PASTEURIZATION OF MILK ARTIFICIALLY INFECTED WITH TWO STRAINS OF BRUCELLA SUIS

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[*Foreword:* Undulant fever as a public health problem is unique in many ways. It has been recognized as such only recently. That the principal source of the disease is in domestic stock no well informed observer doubts. There is no uniformity of opinion concerning the relative importance of channels through which the infection may reach man from animals. No student of the subject denies that infected milk may result in spreading undulant fever among humans.

A recent analysis of 155 cases of sickness that occurred during 1929 and 1930 in Illinois and which were clinically and serologically diagnosed as undulant fever cast a very strong suspicion on raw milk supplies as the agent of transmission in a significant percentage of the total incidence. Observers elsewhere have found evidence that infected milk may be an important means of transmitting the disease.

Furthermore, undulant fever prevalence may be on the up curve, potentially at least. If nothing is done to control the disease a great endemic wave of this ailment among men in the not far distant future is a catastrophe which is well within the realm of the possible. On the other hand a relatively small amount of judicious energy spent now in research and control may offset that possibility.

For these reasons it seems of the greatest importance to bring to light all possible knowledge about the cause of undulant fever and means of controlling the spread of it. The accompanying report is a contribution to an important phase of this knowledge. Some controversy about the efficacy of pasteurization in destroying the causative organisms of undulant fever has arisen. Doubts created by this controversy will survive until the matter is settled by indisputable scientific experimentation. This report might be accepted as closing the chapter on one phase of the necessary experimentation.—ANDY HALL, Director of Public Health, Chairman, State Undulant Fever Committee.]

Results of investigations made in recent years show that (*a*) cattle may be spontaneously infected with Brucella strains of

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the porcine type (Huddleson (1929)), (*b*) cattle may be artificially infected by intravenous injection of porcine cultures (Cotton (1922), Schroeder and Cotton (1925), Carpenter (1927), Graham, Boughton and Tunnicliff (1930)), (*c*) porcine strains may become established in the mammary glands of cows and may be eliminated in the milk (Smith (1929)), Hasseltine (1929), Carpenter and King (1928), Graham (1929)), and (*d*) *Brucella suis* may be isolated from the blood of undulant fever patients (Huddleson (1929), Evans (1927)). Although spontaneous cases of undulant fever have occurred when milk or milk products, so far as could be determined, were not a factor, contaminated milk and dairy products, *via* udder infection, must be considered a possible source of infection in brucelliasis (Carpenter and King (1928), Orr and Huddleson (1928), Murray (1929), Hardy (1929), Illinois Undulant Fever Committee (1929)).

Since porcine Brucella types appear more pathogenic than bovine strains, the intermost susceptibility of cattle and man to porcine Brucella suggests the possible occurrence of a milk-borne, porcine Brucella infection. The possibility of cow's milk producing porcine brucelliasis in man emphasizes the importance of determining the thermal death time of different strains of *Brucella suis* in order to appraise the reliability of milk pasteurization standards in the prevention of porcine Brucella, milk-borne infections.[2]

HISTORICAL

Reported studies on the thermal death time of Brucella strains indicate that a temperature of 140°F. for a shorter time than that prescribed for pasteurization, i.e., thirty minutes, renders Brucella non-viable. McFadyean and Stockman (1909) reported that *Brucella abortus* in the moist state, when exposed to a temperature of 55°C. (131°F.), was destroyed in two hours, and was non-viable after an exposure of ten minutes in water at a

[2] The Illinois Department of Public Health Proposed Milk Ordinance for Municipalities of Illinois designates the heating of milk for thirty minutes at 142 to 145°F. for pasteurization.

temperature of 59 to 61°C. (138.2 to 141.8°F.). Zwick and Wedeman (1912) found that *Brucella abortus* was non-viable after being heated ten to fifteen minutes at 60°C. (140°F.), or five to ten minutes at 65°C. (149°F.). Fabyan (1913) reported that although heat resistance varied with different strains the average strain of *Brucella abortus* was non-viable after ten minutes' exposure to 59°C. (138.2°F.). Park (1927) found that composite cultures prepared from Brucella strains isolated from man, cattle, swine, and goats were killed when exposed for ten minutes to 140°F., seven and one-half minutes to 142°F., and five minutes to 145°F. These thermal death times were determined with artificially contaminated milk which contained 5,000,000,000 Brucella microörganisms per cubic centimeter. Boak and Carpenter (1928) found that an exposure of fifteen minutes to 140°F. destroyed the strains isolated from man, while a porcine strain was destroyed by an exposure of twenty minutes to 140°F. At 142°F. all strains were killed in from ten to fifteen minutes, while at 145°F. the same strains were killed in five minutes. In later experiments by the same authors (1931) temperatures of 142° and 145°F. for twenty and thirty minutes on the most virulent strains of *Brucella abortus* were found satisfactory for the pasteurization of milk. Traum (1930) found that melitensis and abortus strains were killed in fifteen minutes when exposed to 140 to 142°F., while a porcine strain was killed in twenty minutes at 140°F., or in fifteen minutes at 142°F. All three strains were destroyed in ten minutes when exposed to 145°F. Arnold and Gustafson (1930) reported that while caprine and bovine strains were destroyed in thirty minutes at a temperature ranging between 142 and 145°F., porcine strains were more resistant. In milk artificially contaminated with 25,000,000 Brucella microorganisms per cubic centimeter, one porcine strain proved viable after exposure to 146°F. for thirty-five minutes, but was destroyed in forty minutes. Hardy (1930) found strains of *Brucella abortus* non-viable after thirty minutes' exposure to temperatures of 144 to 145°F. Bartram (1931) reported that certain porcine strains may not be destroyed by heating to 140°F. for thirty minutes, to 142°F. for thirty minutes, or to 145°F. for fifteen

minutes. One bovine strain was viable after thirty minutes at 142°F. Murray, McNutt, and Purwin (1932) found that 62 to 63°C. (143.6 to 145.4°F.) in a standard pasteurizer was sufficient to destroy porcine and bovine Brucella strains in three minutes when the pasteurizer was closed. The same degree of temperature applied to the pasteurizer with open lid did not destroy Brucella in the milk foam in thirty minutes. The published data indicate that porcine strains are more resistant than bovine or caprine varieties.

HEAT RESISTANCE OF TWO PORCINE STRAINS

1. Heating of contaminated milk samples

In the study of heat resistance of porcine Brucella, two strains, 2012 and 2872, in artificially contaminated, sterile whole and skim milk samples, were exposed for thirty minutes to temperatures ranging from 134 to 144°F. Strain 2012 was isolated from an aborted, porcine fetus (1922), while strain 2872 was isolated from a spontaneous, osteomyelitic lesion (James and Graham (1930)). Cultures of the two strains grown on agar slants, incubated twenty-four to forty-eight hours at 37°C. and suspended in sterile physiological salt solution, were used as the inoculum. The number of organisms per cubic centimeter was determined by hemocytometer and plate counts. The contaminated milk was heated in approximately 5 cc. amounts in cotton-stoppered tubes, and in 2 cc. amounts in hermetically sealed glass tubes. While it is apparent that Brucella strains might survive a longer period of time in the dried film that sometimes forms on the side of the cotton-stoppered tubes during heating, inasmuch as a comparable milk line may appear in pasteurization vats, this objection may not be of practical significance. However, duplicate contaminated milk samples were heated in sealed tubes for comparison.

The temperatures at which the tubes were heated were maintained in a double boiler and an electrically controlled, constant-temperature water bath. The temperatures in the double boiler bath were determined by a thermometer immersed in a cotton-

stoppered tube of milk, placed in the boiler along with the artificially inoculated tubes. The temperature readings were checked by a second thermometer placed directly in the water. The inoculated tubes and the control tube, with the thermometer, were placed in the bath simultaneously, and the tubes were allowed to reach the specified temperature before beginning the heating period. For sealed tubes the heating period was considered as beginning at the time when the temperature of the water bath became constant after the tubes were added. The temperature did not vary more than one degree during heating. After heating, the tubes were immediately cooled in ice water. To check the accuracy of results obtained with double boiler water bath temperatures, samples of the artificially inoculated milk were heated in an automatic, electrically controlled, water bath. The effect of heat in the double boiler water bath on the two porcine Brucella strains appeared comparable to that obtained with electrically controlled heating.

2. Bacteriologic examination of heated contaminated milk samples

Bacteriologic examination of the artificially contaminated milk, subjected to different temperatures, consisted of plating, on media favoring the growth of Brucella, 0.2 cc. of milk sediment (skim milk) or cream (whole milk) from each sample, immediately after the tubes were cooled. In some cases duplicate cooled samples, or the remainder of the cultured samples, were incubated at 37°C. for four days before culturing. The sediment or cream of the heated samples was streaked on agar plates or inoculated into melted agar and poured into sterile petri dishes. Cultures were incubated for four days at 37°C. Colonies resembling Brucella were transferred from the plates to agar slants which were incubated at 37°C. for two to four days. If the growth, resulting from single colonies picked from the agar streak or poured plate, resembled Brucella culturally (H_2S production and negative carbohydrate reactions) it was identified serologically with Brucella immune and negative serums in dilutions of 1:50, 1:100 and 1:200.

3. Results of bacteriologic examination of heated contaminated milk samples

A. Samples heated in cotton-stoppered tubes

1. Effect of temperatures 140 to 144°F. on strains 2012 and 2872 (5000 Brucella organisms per cubic centimeter) in skim milk. Six samples of milk, inoculated with approximately 5000 Brucella organisms per cubic centimeter, of strain 2012, and 6 samples similarly inoculated with strain 2872 were heated for thirty minutes, 2 samples each at 140, 142, and 144°F. Plate cultures of the heated samples (unincubated) did not yield Brucella. The portions of the milk samples remaining after the plate cultures were made were incubated at 37°C. for two days. The incubated samples inoculated with strain 2012 were administered orally in amounts of 1 cc. to guinea pigs, while the incubated milk samples inoculated with strain 2872 were injected subcutaneously into guinea pigs in 1 cc. doses. The guinea pigs were necropsied at the end of twenty-seven days. No gross, pathologic lesions of brucelliasis were found, while direct cultures of the heart blood, liver, and spleen proved negative. The blood serum failed to agglutinate porcine Brucella antigen

2. Effect of temperature 144°F. on strains 2012 and 2872 (50,000, 1,000,000, and 10,000,000 Brucella organisms per cubic centimeter) in skim and whole milk. Twelve samples of skim milk and 12 of whole milk, in groups of 4, inoculated with strain 2012 and a similar number inoculated with strain 2872 were heated for thirty minutes at 144°F. The amount of inoculum varied so that samples contained 50,000, 1,000,000, and 10,000,000 Brucella organisms per cubic centimeter. Incubated and unincubated, heated, whole milk samples, containing approximately 10,000,000 organisms per cubic centimeter of strain 2012 and of strain 2872, yielded viable organisms. Incubated and unincubated, heated, whole milk samples containing fewer Brucella organisms per cubic centimeter of either strain 2012 or strain 2872 were negative. From incubated, heated, skim milk samples, containing 1,000,000 and 10,000,000 organisms per cubic centimeter of strain 2872, viable Brucella was isolated. However, incubated,

heated, skim milk samples containing fewer Brucella of strain 2872 and those inoculated with strain 2012 did not yield viable Brucella. All unincubated, heated, skim milk samples failed to yield viable Brucella.

3. Effect of temperature 144°F. on strains 2012 and 2872 (100,000,000 Brucella organisms per cubic centimeter) in whole and skim milk. Six skim milk samples and 10 whole milk samples containing approximately 100,000,000 Brucella organisms per cubic centimeter of strain 2012 and a like number of samples, similarly inoculated with strain 2872, were heated thirty minutes at 144°F.[3] Viable Brucella was recovered from incubated and unincubated, heated, whole and skim milk samples inoculated with strains 2012 and 2872.

4. Effect of temperature 144°F. on strains 2012 and 2872 (500,000,000 Brucella organisms per cubic centimeter) in whole milk. Three whole milk samples containing approximately 500,000,000 organisms per cubic centimeter of strain 2012, and 3 samples similarly inoculated with strain 2872 were heated fifty minutes at 144°F.[4] Samples, plated immediately after cooling, yielded viable Brucella. No samples were incubated.

B. Samples heated in hermetically sealed glass tubes

1. Effect of temperature 144°F. on strains 2012 and 2872 (100,000,000 Brucella organisms per cubic centimeter) in whole milk. Whole milk samples, inoculated with strain 2012 and strain 2872, 100,000,000 Brucella organisms per cubic centimeter, were sealed in glass tubes and heated for thirty minutes at 144°F. Three tubes of milk inoculated with each strain were removed at two, five, seven, ten, fifteen, twenty, twenty-five and thirty minutes. These samples were cooled in ice water and cultured immediately. Samples containing strains 2012 and 2872 yielded viable Brucella after being heated two and five minutes but were negative after being heated seven minutes.

[3] The remainder of this contaminated milk was heated in hermetically sealed glass tubes—see Results B 1.

[4] The remainder of this contaminated milk was heated in hermetically sealed glass tubes—see Results B 2.

2. Effect of temperatures 134, 140, 142, and 144°F. on strains 2012 and 2872 (500,000,000 Brucella organisms per cubic centimeter) in whole milk. Whole milk samples artificially contaminated with strain 2012 and with strain 2872, approximately 500,000,000 Brucella organisms per cubic centimeter, were sealed in glass tubes and heated at 134, 140, 142 and 144°F. for thirty minutes. Three samples containing each strain were removed at two, five, seven, ten, fifteen, twenty, twenty-five and thirty minutes, cooled in ice water, and cultured immediately. Viable Brucella was recovered from samples heated at 134°F. for thirty minutes. At 140°F. Brucella was viable for fifteen minutes but non-viable in twenty minutes. Brucella remained viable for ten minutes at 142°F. and five minutes at 144°F., but was non-viable in fifteen and seven minutes, respectively.

DISCUSSION

The data suggest a variance in the thermal death time of two porcine cultures, strain 2012 appearing less heat resistant than strain 2872. Since this was observed only once it is possibly not significant. Variation in thermal death time, however, was noted as the numbers of Brucella organisms placed in the milk varied. The time required to kill Brucella in contaminated milk, therefore, appears dependent upon the degree of contamination, or the number of organisms. This may account for the variation in the thermal death time obtained with the two porcine strains. The thermal death times of the two strains of porcine Brucella were much lower in sealed tubes than in cotton-stoppered tubes. Sealed tubes prevent a milk film from forming during heating. Organisms might survive longer in such a dried film than in the milk sample.

It is conceded that the massive inoculation used in some cases for the artificial infection of milk would seldom be encountered in freshly drawn milk. The number of organisms secreted from the udder appears variable, the greatest number being present in the milk soon after abortion. Brucella organisms appearing spontaneously in milk are greatly reduced or may completely disap-

pear in three or four weeks. In some animals the udder infection persists for months or years. The Mediterranean Fever Commission (1887) reported a variation of the number of Brucella organisms secreted in goat's milk from none to 30,000 per cubic centimeter. Hasseltine (1929) stated that *Brucella abortus* is not present in large numbers in milk—50,000 per cubic centimeter being an exceptionally high number. Carpenter and King (1929) reported 20 to 500 Brucella organisms per cubic centimeter from market milk, while Hasley (1930) obtained an average of 2 organisms per cubic centimeter of certified milk (highest count being 8). Evans (1918) reported 145,000 Brucella organisms per cubic centimeter in the milk of an artificially infected cow, but it was observed that virulent organisms were not continually secreted. Since it is difficult to appraise the conditions in the udder which influence Brucella growth, the numbers encountered when the milk is plated are at best but an index range without reference to a definite degree of infection.

It appears that under certain conditions infected milk may become a medium for the growth and multiplication of Brucella. However, bacterial growth in such samples possibly retards Brucella multiplication and growth by decreasing the pH (below 5) (Carpenter and Boak (1928)).

SUMMARY

1. Two strains of *Brucella suis*, in hermetically sealed glass tubes of whole milk (500,000,000 organisms per cubic centimeter), were non-viable after twenty minutes at 140°F., after fifteen minutes at 142°F., and after seven minutes at 144°F.

2. The same strains proved more resistant to heat in cotton-stoppered tubes of milk. *Brucella suis* survived for thirty minutes at 144°F. in milk containing 10,000,000 to 500,000,000 organisms per cubic centimeter, but the same period of time at the same temperature destroyed *Brucella suis* in milk containing 5,000 to 1,000,000 organisms per cubic centimeter. Therefore, it appears that the thermal death time is influenced by the degree of contamination.

3. The data suggest that efficient pasteurization will prevent milk-borne porcine brucelliasis. However, final conclusions are withheld pending results of studies on commercial pasteurizers.

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