

MILK and FOOD SANITATION

Q FEVER AND ITS RELATION TO DAIRY PRODUCTS*

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Q fever is an infectious disease of man. It is found as an inapparent infection in animals. Cattle, sheep, and goats are found widely infected in nature and are probably the source of the organisms infecting man. These animals shed the organism in their milk which introduces it into the environment of man. It has been demonstrated that the rickettsiae of Q fever may survive present day pasteurization procedures.

This manuscript presents preliminary data of the survival of this organism when suspended in milk and subjected to various time-temperature combinations within the pasteurization range.

HISTORY

Q fever is a rickettsial disease of man. The disease in man may be acute or chronic and induce either a mild or severe illness. The etiological agent, *Coxiella burnetii* (Derrick), is found rather widely distributed in nature where it causes inapparent infections in many species of animals¹. Epidemiological observations have implicated cows, sheep, and goats as important sources of the organism infecting man^{2, 3, 4, 5}. Investigation of outbreaks of Q fever in this country have not revealed arthropods as being important in the transmission of the causative agent from animals to man, neither has man to man nor man to animal transmission been demonstrated as significant in the life history of this organism.

In 1935, Derrick⁶, investigating an outbreak of a febrile illness among packing house workers in Brisbane, Australia, recognized that it represented a disease not previously described. He named it Q fever, the "Q" standing for "query" because at that time the questions surrounding its etiology were still

unanswered. Further investigation in Australia⁷ proved that the etiological agent of this disease was a rickettsia and the name *Rickettsia burneti* was proposed.

Also in 1935, in the Nine Mile Creek area of Montana, Davis and Cox⁸ isolated an infectious agent from the Rocky Mountain wood tick *Dermacentor andersoni*. This agent was identified as a rickettsia and named *Rickettsia diaporica*⁹ because as the name suggests it is filterable. The disease in man caused by this organism was called Nine Mile Fever¹⁰. Subsequent investigations^{11, 12} demonstrated that the causative agent of Nine Mile Fever and that of Q fever were the same.

The etiological agent of Q fever resembles other rickettsiae both morphologically and tinctorially. However, it differs from other members of the genus *Rickettsia* in these important ways: it is filtrable^{8, 9}, it produces no soluble antigen¹³, it does not stimulate the formation of agglutinins of the "X" strains of *Bacillus proteus*^{6, 14} and in addition, the rash observed in other rickettsial diseases is not seen in Q fever. For these reasons a new genus was proposed and the organism is now listed in Bergey's *Manual of Determinative Bacteriology*¹⁵ as *Coxiella burnetii*, (Derrick).

Since these early investigations, Q fever has been reported from many different countries of the world¹⁶. Naturally occurring outbreaks of Q fever in the United States were first recognized in 1946. In Amarillo, Texas, in March 1946, 55 of 136 employees of three meat packing houses became ill of the disease¹⁷. In August of the same year another outbreak occurred in Chicago in which 33 of 81 men on the killing floor of a packing house contacted Q fever¹⁸. In 1947, Dr. Frank Young¹⁹ demonstrated the disease to be present in Southern California, and shortly thereafter it was found to be endemic throughout of California^{2, 3, 5, 20, 21}. Since this time, studies have revealed complement-fixing antibody in the sera of persons residing in Massa-



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chusetts, Minnesota, Oregon, and Texas²² and in Pennsylvania²³. Further elucidation of the geographical distribution of Q fever in the United States will emerge as interest and inquiry develop in various areas of the country.

Infected cows, sheep, and goats shed the organism in their milk^{4, 24, 25} and, therefore, this represents one mode of transmission of the organism from animals to man. The importance of this method of transmission in the epidemiology of the disease needs much more clarification. Most epidemiologic investigations of outbreaks of Q fever have not revealed contaminated milk as being of primary importance in the spread of the disease; instead other routes of transmission have been suggested, such as the air-borne route or contact with contaminated meat, hides, hair, or wool^{2, 3, 4, 5, 23, 26}. It remains for future study to determine exactly

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the role contaminated milk may play in other outbreaks of Q fever and in the sporadic cases of the disease, the total number of which probably far exceeds those occurring in recognized outbreaks. Nevertheless, since *C. burnetii* is found in the milk of naturally-infected cows, and since antibodies to the organism have been demonstrated in the sera of dairy cattle from at least 10 of 48 states²⁷, information on the effect of heat on these organisms is needed.

Early in the study of Q fever there were indications that *C. burnetii* was more resistant to heat and certain chemical agents than most other rickettsiae²⁶. Huebner and the group investigating Q fever in southern California²⁸ found 3 of 32 samples of vat-pasteurized market milk and 1 of 4 specimens of vat-pasteurized market cream, when injected into guinea pigs, induced formation of complement-fixing antibody against *C. burnetii*. They also demonstrated that this rickettsia could be recovered from butter made from the unpasteurized milk of naturally-infected cows²⁹. Lennette³⁰ and the group investigating Q fever in northern California found 1 of 35 samples of commercially vat-pasteurized milk and 2 of 42 specimens of HTST pasteurized milk showed serological evidence in guinea pigs of the presence of the rickettsia of Q fever. In addition, Lennette isolated the organism from one of the milk samples which had stimulated the production of antibodies in guinea pigs. Upon the basis of these findings, a cooperative study of the effect of pasteurization on the organism of Q fever in milk was undertaken by the United States Public Health Service*, the Dairy Industries Supply Association, and the School of Veterinary Medicine, University of California.

It was the consensus that the study should be conducted in three phases. First, a laboratory investigation of the thermal-resistance of *C. burnetii* when suspended in skim-milk, whole milk, and cream. Second, a study of the effects of

commercial pasteurization upon this organism using commercial equipment*. Third, a survey of the efficiency of commercial pasteurization in eliminating viable organisms from the milk of plants receiving milk containing *C. burnetii*. It is the purpose of this paper to present certain information accumulated in this study to date.

LABORATORY STUDIES

Time will not allow a description of the methods used except to refer briefly to certain problems arising when working with *C. burnetii* that are not encountered with the bacterial agents of disease. Foremost among these is the fact that laboratory animals infected with *C. burnetii* do not develop symptoms or lesions that might be used as criteria of infection. The appearance of specific complement-fixing antibody in inoculated guinea pigs, therefore, is usually used to indicate the experience of the experimental host with the etiological agent. Since it is the opinion of some investigators that dead *C. burnetii* may be immunogenic, it becomes necessary to make at least one sub-passage in guinea pigs to ascertain the viability of the organisms in the original inoculum.

Certain laboratory strains of *C. burnetii* have been adapted to growth in the yolk-sac of developing chick embryos. This provides another method of demonstrating the viability of this rickettsia. With the Henzerling strain, organisms may be demonstrated in smears of the yolk-sacs of the third serial egg passage. At the present time, because the Henzerling strain of *C. burnetii* is being used, the guinea pig method, the egg method, and a combination of the two are employed for the demonstration of viable rickettsiae. In this way correlation may be established between the indirect method, in which the appearance of antibody is used as a criterion of infection, and the direct microscopic demonstration of the growth of the rickettsiae in eggs. This is important because in future work with field strains of *C. burnetii*, only the indirect method can be used since field strains of the

organism may not multiply in embryonating eggs immediately.

At the present time skim milk is being used as a diluent for the rickettsiae because it is known that *C. burnetii* survived quite well in this medium. Ten-fold dilutions of the rickettsiae in skim milk were made and each of these dilutions divided into aliquots. One series of these dilutions was stoppered and placed in the refrigerator and the other series heated in the water bath. The samples of milk to be heated were flame-sealed in thin-walled glass ampoules and placed in a test tube rack. The test tube rack was then submerged in a constant temperature water bath of large capacity and agitated during the entire period of observation.

Heat penetration curves were ascertained from the temperature records obtained in two additional thin-walled ampoules containing equal quantities of skim milk in which thermocouples were placed, both in the milk and in the air space above it. Readings of these thermocouples were recorded at twelve-second intervals. The heating up time when plotted on semi-logarithmic paper approximate a straight line.

Thus, the milk sample was heated to the required temperature in less than three minutes. At the end of the holding time the test tube rack containing the milk samples was placed in a cold water bath and chilled to temperatures below 50°F in approximately three minutes.

Subsequent to heating, the heated and unheated dilutions were each inoculated into guinea pigs and embryonating eggs. With the subpassages that were required, 96 guinea pigs and 288 embryonating eggs were necessary for each time-temperature trial. About three months are required to complete the examinations.

Information transmitted to this laboratory from other investigators^{31, 32, 33} concerning the number of *C. burnetii* shed in the milk of infected cows shows that at no time have more rickettsiae been demonstrated than those found in 10,000 infectious guinea pig doses per ml. This information agrees with the findings of this laboratory. It may also be said, that this number of organisms is found in milk only for brief periods during lactation. Some

* Participated in the Communicable Disease Center, Atlanta; The Environmental Health Center, Cincinnati; and the Milk and Food Section, Division of Sanitation, Washington, D.C. The cooperation and assistance of the personnel of these stations is appreciated.

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of the results of experiments in this laboratory, conducted under the conditions outlined above, are presented in table 1.

body in first-passage guinea pigs.

SUMMARY

In summary the following may be said: Most epidemiologic surveys

method of pasteurization as performed in the United States.

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TABLE 1—SURVIVAL OF DIFFERENT CONCENTRATIONS OF *C. burnetii* IN STERILE SKIM MILK WHEN HEATED AT VARIOUS TEMPERATURES FOR 30 MINUTES.

Conc. of <i>C. burnetii</i> in G. pig doses/ml.	Temp heated for 30 min	Survival of viable <i>C. burnetii</i> *
1,000	141	2/2
	142	1/3
	143	0/3
10,000	142	3/3
	143	1/3
	144	0/3
100,000	143	3/3
	144	0/3

*Numerator equals number of times survival was demonstrated. Denominator equals the number of trials.

The data presented in table 1 shows that when a milk sample contains 10,000 infectious guinea pig doses of *C. burnetii*, the maximum thus far demonstrated in the milk of infected cows, enough organisms may survive heating at 143°F for 30 minutes to infect guinea pigs and embryonating eggs. Table 1 also lists the results when 1,000 and 100,000 infectious guinea pig doses of the organism were used. It should be emphasized again that in these trials the organisms were diluted in sterile skim milk.

Experiments in which the organism is contained in whole raw milk heated at different temperatures for different lengths of time are not concluded. Therefore at the present moment it is impossible to predict whether or not whole raw milk will exert a protective effort on the organism when subjected to heat. Experiments using the High-Temperature-Short-Time method of pasteurizing milk have been started, but it will be some time before information regarding this technic will be available.

A note of caution should be included regarding the interpretation of data concerning the thermal resistance of the organism of Q fever unless information on the viability of the heated rickettsiae is included. An evaluation of experiments conducted in this laboratory³⁴ comparing four methods of demonstrating the presence of *C. burnetii* in heated milk specimens indicates that dead rickettsiae are capable of inducing complement-fixing anti-

body have not incriminated milk in the transmission of *C. burnetii* from animals to man. Nevertheless, the organism of Q fever is shed in the milk of infected animals and therefore may come into contact with man. Cows infected with *C. burnetii*, while apparently not exhibiting symptoms of disease may have the organism in their milk in appreciable numbers.

Surveys in the United States of commercially vat-pasteurized milk, in which vats without air-space heaters were used, have shown viable organisms present in 4 of 67 samples, although two of the four samples were phosphatase positive. A survey of HTST pasteurized milk demonstrated that 2 of 42 samples would induce the formation of complement-fixing antibody in 2 of 4 first passage guinea pigs.

The maximum number of *C. burnetii* demonstrated so far in the milk of infected cows is that contained in 10,000 infectious guinea pig doses per ml. It could be demonstrated in 1 of 3 trials, that when this number of organisms was suspended in skim milk and heated at 143°F for 30 minutes enough viable rickettsiae remained to infect guinea pigs and embryonating eggs. At the present time, no experimental data is available regarding the survival of the organism of Q fever when heated in whole milk.

Current information is wholly inadequate to allow evaluation of the efficiency in eliminating viable *C. burnetii* from milk by the HTST

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REPORT OF THE COMMITTEE ON COMMUNICABLE DISEASE AFFECTING MAN

The Committee on Communicable Diseases Affecting Man in its 1952 Annual Report announced that it has undertaken the formulation for adoption by this Association of a manual of epidemiological procedures for the investigation of milk-borne and food-borne disease

outbreaks. The principal objectives in preparing this manual were cited as follows:

1. To provide sanitarians with a procedure to guide them when confronted with milk-borne or food-borne disease outbreaks;
2. To stimulate an active interest on the part of all sanitarians in the epidemiological aspects of their programs; and
3. To improve reporting of such outbreaks in order that sufficient data will be available for use by local, state, and federal agencies and industry in milk and food sanitation program planning.

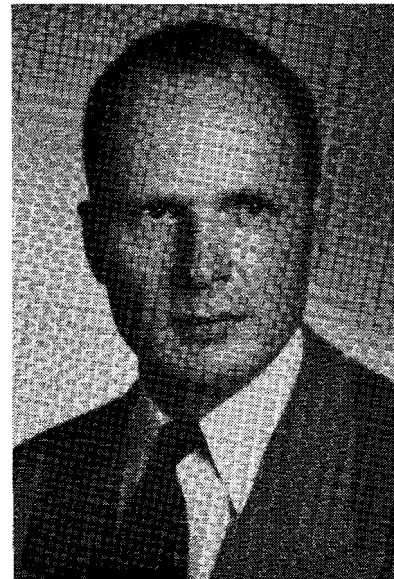
The Committee had planned to have completed the first working draft of the proposed procedure for presentation to the Executive Board of this Association for their review and comment at this 1953 Annual Meeting. However, the Committee regrets that the size of this project has prevented it from proceeding as rapidly as planned. As soon as the first working draft is completed, it will be submitted to the Executive Board for suggestions as to change with respect to format, technical content, proposed procedure for completion of the manual, etc. It will then be submitted to a number of the outstanding epidemiologists in the country and to those members of the Association who, because of their interest in and knowledge of the subject, might wish to contribute to the technical accuracy of this publication. The Committee urges those members of the Association who would wish to review the draft of this manual for the purpose of commenting on it, to so advise the Chairman, or any other member of the Committee.

The Committee hopes to be able to present this procedure in completed form to the Association in 1954 for adoption as its recommended procedure for the investigation of milk-borne and food-borne disease outbreaks.

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RACE APPOINTED FIELD DIRECTOR OF DAIRY PRODUCTS IMPROVEMENT INSTITUTE



Appointment of Donald H. Race as Field Director of the Dairy Products Improvement Institute has been announced by W. A. Wentworth, president of the Institute. At the same time it was announced that the Institute's offices had been moved from Buffalo to a new location at 302 East State Street, Ithaca, New York.

Mr. Race's appointment, which was effective August 1, follows the recent announcement of the appointment of Dr. Arthur C. Dahlberg of Cornell University as Advisor to the Board of Directors of the Institute. Dr. Dahlberg will continue his present duties and activities as Professor of Dairy Industry at Cornell University, while serving in his advisory capacity with the Institute.

Mr. Race comes to his new position after two and a half years with the Pennsylvania Bureau of Milk Sanitation in Harrisburg. Prior to that he was associated with the Stephens Bros. Dairy in Carbondale, Pennsylvania.

He graduated in 1951 from Pennsylvania State College where he majored in dairy manufacturing. From 1942 to 1945 he was an aviator in the U. S. Navy.

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