MILK RECEIVES FEW BACTERIA FROM STABLE AIR.

SUMMARIZED BY
F. H. HALL
FROM BULLETIN BY
G. L. A. RUEHLE AND W. L. KULP.

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*Absent on leave.  †Connected with Grape Culture investigations.
MILK RECEIVES FEW BACTERIA FROM STABLE AIR.

F. H. HALL.

Careful investigation of pure milk problems is proving that many current beliefs and practices are ill founded. The importance of many factors has been overestimated, as shown in recent bulletins of this Station, which prove (1) that over-refinement in securing stable cleanliness and in certain grooming processes is without effect in reducing the number of bacteria in the milk, (2) that cooling and straining the milk need not increase bacterial contamination if the utensils used are carefully sterilized, and (3) that dairy score cards are not reliable indexes to the quality of the milk produced in the stables scored. On the other hand, the value of other agencies has been under-estimated; since researches at this Station and elsewhere prove (1) that reducing the size of the pail mouth lowers very materially the number of germs in the milk, (2) that thorough sterilization of pails and other utensils and their protection from contamination previous to use are more important measures than they have previously been thought; and (3) that, with some cows, the interior of the udder supplies large numbers of bacteria to the milk.

An over-emphasized factor, as proven by investigations here reported, is the influence of stable air on the bacteria content of milk. Practically all the dairy score cards now in use give several points to stable air and cut severely for conditions that tend to increase the quantity of dust therein or to disturb the air so that the dust will move about more freely. Cuts are also given for indications that dust has previously been in the air, such as its presence on ledges, shelves, tops of mangers, feed racks and similar places. Yet the very careful studies here reported prove clearly that the air of the stable, under any ordinary conditions of dustiness or disturbance, is an almost negligible factor so far as it affects the numbers of bacteria in the milk produced.

*This is a brief review of Bulletin No. 409 of this Station on Germ Content of Stable Air and Its Effect Upon the Germ Content of Milk, by G. L., A. Ruehle and W. L. Kulp. Anyone interested in the detailed account of the investigation will be furnished, on application, with a copy of the complete bulletin so long as these are available. Names of those who so desire will be placed on the Station mailing list to receive future bulletins, popular or complete, as issued.
Preliminary work. Before practical studies could be satisfactorily carried on to determine the number of bacteria in stable air and in the milk drawn in the stable it was necessary to test the accuracy of the methods used. Months of careful work were spent in experiments with different types of "aeroscopes" or air filters, and in comparing methods of measuring bacterial precipitation from the air. In air filtration, a fixed quantity of air is drawn through the apparatus by the flow of water from a bottle of known capacity, the air passing through the filter to take the place of the water drawn out. Air filters are of two general types. Very fine sand is used as the filtering material in one form; and water or some other liquid is used in the other type to catch and hold the bacteria.

As air, even under slight pressure, will pass through very minute crevices or holes, many precautions must be taken not only to make an air-filtering apparatus tight so far as outside air is concerned, but also to make the filters perfect. If these are of sand, no crevices or channels must be left through which air can flow instead of filter; and if the bacteria-retaining material be liquid the air must be made to pass through in such a way that no bacteria can escape in large bubbles.

Much care must also be used, with bodies so small as bacteria, lest some of those in the air stop on the way through the aerooscope by adhesion to the walls of the tubes or bottles used and so escape counting. These and many other minor points in connection with the filtering devices were studied in about 400 tests, from two to six or more comparisons being made in each test. Additional work was also required, involving 40 more tests, to compare the counts of bacteria caught either on exposed plates of culture media or in pails of water, from which culture plates were subsequently made.

As a result of these long series of tests, a modified type of aerooscope was developed and adopted for use in measuring the bacteria actually in the air. Apparatus and methods adopted. For estimating the number of germs that would fall from the air in any given time, precipitation into pails of liquid was found preferable to precipitation upon the more commonly used plates of culture media.

Both the standard sand-filter aerooscope and the Rettger liquid-filter type were found to give dependable and accurate results under proper conditions; but because it overcame certain annoying difficulties and was more convenient in work of this character, the modified standard apparatus was selected for the final tests. By this modification, developed in the course of the present investigation, a fused union of two glass tubes is substituted for a cork connection, with its greater chance of leakage; and dry sterilization can be used instead of the steam sterilization necessary with the original standard apparatus, which often leads to caking of the sand and the formation of channels and crevices.
Although the plate-exposure method for counting the bacteria falling from the air has been used in nearly all studies of this kind previously made, it was found to give much smaller counts than the pail-exposure method, consequently the latter was chosen for this work. As the average of all the tests made, ten times as many bacteria were found by the pail method as by the plate method. In the comparisons most favorable to the plate method only one-half as many bacteria were counted as by the pail method, while in the least favorable cases the ratio was as great as 1 to 32. This failure of the plate method to show all the bacteria is undoubtedly due to the fact that on plates only one colony may develop from a dust particle laden with many bacteria, while by the pail method these collected bacteria are separated and more individuals may grow into colonies on the culture plate. These conditions correspond quite closely to those in the milk after dust particles enter it.

From these preliminary investigations and from studies of indirect data derived from several sources, it was believed "that the number of bacteria gaining access to the milk from the air is relatively small in comparison with the numbers derived from the interior of the udder, the exterior of the cow, and the pail," consequently every effort was made during the actual study of the bacteria in, or from, the air to get rid of these other factors so far as possible. An apparatus was devised by which it was possible to shut out contamination from these sources and to distinguish quite satisfactorily between the "washing" effect of milk sprayed through the air in milking and the unaided falling of bacteria into the pail as sources of contamination of the milk. The "artificial cow" used to make these "milking" tests is shown on the title page. This consists of a can or "udder" above the protective platform corresponding to the lower surface of a cow's body, and two glass and rubber "teats" below through which water or other liquid can be sprayed or "milked," either continuously or intermittently as in actual milking, into the pail below. All necessary precautions were taken to make distances and other conditions about this apparatus, when in use, correspond as closely as possible to those beneath the cow. Tests with this apparatus and with the aerosopes were made simultaneously for the pairs or groups of comparisons, sometimes requiring two or three persons to perform the necessary operations and make the records: (1) The air was analyzed for bacteria, using the modified standard aeroscope, which was clamped in position under the platform beside the two pails; (2) the "milking" was carried on, with sterilized water, drawn into sterilized test tubes for a check and then into the pail; (3) a pail under the platform, containing sterilized water, was exposed for a definite period and then protected against further contamination, (4) similar determinations of the amount of precipitation in the open stable were made by placing pails on the floor beside the apparatus.
The plating, incubation of the bacteria and other analytical processes, were made by methods previously found to give reliable results, and were kept as uniform as possible throughout any series of tests.

Practical studies: This "artificial cow" and the aeroscopes were used under many different conditions and in different places in order to study the effect of various stable operations, disturbances of the air and ranges of dustiness upon the numbers of bacteria present or precipitated into the milk.

In the Station stable two series of tests were made during afternoons with the cows in the stable, part of the series when grain was being fed, part while the cows were being milked and one test while the cows were being brushed; and another series was carried through in the morning while the cows were being fed chopped hay.

As none of these tests showed high numbers of bacteria, in the air, falling into the pail from the air, or washed out of it by the milking, conditions were made more severe, equaling or exceeding any that would ever be met in stables. The pieces of apparatus were moved to the stable loft, in the upper story of the large cattle barn, and dust raised artificially.

In the first series a dense dust was raised before each test by stirring up the hay and dirt on the floor of the loft; and in the second series the extremely dusty condition was maintained throughout each test by continuous stirring and sweeping of the refuse on the floor.

As the Station stable presents some unusual conditions, it was thought wise to carry the investigation into commercial dairy stables where surroundings were in every way typical of those ordinarily met, from good to bad; not as complete series could be conducted as in the Station stable, owing to the distance of the stables from the laboratory.

However, analyses of the barn air were made in fifteen tests, five in summer and ten in winter, in one old stable (A) quite well cared for but with dust in the air very evident at the time of each of the tests. Conditions were made very bad during three of the tests by throwing down hay and feeding, while during half of the remaining tests the cows were eating dry hay and in the other half milking was in progress. The second stable (B) had been more recently constructed than the first one; but was poorly cared for; the cows were not brushed, were covered with dust, and occasional ones had dried manure clinging to their flanks. Dry feed was given to the cows after milking, however, and the air was not noticeably dusty at the three visits made to the stable, in July, January and March respectively. The twenty-three tests were made just before milking, during milking, and while the cows were being bedded.
The third stable (C) is one of the most poorly constructed and poorly kept stables in the vicinity, located in the basement of the barn, very poorly lighted and ventilated, the mangers and other fittings are of wood, the floor, except immediately under the cows, of dirt, pulverized into fine dust, the walls back of the cows spattered with dry manure, and dust and cobwebs abundant. Twenty analyses were made here, all in March, but under widely varying conditions, such as with barn empty, empty except for calves and chickens, empty but man removing manure and corn stover, cows coming in, while straw bedding was being placed, cows in stable and receiving grain feed, cows being brushed, and cows being milked.

In this wide range of conditions the variations in the numbers of bacteria found were, of course, great; but except under circumstances that would never long continue in ordinary stables, the number of germs finding their way into the milk was so small as to be almost negligible.

The first table below shows the average number of bacteria in the air of the Station stable or loft in groups of tests, with the interpretation of these numbers in terms of bacteria falling into the milk or "washed" into it by the milking.

**Table I.—Analyses Summarized According to the Number of Bacteria Per Liter of Air.**

| Germ Content Per Liter of Air Between | Number of tests made | Average count per liter of air | Average Precipitation in 5 Minutes | Bacteria washed out of air by "milk" stream. | Total germ content* in "milk."
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0-100</td>
<td>26</td>
<td>51</td>
<td>49</td>
<td>1.8</td>
<td>5.2</td>
</tr>
<tr>
<td>101-1,000</td>
<td>19</td>
<td>312</td>
<td>210</td>
<td>6.6</td>
<td>26.6</td>
</tr>
<tr>
<td>1,001-10,000</td>
<td>32</td>
<td>3,270</td>
<td>3,581</td>
<td>94.0</td>
<td>101.0</td>
</tr>
<tr>
<td>10,000 and upwards</td>
<td>7</td>
<td>18,644</td>
<td>24,284</td>
<td>611.0</td>
<td>226.0</td>
</tr>
</tbody>
</table>

* The figures in this column are the sum of those in two preceding columns.

It will be noted that even when the dustiest possible conditions were secured so that the bacteria averaged more than 10,000 per liter of air, which happened in seven tests only, less than 1000 germs per cubic centimeter got into the milk, while in 78 tests the number was less than 200, and in 45 tests, or more than half of all, the figure was below 50, a quantity so small that the presence or absence of these bacteria could not be detected, except in the very highest grade of milk, by any method of bacterial analysis.

In the next table the analyses made in the Station stable are grouped in accordance with the barn operations then in progress.
### Table II.—Analyses Summarized According to the Barn Operation in Progress at the Time of Making the Analysis.

<table>
<thead>
<tr>
<th>Barn Operation in Progress at the Time the Analysis Was Made</th>
<th>Number of analyses made</th>
<th>Average germ content per liter of air</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Barn empty, everything quiet</td>
<td>60</td>
<td>41</td>
</tr>
<tr>
<td>2. Barn empty, sweeping floor or dusting off stanchions</td>
<td>7</td>
<td>135</td>
</tr>
<tr>
<td>3. Cows in barn, everything quiet</td>
<td>20</td>
<td>94</td>
</tr>
<tr>
<td>4. Brushing off cows</td>
<td>33</td>
<td>130</td>
</tr>
<tr>
<td>5. Feeding dry grain</td>
<td>50</td>
<td>124</td>
</tr>
<tr>
<td>6. Brushing off cows when test was started. Feeding grain at end of test</td>
<td>8</td>
<td>190</td>
</tr>
<tr>
<td>7. Feeding grain at beginning of test. Milking started before test was finished</td>
<td>11</td>
<td>235</td>
</tr>
<tr>
<td>8. Milking in progress</td>
<td>153</td>
<td>111</td>
</tr>
<tr>
<td>9. Feeding silage just after milking was finished</td>
<td>10</td>
<td>271</td>
</tr>
</tbody>
</table>

Under none of these conditions did the average number of bacteria in the air reach 300 per liter, and in no case would the increase in the number of bacteria from the air reaching the milk drawn under these conditions be measurable.

Similar results were secured in the commercial stables; for in the best stable (B) the number of bacteria per liter of air did not reach 1000 in any of the 23 tests, was above 500 in only four tests and was 100 or less in nearly half of them. In A, the bacteria in the air were above 1000 in only two tests, in one case only slightly above this figure when the air was analyzed shortly after the cows had come into the barn and were being milked, but in the other case reaching 16,000, before the cows were milked and while dusty hay was being thrown down for them. In the other tests, the germ content of the air ranged from 4 to 857 per liter and averaged less than 350.

In C, only two analyses showed more than 1000 bacteria per liter of air, and these were both less than 4000, with quite evident reason for the increase over the other analyses through the removal of corn stover and feeding of grain during or shortly before the taking of the air samples. In this stable many tests showed less than 100 bacteria per liter of air, with a very low average for all.

In these stables, except in perhaps one instance, it would have been impossible to detect the addition of bacteria from the air by any analysis made of the milk.

Similar "precipitation" tests were made to compare the numbers of bacteria falling into open-top and small-top pails exposed in the stable during different barn operations, each exposure continuing five minutes, approximately the time required to milk a cow. These
results are summarized in Table III; and in Table IV are given computed data showing the numbers of bacteria in the milk resulting from longer exposure, and with greater numbers of germs in the air, as in the tests in the stable loft.

**Table III.—Results Obtained by Exposing a Sterile Liquid in Pails to the Air of the Open Stable.**

Summarized according to the barn operation in progress at the time the test was made.

<table>
<thead>
<tr>
<th>Barn Operation</th>
<th>Pail.</th>
<th>Number of tests</th>
<th>Precipitations on 1 sq. cm. in 5 min.</th>
<th>Calculated Number of Bacteria Which Would Be Added to the Milk Per C.C. in 1 min.</th>
<th>5 min.</th>
<th>15 min.</th>
<th>30 min.</th>
<th>60 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding dry drain</td>
<td>Loy...</td>
<td>7</td>
<td>71</td>
<td>0.40 2.07 10.35 31.05 62.1 124.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Open...</td>
<td>7</td>
<td>71</td>
<td>0.40 2.07 10.35 31.05 62.1 124.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeding dry</td>
<td>Loy...</td>
<td>22</td>
<td>55</td>
<td>0.30 1.56 7.92 24.06 48.0 96.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chopped hay.</td>
<td>Open...</td>
<td>22</td>
<td>55</td>
<td>0.30 1.56 7.92 24.06 48.0 96.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milking</td>
<td>Loy...</td>
<td>25</td>
<td>114</td>
<td>0.6 3.20 8.65 24.06 48.0 96.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Open...</td>
<td>25</td>
<td>114</td>
<td>0.6 3.20 8.65 24.06 48.0 96.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table IV.—Calculated Number of Bacteria Which Would Fall Into an Open Milk Pail in Given Lengths of Time**

<table>
<thead>
<tr>
<th>Assumed Precipitation on 1 Square Centimeter in 5 Minutes</th>
<th>Calculated Number of Bacteria Which Would Be Added to the Milk Per Cubic Centimeter in 1 minute</th>
<th>5 minutes</th>
<th>15 minutes</th>
<th>30 minutes</th>
<th>60 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1.5</td>
<td>7.3</td>
<td>21.9</td>
<td>43.7</td>
<td>87.5</td>
</tr>
<tr>
<td>100</td>
<td>2.9</td>
<td>14.6</td>
<td>43.7</td>
<td>87.5</td>
<td>180</td>
</tr>
<tr>
<td>250</td>
<td>7.3</td>
<td>36.5</td>
<td>109</td>
<td>218</td>
<td>437</td>
</tr>
<tr>
<td>500</td>
<td>14.6</td>
<td>72.9</td>
<td>219</td>
<td>437</td>
<td>875</td>
</tr>
<tr>
<td>1,000</td>
<td>29.2</td>
<td>146</td>
<td>437</td>
<td>875</td>
<td>1,750</td>
</tr>
<tr>
<td>5,000</td>
<td>146</td>
<td>729</td>
<td>2,187</td>
<td>4,374</td>
<td>8,748</td>
</tr>
<tr>
<td>10,000</td>
<td>292</td>
<td>1,458</td>
<td>4,374</td>
<td>8,748</td>
<td>17,496</td>
</tr>
<tr>
<td>50,000</td>
<td>1,460</td>
<td>7,200</td>
<td>21,870</td>
<td>43,740</td>
<td>87,480</td>
</tr>
</tbody>
</table>

These tests and computations show that under the worst of the assumed normal conditions, where the milk is allowed to stand an hour in the stable in an open 12-inch pail, the average number of bacteria added to the milk would be only 96 when the cows were being fed dry chopped hay, 124 if they were fed dry grain or 199 if they were being milked. These numbers are so small that the addition of the bacteria could scarcely be detected in any but the highest grade of milk by any known method of analysis. An exami-
nation of the results, calculated on the basis of a small-mouthed pail, shows that a five-quart milking could stand in the open stable for one hour or longer without producing any detectable result so far as bacteria added to the milk from the air are concerned.

The lowest and medium results obtained in the stable-loft tests represent conditions that might exist in any stable for a very short time after such dust-producing operations as feeding dry hay or grain, when exposure of milk in the open pail for any considerable time would result in an appreciable addition of bacteria to the milk. The effect of such operations passes quickly; for tests made within 15 minutes after some dust-producing disturbance showed marked reduction of the numbers of bacteria either in air or falling into the milk in that time. The highest of the results secured in the stable loft represent conditions that are scarcely conceivable even in the worst commercial dairies. They could be obtained only by attempting to milk within a few feet of the dusty end of a threshing machine or fanning mill. The precipitation pails exposed under these conditions were covered with a layer of dirt at the end of five minutes.

Of course the numbers of bacteria increase very rapidly in exposures under such conditions.

Conclusion. While these investigations show that the numbers of bacteria present in stable air markedly exceed those found for country air, city street air, air of offices, schools or factories, they prove very clearly that the number falling into the milk during milking or during any short exposure in the stable, under conditions allowable in any respectable dairy, is so small as to be negligible. Milking under extremely adverse conditions, like feeding very dusty hay or grain during milking, and especially exposure of milk in the open pail for any considerable time under such conditions, should, of course, be avoided.
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