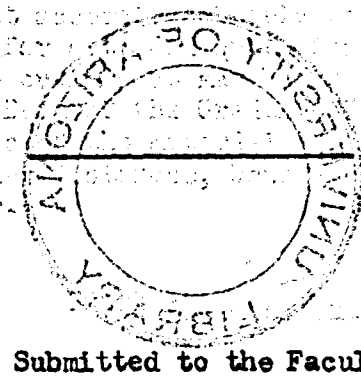
A SEROLOGICAL SURVEY OF Q FEVER IN ARIZONA

by

Arthur F. Di Salvo



A Thesis Submitted to the Faculty of the

DEPARTMENT OF BACTERIOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

UNIVERSITY OF ARIZONA

1958


UNIVERSITY OF ARIZONA
LIBRARY

E979l
1958
2

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in their judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Arthur F. DeSalvo

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Kenneth Wertman — 2/4/58

KENNETH WERTMAN
Professor of Bacteriology
and Head of the Department

Date

TABLE OF CONTENTS

LIST OF TABLES AND MAP

INTRODUCTION

Q fever is a recent addition to the list of diseases of animals transmitted to man. It is a febrile illness, caused by the rickettsia Coxiella burnetii, often mis-diagnosed as atypical pneumonia or pneumonitis. The first case of Q fever was observed by Derrick in 1935 (1), but there was no interest in the disease until 1946. At this time there were outbreaks in Amarillo, Texas (2) and Chicago, Illinois (3). In 1947 there were indications that Q fever was endemic in Southern California.

Since that time, extensive studies in California have shown that cattle, sheep and goats are a source of the causative organism. The infected cattle do not show any pathological symptoms nor is there any loss of production in dairy cows (4). The United States Department of Agriculture has not conducted any investigational work on this disease because it is not an economical problem in the livestock industry (5).

Man is the incidental host through contact with these animals or some of their products. To effectively control the human infections of Q fever it is necessary to control the spread of the organism by its host, the domestic livestock.

It was the purpose of this study to determine whether or not complement fixing antibodies to Q fever are present in the domestic livestock of the State of Arizona. Evidence of contact with C. burnetii was found in some areas.

A REVIEW OF Q FEVER

Q fever is a relatively new disease among the infections of man. The first case was investigated by Derrick in Australia in 1935. He named the organism Rickettsia burneti after F.M. Burnet who succeeded in isolating the organism and found it to be a rickettsial agent. Abattoir workers composed a large portion of the infected group. It was established that livestock, particularly cattle, were involved in the transmission. After much deliberation, Derrick named the new clinical entity Q fever. The Q standing for query -- as there were still many unknown factors.

At the same time, in the United States, Davis and Cox isolated a filter passing agent from the tick Dermacentor andersoni. The organism was first found in 1935 at Nine Mile Creek near Missoula, Montana (6). While this organism was being studied at the National Institute of Health, a member of the staff contracted an illness in May, 1938. The course of the illness was similar to that described by the Australian workers as Q fever. At the same time the National Institute of Health was studying a strain of R. burneti supplied by Dr. Burnet. Five guinea pigs which had recovered from infection with the Australian Q fever organism were challenged with the rickettsiae isolated in Montana and were found to be immune. Further investigations by Dyer proved them to be the same organism (7). This was later confirmed by Burnet and Freeman in Australia (8).

During the Second World War, Q fever again came to the attention of medical investigators. In 1943 and 1944, Q fever was endemic in

Athens, Greece (9). The disease was termed "Balkan Grippe" by the Germans who were occupying Greece at the time. Caminopetros isolated the causative organism and this was later shown to be a strain of R. burneti.

In the winter of 1944-1945 there were several outbreaks among the Allied troops in Italy. During the following spring and summer Q fever occurred among the troops returning to the United States from Europe (10).

Several laboratories which were studying this rickettsial disease experienced laboratory infection among the personnel (7, 11, 12). Prior to this time Q fever was considered a medical and laboratory curiosity in this country.

In 1946, Q fever became a clinical problem in the United States. The first outbreak occurred among a group of livestock workers in Amarillo, Texas in March (2). In August of the same year a second area was affected. This outbreak occurred among the workers in a slaughterhouse in Chicago (3). These two incidents were similar in the following respects: a) They both occurred among slaughterhouse employees, b) were explosive, c) infected a large percentage of workers, and d) were transmitted by contact with infected body fluids and tissue.

In the fall of 1946 an outbreak of Q fever occurred in Los Angeles County, California. These cases occurred in a milk shed area (13). A Q Fever Laboratory was established in this region and extensive studies of this rickettsial infection were initiated.

It appears as though the epidemiological pattern of Q fever can vary with the geographic area involved. The studies performed in

the State of California bears out this statement. In Southern California the highest prevalence of Q fever antibodies were found in dairy cattle (14), while in Northern California sheep and goats were incriminated (15). It was interesting to note that in Los Angeles County, where the first California outbreak occurred, most of the cows were imported into that area because of the high cost of feed and land (13). This may indicate that the original foci of infection was elsewhere.

A Q fever epidemiological survey was conducted among dairy cows in the State of Wisconsin in 1957 (16). A high degree of positive reactions, 8.0 percent, were found in the southeast counties of the State as compared to an average of 0.35 percent in the other counties. This might indicate a spread of Q fever from the Chicago area, where one of the first outbreaks of this disease occurred in the United States. Human sera, taken from the vicinity of high titer cattle sera, also showed a high incidence (22.3 percent) of Q fever antibodies.

This is interesting in view of the fact that in 1955 Schmidt and Harding reported that 39.8 percent of a population group in Illinois had substances in their blood that "inhibited" complement fixation when they were tested against the Q fever antigen (17). If this is not an inhibition of the complement fixation, then there might possibly be a higher incidence than usually recognized of subclinical infection of Q fever.

In the past decade, reports of Q fever have come from around the world. In 1946 R. burneti was isolated and identified as the causative agent of a series of pneumonias in Panama (18). Q fever has also been reported in Switzerland (19) and in Great Britain (20). A survey in 1956

reported antibodies to R. burneti in Lebanon where there was a high incidence among humans (21). This rickettsial infection has now been described from all continents with the exception of South America.

THE ETIOLOGIC AGENT

The causative organism of Q fever is Coxiella burnetii (Derrick) Phillip (22). Derrick named it Rickettsia burneti after F. M. Burnet who first studied the infectious agent and recognized it as a rickettsia. The organism was also isolated in Montana and named Rickettsia diaporica by Cox (23). After further study, these organisms were found to be one and the same, and enough characteristics set it apart from other rickettsiae, thus justifying a new genus. The genus Coxiella (after Hereld R. Cox, the co-discoverer in the United States) was then proposed by Phillip (24).

Coxiella burnetii is a minute, plump, pleomorphic rod, morphologically typical of other rickettsiae. It is often coccoid and occasionally filamentous. It stains red in Macchiavello's stain, purple in Giemsa, and is gram negative (9).

It differs from the type organism Rickettsia prowazeki in some properties. It readily passes through bacterial filters (6), it remains viable in cell-free media for long periods at room temperature (23), and is more resistant to heat and chemicals. The Weil-Felix Reaction is negative and the rash, characteristic of other rickettsial diseases, is absent.

Other rickettsiae are usually inactivated following exposure to 50°C for 15 minutes. Several strains of C. burnetii were found to survive 63°C for 30 minutes. The rickettsiae of Q fever also showed exceptional resistance to formalin and phenol (25).

Oval inclusion bodies, 20-30 u in diameter, can be demonstrated in infected tissue. These have been shown to be large masses of rickettsiae so tightly packed, that the organisms appear as granules. In the organs of infected mice the agent has been found intracellularly and extracellularly, singly or in masses (9).

Complete reciprocal cross immunity has been found among the several strains of C. burnetii isolated from human cases. The antigens prepared from these strains showed some variation in sensitivity (26).

a dairy demonstrated a high incidence of Q fever antibody. In specific area studies, the occurrence increased with persons who lived closest to certain dairies (27).

Raw milk has been incriminated as a possible carrier of Q fever (24). Buchner et al. collected milk samples from dairies which were suspected as a possible source of infection. These specimens of raw milk were inoculated into guinea pigs and rickettsiae identified as C. burnetii were isolated (25).

In testing the efficacy of pasteurization, lots of milk were divided into three portions. Two portions were pasteurized and the third portion was tested raw. These samples were inoculated into a series of guinea pigs. The complement fixation tests on these animals showed that 58.3 percent of those inoculated with raw milk gave a positive complement fixation reaction, while only 5.2 percent of the guinea pigs inoculated with pasteurized milk gave a positive complement fixation reaction (26). These tests seem to indicate that pasteurization will reduce, if not eliminate, C. burnetii from milk.

EPIDEMIOLOGY

The role of milk as a carrier of C. burnetii has been studied intensively in Southern California. It has been shown that individuals in the livestock industry have a high proportion of complement-fixing antibodies to Q fever. Within this group, those people who are in more intimate contact with the live animals or certain raw products have a higher incidence of infection. A population study of close proximity to a dairy demonstrated a high incidence of Q fever antibody. In specific area studies, the occurrence increased with persons who lived closest to certain dairies (27).

Raw milk has been incriminated as a possible carrier of Q fever (28). Huebner et al. collected milk samples from dairies which were suspected as a possible source of infection. These specimens of raw milk were inoculated into guinea pigs and rickettsiae identified as C. burnetii were isolated (29).

In testing the efficacy of pasteurization, lots of milk were divided into three portions. Two portions were pasteurized and the third portion was tested raw. These samples were inoculated into a series of guinea pigs. The complement fixation tests on these animals showed that 92.3 percent of those inoculated with raw milk gave a positive complement fixation reaction, while only 6.2 percent of the guinea pigs inoculated with pasteurized milk gave a positive complement fixation reaction (30). These tests seem to indicate that pasteurization will reduce, if not eliminate, C. burnetii from milk.

Recent studies show that an increase of 2°F in the standard pasteurization temperature is effective in destroying the infectious agent (31). It has also been shown that calves ingesting infected milk will disseminate the rickettsiae in their feces (32).

The meat packing industry has been involved in many explosive outbreaks of Q fever (2, 3, 27). The rickettsiae seem to spread from the infected tissues and body fluids of the animals being slaughtered. There is also evidence that certain of the raw products will disseminate the organism. The highest incidence of infection occurred among those workers on the killing floor (2).

Dust probably plays an important role in the dissemination of C. burnetii. In tests performed on dust-laden air, the organism was isolated from a dairy barn and a sheep ranch (33).

Dessication apparently has little effect on the rickettsiae. In an outbreak of Q fever in a wool and hair processing plant, the organism was isolated from the wool (34).

Coxiella burnetii has also been isolated from the placentas of naturally infected sheep (35) and dairy cows (36). This indicates the importance of this organ as a means of dispersing the infectious agent. The hide of the newborn calf is also contaminated in this manner. It appears as though domestic livestock play an important role in the spread of this disease among human contacts.

It has been the only successful treatment for Q fever has been with [illegible]. Although the drug is not highly effective, it usually results in a significant reduction in the duration of the fever (39).

A vaccine against Q fever has been tried on dairy cattle. It does have some effect of inducing resistance to C. burnetii (40). Immunity to a second infection has been demonstrated in guinea pigs.

CLINICAL FEATURES

The outstanding characteristic of Q fever is its sudden onset. Many patients are able to give the hour at which they first became ill (37). The temperature is usually elevated, from 103°F to 105°F, and persisting for 4 to 15 days. It usually subsides by lysis. In some cases the fever will be prolonged and relapses and sequelae will occasionally occur (27). This is accompanied by anorexia, chills and headache with retro-orbital pain.

Roentgenograms will show consolidation in many cases (37, 28). Patchy areas of consolidation occur in a small portion of the lobe, and these areas are homogenous and ground glass in appearance. There has been some collapse of lung tissue noted.

Later work by Clark et al. has shown that respiratory symptoms were not always present and pulmonary lesions are not essential to a diagnosis of Q fever (38). It is of interest to note that in 35 cases of lung involvement, 31, or 88% of the films showed the lesions to be in the lower lobes (37, 38).

The incubation period, from 2 to 3 weeks, and the explosive nature of Q fever, gives evidence of a common source of infection. The combination of these two factors seems to indicate that there is no direct person-to-person spread of the disease.

The rash, which is characteristic of most rickettsial diseases, is absent in the case of Q fever.

There have been some deaths attributed to this infectious agent.

To date the only successful treatment for Q fever has been with aureomycin. Although the drug is not highly effective, it usually results in a significant reduction in the duration of the fever (39).

A vaccine against Q fever has been tried on dairy cattle. It does have some affect of inferring resistance to C. burnetii (40).

Immunity to a second infection has been demonstrated in guinea pigs.

LABORATORY DIAGNOSIS

The only laboratory technics that can be used to diagnose Q fever are a demonstration of a substantial rise in the titer of specific complement-fixing antibodies or by recovery of the organism from the patient. To be of any clinical significance, paired specimens of blood serum should be tested. These should be taken during the acute and convalescent stage of the illness. A fourfold rise in antibody titer is considered significant of present infection.

Infected material from patients may be inoculated into guinea pigs to recover the causative organism. Milk, from cows suspected of having Q fever, has also been inoculated into guinea pigs and the organism successfully isolated. The infectifity of these animals has been determined by the demonstration of Q fever antibodies in their blood serum 30 to 38 days after infection.

The routine laboratory tests cannot be used as diagnostic tools. The usual Proteus OX-19 agglutinins (Weil-Felix Reaction) are absent. The red cell count and the hemoglobin concentration do not show any change. The white cell count and the differential also tend to remain normal; however, there is a moderate elevation of the sedimentation rate during the acute stage of illness (28, 37).

MATERIALS AND METHODS

The complement fixation technic employed was described by Plotz and Wertman (41). This method has been shown to be both sensitive and specific. No other infectious agent has been found that will elicit the specific complement fixing antibodies of Q fever. Neither brucellosis nor syphilis antibodies will give a positive reaction with C. burnetii (42). It was found that on rare occasions an anamnesis of brucella antibodies may occur in Q fever but disappear early in the illness (43). The purified rickettsial antigens will not produce positive complement-fixing reactions with convalescent sera other than Q fever.

According to Sulkin and Strauss, the complement-fixing antibodies of Q fever in the human will persist for at least as long as 17 months (45). A positive reaction to C. burnetii in the complement fixation test is regarded as representing previous effective contact with the organism, and a high titer suggests current infection of Q fever (46).

The titer is expressed as the highest serum dilution giving 3 plus or 4 plus fixation. Titers of 1:5 or greater have been considered of diagnostic value in Q fever (47).

A screen test of three dilutions, 1:5, 1:10, and 1:20 was performed on each serum. This procedure used to eliminate any "false negatives" due to the prozone effect. When a serum reacted in the 1:20 dilution, further dilutions were carried out to establish the endpoint.

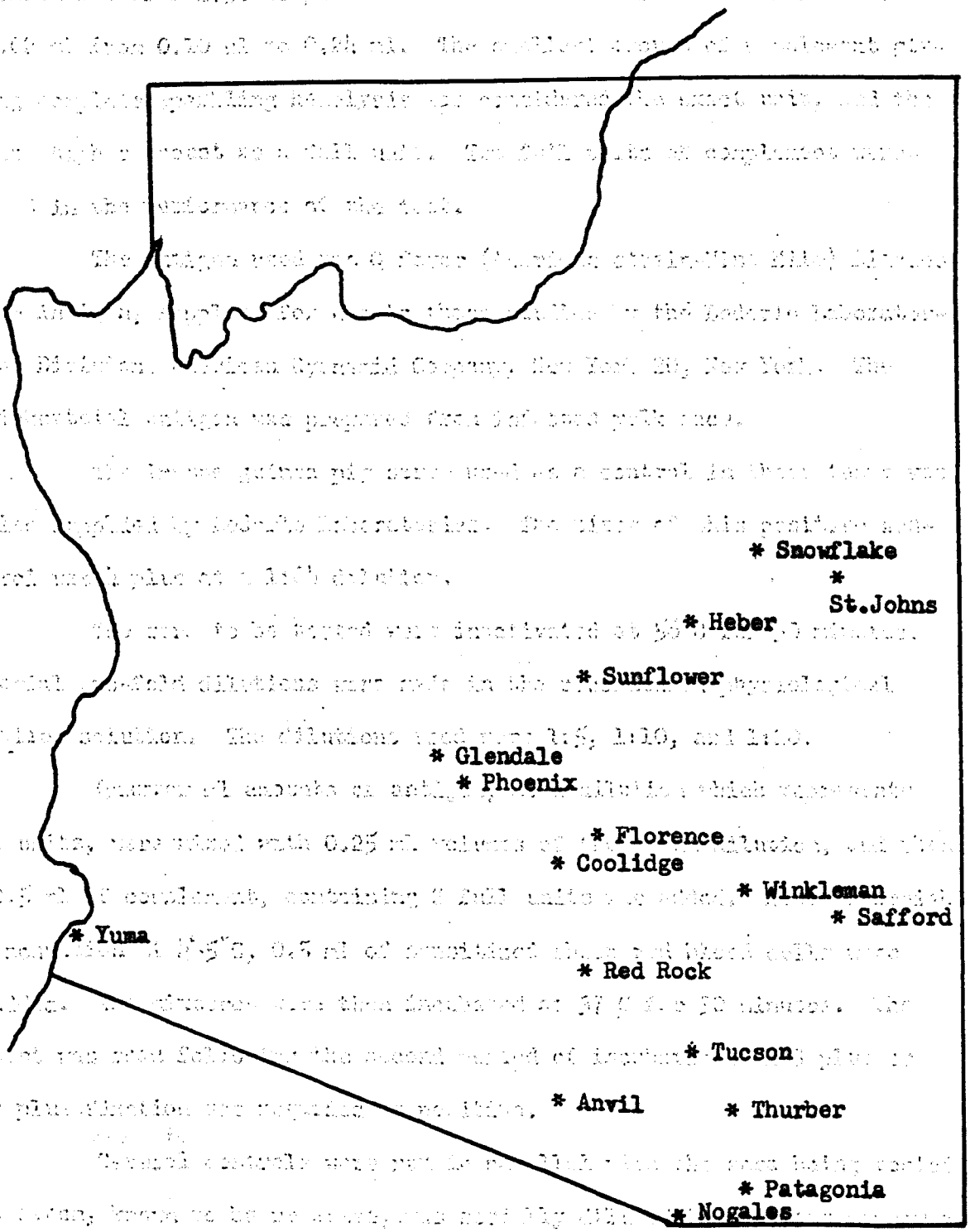
The Agricultural Research Service of the United States Department of Agriculture, Phoenix, Arizona, cooperated in obtaining specimens for study. They drew blood from livestock for the routine diagnosis of brucellosis. The sera remaining after the completion of the tests were used for this survey. It was desired that tests should be performed on animals from a wide variety of geographical areas within the State. The map on the following page shows the distribution of the tested herds.

A suspension of commercially prepared sheep red blood cells, obtained from Hyland Laboratories, Los Angeles, California, was used in the hemolytic system. The cells were washed by centrifugation three times or until the supernatant was clear. A 3 percent suspension of the sheep cells was made by resuspending the packed cells in the physiological saline solution containing 0.01 percent magnesium sulphate and 0.004 percent calcium chloride.

A commercial preparation of glycerinated anti-sheep hemolysin (Standardized Anti-Sheep Hemolysin) was purchased from Cappel Laboratories, West Chester, Pennsylvania, and used in these tests. The hemolysin was diluted to contain 2 units in 0.25 ml.

Sensitized red blood cells were then prepared by mixing equal quantities of hemolysin dilution and a 3 percent suspension of washed sheep erythrocytes.

The complement used was a commercial preparation of dried pooled serum from healthy male or non-pregnant female guinea pigs, a product of Travenol Laboratories, Inc., distributed by Scientific Products Division, American Hospital Supply Corporation, Los Angeles, California. Fresh complement was titrated each day the tests were performed. Varying


* Snowflake
*
St.Johns
* Heber
* Sunflower
* Glendale
* Phoenix
* Florence
* Coolidge
* Winkleman
* Safford
* Yuma
* Red Rock
* Tucson
* Anvil
* Thurber
* Patagonia
* Nogales

quantities of a 1:30 complement dilution were titrated in increments of 0.02 ml from 0.10 ml to 0.24 ml. The smallest amount of complement giving complete sparkling hemolysis was considered the exact unit, and the next higher amount as a full unit. Two full units of complement were used in the performance of the test.

The antigen used was Q fever (American strain-Nine Mile) Diagnostic Antigen, supplied for use in these studies by the Lederle Laboratories Division, American Cyanamid Company, New York 20, New York. The rickettsial antigen was prepared from infected yolk sacs.

The immune guinea pig serum used as a control in these tests was also supplied by Lederle Laboratories. The titer of this positive control was 4 plus at a 1:64 dilution.

The sera to be tested were inactivated at 56°C for 30 minutes. Serial two-fold dilutions were made in the recommended physiological saline solution. The dilutions used were 1:5, 1:10, and 1:20.

Quarter ml amounts of antigen, at a dilution which represents 2 units, were mixed with 0.25 ml volumes of the serum dilution, and then 0.5 ml of complement, containing 2 full units was added. After overnight incubation at 4°-5°C, 0.5 ml of sensitized sheep red blood cells were added. The mixtures were then incubated at 37 C for 30 minutes. The test was read following the second period of incubation. A 3 plus or 4 plus fixation was regarded as positive.

Several adequate controls were run in parallel with the sera being tested. A serum, known to be negative, was serially diluted in the same manner as the unknowns.

Two-fold dilutions of the positive serum from 1:5 to 1:160 were also included. This serum gave a 4 plus reading in the 1:40 but not in the 1:80 dilution.

A serum control to determine any anti-complementary effects of each serum was also included. This tube, with a serum dilution of 1:2.5, was the same as the unknown samples except that saline was substituted for the antigen. This should always show complete hemolysis.

A complement titration was incubated overnight with the sera being tested to determine the amount of complement available after incubation. The complement control consisted of complement in 0, 0.1, 0.25, 0.3, and 0.5 ml amounts. The first two tubes containing less than one full unit of complement, show no hemolysis. The third tube, representing one full unit of complement has 3 plus or 4 plus hemolysis, and the last two tubes should show complete hemolysis.

RESULTS

The complement fixation tests on 751 sera from domestic livestock indicate an incidence of antibodies to Coxiella burnetii in the animals tested in the State of Arizona. As is shown in Table 1, 229 or 30.5 percent of the animals tested gave a positive reaction.

TABLE 1

Results of complement fixation tests to C. burnetii in the State of Arizona, 1957.

Animal	Number Tested	Number Positive	Percent Positive
Cattle	674	225	33.4
Sheep	62	3	4.8
Goats	15	1	6.6
Total	751	229	30.5

The geographic distribution of Q fever antibodies and the degree of infection in the cattle tested is shown in Table 2. Of 674 cattle tested, 225 representing 33.4 percent were found to posses specific antibodies. It will be noted from this table that these herds fall into three general groups. One group are those animals that had a very low percentage of Coxiella antibodies, or in some instances, none at all. The Yuma and Phoenix areas had a high percentage of positive reactions, and many of these had a high titer. The third group had a high percent of reactors, but these were of a low titer. The Florence area seems to be an exception. It had 23.4 percent reactors, two of which had a titer of 1:80.

TABLE 2

Geographic distribution of complement-fixing antibodies to C. burnetii in cattle according to titer*, 1957

Area	Number Tested	Number Negative	5	10	20	40	80	160	320	640	1280	2560	Number Positive	Percent Positive
Yuma	106	26	20	10	11	24	5	6	2	1		1	80	75.5
Patagonia	47	27	14	3	3								20	42.5
Nogales	33	31	2										2	6.0
Thurber	68	53	13	2									15	22.0
Anvil	60	57	2	1									3	5.0
Tucson	74	67	4	1	1	1							7	9.5
Red Rock	35	26	8	1									9	25.7
Winkleman	34	19	14	1									15	44.1
Florence	47	36	4	3	2		2						11	23.4
Coolidge	3	3											0	0
Phoenix	91	55	13	9	3	3	4	2	2				36	39.6
Sunflower	29	4	13	9	3								25	86.2
Heber	4	4											0	0
Snowflake	11	11											0	0
St. Johns	32	30	2										2	6.2
Total	674	449	109	40	23	28	11	8	4	1		1	225	33.4

* Titer is expressed as the reciprocal of the highest serum dilution giving 3 plus or 4 plus fixation.

Univ. of Arizona Library

Table 3 compares the prevalence of complement-fixing antibodies among cattle, sheep and goats from the Tucson area. The percentage of positive reactors was low in cattle and sheep and it was negative in the goats of this area. Unfortunately, it was not possible to test sheep and goats in the endemic localities in order to compare with the high incidence of antibodies in cattle.

TABLE 3

A comparison of the incidence of antibodies to C. burnetii in cattle, sheep, and goats in the Tucson area, 1957.

Animal	Number tested	Number Positive	Percent Positive
Cattle	74	7	9.5
Sheep	62	3	4.8
Goats	10	0	0
Total	146	10	6.8

DISCUSSION

There is no evidence in the literature of any investigation of the possibility of Q fever in Arizona. Since cattle raising is an important industry here, and herds are located throughout the State, it was decided that a serological survey of the domestic animals would give evidence of any endemic areas of the disease.

The dry climate in Arizona would not lessen the possibility of the disease being prevalent. In Los Angeles County, where Q fever is endemic, the yearly rainfall is 15.5 inches and the climate is warm and dry (13). In Arizona, the average yearly rainfall is about 10.5 inches (48). The lack of constant humidity would not be of much consequence because of the resistance of the organism to dessication. The mild climate of Arizona seems even more suitable for Q fever because there is no seasonal incidence of the disease (28).

The results of this survey have shown that there are two areas in the State that show a large percentage of reactors with a high titer. In Phoenix, 36 of the 91 cattle tested (39.6 percent) gave a positive reaction to Q fever. In Yuma, 80 specimens of the 106 sera tested (75.5 percent) gave a positive reaction. According to other investigators, the high titer may be indicative of current infection (47).

Three other areas, Patagonia, Winkleman, and Sunflower were found to have a high percentage of reactors, but these specimens only fixed complement in a low dilution. This may be evidence of previous effective contact with the causative organism, C. burnetii.

When considering these findings it is important to note that the Yuma and Phoenix area have many feedlot operations. Therefore there are many cattle kept together in close quarters. This would greatly facilitate the spread of the infectious organism and account for the high number of positive reactions from the cattle in these areas.

Conversely, many of the cattle in the areas with a small percentage of reactors are range cattle. Here the dissemination of the rickettsiae is more difficult. In Tucson, where there is a low percentage of reactors, the results of the complement fixation tests on cattle are corroborated by similar results of sheep which are kept in close quarters. This is shown in Table 3.

Fifteen goats were included in the survey. Ten from Tucson were negative. One goat, of the three from Safford, gave a positive reaction in the 1:10 dilution. Two goats from Glendale were negative.

This has been the first work of this nature in the State of Arizona. It may not be conclusive due to the relatively small number of animals tested. However, it does give some evidence upon which to base further investigations.

ACKNOWLEDGMENT

The interest of Keith Maddy, D.V.M. and his aid in obtaining the specimens for this study is gratefully acknowledged.

SUMMARY

A complement fixation test was employed to determine the incidence of antibodies to C. burnetii, the causative agent of Q fever, in the sera of domestic livestock in Arizona. These tests were performed on 751 cattle, sheep and goats. On these, 229 or 30.5 percent, fixed complement in a dilution of 1:5 or greater.

The sera of cattle from Yuma and Phoenix had a high percentage of reactors with high titers. Patagonia, Winkleman, and Sunflower had a high percentage of complement-fixing antibodies, but these were all in low titers.

Although there were only a few sheep and goats tested, the reactions confirmed those that were found in cattle of the same area.

BIBLIOGRAPHY

1. Derrick, E.H., Med. J. Australia, 2:281, 1937.
2. Topping. N.H., Shepard, C.C., Irons, J.V., J. Am. Med. Assoc., 133:813, 1947.
3. Shepard, C.C., Amer. J. Hyg., 46:185, 1947.
4. Huebner, R.J., Bell, J.A., J. Am. Med. Assoc., 145:301, 1951.
5. Osteen, O.L., Acting Head, Viral and Rickettsial Disease Section, Agricultural Research Service, U.S.D.A., Personal communication.
6. Davis, G.E., Cox, H.R., Pub. Health Rep., 53:2259, 1938.
7. Dyer, R.E., Pub. Health Rep., 54:1229, 1939.
8. Burnet, F.M., Freeman, M., Med. J. Australia, 1:887, 1939.
9. Commission on Acute Respiratory Diseases, Am. J. Hyg., 44:110, 1946.
10. Feinstein, M., Yesner, R., Marks, J.L., Am. J. Hyg., 44:72, 1946.
11. Robbins, F.C., Rustigan, R., Am. J. Hyg., 44:64, 1946.
12. Commission on Acute Respiratory Diseases, Am. J. Hyg., 44:123, 1946.
13. Shepard, C.C., Huebner, R.J., Am. J. Pub. Health, 38:781, 1948.
14. Lennette, E.H., Dean. B.H., Abinanti, F.R., Clark, W.H., Winn, J.F., Holmes, M.A., Am. J. Hyg., 54:1, 1951.
15. Lennette, E.H., Clark, W.H., Dean, B.H., Am. J. Trop. Med., 29:527, 1949.
16. Kitze, L.K., Am. J. Hyg., 65:239, 1957.
17. Schmidt, N.J., Harding, H.B., J. Bact., 71:217, 1955.
18. Cheney, G., Geib, W.A., Am. J. Hyg., 44:158, 1946.
19. Gsell, O., Med. and Hyg., 5:317, 1947.

20. Marmion, B.P. and others, Lancet, 1:503, 1953.

21. Garabedian, G.A., Djanian, A.Y., Johnston, E.H., Am. J. Hyg., 63:308, 1956.

22. Breed, R.S., Murray, E.G.D., Smith, N.R., Bergey's Manual of Determinative Bacteriology, Seventh Edition, The Williams and Wilkins Company, Baltimore, Maryland, 1957.

23. Cox, H.R., Pub. Health Rep., 54:1822, 1939.

24. Phillip, C.B., Pub. Health Rep., 63:58, 1948.

25. Ransom, S., Huebner, R.J., Am. J. Hyg., 53:110, 1950.

26. Topping, N.H., Shepard, C.C., Huebner, R.J., Am. J. Hyg., 44:173, 1946.

27. Bell, J.A., Beck, M.D., Huebner, R.J., J. Am. Med. Assoc., 142:868, 1950.

28. Beck, M.D., Bell, J.A., Shaw, E.W., Huebner, R.J., Pub. Health Rep., 64:41, 1949.

29. Huebner, R.J., Jellison, W.L., Beck, M.D., Parker, R.R., Shepard, C.C., Pub. Health Rep., 63:214, 1948.

30. Huebner, R.J., Jellison, W.L., Beck, M.D., Wilcox, F.P., Pub. Health Rep., 64:499, 1949.

31. Enright, J.B., Sadler, W.W., Thomas, R.C., Public Health Monograph No. 47, United States Government Printing Office, Washington, D.C., 1957.

32. Bell, J.A., Parker, R.R., Stoenner, H.G., Am. J. Pub. Health, 39:478, 1949.

33. DeLay, P.D., Lennette, E.H., DeOme, K.B., J. Immunol., 65:211, 1950.

34. Sigel, M.M., el al., Am. J. Pub. Health, 40:524, 1950.

35. Welsh, H.H., Lennette, E.H., Abinanti, F.R., Winn, J.F., Pub. Health Rep., 66:1473, 1951.

36. Luoto, L., Huebner, R.J., Pub. Health Rep., 65:541, 1950.

37. Robbins, F.C., Ragan, C.A., Am. J. Hyg., 44:6, 1946.

38. Clark, W.H., Lennette, E.H., Railsback, O.C., Romer, M.S., A.M.A. Arch. Int. Med., 88:155, 1951.

39. Clark, W.H., Lennette, E.H., Meiklejohn, G., A.M.A. Arch. Int. Med., 87:204, 1951.

40. Luoto, L., Winn, J.F., Huebner, R.J., Am. J. Hyg., 55:190, 1952.

41. Plotz, H., Wertman, K., Science, 95:441, 1942.

42. Lennette, E.H., Clark, W.H., Jensen, F.W., Am. J. Pub. Health, 42, 12, 1952.

43. Meyer, K.F., Eddie, B., Calif. Med., 70:292, 1949.

44. Wertman, K., J. Lab. and Clinical Med., 30:112, 1945.

45. Sulkin, S.E., Strauss, E., Proc. Soc. Exper. Biol. and Med., 67:142, 1948.

46. Huebner, R.J., Jellison, W.L., Beck, M.D., Ann. Int. Med., 30:495, 1949.

47. Diagnostic Procedures for Virus and Rickettsial Diseases, Second edition, p. 543, American Public Health Association, New York, 1956.

48. United States Weather Bureau, Tucson, Arizona, Personal communication, 1957.