packinghouse workers. However, as Shepard had already pointed out, the infectious agent must reside rarely, not commonly, in such an environment.[9] Among the Minnesota packinghouse workers the incidence of positive reactors for *C. burnetii* was negligible, and yet these workers had had contact with many thousands of animals over the course of years.

Unlike recent experiences in Los Angeles County, the incidence of rickettsial antibodies among dairy workers in the Dallas area was not higher than in a sample of the general population. Many of the persons studied had had years of close association with dairy cattle. As with meat packers, one must conclude that the epidemiological association between Q fever and occupation or environment seems to be an intermittent, and not a constantly operating phenomenon.

In one of the studies of the Los Angeles outbreak recently reported, data are presented indicating that 1.8 per cent of a group of sera with negative STS from individuals residing in the milk shed area exhibited complement-fixing rickettsial antibodies in a titer of 8 or 16.[11] In the present study, a similar incidence of antibodies in low titer was also found in Dallas, Boston, and Oregon, areas where Q fever has not been recognized clinically. In Texas (Dallas, Amarillo, and Fort Worth) the incidence of antibodies for *C. burnetii* appears to be higher than elsewhere. These data may suggest that Q fever

may be widely distributed throughout the United States.

A question has arisen in recent publications as to the selection of the proper strain of *C. burnetii* for use as an antigen in complement-fixation tests. Patients infected in Italy with Q fever developed complement-fixing antibodies to the Italian (Henzerling) strain but not to the American (Dyer) strain.[2] The latter has been said to be a less sensitive antigen than the former.[22] Immunized guinea pigs developed an early rise in titer to the Henzerling strain, and a delayed response to the Dyer strain, but 2 months later the titers became comparable. Similarly, human beings immunized with vaccines prepared with these strains exhibited better antibody responses with the Henzerling than with the Dyer strains.[23] It must be pointed out however, that successful, and diagnostically useful, complement-fixation tests were performed with the Dyer strain in the investigation of the Amarillo epidemic,[8] and in a follow-up study of this epidemic, carried out at this laboratory, dealing with the persistence of antibodies.[17] The problem of the choice of antigens cannot be considered settled. The present study was conducted entirely with the use of the Dyer strain and no comparisons with the 2 antigens could be made. If the results of the studies mentioned above are applicable to the present circumstances it may be implied that an even greater number of sera would have fixed complement had the Henzerling strain been employed.

SUMMARY

1. Complement-fixation tests were performed in 5,470 sera using yolk-sac antigen of *C. burnetii* (American Nine Mile strain). The sera were obtained from persons residing in Massachusetts, Minnesota, Oregon, and Texas. Sera were obtained from meat packers in Fort Worth, Tex., and Austin, Minn., and from dairy workers in the Dallas area.

2. Sporadic instances of complement-fixation, which may indicate Q fever, were found in sera from all geographic areas included in the study, suggesting that Q fever may occur in low incidence throughout the country. Evidence for its occurrence in appreciable numbers among packinghouse workers in Fort Worth was presented. The evidence also suggested that residents of the southwestern part of the United States may have a higher incidence of complement-fixing antibodies with *C. burnetii* than in other areas of the country.

ACKNOWLEDGMENTS—The serological studies in this laboratory were performed by Elizabeth L. Watson and Helen Leonard. Grateful acknowledgment is made to: Drs. Harold M. Williams and D. A. Reekie for supplying serum specimens from Fort Worth, and to Miss Edith Mazurek for performing STS on these specimens; Dr. William A. Hinton for supplying the serum specimens from Boston, performing STS on these sera, and for supplying clinical data and second specimens on positive cases; Dr. H. R. Allen, for supplying sera from Austin, Minn.; Jack Wyatt for sera from Amarillo and STS on these sera; Dorothy Lee for supplying sera from Dallas and for STS on these and other sera; Earl Sudderth and his staff for assistance in collecting sera from Dallas dairy workers; and Dr. A. W. Frisch of Portland, Ore., for generously permitting the inclusion in this report of data obtained in his laboratory.

We also wish to acknowledge the generous assistance of Dr. Herald R. Cox in supplying the antigens used in this study and in testing selected sera in his laboratory.

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