The Value of the Milk Serum Agglutination Test in Safeguarding Raw Milk Supplies*

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Purpose of the Milk Serum Test

The milk serum agglutination test is one of the comparatively recent additions to the list of milk tests. The purpose of the test is to determine whether or not there is evidence of Bang’s infection in the milk. Since the Bang’s disease organism is destroyed by pasteurization, there is little if any value in applying the test to pasteurized milk. The fact that approximately two-thirds of the milk sold by licensed dealers in Vermont is now being pasteurized limits the application of the test to that third of the supply that is sold as raw and in addition to the raw used on farms. There are now about 350 herds from which raw milk is being sold by licensed dealers.

Early Use of the Test

The Committee on Standard Methods for the Examination of Dairy Products included the milk serum test in its 1939 edition of Standard Methods. That action gave official recognition to the value of the test. Dr. I. F. Hud­dleson, Research Professor in Bacteriology at Michigan State College did a vast amount of experimental work in the method of making the test and he wrote a book, “Brucellosis in Man and Animals,” which covers many phases of the Bang’s disease problem. Dr. R. T. Westman of Kansas City, Kansas, was one of the first health officers to make extensive use of the milk serum test.

On May 13, 1941, Dr. Edwin M. Knights of the Providence, R. I., Health Department reported his use of the milk serum agglutination test. This report was given at Bridgeport, Connecticut, at the meeting of the Connecticut Association of Dairy and Milk Inspectors. The test had been con­ducted on individual samples of milk produced by 1,097 cows in 31 herds that furnished the raw milk to Providence. These were the herds that fur­nished 3 percent of raw milk to the city. and were considered the best herds in Rhode Island. They were therefore permitted to furnish the only raw milk sold.

One of these herds supplying milk to a boy who contracted undulant fever contained four reactors to the milk serum test and these results were con­firmed by an official blood test of the same cows.

An average infection of 14.3 percent was found in the thirty-one herds. The net result was that twelve herd milk supplies were changed to pasteurized milk, fifteen others to the pasteuriza­tion of a large portion of their supply and only three remained as raw milk dealers. Any herds from which any raw milk was sold had removed the reactors. Only one of the thirty-one herds contained no reactors.

So far as I have been able to learn, Vermont is the first state to apply the use of the milk serum test to all raw milk sold by licensed dealers in the state.

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OUR PROBLEM

Two months after I heard Dr. Knights render his report at Bridgeport, the Vermont Department of Public Health reported three cases of undulant fever to us on a Vermont raw milk route with a request to test the herd as soon as possible. The owner had already applied for the test and ten days later the result showed 24 reactors, 12 suspects, and 21 healthy animals. The results of this blood test aroused our interest in the possibility of locating Bang infection in our raw milk supplies.

At that time less than a third of our retail milk was being pasteurized and about a thousand herds contributed to our raw milk supply. Comparatively few of these herds had been blood tested. The state Bang's control law permitted reactors to be retained in the herds. The new state milk inspection law, however, and the regulations required by that law, had been in effect less than a year and these provided that no milk should be sold for consumption within the state unless it was obtained from healthy cows. It therefore followed that no milk could legally be sold from cows reacting to the Bang test. Before describing the plan that we adopted in the use of the milk serum test, permit me to discuss the importance of Bang's disease and the occurrence of undulant fever in Vermont.

IMPORTANCE OF BANG'S DISEASE

Results of official blood testing in Vermont show that two-thirds of the herds are infected. The initial tests of 2,390 herds show 17 percent of the animals tested were reactors.

To show the possible relationship of Bang's disease and undulant fever, I will again quote Dr. Huddleson, who says, "The two important channels through which the Brucella (the Bang's organism) is eliminated from the infected cow are the uterus at the time and shortly after abortion and the infected udder. The organism may be eliminated in the milk from the infected udder during the life of the animal."

It is not strange, therefore, that cases of undulant fever have occurred where raw milk from infected animals was being used. It is probably not the job of a milk inspector to attempt to control the occurrence of contact cases of undulant fever that are due to handling animals and carcasses infected with Bang's disease.

OCCURRENCE OF UNDULANT FEVER

The first cases of undulant fever were reported in the United States in 1904 and in Vermont in 1928. Thirty-two states have reported a peak in the number of cases during the past three years. The importance of undulant fever in recent years is therefore emphasized. One state reported 354 cases last year. For Vermont the number reported by the State Department of Health are listed in Table 1.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1928</td>
<td>2</td>
</tr>
<tr>
<td>1931</td>
<td>13</td>
</tr>
<tr>
<td>1934</td>
<td>24</td>
</tr>
<tr>
<td>1938</td>
<td>42</td>
</tr>
<tr>
<td>1941</td>
<td>58</td>
</tr>
<tr>
<td>1943</td>
<td>57</td>
</tr>
</tbody>
</table>

Half of the cases that have occurred in Vermont during the calendar years 1941 and 1942 were on the routes of licensed raw milk dealers and the remainder were on dairy farms where raw milk was used and where cattle were handled and in some cases slaughtering done. The cases have been widely scattered. They have included several lawyers, a school teacher, the owner of a defense plant, a post office employee, a recruiting officer, laborers, housewives, farmers, school children, and four retail raw milk dealers. There have been no sporadic outbreaks although in several instances more than one case has occurred on the same milk route. Only
three contact cases, supposedly due to the handling or butchering of cattle, have been reported in Vermont during the last three years. Four cases have been fatal during this period.

It is evident from Table 2 that there are many mild cases of undulant fever that escape diagnosis inasmuch as several cases have been found in persons who were required to submit to examinations when seeking employment.

**Our Experience with the Milk Serum Test**

Two years ago we began the use of the milk serum agglutination test. A sample of raw milk was secured for test from each raw milk dealer. In some cases these samples were mixed milk from different farms and it became necessary to obtain a separate sample from each herd supply. A summary was made after we had tested 690 herd samples and it was found that 12 percent of the samples showed infection. We thought it was desirable to know which cows were infected in addition to knowing that there was infection in the herd. Consequently we sampled the individual cows in the herds that showed infection. In this work separate samples were tested from 1,853 cows in 92 herds with the result that there were 15 percent reactors and 6 percent suspects on the milk serum test.

Since the milk serum test shows evidence of Bang's infection in the milk, it gives evidence of potential danger in many of our raw milk supplies.

The owners of infected herds were at once given the option of having their herds officially blood tested and to eliminate reactors, if any were found, or to pasteurize their entire milk supply that was sold for local use. We wrote them the following letter:

"**Dear Sir:**

On ............ the Vermont Department of Agriculture tested milk from ............ of your milking cows for evidence of Bang's disease.

"Bang's disease in dairy cows may infect the milk and when the milk is so infected may cause undulant fever in the persons drinking such raw milk.

"Milk produced by ............ of your cows showed infection. Infected milk is a menace to public health and we therefore recommend that you have your entire herd tested at once for Bang's disease and remove all reactors from your herd or that you arrange promptly to pasteurize your entire milk supply.

"An application blank for the blood test of your herd is enclosed. Please sign it and return to this office within ten days or arrange within ten days to have your milk which is sold in local markets pasteurized. Unless you follow one of these two options,—namely, blood test your herd and remove reactors if any or pasteurize all of your locally sold milk, we shall be compelled to ask you, or the dealer who sells your milk, to show cause why the state license authorizing sale of this milk should not be suspended.

"The elimination of infected cattle from your herd or the pasteurization of milk tends to protect you against a claim that may arise due to the sale of infected raw milk as well as to protect the health of your family and of your milk consumers. Your reply by return mail is requested."

Following out this program, 40 of the 92 herds were then blood tested under the State-Federal Cooperative Control Plan. Of the 1,181 cows in these 40 herds there were 22 percent reactors and 9 percent suspects. Six of these herd owners discontinued selling milk in local markets. They then sold their milk to the shipping station.
since all milk shipped into the larger cities from this area is pasteurized at destination. Incidentally pasteurization does destroy the Bang organism if present in the milk. Six other dealers started to pasteurize their entire milk supply and the other 28 herd owners removed the reactors from their herds and continued to sell raw milk.

This program is being continued at the present time except that we secure a sample of milk for the milk serum test from each can of milk produced for sale as raw milk. It is recognized that we miss infection due to the fact that there are frequent herd additions.

The milk serum test is being used as a screen test toward securing a safe milk. A positive or suspicious test demands either that the herd be officially blood tested and reactors removed or that the milk be pasteurized.

Undulant Fever Investigations

It has been our privilege to comply with the request from the State Board of Health to investigate the sources of milk used by many persons who have had undulant fever during the past three years. Table 3 summarizes the results of this work.

There is apparently some decrease this year in the number of cases occurring on raw milk routes. Approximately half of the herds supplying raw milk dealers in the state have now been blood tested.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number cases of undulant fever reported</th>
<th>Number cases on raw milk routes</th>
<th>Number supplying herds</th>
<th>Number Bang reactors in these herds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1941</td>
<td>58</td>
<td>29</td>
<td>17</td>
<td>149</td>
</tr>
<tr>
<td>1942</td>
<td>57</td>
<td>28</td>
<td>21</td>
<td>188</td>
</tr>
<tr>
<td>1943 (to Nov. 1)</td>
<td>57</td>
<td>19</td>
<td>15</td>
<td>73</td>
</tr>
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</table>

Comparison of Milk Serum and Blood Serum Agglutination Tests for Bang's Disease

The blood serum agglutination test, commonly called the "blood test," is the official test employed in the cooperative state-federal control of Bang's disease in dairy herds. Samples of the blood are drawn and identified with the ear-tag numbers corresponding to the cows from which the samples are taken. After the blood coagulates the clear serum is used to make the test.

The milk serum agglutination test is similarly made by using the clear serum after the milk has coagulated. Coagulation may be hastened by the addition of a small amount of rennet to each sample of milk.

Comparisons of the two tests have been made on the same cows in 31 Vermont herds. These tests were made between October, 1941, and September, 1942, and all the herds were producing milk sold by retail raw milk distributors. A total of 472 cows were compared. The majority of the milk serum and blood serum tests of these 31 herds were less than 60 days apart although a few of the tests were more than 90 days apart. All tests were made in the same routine and by the same technicians as are employed in official Bang's disease control work. Comparisons follow:

71.8 percent (339 cows) of the milk and blood tests checked.
10.8 percent (51 cows) of the negative tests on milk were suspicious on blood.
12.1 percent (57 cows) of the negative tests on milk were reactors on blood.
4.4 percent (21 cows) of the suspicious tests on milk were reactors on blood.
0.9 percent (4 cows) of the reacting tests on milk were suspicious on blood.

Many cows, as shown above, that are negative on the milk serum test may be suspects or even reactors on the blood serum test. The above results show that 99.1 percent of the official blood tests either checked or were higher in titer than the milk tests.
Serum Agglutination in Milk Control

of the corresponding cows. This indicates that many reacting animals would be overlooked if the milk serum test were used as the basis for the determination of reactors. When the milk serum test shows even a slight reaction, it is reasonably sure that a blood test of the animal would also show as strong a reaction and very probably a stronger one. The above comparison shows that the milk serum test gave the stronger reaction on only 4 cows or less than 1 percent of the total number of animals tested.

In both the blood and the milk serum tests, cows are classified as “negative,” “suspicious,” or “reactors” depending on the results obtained by testing various amounts of the blood or milk serum respectively in combination with a prepared antigen which is a product made from Brucella or Bang’s organisms. The agglutination results are read in accordance with Table 4.

The presence of an appreciable amount of agglutinins in the serum caused by the infection results in an agglutination or clumping together of the organisms contained in the antigen. These agglutinated organisms settle to the bottom of the tube and can be plainly seen with the naked eye. A partial agglutination in a dilution of 1:100 is read 5 and is shown above as a suspicious result. A complete agglutination in a 1:100 dilution indicates a reacting animal and is recorded as 6.

Conclusions

The use of raw milk from Bang infected herds presents an important health control problem. The milk serum test furnishes information that assists control officials to combat this problem. Our milk supplies can be made safe from Bang’s infection by proper testing of cattle and removal of reactors or by pasteurization of the milk.

Table 4

Reading the Agglutination Test

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Reaction</th>
<th>Code</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:25</td>
<td>0.08 c.c. serum to 2 c.c. antigen...</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>1:25</td>
<td>0.04 c.c. serum to 2 c.c. antigen...</td>
<td>Partial agglutination</td>
<td>1</td>
</tr>
<tr>
<td>1:50</td>
<td>0.04 c.c. serum to 2 c.c. antigen...</td>
<td>Complete</td>
<td>2</td>
</tr>
<tr>
<td>1:50</td>
<td>0.02 c.c. serum to 2 c.c. antigen...</td>
<td>Partial</td>
<td>3</td>
</tr>
<tr>
<td>1:100</td>
<td>0.02 c.c. serum to 2 c.c. antigen...</td>
<td>Complete</td>
<td>4</td>
</tr>
<tr>
<td>1:200</td>
<td>0.01 c.c. serum to 2 c.c. antigen...</td>
<td>Partial</td>
<td>5</td>
</tr>
<tr>
<td>1:200</td>
<td>0.01 c.c. serum to 2 c.c. antigen...</td>
<td>Complete</td>
<td>6</td>
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