OBSERVATIONS ON THE THERMAL INACTIVATION OF THE ORGANISM OF Q FEVER IN MILK.[1]

J. B. ENRIGHT[2] W. W. SADLER[2] AND R. C. THOMAS[3]

School Veterinary Public Health, University of California, Davis and Robert A. Taft Sanitary Engineering Center, U. S. Public Health Service, Cincinnati, Ohio

Q Fever is an infectious disease of man. Cattle, sheep and goats, who for the most part suffer inapparent infections with the organism, are the important sources of infection for man. These animals shed the organism in their milk.

This manuscript reports on the cooperative studies designed to determine the times and temperatures needed to eliminate the causative rickettsiae, Coxiella burnetii, **from cows milk. It is reported that the present minimum standard of pasteurization by the vat method of 143° F. for 30 minutes is inadequate, but the temperature of 145° F. for 30 minutes will eliminate the organism. The pasteurization of milk according to the present standards for HTST equipment of 161° F. for 15 seconds seems adequate to destroy** C. burnetii.

Q Fever is a rickettsial disease of man which may be acute or chronic in course and mild or severe in reaction. In the short time since the disease was first described by Derrick (*7*), the disease or the causative organism has been identified in many countries of the world (*5*). The etiological agent, *Coxiella burnetii* (Derrick) is found rather widely distributed in nature where it causes infection in many species of animals (*8*). In the majority of animals the infection is not associated with illness and it is not unusual to find an infected dairy cow one of the dairyman's better producers.

Investigation of various outbreaks of Q Fever in the United States has revealed that cows, sheep and goats are the important sources of infection for man (*2, 3, 17, 21*). The rickettsiae are shed in the milk of the infected animal (*4, 10, 12, 16*) and from other body orifices (*1, 14, 18, 22, 23*). Since *C. burnetii* may be found in great numbers in the milk of infected dairy cows, this food becomes one of the vehicles responsible for transmission of the organism from the infected cow to man. The organism is also found on dust and other particles in the environment of the infected animal and the smaller of these particles may become air-borne (*6*). Inhalation of these infected air-borne particles constitutes another means of exposing man to the organism.

Dr. John B. Enright is Chairman of the Department of Veterinary Public Health, School of Veterinary Medicine, University of California. He received his Ph. D. degree from Stanford University in 1947. Later he was acting assistant chief of the Viral and Rickettsial Disease Laboratory California State Department of Public Health.

His work has been in the field of viral and rickettsial diseases, more specifically poliomyelitis, encephalitis, Q Fever and rabies.

The early studies of Q Fever in California revealed *C. burnetii* to be relatively more resistant to heat than other vegetative pathogens. Evidence of this characteristic was obtained with the isolation of the organism from milk pasteurized according to the recommended minimum standards (*11, 15*). This observation was confirmed in the laboratory together with indications that *C. burnetii* was also quite resistant to some of the more common disinfecting agents (*19*). The rickettsiae were shown to be in the butter and the buttermilk made from the unpasteurized milk of infected dairy cows and this butter was still infectious for guinea pigs 41 days after preparation (*13*).

Because of these findings and their implications to the public health, the Director of the California State Department of Public Health inquired as to the possibility of a study to determine the times and temperatures necessary in the pasteurization of milk to elimi-

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[2]Department Veterinary Public Health, School of Veterinary Medicine, University of California, Davis, California.

[3]Robert A. Taft Sanitary Engineering Center, Division of Sanitary Engineering Services, U. S. P. H. S., Cincinnati, Ohio.

nate viable *C. burnetii*. Upon this basis a cooperative study was undertaken by the United States Public Health Service[4], the Dairy Industry Supply Association, The Milk Industry Foundation and the School of Veterinary Medicine of the University of California with the close support of the California State Departments of Agriculture and Public Health[5].

The study required better identification of the problem in the field, determination of the most suitable quantitative methods, laboratory thermal-resistance studies and application of the laboratory findings to commercial equipment and methods. The results of this study are published in detail (9). The purposes of this communication is to present some of the findings of this study of pertinent interest to the International Association together with some information obtained during the course of the study not included in the detailed report.

EXPERIMENTAL

Before a realistic thermal-resistance study could be conducted it was necessary to obtain answers to two fundamental questions.

The first of these questions concerned the maximum number of *C. burnetii* that might be found in the milk of an infected cow. To obtain information on this point the milk supplies of three communities were sampled; samples of milk were obtained from widely-separated areas of California; the milk of individual dairy cows within an infected dairy herd was tested at intervals during which time some of the animals calved; a dairy cow was infected and the number of rickettsiae in her milk determined; and information from investigators in other parts of the United States was obtained. It was necessary to establish this point because the heat necessary to eliminate the number of rickettsiae found in the placental fluids of an infected cow might result in objectionable flavors in the milk, while the temperature required to kill a lesser population might result in an unsafe product.

The second question concerned the evalution of several methods to ascertain the most sensitive and accurate procedure for determining the presence of small numbers of viable rickettsiae in a test sample. Since *C. burnetii* will not grow on artificial media and because it neither induces a constant symptomatology nor pathology in animals, the criterion for infection is the appearance of specific complement-fixing antibody in the serum of the experimental host. The question arose as to the possibility of the heat-killed rickettsiae also inducing the formation of specific antibody.

When the best answers to these questions were obtained a thermal-resistance study was conducted in the laboratory with the proper concentration of *C. burnetii* suspended in whole raw milk. This was followed by observations on the thermal-inactivation of the rickettsiae suspended in whole raw milk and subjected to different combinations of time and temperature in regular commercial heat exchangers installed in a complete modern pasteurization plant using standard procedures and controls[6].

Limitations of both time and space prevent the description and identification of the materials and methods used in this study. These are presented in detail elsewhere (9).

RESULTS

A total of 109 samples of the raw milk supply of three California cities was tested for the presence of *C. burnetii*. Each sample was obtained from the milk of a different producer except for one sample that was obtained from a tank truck that contained the milk from three large dairies. These samples were obtained at the plant upon delivery from the dairy farm and were composite samples of the milk from each farm. It was found that the milk from 1 of 5, 1 of 10 and 1 of 13 of the producers supplying the respective cities contained the organism of Q Fever. Of the 109 samples 8 or 7.3 per cent contained the organism in demonstrable numbers.

In a second survey 376 retail milk or cream samples were obtained from different areas of California. While each of these samples was from a different creamery and represents a composite from different producers, this should not be considered as a representative sampling of the milk supply of California – only as a large number of samples representing several geographical areas and many different breeds of dairy cattle. Of the 376 samples 14 raw and one pasteurized contained the organism of Q Fever. Titration of these positive samples revealed that two had 1000, six had 100, four had 10 and three had one infective guinea pig doses[7] per 2 ml. These composite samples indi-

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[7]An infective guinea pig dose may be defined as the minimum number of *C. burnetii* required to infect a guinea pig by intraperitoneal inoculation. In this study these organisms were always contained in 2 ml. of inoculum. This unit of measurement of the concentration of rickettsiae, the infective dose, is composed of many individual organisms, but is treated as a unit mathematically.

cated no difference in the numbers of rickettsiae in the milk from different geographical areas of California.

The milk from 137 individual cows in a dairy herd known to be infected with *C. burnetii* was obtained. Eighteen of these 137 samples were found to contain the rickettsiae. Titration of these positive samples showed three contained 1000, five contained 100, five contained 10 and five contained one infective guinea pig dose per 2 ml. Serological evidence indicated that 12 of the 18 positive cows were infected at least 200 days prior to the sampling of their milk. The milk from four of these positive animals was collected 205 days later after each had calved. One of these was found to be shedding the same number of *C. burnetii* as previously. This animal's milk contained 1000 infective guinea pig doses per 2 ml. and serological evidence indicated that she had been infected at least 405 days prior to the collecting of this second sample of milk.

A lactating dairy cow was inoculated by way of the teat canal with the Henzerling strain of the organism. She was nursing two calves and presumably milked out each day. On the ninth day after inoculation a milk sample was taken, immediately diluted and inoculated into guinea pigs. This milk sample was found to contain 10,000 infective guinea pig doses per ml.

In the studies of other investigators, *C. burnetii* in concentrations of 10,000 infective guinea pig doses had been demonstrated in the milk of infected dairy cows.

Since *C. burnetii* in concentrations to be found in 1000 infective guinea pig doses had been found in milk samples that were composites of the milk of many individual cows, either all the animals in the infected herd from which the composite was derived were shedding the same number of organisms in their milk or a few individuals were shedding tremendous numbers. Investigation of the individual cows in an infected dairy herd revealed only 13.1 per cent of the animals were shedding these organism in their milk. Furthermore, since all dairy breeds were not tested, and some might secrete more organisms in their milk than others, it was deemed prudent to use a test population of 100,000 infective guinea pig doses in the thermal-resistance studies.

Four methods of determining the presence of small numbers of *C. burnetii* were compared. Each method was compared using both unheated and heated samples of milk containing the organism. Evaluation of the results of this experiment showed that two consecutive passages in guinea pigs was the most sensitive and specific test compared. Results using one passage in guinea pigs were influenced by the killed rickettsia contained in relatively high proportion in the heated samples.

In the thermal-resistance study conducted in the laboratory a population of *C. burnetii* represented by that number contained in 100,000 infective guinea pig doses was suspended in whole raw milk and subjected to various combinations of time and temperature. Well-controlled, experimentation was conducted through the temperature range from 141° F. to 151° F. The absence of good sampling devices prevented accurate observations in the temperature zone 153° F. to 163° F., with the extremely short time intervals necessary. While certain statistical considerations limit the reliability of extrapolation, this was done as the only method available of indicating the time-temperature relationships to be found in this higher temperature zone. The statistical treatment of the data, the use of a 2 *sigma* or 97.7 percent confidence interval added to the minimum time of destruction, and the relatively large population subjected to heat all tend towards minimizing the dangers of this extrapolation. Nevertheless, the time-temperature relationships expressed by the extrapolated regression line may be accepted as indications only for application to the data obtained in this higher temperature range with the high-temperature-short-time commercial pasteurization equipment.

In Table 1 the pertinent results of calculations from the data obtained in the laboratory study are presented. These are arranged according to certain selected temperatures and the time point on the LD_{50} percent, and the minimum time of destruction regression line intercepted by these temperatures. In addition, the intercept of the minimum time of destruction regression line plus a 2 *sigma* or 97.7 percent confidence level (as a margin of safety) is also listed for each temperature. The maximum times of survival and minimum times of destruction from which the regression lines were derived include the factor representing the lethal effect occuring during the heating-

TABLE 1 — TIMES AT PERTINENT TEMPERATURES ON THE REGRESSION LINES DERIVED FROM THE LABORATORY DATA.

Temp. °F.	50 percent endpoint	Minimum time of destruction	Minimum time of destruction plus 2 sigmas
143	29.39 min.	33.02 min.	46.03 min.
145	16.29 min.	18.31 min.	25.42 min.
160	11.7 sec.	13.2 sec.	20.4 sec
161	8.7 sec.	9.8 sec.	15.4 sec.
162	6.5 sec.	7.3 sec.	11.6 sec.

up and cooling-down periods expressed in equivalents of the times at the holding temperature.

In Graph I are presented the regression lines derived from the data obtained in the laboratory thermal-resistance study together with the presently recommended pasteurization line. There is also plotted on this graph the results of the study in which *C. burnetii* suspended in whole raw milk was subjected to different combinations of time and temperature in a commercial Vat-Type pasteurizer. The dots represent points of maximum survival while the circles represent the points of minimum destruction obtained with this type of equipment. The times are those at the holding temperature only.

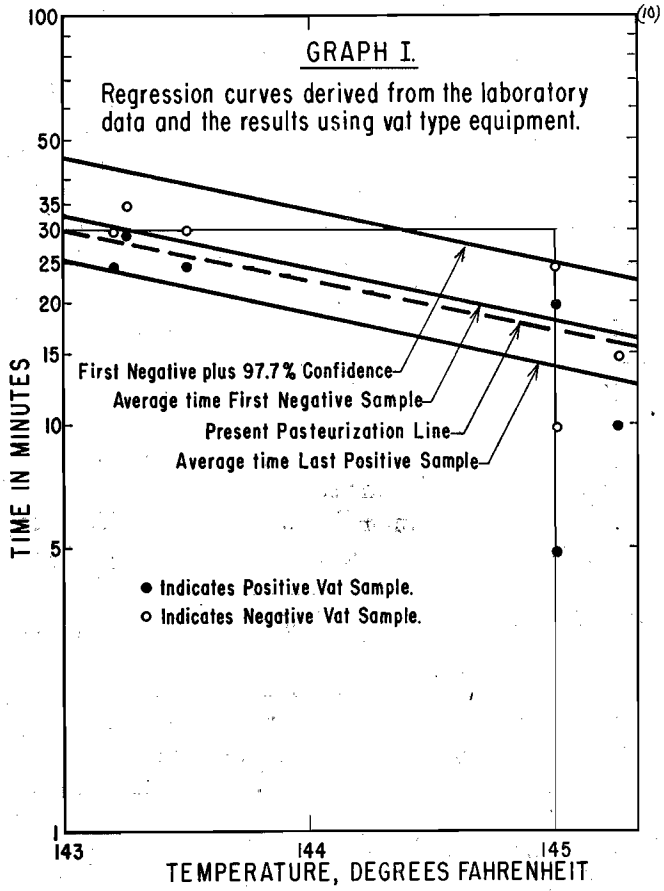
In Graph II are plotted the results obtained when the organism of Q Fever suspended in whole raw milk was subjected to heat in a commercial type high-temperature-short-time heat exchanger. In this study fifteen temperature points were studied in the range 155.5° F. to 163° F. For each temperature, the total time the organisms were subjected to heat (expressed in equivalents of the holding time) and the results of animal inoculation are plotted for samples obtained at the entrance to the holding tube, at the end of the holding tube and at the exit from the cooling section. The dots represent the presence of viable rickettsiae while the circles indicate no viable organisms could be demonstrated. In this way 45 samples were obtained.

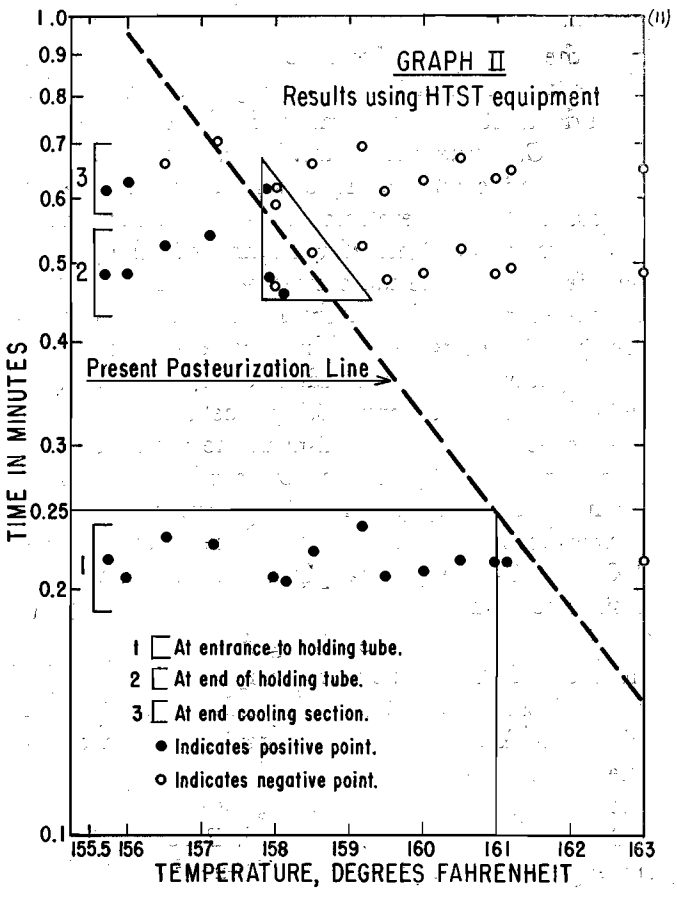
It should be pointed out that the concentration of organisms heated in the HTST equipment was considerably higher than that used in the laboratory studies. Populations of one to ten million infective guinea pig doses were used. This error in dilution of the 100 gallons of milk used in each trial was not discovered until the experiment was finished due to the length of time required for its determination. However the larger concentration of organisms may be considered as an additional safety factor.

DISCUSSION

The collection of milk samples from different areas of California revealed the presence of *C. burnetii* in many of the raw milk samples collected. The samples were either composites of the milk produced on one dairy or composites of the milk received from several producers by a creamery. Even though the samples represented milk from many animals some were found to contain the organism in concentrations represented by that number found in 1000 infective guinea pig doses. This indicates that all the animals whose milk contributed to the composite were secreting this number of organisms in their milk or some were shedding many more.

Investigation of the individual animals in a dairy herd in which the infection had been shown to be





present for at least 200 days showed only 13.1 percent of the animals shedding the organism in their milk. However, the milk of none of these animals contained over 1000 infective guinea pig doses of the organism in their milk. One infected animal in this herd was continuing to shed this number of organisms 205 days later and after a subsequent calving. The persistence of the infection is indicated by the fact that serological evidence showed this animal to be infected 405 days prior to the collection of this last sample of milk.

Information from other investigators and determination of the concentration of *C. burnetii* in the milk of a dairy cow infected by way of the teat canal demonstrated that the milk of an infected dairy animal could contain as many as 10,000 infective doses of the organism in her milk. Not all breeds of dairy cows were tested and some might be capable of shedding greater numbers of organisms in their milk. This factor could be of importance in the pasteurization of milk from some pure bred dairy herds. Other investigators had indicated the possibility that the greatest number of organisms would appear in the milk shortly after freshening. In the investigation of the individual animals in the infected dairy herd many different time intervals after parturition were encountered, but not all. For the above reasons it was thought safer for the purposes of the thermal-resistance studies to consider 100,000 infective guinea pig doses as representing the maximum number of *C. burnetii* to be found in the milk of dairy cows. Through an error in dilution that could not be discovered until after the experiment was concluded, because of the time required to determine the number of organisms at risk, the concentration of rickettsiae subjected to heat in the HT ST runs was in the neighborhood of 1 to 10 million infective guinea pig doses. This greater number of organisms can only be considered as increasing the margin of safety of these findings.

The results of the laboratory conducted thermal resistance studies are briefly outlined in Table 1. It may be seen that the present minimum standards recommended for the pasteurization of milk by the vat method will not kill *C. burnetii*. Elevating the temperature from 143° F. to 145° F. and maintaining the 30 minute hold interval results in the destruction of the number of *C. burnetii* apt to be found in cows milk. This time and temperature combination of 145° F. for 30 minutes offers more than the 97.7 percent confidence level as a margin of safety as indicated in Graph I. Application of the findings of the laboratory study to commercial vat type pasteurization equipment equipped with a spaceheater confirm these conclusions. It may be seen in Graph I that *C. burnetii* did survive at time and temperature combinations greater than those represented by the present pasteurization curve, but never at combinations greater than the regression line derived from the minimum time of destruction (first negative sample) points plus a two *sigma* or 97.7 percent confidence interval.

The points plotted on Graph II show the results of heating large numbers of *C. burnetii* suspended in whole raw milk in HTST pasteurization equipment. These points are presented in relation to the presently recommended pasteurization curve. Since extrapolation of the regression lines calculated from the data collected in the laboratory in a lower temperature range might be misleading these lines are not included in Graph II. Certain pertinent points of time and temperature on the extrapolated lines are presented in Table 1. The use of a 15 second holding time and a two degree temperature interval does not allow precise determination of either the maximum survival of the minimum destruction endpoints. It may be seen by examination of the 44 points on Graph II that only one maximum survival endpoint falls above the present pasteurization line, while two minimum time of destruction endpoints are below it. Further strength is given to the impression that the present pasteurization line approximates the thermal-inactivation of *C. burnetii* by consideration of the seven points located within the triangle on Graph II. Here there are positive and negative points within a 0.5° F. and 0.12 minute range. This relatively narrow range straddles the present pasteurization line, but is well below the minimum destruction plus the 97.7 confidence interval regression line extrapolated from the laboratory data.

The data obtained with the HTST equipment include the total heat treatment of the organism. However, it must be kept in mind that in practice the only time controlled is that in the holding tube. The interpretation of the data must be qualified by these facts and presented with reservations as conditions under which different heat-exchangers in the hands of different operators will vary. These variations will influence the lethal effect in the heat-up and cool-down periods. However if the 15 second interval in the holding tube is strictly adhered to and if the regression line derived from the laboratory data which intercepts 161° F. at 15.4 seconds is accepted it is highly improbable that the total equivalent heat treatment will fall below this time-temperature combination. Furthermore, the number of safety factors and the use of a concentration of organisms 100 to 1000 times greater than the maximum concentration demonstrated in the milk of infected dairy cows still further reduces the probability of *C. burnetii* surving HTST pasteurization at the minimum recommended standards when suspended in whole raw milk.

Phosphatase tests run by the method of Sanders and

Sager (*20*) were run on 36 of the 44 samples obtained in this experiment. None of the samples collected from the end of the holding tube at temperatures above 158° F. were positive. This again emphasizes the important point that phosphatase positive milk indicates greatly inadequate heat treatment, but phosphatase negative milk does not mean that the milk was pasteurized according to the minimum recommended standards for HTST equipment.

SUMMARY

Observations relating the number of organisms apt to be found in the milk of infected cows to the population of *C. burnetii* to place at risk in studies of the effectiveness of pasteurization of milk from cows infected with this rickettsiae have been presented.

Results using the vat type pasteurization equipment support the findings of the laboratory study that 143° F. for 30 minutes is wholly inadequate to eliminate viable *C. burnetii* from whole raw milk, while heating for the same time at 145° F. insures elimination of these organisms with a high level of confidence.

The observations using HTST pasteurization equipment tend to confirm the extrapolated regression line derived from the laboratory data and strongly support the presently recommended standard of pasteurization of 161° F. for 15 seconds as adequate to eliminate viable *C. burnetii* from whole raw milk.

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