OBSERVATIONS ON "PIN POINT COLONY" ORGANISMS IN THE BALTIMORE MILK SUPPLY

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INTRODUCTION AND LITERATURE

It seems to be quite generally agreed that the "pin point colonies" which occasionally appear on plain agar plates inoculated with diluted milk for the purpose of estimating the number of organisms present may be due to a variety of causes.

Yates (1923) suggested that since the trouble was first recognized, coincident with the change in the method of adjusting the reaction of culture media and the more or less general use of chlorine solutions in dairies and on farms, there might possibly be a connection between one or both of these procedures and "pin point colonies."

Ayers and Johnson (1924) found the appearance of these colonies from pasteurized milk from one milk plant to be due to a thermophilic organism which they named *Lactobacillus thermophyles*. They also suggested that crowded plates, the reaction of the medium and heat resistant non-thermophilic bacteria might be possible causes.

Cooledge (1924) by a special technique isolated alkali producing thermophilic bacteria from milk and found that these organisms when present in great numbers overcame the unfavorable reaction of the medium and developed as typical pin point colonies.

Harding and his collaborators (1924) have demonstrated that there are present in all classes of milk organisms which will grow at the pasteurizing temperature. We are not sure whether these organisms produced pin point colonies or not.

OBSERVATIONAL AND EXPERIMENTAL

For a little over two years we have observed in connection with our routine milk control counts that we believe to be typical cases of "pin point colonies." What we call typical cases are not due to crowded plates, for counts made from low dilutions (in so far as they can be counted at all because of their extreme minuteness) are much higher than those from higher dilutions. This would not be true if the "pin point colonies" were due to crowding. They are not due to differences in the reaction of the culture medium in so far as these differences exist prior to incubation. However, in this connection it must be borne in mind that the reaction of the medium in the plates after incubation is rarely the same as it was before incubation. Due to the activity of the developing organisms it is practically always changed. Usually the low dilutions become more alkaline than the higher dilutions, although there may be a change in the acid direction.

Late in the year 1922 we became convinced of the typical appearance of these plates. In order to report our findings as accurately as possible we estimated the number of colonies as best we could and reported the counts with the notation "pin point colonies." This signified that there were at least as many organisms present as reported and probably a great many more. This procedure has been in operation for a little over two years.

It has been our general observation that these cases of "typical pin point colonies" were much more numerous in the winter. In order to establish our observation in this regard we went over our records and calculated the percentage of the total number of examinations made each month which had shown "typical pin point colonies." This was done for each of the three main classes of milk which we analyze. Figure 1 shows the curves constructed ed from these figures for the last two years.

In order to determine if possible the organism or organisms producing these colonies we collected cultures from typical plates over a period of two months (December 15, 1923 to February 15, 1924). One or two colonies from each plate were fished under the microscope and inoculated into litmus milk. These cultures

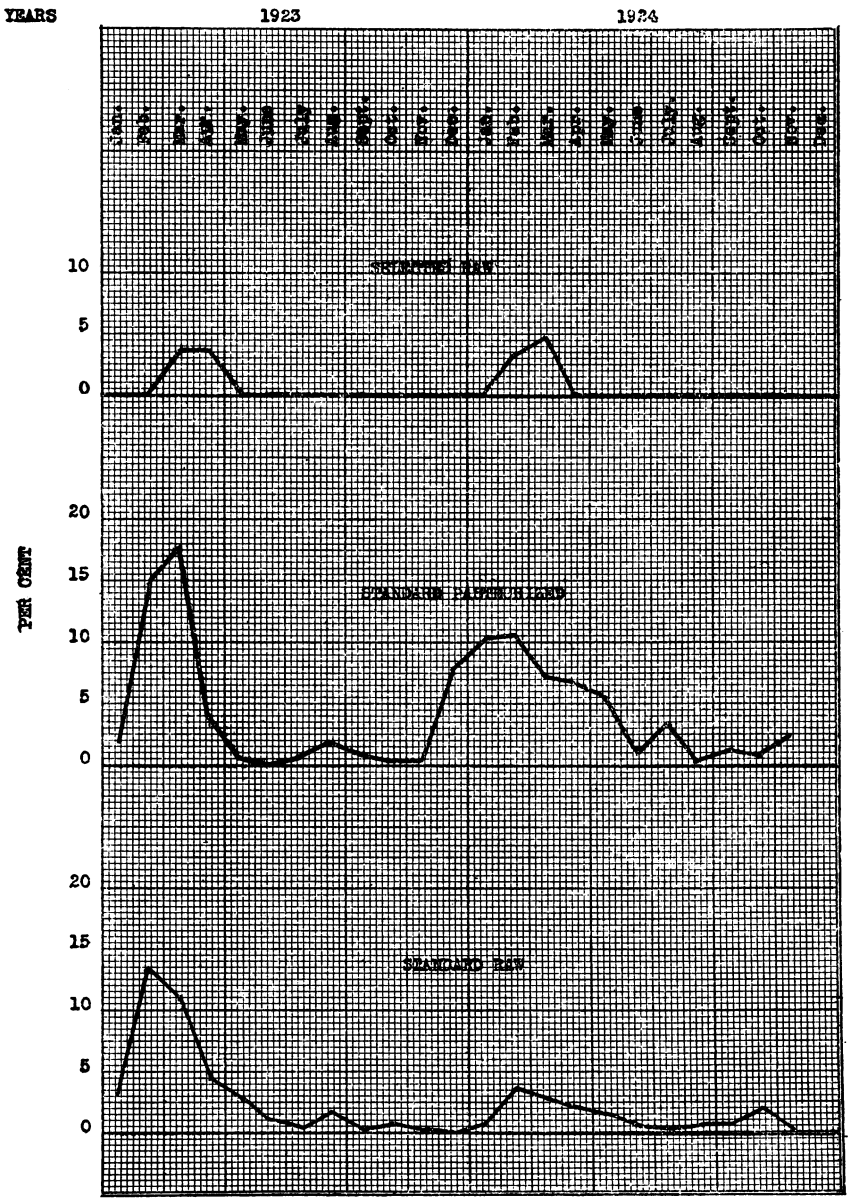

FIG. 1

were incubated for twenty-four hours at 37°C. and then plated on Ayer's milk powder Agar A. In this way pure cultures were secured and were kept growing in milk by transferring every two or three weeks. In all, 52 cultures were secured. Twenty-one of these came from raw milk, 26 from pasteurized milk, and 5 from ice cream. It should be mentioned that the five cultures from ice cream were from plates which were not typical. The colonies were quite small, but they developed in such a manner that approximate check counts could be made from the different dilutions.

The 21 cultures from raw milk included cultures from the raw milk of 5 different pasteurizing plants. The 26 cultures from pasteurized milk included cultures from the pasteurized milk of 7 different pasteurizing plants. The 5 cultures from ice cream included cultures from one plant only.

On morphological examination 50 of these cultures proved to be streptococci, one was a staphylococcus and one a spore former. These last two cultures when replated on plain agar produced large colonies. All of the streptococcus cultures when plated in pure culture on plain agar at pH 6.8 to 7.0 either produced typical small colonies or failed to show up as colonies. Sixteen of the streptococcus cultures were studied further. We have not attempted to identify these cultures because they do not seem to be typical of any well defined type. The characteristics of these organisms are given in table 1.

In so far as we have examined these cultures we have for the most part followed the procedure of Ayers and his collaborators in their studies of the streptococcus from different sources. (Exceptions which should be noted are the use of rabbits' blood and North's medium in our blood agar plates, and of National Aniline and Chemical Co. methylene blue in concentration of 0.1 cc. of 0.5 per cent solution in 10 cc. of milk.) This was done in order that we might determine if possible by comparison with the organisms which they studied the probable source of these "pin point colony" producing streptococci. In this we were disappointed since our cultures do not seem to be typical of any class which they studied, with the possible exception of the 7

TABLE 1

Characteristics of "pin point colony" streptococci

NUMBER OF CULTURES	SOURCE	HEMOLYSIS	CHAINS	METHYLENE BLUE 37°C.	LITMUS MILK	FERMENTATION*							ACID PRODUCTION IN LITMUS MILK†			COLONIES ON PLAIN AGAR WHEN IN PURE CULTURE
						Glucose	Lactose	Sucrose	Salicin	Mannitol	Raffinose	Inulin	20°C.	37°C.	50°C.	
2	Ice cream	Alpha green	Short	Complete reduction 18 hours, coagulated 24 hours	Acid coagulated	+	+	+	+	+	+	−	++	+++	−	+
1	Pasteurized milk	Alpha green	Long	Complete reduction 18 hours, coagulated 24 hours	Acid coagulated	+	+	+	−	−	−	−	−	++	−	−
2	Pasteurized milk	Alpha green	One long; one short	Partial reduction 48 hours, coagulated 72 hours	Acid coagulated	+	+	+	−	−	−	−	−	++	−	-
3	Pasteurized milk	Gamma green	Long		Acid coagulated slowly	+	+	+	−	−	−	−	−	+	−	-
1	Pasteurized milk	Alpha green	Short		Acid	±	±	−	−	−	−	−	±	+	−	-
7	Raw milk	Alpha green	Long		Weak acid	−	−	−	−	−	−	−	±	+	−	+

* Culture tested with methyl red. + indicates final acid color pH 4.6 or lower. ± indicates neutral range approximately pH 5.4 to 4.8. − indicates that cultures did not develop enough acid to appreciably change the color of methyl red pH 5.8 or higher.

† − indicates no change in the color of litmus after five days' incubation. ± indicates the first slight change in three to five days. + indicates a definite change in three days. ++ indicates a definite change in two days. +++ indicates a definite change in one day.

cultures from raw milk, which show some similarity to their cultures of *Streptococcus acidominimus*, which they found was one of the minor udder types.

In order to approximate the optimum growing temperature for these organisms, three sets of litmus milk tubes were inoculated in the same manner. One set was incubated at 20°C., one set at 37°C. and one set at 50°C. The rapidity with which acid was produced as determined by a change in the color of litmus, was taken as indicative of the comparative rate of growth at the different temperatures. The results of these tests are included in the table.

In the last column of the table is indicated the ability of these organisms to produce colonies on plain agar (pH 6.8 to 7.0) when inoculated in pure cultures and without the addition of milk.

DISCUSSION

It would appear from the data presented above that what we have considered "typical pin point colonies" have a fairly definite seasonal occurrence. In each of two years we have found them to occur with the greatest frequency in the months of January, February, March, and April. They occurred at the same time in both raw and pasteurized milk. This fact would indicate that the "pin point colony trouble" which we have been observing has its origin at the farm. However, in examining the organisms responsible for these colonies we found several varieties of streptococci, and those from pasteurized milk were different than those from raw milk. Our cultures were not collected with the idea of determining the predominant types in raw milk and pasteurized milk. To establish a difference in type would require the study of a great many more cultures. Other points to consider are the dilutions of the milk in the original plates and the flora (other than "pin point" colony organisms) present in the milk. What part other organisms may play in making conditions suitable for the growth of a particular organism when they are associated on the same plate is a subject we know little about.

It should be noted that these organisms occur in the milk

supply of Baltimore coincident with the spring freshening season for cows on the Baltimore milk shed. Whether streptococci are more numerous in the udder of cows or about the farm at this time of the year we do not know. However, on purely theoretical grounds, udder trouble would be expected to be quite prevalent at this time of the year.

We do not propose to draw any conclusions from this work as to the connection between these organisms and mastitis in cattle. Much more work would be necessary to determine such a fact. However, it may be said that should such a relationship be established, the occurrence of "typical pin point colonies" when due to streptococci might prove to be a valuable index by means of which udder trouble could be located; just as "pin point colonies" when due to thermophiles have proven of value in locating trouble in pasteurizing plants.

SUMMARY

The literature indicates that "pin point colonies" on milk plates may be due to a variety of causes, such as crowded plates, reaction of medium, thermophilic organisms, and streptococci.

We have found "pin point colonies" to occur on our routine plates for the control of milk with the greatest frequency in the months of January, February, March and April.

The organisms responsible for the colonies were found to be streptococci.

The possible relationship of these organisms to udder trouble and the significance which could be attached to "pin point colonies" when due to streptococci should it be shown that these organisms indicate mastitis have been discussed.

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