THE FREQUENCY OF ISOLATION OF BRUCELLAE FROM THE MILK OF FAMILY COWS

by

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B. S., Kansas State Teachers College
Emporia, Kansas, 1948

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Bacteriology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1952

TABLE OF CONTENTS

INTRODUCTION

The Bureau of Animal Industry reports that approximately five percent of the cattle in the United States are infected with brucellosis. Though large inroads have been made on this disease, there is still much work to be done before the situation can be considered under control. At one time many considered the calfhood vaccination and blood testing program as a satisfactory method of eradicating and controlling brucellosis. However, ten years after the institution of the vaccination and blood testing program, the incidence of brucellosis in cattle still remains an economic and public health problem. Economically, the loss in the production of milk, meat, and young stock is serious; hygienically the transmission of the disease to humans, either by consumption of contaminated products or by handling the carcasses of the infected animals, constitutes a serious public health problem.

This investigative survey deals with one phase of the public health problem; i. e., the possible role of the "family cow" in the transmission of brucellosis to man. An attempt has been made, through the studying of samples of milk from "family cows", to detect the possibility of infected animals shedding the brucella bacilli in the milk and thereby serving as agents in the spread of the disease. Interest was centered on the milk of the "family cow" as this milk is in most cases consumed raw; whereas, the milk from the commercial herds is usually sold to a distributor and therefore finds its way on to the retail market as pasteurized milk.

If a program designed to control an infectious disease is to be effective, one of the first considerations should be the deter-

mination of how the disease is spread. In the case of brucellosis, a lack of correlation of data from various workers would indicate that this factor is not too well understood. The first suspected agent was the mosquito. The Mediterranean Commission, set up to study the malady, then known as Malta fever, spent a good deal of time and money working with the mosquito only to discover that the soldiers stationed on the isle of Malta were being made sick as the result of consumption of raw goat's milk, rather than the bite of a mosquito. It was not too long after that before the etiological agent was isolated and given a name. A question that confronted the members of that commission, and one that still faces the public today, is how does the organism get established in the goat, cow, sheep, or swine. Furthermore, once the organism is stamped out of a herd, what is the reservoir from which the organism once again can become established in a herd and cause the extensive damage of which it is capable.

Washko and Hutchings (1948), while studying the pathogenicity of Brucella suis for cows, showed that one of the most effective routes of entry for this organism was through the teat canal. They further showed that feeding the placenta of an infected sow to a cow resulted in the establishment of an infection with this organism. Though the organism that most often infects cattle (Brucella abortus) is somewhat less invasive than Brucella suis, it seems logical that the less invasive Brucella abortus might gain entrance at least through the teat canal. Though not a proven fact, it is possible to visualize the disease causing organism being transmitted from one female to another, or even from one species to

another.

It has long been the consensus of the laity and some investigators that the organism is not transmitted at the time of natural mating. Bendixen and Blom (1947) made a study of the males from which semen was being shipped or distributed for artificial insemination purposes and found that fourteen percent of the bulls reacted to the blood test for brucellosis. Of these, about three percent of the total were culturally positive when the semen was injected into cavies. Furthermore, the organisms were isolated from various parts of the genital tract, which would indicate that even by natural mating the bull could transmit the etiological agent.

Organisms that have at some time or other been suspected of harboring and transmitting the brucellae are legion. Ruhlland and Huddleson (1941) proved to their own satisfaction that the cockroach is not capable of harboring the organisms, since when fed a pure culture of Brucella abortus the cockroach excreted the organisms almost immediately and the longest that the organism remained viable in the intestine of the cockroach was twenty-four hours. On the other hand, these same two investigators showed that the species of flies found around the house and barn are very good harborers of the organism, as they were able to isolate Brucella abortus five days after the flies were fed a pure culture of the organism. This experiment was not carried further.

Another investigation on the role of insects and related species, especially of an ectoparasitic nature, in the harboring and transmission of the brucellae is that of Tovar (1947). Tovar first

worked experimentally with ticks, fleas, and bedbugs, of which he found all three susceptible to infection with Brucella. Ticks and bedbugs eliminated the organism in the feces as long as three months after feeding on contaminated material; however, ticks were the only one of the three arthropods that were able to transmit the organism. Ticks were also the only one of the three that were able to transmit the organism through their eggs to larvae, a fact of no small epidemiological significance. Following this experimental work Tovar went into the field where brucellosis was endemic and collected ticks from animals that reacted positively to the rapid serum agglutination test. From these ticks he isolated one strain of Brucella abortus and two strains of Brucella meletensis. It seems that this may be the answer to the question of the reservoir; this is the only report of such a nature found in the literature and it would not be good logic to draw conclusions on a few isolated cases.

The infection of humans is almost without exception either directly or indirectly from animals, Bort et al.(1944). A person may become infected directly from handling infected carcasses. In fact, the incidence of human brucellosis is so high among packing house workers that some states, Iowa for one, have declared brucellosis an occupational disease of packing house workers. Contact of this nature involves a comparatively few people, though, therefore, an indirect means of transmission must be considered.

Of the means of transmitting brucellae from the animal to man, the most important and the one with which this study is concerned is through the medium of milk. For the animal to be able to trans-

mit the organism she must harbor an active infection or exist as a healthy carrier. To transmit the organism in the milk the most logical site of this infection, or localization in the case of the carrier, would be the udder. Because of the lack of characteristic pathological conditions associated with the udder infection it has been hard to establish definitely that the udder is a site of infection. Mitchell and Duthie (1930) removed the udder of two reactors and followed the blood picture over a period of time that they considered adequate. These workers found that the titers of these two animals decreased, slowly at first and rapidly later on, and after some time they were unable to isolate the organisms from the blood. They interpreted this as indicative of the udder being the site of infection, subacute though it may be.

Whether or not the udder is the site of the infection that serves as the source of the organism eliminated in the milk has not been proven. Nevertheless, it is a proven fact that the organisms causing brucellosis are shed in the milk of cows (Smith, 1934) and remain viable therein varying lengths of time, depending upon the storage conditions and environment (Anonymous, 1928) and (Fulton, 1941).

It was mentioned above that it is possible for the cow to exist in an apparently healthy, carrier state. The basis for this statement is the paper by Thompson, 1934, in which he reports the isolation by cavy inoculation of Brucella abortus from ten cows that gave no previous history of the infection.

The importance of milk in the transmission of brucellosis from the infected animal to man necessarily makes the conditions

under which the organisms are shed from the udder of the infected animal important. As in most of the areas concerned with brucellosis there is much disagreement on the conditions and predisposing factors involved in the shedding of brucellae from the udder.

A factor apparently apart from the functioning of the animal is a seasonal variation in the brucellae content of the milk. Jones (1943) has reported that there is a distinct correlation between the season of the year and the incidence of Brucella abortus in raw milk. In a limited number of cases he found that generally there is a higher occurrence of Brucella abortus in the winter months than at other times, and specifically the highest incidence occurred in January and the lowest in August.

Many studies have dealt with the relationships between the appearance of the brucellae organisms in the milk and the agglutination titers of the milk serum and blood serum from the animal. The earlier investigations of this nature supported the view that there was a higher correlation between the milk serum titer and the shedding of the organisms from the udder than there was between the blood serum titer and the presence of the organisms in the milk. Mitchell and Humphreys (1931) reported that they were unable to isolate Brucella organisms from milk that gave a negative agglutination test when the tube test was used; however, all positive agglutinations did not give isolations. This latter fact must not be given too much significance, as even with improved techniques isolations are never 100 per cent. Gilman (1931) also supports the view that there is a higher degree of correlation between the milk serum titers and the isolation of the organism than between the

blood serum titers and the isolation of the organism on laboratory media. He reported that of 113 cows from which he was able to isolate the organisms by cavy inoculation with cream, 78 per cent of those that showed a milk serum titer of 1:80 gave isolations via laboratory media; whereas, only 63 per cent of those that showed a blood serum titer of 1:80 gave isolations by use of laboratory media.

In spite of these earlier reports, it is generally accepted that the blood serum agglutination test is of more value than the milk serum agglutination test. Meyer and Huddleson (1938) showed that it was often possible to isolate the organism through the use of the cavy inoculation sometime before agglutinins appeared in the milk serum. It was reported by Hayes and Barger (1935) that the organisms may be isolated from milk up to eight months before the appearance of agglutinins in the milk serum.

MATERIALS AND METHODS

Samples

Sources of Samples. The samples of milk that were used in this investigation were collected from cows whose milk was used primarily by the owner; i. e., "family cows," in Rice County, Riley County, and areas of Wabaunsee County proximal to Manhattan, Kansas.

Collection of the Samples. A composite sample of the milk to be examined was collected at the time of milking. A composite sample is to be construed as a sample from an individual animal, as opposed to quarter samples. Though for both the isolation and immunological examination quarter samples are recommended, it was felt

that the nature of the information desired and for the sake of expediency the collection of composite samples was justified (Prichett and Walton, 1940). The amount of sample was 40 to 50 milliliters taken from the pail into which it had been milked, following stirring. The containers were sterile large screw-cap test tubes which were used to facilitate the removal of the cream, since in a tall column the cream layer is deeper.

Treatment of the Samples. The sample was refrigerated immediately following collection and maintained in that state for twenty-four hours to allow the cream layer to collect at the top of the sample. Following refrigeration the cream layer was removed for the inoculation of the media (described below) and five milliliters of the skim milk were coagulated with rennet and the serum allowed to separate by placing the tubes in a slanting position in the refrigerator. The treatment with rennet involved the addition of two drops of a one per cent solution of rennet to each five-milliliter sample. Samples collected in Rice County were treated slightly different in that the cream was frozen at the time of removal and cultured later rather than at the time it was removed (Fulton, 1941).

Culturing

Media Used. The media that was used throughout the examinations was dehydrated tryptose agar prepared by the Digests Ferments Company. This medium was given selectivity by the addition of crystal violet in a final concentration of one part crystal violet to five hundred thousand parts of media. This concentration was arrived at as a result of literature reports and some preliminary experimenta-

tion. It had been learned by past experience that the concentration most often recommended; i. e., one part crystal violet dye to seven hundred thousand parts of media, was not sufficient to inhibit the growth of many of the gram positive organisms encountered in milk, especially a short chain streptococcus that grows rapidly under increased carbon dioxide tension. Bradley et al. (1941) recommended the use of crystal violet in a concentration of one part crystal violet dye to two hundred thousand parts of media. In using this concentration with laboratory strains of Brucella abortus, melitensis, and suis mixed in unpasteurized milk it was found that the growth of these organisms was inhibited to such a point that if they were not present in numbers far above those normally found in infected milk the chances for isolation were decreased drastically. Through trial and error the concentration of one part crystal violet dye to five hundred thousand parts of media was found to give the best selectivity for the brucellae.

Inoculation of the Media. Except in the case of the samples collected in Rice County, at the time of the removal of the cream from the samples two-tenths milliliter of the cream was placed on ten milliliters of the above described media that had been poured into sterile petri dishes and allowed to solidify. The cream was then spread evenly over the plated media with a sterile piece of glass rod that had been bent at right angles. It was felt that this was more satisfactory than using a similarly bent inoculating needle as there was less danger of tearing the agar surfaces; however, there is the disadvantage of increasing the number of pieces of equipment since the glass rod can be used but once without re-

sterilizing, whereas the needle need only be well flamed in the bunsen burner flame.

Incubation of the Inoculated Media. All inoculated media were incubated in an air-tight container in which a lighted candle was allowed to burn out. The temperature of incubation was 35 degrees to 37 degrees Centigrade. The time of incubation was five days.

Examination of Cultures. Following incubation the inoculated plates were removed from the air-tight container and examined for colonies that were characteristic of Brucella. On media containing crystal violet the colonies were spheroidal, blue violet in color, and two to three millimeters in diameter. Occasionally colonies isolated from milk will be flattened and up to seven millimeters in diameter. Colonies approximating either of these descriptions were examined by gram staining and all colonies showing gram negative coccobacilli were subcultured under increased carbon dioxide tension. After incubation of the subculture for five days it was examined for purity. If a pure culture was indicated a rapid slide agglutination test was performed as a means of final identification.

Immunological Studies of the Samples

In addition to the attempted isolation, a tube agglutination test was performed on each sample of milk as both a screening measure and an additional check. In addition to the preparation of the sample (see treatment of sample) this procedure involved two general steps: (1) the preparation of an antigen and (2) setting up and reading the test.

Preparation of the Antigen for the Tube Agglutination Test. A smooth strain of Brucella abortus was grown on tryptose agar for seventy-two hours at 37 degrees Centigrade. The growth was then examined for purity by Gram staining a smear made from the growth. After purity had been established, the growth was suspended in physiological saline containing five tenths per cent phenol. The turbidity of the cell suspension was adjusted to McFarland nephelometer tube I at the time of use.

Setting Up and Reading the Tube Agglutination Test. Two milliliters of the cell suspension adjusted to McFarland nephelometer tube I was placed into each of five agglutination tubes. To this cell suspension milk serum from the coagulated portions of the samples was added with a Bureau of Animal Industry pipette to give the following serum dilutions 1:25, 1:50, 1:100, 1:200, and 1:400. The cell suspension and the serum were well mixed by inverting the tubes, and were then incubated at 37 degrees Centigrade for forty-eight hours. Following incubation the tubes were examined for complete sedimentation and aggregation of the cells, partial sedimentation and aggregation of the cells, and no sedimentations. With each group of agglutination tests performed a tube of antigen without serum added was incubated as a control for spontaneous agglutination.

Survey Questions

At the time that the samples were collected the following questions were asked the party contributing the sample:

1. Was the cow from the sample was obtained calfhood vaccinated?

2. Had the cow from which the sample was obtained ever aborted?

3. Had the cow from which the sample was obtained been blood tested for Bang's disease? If so, when?

RESULTS

Experimental Results

Isolation of Brucella. A total of one hundred sixty-one samples were collected and submitted to the examinations as described above. From these samples a total of two positive isolations were obtained. Table 1 lists the samples and isolations by the counties from which they were collected.

Table 1. Samples and isolations tabulated by counties.

Number tested and isolation records	Rice	Riley	Wabaunsee
Number of samples	111	34	16
Number of isolations	2	0	0

Immunological Reactions of the Milk Serum. Of the samples, ten gave positive reactions in dilutions of 1:25 and six gave positive reactions in dilutions of 1:50. There were no samples that gave positive reactions in dilutions higher than 1:50. As is shown in Table 2, there were no isolations from any sample that did not give a positive reaction to the tube agglutinations test.

Table 2. Correlation of milk serum reactions and isolations tabulated by counties.

Titration records of cows	Rice	Riley	Wabaunsee
Number of positive samples in:			
Dilution 1:25	10	0	0
Dilution 1:50	6	0	0
Number of positive samples giving isolations	2	0	0

Survey Results

The results of the questions asked the contributors of the samples regarding the history of the cows from which the samples were taken can best be shown in tabular form. This is done in Table 3.

Table 3. Results of the survey question answers tabulated by counties.

History of cows tested	Rice	Riley	Wabaunsee
Total samples	111	34	16
Calfhood vaccination			
Number answering yes	26	12	8
Number not knowing	24	16	6
Number of abortions	3	0	0
Number blood tested	34	14	7
Time elapsed since blood tested			
Less than 6 months	9	4	2
Less than 1 year	20	10	4
More than 1 year	5	0	0

DISCUSSION

Using the Bureau of Animal Industry as the authority, it was

stated before that the incidence of brucellosis among cattle is about five per cent. The incidence as reported in the results of this work is much lower, being slightly in excess of one percent Though the epidemiology of the disease among cattle has not been worked out in detail, it is well known that transmission from one animal to another occurs quite readily. This work having been done entirely with the "family cow" where only one or two cows are kept on the premises, with little or no contact with other animals, it is easily seen why the incidence should be lower than a national average where mostly large herds are involved. In fact it would not have been surprising if no positive isolations had been obtained.

There has been much discussion of the merits of the whey agglutination test. Colien (1940), Gilman (1931), and Mitchell and Humphreys (1931) tend to support the validity of the whey agglutination test; whereas, the work of such people as Hayes and Barger (1935), Henry et al. (1935), and Meyer and Huddleson (1938) tends to discredit the validity of this test. Though a comparatively few animals were involved in this project, the data presented certainly uphold the validity of the test; i. e., there were no positive isolations with the corresponding absence of a positive agglutination test. The most convincing evidence against this test is that presented by Meyer and Huddleson (1938), who found that there was a period following infection (experimentally) that agglutinin could not be demonstrated in the milk serum of the animal known to be infected.

That there is a need of education on this problem is evidenced

by the answers received for the survey questions. Approximately fifty per cent of the cows had not been blood tested for brucellosis, which is the Bureau of Animal Industry approved method for detecting infections. In practically all of these cases the milk from these cows was being used in the raw state, which magnifies the public health problem. It was noticed that once an owner had been made aware of the necessity of blood testing that owner kept up the testing program as is the recommended practice. The data show that, of the 55 that had their animals tested for brucellosis, 49 or 89 per cent had them retested or tested within the last year.

With the modernization of the rural areas and the close proximity of many of the keepers of "family cows" to the cities and towns, another approach to the methods of control of the transmission of the disease to the user of milk from such a source would be to educate these users to the practicability of the "home pasteurizer." Huddleson et al. (1949) have performed many carefully controlled experiments of the effectiveness of a number of these commercial models and found that, if they are used as prescribed by the manufacturer, they are effective in eliminating all three of the pathogenic Brucella from milk inoculated with as many as 100,000 viable organisms per milliliter.

SUMMARY

Of 161 samples from cows designated as "family cows," two isolations of Brucella species were made. This is about one-fourth of the incidence reported by the Bureau of Animal Industry for the entire United States.

More than fifty per cent of the contributors of samples were using the milk in a raw state from cows that had not been blood tested for brucellosis.

ACKNOWLEDGMENTS

The author wishes to thank Professor V. D. Foltz, Major Instructor, for his help and guidance while working on this problem, and to express appreciation to Dr. P. L. Gainey, Head of the Department of Bacteriology, for many helpful suggestions, especially those pertaining to the construction of the manuscript.

REFERENCES

Anonymous.
Brucella abortus in milk and other dairy products. Amer. Jour. Pub. Hlth. 18 (6): 743-751. 1928.

Bendixen, H. C., and Erik Blom.
The spread of brucellosis in the semen transported and distributed for artificial insemination purposes. Jour. Amer. Vet. Med. Assoc. 103 (10): 337-345. 1947.

Berman, D. T., and B. A. Beach.
Studies on the repeated vaccination of cattle with Brucella abortus: strain 19. I. Agglutination response of animals vaccinated as calves and revaccinated as young adults. Amer. Jour. Vet. Res. 10 (36): 208-213. 1949.

Borts, I. H. et al.
Some observations regarding the epidemiology, spread, and the diagnosis of brucellosis. Jour. Kans. Med. Soc. 46 (12): 399-405. 1945.

Bradley, Robert et al.
Isolation of Brucella abortus from milk. Jour. Bact. 41 (9): 286. 1941.

Colien, F. E.
Significance of Brucella abortus agglueinins in the whey of raw milk. Jour. Bact. 39 (3): 345-346. 1940.

Donham, C. R.
Sources of error in the agglutination test for Bang's disease. Jour. Amer. Vet. Med. Assoc. 95 (749): 136-139. 1939.

Feinberg, Richard et al.
Observations on the factors influencing the diagnostic agglutination titration in brucellosis. Fed. Proc. 9 (1): 381.

Fulton, J. S.
Contagious abortion of cattle and undulant fever in man. Canadian Jour. Pub. Hlth. 32: 194-198. 1941.

Gilman, Herbert.
Further studies on the relationship of the milk agglutinin titer to the elimination of Brucella abortus from the udder. Cornell Vet. 21 (3): 243-246. 1931.

Hayes, F. M., and C. Barger.
Bang's disease in a naturally infected herd. Hilgardia. 9 (11): 543-566. 1935.

Henry, B. S., and Jacob Traum.
A comparison of the factors influencing the agglutination test for Brucella abortus. Jour. Infect. Dis. 47 (5): 367-379. 1930.

Henry, B. S. et al.
Brucella abortus shedder conditions in twenty cows. Hilgardia. 9 (11): 543-566. 1935.

Huddleson, I.
Brucellosis in man and animals. London: Oxford University Press. 1934.

Huddleson, I. et al.
The viability of Brucella abortus in milk and cream heated in electrically operated home pasteurized milk. Jour. Dairy Sci. 32 (1): 29-32. 1949.

Hull, Thomas, and Luther Black.
Undulant fever as a public health problem. Jour. Amer. Med. Assoc. 88 (7): 463. 1927.

Jones, E. R.
The seasonal variation in the incidence of Brucella abortus in raw milk. Jour. Path. and Bact. 55 (3): 357. 1943.

Jordan, Carl et al.
Brucellosis: considerations of its epidemiology, diagnosis, and control. Amer. Jour. Pub. Hlth. 33: 773-778. 1943.

Meyer, D. B., and I. Forrest Huddleson.
The relationship between the presence of Brucella abortus and its agglutinins in milk in cattle showing blood agglutinin titer greater than 1:200. Cornell Vet. 28 (4): 293-295. 1938.

Mitchell, Charles, and R. C. Duthie.
The udder as the reservoir for Brucella infection in cattle. Canadian Jour. Res. 2 (6): 403-405. 1930.

Mitchell, Charles, and F. A. Humphreys.
Studies in Brucella infection of cattle. Cornell Vet. 21 (1): 57-67. 1931.

Norton, John F., and L. R. Pless.
Agglutinins of Brucella abortus in blood and milk: Use of the agglutination reaction in control of certified milk. Amer. Jour. Pub. Hlth. 21 (5): 499-502. 1931.

Prichett, H. D., and S. T. Walton.
A study of the agglutinin titer of milk serum from known infectious abortion reactors. Jour. Amer. Vet. Med. Assoc. 96 (759): 709-713. 1940.

Ruhlland, H. H., and I. Forrest Huddleson.
The role of species cockroaches and flies in the dissemination of Brucella abortus. Amer. Jour. Vet. Res. 2 (5): 371-372. 1941.

Smith, J.
Excretion of Brucella abortus in milk. Jour. Comp. Path. and Therapy. 47 (2): 125-133. 1934.

Special Committee of the United States Livestock Sanitary Assn.
What is known about brucellosis? United States Livestock Sanitary Assn. 1949.

Thompson, R.
Elimination of Brucella abortus with milk of carrier cows. Jour. Infect. Dis. 55 (1): 7-11. 1934.

Tovar, Raul M.
Infection and transmission of Brucella abortus by ectoparasites. Amer. Jour. Vet. Res. 8 (26): 138-140. 1947.

United States Department of Agriculture, National Research Council, and Federal Security Agency. Brucellosis: A Symposium. Amer. Assn. for Advancement of Science. Washington. 1950.

Washko, F. V., and L. M. Hutchings.
Studies on the pathogenicity of Brucella suis for cattle. Amer. Jour. Vet. Res. 9 (33): 342. 1948.

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AN ABSTRACT

This investigative survey was undertaken to ascertain the possible role of the "family cow" in the epidemiology of brucellosis. Attention was focused on the family cow since this milk is most often consumed in the raw state and because the family cow is usually the only animal on the premises that is capable of harboring brucellae.

Samples of milk were collected from family cows and stored at refrigeration temperatures for eighteen to twenty-four hours. The cream from these samples was cultured in an atmosphere of increased carbon dioxide tension for five days on tryptose agar containing crystal violet in a final concentration of one part crystal violet to five hundred thousand parts of medium. Following incubation the cultures were examined for typical Brucella colonies. Colonies that showed gram negative coccobacilli when stained by the Gram's method were subcultured under increased carbon dioxide tension. When sufficient growth had appeared on the subculture a slide agglutination test was performed as a means of positive identification.

In addition to the culturing procedure, tube agglutination tests were performed on the serum of the milk samples that were cultured. This was done as a screening measure and to check for possible misses in the culturing of the organisms.

Of the 161 samples cultured for the isolation of brucellae and tested for the presence of antibodies to Brucella abortus, two positive isolations were obtained. A certain degree of correlation between the presence of antibodies in the milk serum and the

presence of viable brucellae in the cream from the same sample was noted. This was indicated by the fact that in no sample which yielded viable organisms was the milk serum agglutination test negative in a titer of 1:50. However, it must be noted that there were milk samples the serum of which gave positive agglutination test in a titer of 1:50, but failed to yield positive isolations.

From the results obtained in this project and results reported in the publications of other work the following conclusions were reached by the author:

1. The incidence of brucellosis among the animals tested is about one-fourth as high as that reported by the Bureau of Animal Industry for dairy herds.

2. The explanation tendered for this lowered incidence is that the animals tested were for the most part not in contact with other susceptible animals.

3. There is still a need for education on the subject of brucellosis. This was indicated by the fact that more than 50 percent of the sample contributors were using milk from cows that had not been blood tested for brucellosis, which is the method of detection accepted by the Bureau of Animal Industry.