Serologic Analysis of a Penitentiary Group Using Raw Milk From a Q Fever Infected Herd

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SINCE the early investigations by Huebner and his associates (*1*), raw milk has been considered a potential source of Q fever infection in man. Experimental studies by Fonseca and his co-workers (*2*) have indicated that infection in man by oral ingestion of the infectious agent is difficult to attain. Epidemiologic investigations in the United States of patients with clinical disease have shown that Q fever infection is transmitted chiefly through the air (*3–5*). However, studies of sporadic occurrences of Q fever in England have been credited to the ingestion of infected raw milk (*6*).

The potential danger of acquiring clinical disease from consuming raw milk seems minimal in view of the high infection rates in the dairy herds of Idaho and the many people in the rural areas there who continue to consume raw milk without evidence of clinical disease (*7*).

Our study of a penitentiary group in Idaho adds information on the effects of consuming raw milk containing viable *Coxiella burnetii.*

Dairy Herd Tests

Milk samples from individual cows in the prison farm dairy herd were tested in February, September, and October 1959 by the Luoto capillary agglutination test (*8*). In February, positive tests were obtained on the undiluted milk samples from 52 (90 percent) of 58 cows. In September, 39 (51 percent) of 76 such tests were positive, and in October, 26 (35 percent) of 74 were positive. Eleven of 39 cows testing positive in September had capillary agglutination titers of 1:64 or greater. A pooled milk sample from the herd also had a titer of 1:64. In the October test, 9 of 26 cows testing positive had titers of 1:64 or greater. The pooled milk sample had a titer of 1:32.

Isolation attempts were made on nine pools of milk samples collected in September; the samples had capillary agglutination titers ranging from 1:8 to 1:256. Each pooled sample was inoculated into four hamsters, and *C. burnetii* was isolated from all nine pools.

Seventeen percent of the 69 cows in a control herd at another State institution (Nampa State School) had positive agglutination tests. No isolation attempts were made from this control herd.

Test Groups

Initially, 300 male prisoners at the Idaho State Penitentiary were given a serologic test for Q fever. From these, a study group of 120 men were selected for sufficient testing to allow determination of their Q fever antibody titers. A minimum of three tests for each person was considered necessary in a 6- to 10-month period. The tests were made from February through December 1959. Prior to and during this period, the milk supply at the prison was unpasteurized and originated from the prison farm dairy herd, 10 miles from the prison. The prisoners who worked at the farm were excluded from the study group. Histories of

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illnesses, consumption of milk, and contact with animals were elicited from as many of the group as possible by questionnaires and interviews.

Beginning in March 1960, the milk supply at the prison was pasteurized adequately. Serologic tests were made of 112 new prisoners institutionalized after the pasteurization procedures were started. This group comprised one of the two control groups for comparing the normal distribution of antibody titers with that of the study group.

In addition to the 112 prisoners in the control group, 99 males at the Nampa State School for the mentally retarded, who had consumed only the pasteurized milk from the school's dairy herd, were tested three times during a 6-month period. These two control groups provided information on the expectancy of antibody titers influenced by a pasteurized product from a dairy herd infected with Q fever.

Serologic Test

The complement fixation test (*9*) was used for all Q fever blood tests. American Nine Mile strain CF antigen, obtained from Lederle Laboratories, was used throughout this study.

Serial twofold dilutions of inactivated serums in 0.1 ml. volume were tested by using 2 units of antigen in 0.1 ml. volume and 2 exact units of complement in 0.2 ml., with overnight fixation. The hemolytic system consisted of 0.2 ml. of 2 percent sensitized sheep erythrocyte suspension. The highest serum dilution giving a 4+ or 3+ (25 percent hemolysis) reading was taken as the titer.

Readings were made when aliquots of pooled positive control serums reproduced the same titer as previously determined in numerous testing procedures. An antigen complement control, consisting of 0.1 ml. of antigen added to tubes containing 2, 1½, 1, ½, and ¼ units of complement, was also included in each test run. The Q fever complement fixation test was found to be quite reproducible under the conditions used in this study.

An interpretation of positive significance was attached to individuals who had titers of 1:8 or greater. Positive conversions noted in individuals during the testing period were considered significant if the reciprocal titers increased from 0 to 1:16 or greater, or a fourfold or greater increase from the original titer was demonstrated.

Results

Initial antibody levels of reciprocal titers 1:8 or greater were noted in 35 (11.7 percent) of the 300 men tested. Of the 120 men tested 3 times or more, 42 (35 percent) were positive in at least 1 test; 12 (28.6 percent) of the 42 had positive conversions. According to the above criteria, distribution of positive titers for the 42 individuals was as follows:

CF reciprocal titer	*Number*
1:8	12
1:16	13
1:32	11
1:64	3
1:128	2
1:256	1
Total	42

Of the 99 men tested at the Nampa institution, 4 were positive. Three of the men had titers of 1:8 and the other a titer of 1:16.

Tests of the 112 new inmates who consumed only the pasteurized milk revealed 10 (8.9 percent) with positive titers. None of the men in these two control groups had positive conversions.

Histories of febrile or respiratory illnesses were found as frequently among the prisoners with negative titers as among those with positive titers. Many of the illnesses recorded had occurred during influenza outbreaks in Idaho. The group with positive conversion did not have any illness which could be associated with the rise in antibody titer. Many inmates in the test group had preexisting Q fever antibodies when imprisoned.

All 120 men in the study group were questioned personally regarding contact with animals before imprisonment; 56 were able to give definite histories. The relationship between the CF titers and animal contact is shown in table 1. No significant difference exists between the men with positive and negative CF titers with regard to animal contact or length of time since animal contact.

Table 1. Animal contact histories of 56 prisoners before entering prison

Animal contact	Number with positive CF titers	Number with negative CF titers	Total	Percent positive
No contact	7	14	21	33
Less than 5 years ago	5	7	12	41. 7
More than 5 years ago	6	17	23	26. 1
Total	18	38	56	32. 1

Table 2. Length of time spent in prison prior to tests

Length of stay	Number positive	Number negative	Total	Percent positive
Less than 1 year	8	22	30	26. 6
More than 1 year	10	16	26	38. 5
Total	18	38	56	32. 1

An attempt was made to determine if length of imprisonment had any influence on the results of the serologic tests for Q fever by comparing the tests of the 56 males who had adequate histories of animal contact. The data in table 2 indicate that inmates confined for 1 year or longer had a higher incidence of positive serologic tests for Q fever. However, the differences are not significant.

All but four men in the study group had consumed milk; therefore, it was impossible to select a non-milk-drinking control group large enough to be significant. All four men who did not drink milk had negative titers.

Discussion

Although there was no evidence of clinical disease in the study group, the presence of complement fixation antibodies must be accepted as an indication that exposure to a specific agent had occurred.

Twelve inmates of the 120 tested 3 times or more demonstrated fourfold or greater increases in CF titers during the study. As a diagnostic tool, a fourfold increase in titer, along with typical clinical symptoms, is satisfactory evidence of a specific clinical infection. Normal fluctuations in titers can be expected at lower dilutions, and reexposure to the agent should exert a stimulating effect on the production of antibodies.

In clinical cases in Idaho, titers frequently have risen from negative during the acute phase to a titer of 1:256 and greater.

Nearly all the titers of the study group were in the magnitude of 1:16 and 1:32. The 12 inmates who demonstrated fourfold or greater increases in the CF antibody titer experienced an inapparent primary infection or an anamnestic response to the organism.

Antibody levels of 1:8 and 1:16 persisted in some males of the group throughout the 2-year test period. This would indicate a persistent titer that did not change after pasteurized milk was consumed.

The results show that individuals who routinely drank raw milk containing viable *C. burnetii* had a higher incidence of positive Q fever blood tests than did two similar control groups who drank only pasteurized milk. Thirty-five percent of the 120 individuals routinely drinking raw milk had positive blood tests, while only 8.9 percent of the 112 individuals committed after pasteurization was instituted gave positive tests. Only 4 of 99 individuals in another control group drinking only pasteurized milk had positive Q fever complement fixation tests.

This study indicates that consuming raw milk from a dairy herd naturally infected with Q fever did not produce detectable clinical disease in an adult male population. The data indicate that a significant number in the group demonstrated a positive serologic response during the test period. Increasing numbers in the group became serologically positive after a period of time in prison. Of the 42 males considered to have positive serologic tests, 28.6 percent were serologically negative originally or developed fourfold or greater increases in Q fever titers during the test period.

The available evidence suggests that, in the absence of clinical disease, the ingestion of viable *C. burnetii* in raw milk may give rise to detectable antibodies. If this observation is valid, then it should be considered in evaluating the results of Q fever antibody surveys of humans. More definite studies of a serologically

negative group from Q fever-free areas being fed known concentrations of infected raw milk are needed to define more specifically the relationship between infected milk, production of antibodies, and clinical disease.

Further studies also should be made to determine if oral ingestion of viable *C. burnetii* will protect against subsequent clinical infection via the upper respiratory route.

Summary

Positive complement fixation titers were noted in 42 (35 percent) of 120 men in at least 1 test of a penitentiary group in Idaho using raw milk infected with *Coxiella burnetii.* Twelve of the 120 individuals had positive conversions or showed a significant increase in antibody titer during the testing period. This group had a higher incidence of positive Q fever complement fixation tests than did two similar control groups who drank only pasteurized milk. Tests of a control group of 112 prisoners consuming only pasteurized milk revealed positive complement fixation tests in 10 (8.9 percent). Positive blood tests were obtained in 4 of 99 men in another control group who drank pasteurized milk. There was no evidence of infection or stimulating effect on production of antibodies in either of the two control groups.

No clinical disease attributable to Q fever infection was recognized in the prison group. Interpretation of true infection or recall phenomenon from reexposure could not be made because many of the prisoners had antibody titers before commitment.

Further studies using a serologically negative group are needed.

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