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OBSERVATIONS ON THE PRESENCE OF THE BACILLUS ABORTUS BOVINUS IN CERTIFIED MILK

PRELIMINARY NOTES *

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A large amount of experimental work has been done during the past decade to impress on the public and profession the fact that raw milk is never a safe milk, and that in order to be fit for consumption all milk must be pasteurized. The effect has been that in many states legislation has been enacted governing and requiring the pasteurization of all milk with one exception, and this exception is certified milk. In view of the enthusiasm, entirely justified, of public health experts everywhere as to the value of pasteurization, suggestions have been rather numerous, particularly during the past two years, that even certified milk is not a safe raw product, and that it, too, should be heated. Without entering into the relative values of raw and heated milk in infant feeding, it is unquestionably permissible to say that with our present knowledge most pediatricists are still of the opinion that raw milk plays a very important and needed rôle during the first two years of life, provided such a product can be obtained as is not a potential source of danger to the infants consuming it.

It has always been the proud opinion of those medical men interested in the certified milk problem that in this product conditions as ideal as were possible had been met, and that for practical purposes certified milk was a safe raw milk. When the literature, however, began to offer suggestions here and there doubting its efficiency and safety, it became necessary for the problem to be attacked by those interested in it, in order that the foundation on which the movement had been built might be forever strengthened and its value be definitely proved, or, if it was not a dependable product, that this even more important fact should be elicited.

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Within certain human limitations it has always been felt by certified commissions that a very thorough safeguard had been built against the ordinary milk-borne infections due to human contamination by a fairly perfect system of dairy hygiene. The weak link in the chain, therefore, lay in that group of diseases to which man is heir, emanating from the cattle themselves. And in this group bovine tuberculosis plays the leading rôle. Tuberculin testing, performed twice a year, has succeeded in reducing the disease to a minimum, but even with this precaution some reactors are usually present, and occasionally a severe infection will decimate a herd under the best of conditions.

The following question, therefore, suggested itself. By semi-annual tuberculin tests, can we discover reactors so early in the course of their disease that they are not throwing out tubercle bacilli in their milk and in this way disseminating bovine tuberculosis? To determine this fact but one method seemed satisfactory, namely, to inject samples of certified milk into guinea-pigs in an effort to produce tuberculous lesions, if tubercle bacilli were present. A careful review of the literature has failed to show any record of this determination having ever been made with certified milk, although the number of men who have demonstrated the presence of tubercle bacilli in market milk is too large to mention. With this idea in mind, samples of certified milk were collected over a period of several months from the regular distributors so that the product as it was ordinarily consumed should be investigated.

TECHNIC

The following technic was carried out: Eight hundred cubic centimeters of milk were centrifuged for one-half hour, 2,000 to 3,000 revolutions per minute, 100 c.c. in each of eight tubes. At the end of that time the cream and skim milk were poured off and the sediments were collected in about 20 c.c. of normal saline solution. On account of centrifugalization for one-half hour, the high speed, and some experiments of one of us, it was not considered necessary to inject the cream layer, which otherwise frequently retains tubercle bacilli. Five cubic centimeters of the sediment suspended in saline were then injected intramuscularly into the upper and inner surface of the right hind thigh of a guinea-pig. Four guinea-pigs were injected for each sample of milk.

Without giving much thought to the problem when the experiments started, beyond the question as to whether or not tuberculous lesions would be discovered at the time of the necropsy, the animals in the first experiment were not disturbed for about twelve weeks. At this time two of them were chloroformed, and, as is not infrequently the case, in gathering experimental data, a very unexpected situation was discovered. The animals were not appreciably emaciated. On post-mortem examination the spleens were found markedly enlarged and studded with small grayish nodules. There was a general adenopathy, but no caseation. The testes of both animals were atrophic and the

epididymis of one contained pus. The lungs, liver and kidneys were apparently normal. Careful examination of the spleen nodules after maceration failed to show any acid-fast bacilli. We were, therefore, apparently dealing with a disease simulating tuberculosis but having its most marked effect in the spleen, lymph nodes and sex organs. The condition was immediately recognized by one of us as abortion disease of guinea-pigs, and it was evident that we had to deal with two problems, tuberculosis, for the determination of which the experiments were started, and abortion disease, the determination of which the experiments elicited.

The literature pertaining to the subject of this paper as announced in the title has been purposely not even referred to up to the present, because it seemed more logical to state first the object of the work, and then elaborate on the literature after emphasis had been laid on the peculiar manner in which our attention was focused on the positive findings.

In discussing the literature it will be desirable to consider it in the following manner: First, those investigations bearing on milk as a cause of abortion disease; second, those investigations bearing on methods of diagnosis; third, those bearing on the possible result that the presence of these organisms in milk may have on public health.

Ever since Bang,¹ in 1907, fulfilled Koch's laws in regard to the *Bacillus abortus bovinus* as the etiologic agent in abortion disease of cattle, countless investigators have been attacking the problem because of its fascinating biologic possibilities, and on account of its very great economic importance.

Theobald Smith,² in 1894, first called attention to a peculiar disease occurring in guinea-pigs, following the inoculation of milk, that resembled tuberculosis, but it was not until seventeen years later, in 1911, that he, in association with Marshal Fabyan,² determined beyond question that these lesions were due to *B. abortus*. Their conclusions at this time were:

1. *B. abortus* (Bang) is most likely the only cause of infectious abortion among cattle; 2, *B. abortus* produces in guinea-pigs a peculiar general disease that very seldom kills the animal. It resembles tuberculosis and is characterized by chronic interstitial new tissue, consisting for the most part of epithelioid and lymphoid cells; 3, *B. abortus* occurs in milk, and therefore it is indicated to determine whether it can have any causative relationship to sclerosis of tissues or organs, or to the chronic diseases of man or domestic animals.

1. Bang, B.: Die Aetiologie des seuchenhaften ("infektiösen") Verwerfens. Ztschr. f. Tiermed., Jena, 1897, **1**, 241.

2. Smith, Theobald, and Fabyan, Marshal: Ueber die pathogene Wirkung des *Bacillus abortus* Bang, Centralbl. f. Bakteriologie u. Parasitenkunde, Abt. I, 1912, **61**, 549.

Schroeder and Cotton³ state that the udder is the favorite habitat of *B. abortus*, and in the nonpregnant cow it is the only habitat. One animal in their work showed bacilli in her milk for seven years. They injected *B. abortus* intravenously into a nonpregnant animal and the organisms disappeared in a few hours from the circulation and were present only in the udders and neighboring lymph nodes. They gave an intravenous injection to a virgin heifer 4 years old, and later the bacilli were found in the udders which had never functioned. They examined all the organs of infected animals by guinea-pig inoculation and cultural methods and found *B. abortus* only in the udders or neighboring lymph nodes, and in one instance in the pelvic lymph nodes. They assert, first, that the udders of cows are a common habitat of abortion bacilli; second, that abortion bacilli do not maintain themselves in the bodies of nonpregnant animals elsewhere than in their udders. They, furthermore, experimentally produced the disease by injecting the organisms into the udders, and call attention to the fact that milkers may perpetuate the disease by carrying it on their hands. If other workers prove their claims to be well founded, little wonder can be attached to the presence of *B. abortus* in milk. Previous to those experiments, the same investigators had examined the milk from a number of different dairies. Of 77 samples from 38 dairies, 8 samples from 6 different dairies were infected. In a second series of 140 samples from 4 dairies, Dairy A, 35 samples, showed 11 infected; Dairy B, 33 samples, 7 infected; Dairy C, 34 samples, 2 infected; Dairy D, 38 samples, 2 infected; a total of 214 samples with 30, or 14 per cent., infected.

In another series of 140 cows free from tuberculosis, the milk of nineteen was infected with *B. abortus*. In another series of thirty-six cows with a large proportion tuberculous, the milk of eleven showed *B. abortus*.

Zwick and Krage⁴ isolated organisms from the milk of three cows and proved its identity by agglutination tests. They noted no changes in the udders or milk macroscopically, and concluded that *B. abortus* may pass through the udders without causing lesions. They likewise injected strains into the udders of goats and produced the disease in pregnant animals, and further injected them, both by the subcutaneous and intravenous routes, recovering the bacilli in the milk at the end of twenty-four hours, in which they were still present at the end of eight weeks' observation without demonstrable lesions in the udders.

The result of the most comprehensive, purely bacteriologic work

3. Schroeder and Cotton: The Bacillus of Infectious Abortion Found in Milk, U. S. Dept. of Agric. Rep. Bureau of Animal Indust., Circ. 216.

4. Zwick and Krage: Ueber die Ausscheidung von Abortusbacillen mit der Milch infizierter Tiere, Berl. tierärztl. Wehnschr., 1913, **29**, 41.

that has been done on milk was published by Alice C. Evans⁵ in 1916. She described three groups of organisms, streptococci, staphylococci and bacilli of the abortus group. A special method of plating was necessary for isolating these bacilli, because a milk containing hundreds of thousands of *B. abortus* might pass unrecognized by the ordinary laboratory method of examination. She studied 192 samples from 161 different cows. In 45, or about 23 per cent., she discovered *B. abortus*, and in 33 of these 45, or 17 per cent. of all the samples, she isolated what she has termed the *B. abortus* var. *lypolyticus* on account of its fat-splitting character. Two other varieties that she isolated, B and C, resembled cultures obtained from pathogenic sources. Evans also examined by plating, two bottles of certified milk, and found that the *B. abortus* represented 25 per cent. of the total bacteria. This year, 1917, Evans⁶ has reported on the further examination of the milk of 23 individual cows and the *B. abortus* developed in 17, or 73.9 per cent. The cultures of 11 samples were studied in detail, and in 9 *B. abortus* var. *lypolyticus* was found, while in the remaining 2, the bacilli resembled cultures from pathologic material.

The importance of the *B. abortus* var. *lypolyticus* has not been determined from a pathogenic standpoint.

A. Eichorn and G. M. Potter,⁷ referring to them as mutation forms that morphologically and culturally resemble *B. abortus*, say that testing them against pathogenic strains by agglutination and complement fixation failed to give positive reactions. Feeding and inoculation experiments were also inconclusive. One of Evans' most interesting findings is that *B. abortus* from pathogenic sources when grown on mediums containing butter fat for nine and one-half months, has acquired a fat-splitting quality. Eichorn and Potter say that they have had several herds under observation in which abortion occurred occasionally, and in which repeated serologic tests failed to reveal the presence of *B. abortus*. Several questions are suggested to them: (1) Can it be that these fat-splitting organisms play a rôle in such cases, or are they entirely harmless? (2) Are they attenuated forms of the pathogenic variety which have lost some of their characteristics as they acquired the fat-splitting quality? (3) If this is so, may they not under certain circumstances regain their pathogenic properties? (4) Are they detrimental to human health?

5. Evans, Alice C.: The Bacteria of Milk Freshly Drawn from Normal Udders. Jour. Infect. Dis., 1916, **18**, 437.

6. Evans, Alice C.: The Large Number of Bacterium Abortus var. Lypolyticus Which May Be Found in Milk, Jour. Bact., 1917, **2**, 185.

7. Eichorn, A., and Potter, G. M.: The Present Status of the Abortion Question. Jour. Am. Vet. Med. Assn., December, 1916, **50**, 295.

E. C. Schroeder⁸ in 9 series of milk tests, injected 516 samples of milk from 90 dairies into 1,068 guinea-pigs; 103, or about 10 per cent., of the guinea-pigs developed abortus disease and the milk distributed by 29 dairies proved from time to time to be infected with abortion bacilli. He likewise fed guinea-pigs raw, pasteurized and boiled milk, and says that several of the pigs receiving raw milk developed abortion disease.

Ever since 1909, when Holth⁹ and Grinstedt,¹⁰ working independently, obtained positive agglutination tests in the serum of cattle infected with abortion disease, serum diagnosis has been generally employed in the determination of this condition. About the same time McFadyean and Stockman¹¹ showed the presence of antibodies in the blood of infected animals by agglutination tests.

Sven Wall,¹² after 771 examinations, concluded that the agglutination and complement fixation reactions are practical to determine infection if properly carried out. These reactions persist over six months in most animals. He infected a horse intravenously and obtained positive reactions in five days.

Hadley and Beach¹³ came to the same conclusion. Brüll¹⁴ found these tests very valuable. Zwick and Zeller¹⁵ got positive results in a very large number of cases. Larson¹⁶ in 1912 in this country established that contagious abortion is due to the bacillus of Bang by the complement fixation test. With the exception of the English commission, all workers agree on the value of these tests on the blood

8. Schroeder, E. C.: An Experiment with Raw and Heated Cow's Milk and Its Lesson, with Comments on *Bacillus Abortus*, *AM. JOUR. DIS. CHILD.*, 1913, **6**, 334.

9. Holth, Halfdan: Die Agglutination und die Komplementbindungsmethode in der Diagnose des seuchenhaften Verwerfens der Kühe. *Berl. tierärztl. Wchnschr.*, 1909, **25**, 686.

10. Grinstedt, P.: The Agglutination Test in the Diagnosis of Contagious Abortion of Cattle, *Jour. Compar. Path. and Therap.*, 1910, **23**, 279.

11. McFadyean, J., and Stockman, Stewart: Epizootic Abortion in Cattle, Report of the Departmental Committee Appointed by the Board of Agriculture and Fisheries to Inquire Into Epizootic Abortion, App. to Part I, London, 1909.

12. Wall, Sven: Ueber die Feststellung des seuchenhaften Abortus beim Rinde durch Agglutination und Komplementbindung, *Ztschr. f. Infektionskrankh.*, 1911, **10**, 23 and 132.

13. Infectious Abortion in Cattle. *Jour. Compar. Path. and Therap.*, 1906, **19**, 191.

14. Brüll, Ziga: Beitrag zur Diagnostik des infektiösen Abortus des Rindes. *Berl. tierärztl. Wchnschr.*, 1911, **27**, 721.

15. Zwick and Zeller: Ueber den infektiösen Abortus des Rindes. *Arb. a. d. k. Gsndhtsamte.*, 1912, **43**, 1.

16. Larson, W. P., and Sedgwick, J. P.: The Complement Fixation Reaction of the Blood of Children and Infants, Using the *Bacillus Abortus* as Antigen, *AM. JOUR. DIS. CHILD.*, 1913, **6**, 326.

serum of infected animals. Schulz,¹⁷ working in Dresden, in 1912 combined the agglutination test with the intradermal test. His results in the agglutination reactions are identical with those of his predecessors in the work. He found one previous record in the literature of the use of the intradermal test as a diagnostic aid, and he lays great emphasis on its value. He considers as a positive reaction any swelling on the third day of at least 5 mm. diameter. The results, however, vary with the character of the antigen used between 45 per cent. and 100 per cent.

Reinhardt and Gauss,¹⁸ working in 1914 and 1915, report results absolutely in accord with those previously given. They worked in addition, however, with the reactions in the milk serum of infected animals with which Sven Wall had previously worked. Wall found in 25 cows, 23 reacting to blood serum tests. In 11, or about 50 per cent., the milk showed corresponding reactions. Reinhardt and Gauss, examining 27 milk serums, got positive agglutination and complement fixation tests in 21. In 15 cows, when the blood and milk were tested, they got corresponding results in 11.

To determine the time of appearance of antibodies in the blood and milk, Reinhardt and Gauss injected two goats intravenously with a culture of *B. abortus*, the first, half through her pregnancy, and the second, shortly after the birth of two kids. The first goat aborted in seven days. They got positive reactions in the blood and milk of both animals, which lasted longer and with higher dilution in Goat 1 than in Goat 2. No positive reactions were obtained with the blood of the two kids, even though they were with their mother constantly and nursed ten weeks. They conclude that in infected animals antibodies go over into the milk from the blood and last a long time.

Giltner, Cooleage and Huddleston¹⁹ introduced pure cultures of *B. abortus* into the udders of cows and got positive milk serum tests on the following day without any evidence of gross lesions in the udders. They believe that the abortus bacillus has antigenic without pathogenic action. In every case in which *B. abortus* was found by inoculating milk into guinea-pigs, agglutinins were found in the milk, but *B. abortus* was not found in every case that gave positive tests for milk serums. Either the bacilli were present in too small numbers

17. Schulz, W.: Ueber den diagnostischen Wert der Agglutination und der Intrakutanreaktion beim infektiösen Abortus der Kühe, Inaug. Dissert., Dresden, 1912.

18. Reinhardt, R., and Gauss, G.: Untersuchungen über das Vorkommen von Antikörpern gegenüber dem Bacillus abortus infectiosi im Blut und in der Milch abortuskranker Tiere, Ztschr. f. Infektionskr. Haustiere, 1914 and 1915.

19. Giltner, Ward: Contagious Abortion. Rep. Sec. State Board Agric. and Ann. of Exper. Station, Michigan, 1911, p. 155.

to give lesions, or the presence of antibodies in the milk is a passive result from their presence in the blood of the animal.

Investigations bearing on the influence of *B. abortus* on public health have unfortunately been very few.

Mohler and Traum²⁰ examined the serums of forty-two persons without getting any positive complement fixation or agglutination reactions, although Larson had previously found three positive reactions in 100 serums examined. Out of fifty-six instances in which tonsils and adenoids were inoculated into guinea-pigs by Mohler and Traum, one animal showed the presence of *B. abortus bovinus*.

Larson and Sedgwick,¹⁶ in 1913, assert that after systematically examining the serums of women who had aborted, they found that a larger number gave a positive complement fixation reaction when the *B. abortus* was used as antigen than when the ordinary syphilitic antigen was employed. They had examined, up to that time, the blood of 425 children and found seventy-three, or 17 per cent., positive and 352 negative. They also made agglutination tests which ran parallel. They concluded that the positive reactions were the result of an active immunity due to antibodies generated by the patient, and did not necessarily mean active infection of the individual. In 1915 Larson and Sedgwick²¹ further report that it was observed in their original group of 425 children that those receiving milk from an abortus-free herd gave no reaction, while those fed on market milk gave a high percentage. Over 40 per cent. of the children in one institution gave positive reactions, and in one group as high as 48 per cent. reacted. In forty-two new-born children they got no reactions, but one infant in whom artificial feeding was employed on the seventh day, gave a positive reaction on the twenty-first day.

Ramsey²² examined the blood of fifty-eight boys and an equal number of girls; six boys and one girl gave positive reactions.

Nicoll and Pratt²³ call attention to the fact that several investigators have shown that the ingestion of a large number of bacilli may give antibodies in the blood. The authors had the same results feeding guinea-pigs typhoid bacilli. In testing out a large number of infants and children in the New York Foundling Asylum with agglutination reactions, with very few exceptions they got negative results. One patient in the obstetric ward, having had a miscarriage at the seventh month, gave a positive agglutination reaction of 1 to 300, using

20. Mohler and Traum: Infectious Abortion of Cattle, U. S. Dept. of Agric., Bureau of Animal Indust., Circ. 216, 1911, p. 147.

21. Larson, W. P., and Sedgwick, J. P.: Further Studies on the Epidemic Abortion Reaction in Children, AM. JOUR. DIS. CHILD., 1915, **10**, 197.

22. AM. JOUR. DIS. CHILD., 1915, **10**, 201.

23. Nicoll, M., Jr., and Pratt, J. S.: Does the Bacillus Abortus (Bang) Infect Man? AM. JOUR. DIS. CHILD., 1916, **10**, 203.

B. abortus as an antigen. Two months later her serum still gave a positive reaction 1 to 100. Her infant gave the same reaction. A fosterchild that she was nursing gave a negative reaction. The vaginal discharge, the milk of this mother and the feces of the child were injected into guinea-pigs, with negative results.

Coolidge,²⁴ in an effort to prove whether or not the *B. abortus* is pathogenic for man, made a number of interesting experiments. He fed 18 guinea-pigs naturally infected milk from 33 to 287 days daily (the milk being tested every four days for antibodies). Two, or 11 per cent., developed antibodies, but no lesions. There occurred 1 abortion and 2 abnormal parturitions. Two controls in noninfected milk, 2 on no milk, remained negative. Two guinea-pigs fed on negative milk, plus cultures, developed antibodies, but no lesions. There is not sufficient evidence from this experiment to warrant the conclusion that infected milk is dangerous to guinea-pigs by ingestion. It has slight antigenic, but no pathogenic, action. Fourteen normal rabbits were fed naturally infected milk for 124 days. Coolidge used 4 controls. Two were fed noninfected milk, 2 no milk. The control gave the same reactions as those receiving infected milk. Only 1 of the 14 gave an increase in antibody content during the 124 days, which persisted 84 days after stopping the feedings. There were no abortions and no postpartum lesions. Six rabbits were fed noninfected milk, plus a culture of *B. abortus*; antibodies developed to a maximum on the forty-second day, weakened on the fifty-second day and were negative in 4 of the 6 on the eighty-fourth day, although the feeding still went on. There were no abortions. The animals had two litters of young, and no postpartum lesions were evident. Infected milk is probably, therefore, not dangerous to rabbits on ingestion. It is not pathogenic, very slightly antigenic. The investigator also fed calves infected milk and got antibodies in the blood, but was not able to say whether they were due to active infection, or active or passive immunity.

Coolidge²⁵ examined the blood of persons drinking raw milk, pasteurized milk and no milk. Of the 6 drinking raw milk previous to the test, 3 gave positive complement fixation and agglutination tests. Of the 4 persons drinking pasteurized milk, one gave a positive complement fixation test. Those drinking no milk gave negative reactions. He then made a feeding experiment. He gave each of 7 persons 1½ pints daily for 8 weeks of a milk high in *B. abortus* and antibodies. The antibody content of the human serum was determined before

24. Coolidge, L. H.: Is Bacterium Abortus (Bang) Pathogenic for Human Beings? Jour. Med. Research, July, 1916.

25. Coolidge, L. H.: Agglutination Test as a Means of Studying the Presence of the Bacterium Abortus in Milk, Jour. Agric. Research, February, 1916, 5, 871.

giving the milk, during the consumption, and four weeks after this period. Of the 7, 5 showed an increase in the antibody content, but in no case was this marked. He made another feeding experiment. He gave to 2 adult males daily for six weeks a negative milk, to which was added 10 c.c. of a forty-eight-hour culture of *B. abortus*. No increase in the antibody content was noted, nor were any untoward effects in evidence. Cooledge concludes:

If the abortus antibodies in the blood were due to an infection of the individual by the organisms being present in the milk, one would expect a fairly high and lasting antibody content; but if the antibodies were due to a passive immunity, one would expect a weak reaction, persisting but a short time after stopping the ingestion of the milk. It is probable, therefore, that the antibodies represent a passive immunity due to absorption from the milk. There is very little evidence that *B. abortus* is either pathogenic or antigenic for human adults at least.

Referring to the reaction present in the blood of young children, Cooledge explains this as perhaps due to the absorption of the antibodies from the milk through the intestinal mucosa as exemplified by the work of Ehrlich and Wassermann, who found young mice capable of assimilating antitoxin through the intestinal tract and developing passive immunity to a certain degree. He does not, therefore, feel that there is sufficient proof to warrant the statement that the *B. abortus* is pathogenic to man.

EXPERIMENTAL DATA

The milk studied in our experiments was obtained from five dairies, called, for convenience, Dairy A, B, C, D and E, over a period of several months, and the number of samples examined with the dates and findings is shown in Table 1.

In order to gather some data as to the prevalence of abortus bacilli in the milk of individual cows, and at the same time to note the possible occurrence of tubercle bacilli in the milk of animals that have reacted to the tuberculin test, which six months previously had failed to react, the milk of nine recent reactors was examined, using the same technic that had been used in examining the mixed milk of the herds. The results are shown in Table 2.

Realizing that we had to deal with a condition which could only be differentiated from tuberculosis in guinea-pigs by most careful examination, a scheme was devised for studying the animals, into which the milk samples were injected, that can be best described by giving a careful history of one of the infected guinea-pigs.

TABLE 1.—DATA CONCERNING MILK SAMPLES STUDIED

Dairy	Test	Date	Guinea-Pig	Result	Percentage Positive
A	1	8/21/16	13	Negative	0.
			14	Negative	
			15	Negative	
			16	Negative	
A	2	8/25/16	31	Negative	25
			32	Negative	
			33	Negative	
			34	Typ. Ab. Dis.	
A	3	9/ 2/16	55	Negative	50
			56	Negative	
			57	Typ. Ab. Dis.	
			58	Typ. Ab. Dis.	
A	4	9/23/16	59	Negative	75
			60	Typ. Ab. Dis.	
			61	Typ. Ab. Dis.	
			62	Typ. Ab. Dis.	
A	5	9/26/16	63	Typ. Ab. Dis.	75
			64	Typ. Ab. Dis.	
			65	Typ. Ab. Dis.	
			66	Negative	
A	6	10/ 2/16	71	Typ. Ab. Dis.	75
			72	Typ. Ab. Dis.	
			73	Doubtful	
			74	Typ. Ab. Dis.	
B	7	8/18/16	9	Negative	25
			10	Acute Inf.	
			11	Negative	
			12	Typ. Ab. Dis.	
B	2	8/22/16	19	Typ. Ab. Dis.	75
			20	Doubtful	
			21	Typ. Ab. Dis.	
			22	Typ. Ab. Dis.	
B	3	8/29/16	39	Typ. Ab. Dis.	100
			40	Typ. Ab. Dis.	
			41	Typ. Ab. Dis.	
			42	Typ. Ab. Dis.	
B	4	1/ 5/17	124	Typ. Ab. Dis.	100
			125	Typ. Ab. Dis.	
			126	Typ. Ab. Dis.	
			127	Typ. Ab. Dis.	
C	1	8/15/16	5	Typ. Ab. Dis.	100
			6	Typ. Ab. Dis.	
			7	Typ. Ab. Dis.	
			8	Typ. Ab. Dis.	
C	2	8/31/16	43	Negative	75
			44	Typ. Ab. Dis.	
			45	Typ. Ab. Dis.	
			46	Typ. Ab. Dis.	
C	3	9/13/16	51	Typ. Ab. Dis.	100
			52	Typ. Ab. Dis.	
			53	Typ. Ab. Dis.	
			54	Typ. Ab. Dis.	
C	4	9/28/16	67	Doubtful	75
			68	Typ. Ab. Dis.	
			69	Typ. Ab. Dis.	
			70	Typ. Ab. Dis.	
D	1	8/11/16	1	Typ. Ab. Dis.	100
			2	Typ. Ab. Dis.	
			3	Typ. Ab. Dis.	
			4	Typ. Ab. Dis.	

TABLE 1.—DATA CONCERNING MILK SAMPLES STUDIED—(Continued)

Dairy	Test	Date	Guinea-Pig	Result	Percent. Positive
D	2	8/23/16	23	Typ. Ab. Dis.	100
			24	Typ. Ab. Dis.	
			25	Typ. Ab. Dis.	
			26	Typ. Ab. Dis.	
D	3	8/24/16	27	Negative	75
			28	Typ. Ab. Dis.	
			29	Typ. Ab. Dis.	
			30	Typ. Ab. Dis.	
D	4	8/28/16	35	Typ. Ab. Dis.	100
			36	Typ. Ab. Dis.	
			37	Typ. Ab. Dis.	
			38	Typ. Ab. Dis.	
D	5	1/ 5/17	128	Typ. Ab. Dis.	100
			129	Typ. Ab. Dis.	
			131	Typ. Ab. Dis.	
E	1	1/ 6/17	132	Typ. Ab. Dis.	75
			133	Typ. Ab. Dis.	
			134	Negative	
			135	Typ. Ab. Dis.	
E	2	1/18/17	139	Typ. Ab. Dis.	100
			140	Typ. Ab. Dis.	
			141	Typ. Ab. Dis.	
			142	Typ. Ab. Dis.	
E	3	1/22/17	143	Negative	25
			144	Typ. Ab. Dis.	
			145	Negative	
			146	Negative	

TABLE 2.—RESULTS OF EXAMINATION OF MILK OF COWS REACTING TO B. ABORTUS AND TO TUBERCULIN—INDIVIDUAL COWS—TUBERCULIN REACTORS

Milk Sample	Guinea-Pig	Necropsy Findings
Dairy C, Cow 1	92	Negative
	93	Negative
	94	Negative
	95	Negative
Dairy C, Cow 2	96	Negative
	97	Negative
	98	Negative
	99	Negative
Dairy C, Cow 3	100	Negative
	101	Negative
	102	Negative
	103	Negative
Dairy C, Cow 4	104	Negative
	105	Negative
	106	Negative
	107	Negative
Dairy C, Cow 5	108	Negative
	109	Negative
	110	Negative
	111	Negative
Dairy C, Cow 6	112	Typ. Ab. Dis.
	113	Negative
	114	Typ. Ab. Dis.
	115	Typ. Ab. Dis.
Dairy C, Cow 7	116	Negative
	117	Negative
	118	Negative
	119	Negative
Dairy C, Cow 8	120	Negative
	121	Negative
	122	Typ. Ab. Dis.
	123	Negative
Dairy F, Cow 9	199	Negative
	200	Negative
	201	Negative
		Negative

SAMPLE EXPERIMENT

Guinea-Pig 71. Male. Oct. 3, 1915. Weight, 272 gm. Injected intramuscularly into the inner and upper surface of right hind thigh, 5 c.c. of sediment obtained from 800 c.c. of whole milk from Dairy A, suspended in a little saline. Jan. 27, 1917, 116 days after injection, intradermal tests were made, using as antigens abortin, tuberculin, bovine, tuberculin, avian, colin, and typhoidin. Jan. 29, 1917, forty-eight hour readings were as follows:

Colin, N. P.

Abortin, 0; 3.9 by 3.9; marked edema; center yellow; necrosis.

Tuberculin, avian, N. P.

Typhoidin, N. P.

Tuberculin, bovine, 0; 0.6 by 0.6.

Feb. 2, 1917, 122 days after the injection of the milk, the animal was first injected with trypan blue for vital staining, and the dye injections were repeated daily until Feb. 24, 1917. Feb. 16, 1917, 136 days after the milk sediment was injected, the guinea-pig weighed 490 gm., a gain of 218 gm. March 5, the animal was chloroformed, 153 days after the experiment was begun. The following findings were noted:

There is a general adenopathy. The spleen, 5 by 4 cm., is enlarged and hard, contains numerous small nodules. The liver contains a few nodules. The kidneys and lungs are negative. There is an abscess in the right testicle with a firm wall. The left testicle is small and atrophic.

Bone lesions: The left ninth rib is the seat of a tumor mass, the size of a large pea, containing deep blue cheesy matter surrounded by a dense layer of tissue. The right ninth and tenth ribs are the seats of small tumors, the size of a grain. The left seventh rib at the point of insertion to the vertebra has a tumor the size of a small pea, containing cheesy matter. Spleen culture on potato glycerin serum agar, positive in eight days.

Agglutination Tests: Blood taken from the heart of the guinea-pig before chloroforming, March 15, 153 days after the milk injection, gave a positive reaction in a dilution of 1 to 1,000 to 1 to 4,000.

Recapitulating, the procedure was briefly as follows: Several weeks after the milk had been injected, intradermal tests were made, using various antigens; at this time blood was taken from the heart for agglutination determinations. Some animals were then repeatedly injected with a vital stain, following which they were chloroformed. The various organs were studied macroscopically as far as the vital staining permitted, and microscopically, and in most cases an effort was made to isolate the invading organism. The results have been carefully tabulated in a manner shown in Table 3 and the more minute details, referring particularly to cutaneous hypersensitiveness, agglutination reactions, gross and microscopic lesions and cultural characteristics of the invading organisms, will be reported in later communications.

Studying the results in detail leads to some interesting observations. Of the twenty-two samples of milk obtained from the mixed herds of five different dairies, twenty-one, or over 95 per cent., showed definite *B. abortus* infection. None showed the presence of tubercle bacilli in sufficient number to cause tuberculosis in guinea-pigs. The

milk of Dairy A, from which the one sample was obtained that produced no lesions, infected 50 per cent. of the animals that were injected, the experiments extending over three months. *The milk of Dairy B* infected about 80 per cent. of the animals injected, the experiments extending over a period of six months. *The milk of Dairy C* infected about 88 per cent. of the animals injected, the tests extending over a period of two months. *The milk of Dairy D* infected 95 per cent. of the animals injected, the experiments extending over a period of six months. *The milk of Dairy E* infected 66 per cent. of the animals injected, the tests having been made with only a short interval between them.

It is interesting to note that Dairy A, from which the one negative sample was obtained, was evidently a less seriously infected herd, or harbored a less virulent type of organism, than the other dairies, because in no single experiment were 100 per cent. of the guinea-pigs infected. The importance, in this connection, of using large quantities of milk and a sufficient number of animals cannot be overestimated. The quantity used, 800 c.c., is much larger than is usually recommended in the literature, and this unquestionably accounts for the large percentage of positive results. Furthermore, if fewer animals had been used in the individual tests, the results would not have been nearly as striking. In several instances the first two or three guinea-pigs were apparently uninfected, while the last animals examined were overwhelmed with the disease, showing in guinea-pigs, as in cattle, the marked individual susceptibility to the abortus bacillus.

A study of the results obtained by injecting the milk of individual cows reacting to the tuberculin test, which had not reacted to it six months before, shows that none of these animals was excreting tubercle bacilli in sufficient quantity in their milk to infect guinea-pigs with tuberculosis. About 22 per cent. of them were harboring pathogenic *B. abortus*, which corresponds fairly well with previous observations where the milk of individual cows was tested in this connection. The number tested, however, was too small to draw positive conclusions, but the result is at least suggestive.

At this point it may be well to discuss a little more in detail the methods that were used to establish a correct diagnosis in each animal, and briefly to summarize the results. The agglutination test was made according to the usual technic. Reactions under 1 to 100 were not considered positive. In the serum of the infected animals the reaction was positive in dilutions of from 1 to 600 to 1 to 1,000, and sometimes in dilutions greater than 1 to 2,000. The complement fixation test was not made because most investigators are of the opinion that the agglutination test is quite as reliable and its technic is much more simple.

The intradermal tests were always positive in infected animals, the

characteristics of the reaction being marked induration, frequently with central necrosis, an areola at least 1 cm. in diameter, with persistency of the reaction over the forty-eighth hour. The organisms were isolated in over 20 per cent. of the infected animals. In the remaining animals, either no attempt was made to isolate the organisms, or, on account of the special vital staining dye that had been injected, the bacilli did not grow on the mediums incidental to conditions the exact nature of which is being studied. By reinoculation of the spleen or isolated organisms from infected animals, the identical disease was produced in guinea-pigs. The isolated organisms were further identified by an abortus serum produced by a known *B. abortus* strain. It is of profoundest interest to note that where animals had been mated for months, infection in either the male or female practically always resulted in a failure of impregnation.

The pathologic lesions, both macroscopic, which have been referred to earlier in this paper, and microscopic will be further studied and reported in a later communication. The disease attacks principally the lymph nodes, spleen and sex organs, with a special selective action toward the last, but it may also manifest itself in the liver, bones, lungs, eyes and kidneys in some cases.

CONCLUSIONS

As far as can be concluded in a preliminary report from a limited amount of material, it may be said:

1. *B. abortus* is, for practical purposes, always present in the certified milk produced in the San Francisco Bay regions.

2. Tubercle bacilli are not present in this same milk in sufficient number to give tuberculosis to guinea-pigs, although this conclusion may prove incorrect on further experimentation.

3. If the above conclusion is correct, there is no necessity for pasteurizing certified milk on account of any danger that it may possess as a disseminator of bovine tuberculosis to infants.

4. It is not unlikely that in many previous milk tests for tubercle bacilli, the anatomic lesions of bovine abortion disease in the guinea-pig were mistaken for tuberculosis.

5. If the *B. abortus* is present in certified milk to the extent evident from these experiments, it is difficult to consider it pathogenic for infants, without, so far as is known, ever having produced recognizable lesions on post mortem examination.

6. The result of this work, however, is one more definite indication that it is of greatest importance to study the abortus problem from every angle to be absolutely certain of its bearing on the health of infants.

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TABLE 3.—RESULTS OF INTRADERMAL INJECTIONS OF MILK SEDIMENT IN GUINEA-PIGS

Guinea-Pig	Sex	Weight, Gm.	How and When Injected	Time Interval, Days	Cutaneous Hypersensitiveness				Agglutination	Necropsy Findings	Cultural Results	End Weight, Gm.	Remarks
					B. Abortus Broth, 1-2	B. Abortus Protein 1-1,000	B. Typhosus Protein 1-1,000	Tuberculin bovine, glycerin 1-2 human; Alc. Precip. 1-3					
126	M	294	Milk from Dairy E, 1/5/17	54	2.8 by 2.8 M. I. R.	1.4 by 1.4	N. P.		1-200 1-1,000	General lymphadenitis; spleen 3.5 by 2; scarred, nodular; testes atrophic; other organs negative.	Spleen cultures positive in 5 days	480	4/7/17, trypan blue injections begun 4/24/17, chloroformed 3/5/17, injection of dia mil blue 3/26/17 chloroformed
128	M	382	Milk from Dairy C, 1/5/17	54	3.1 by 3.4	1.6 by 1.6	N. P.		1-2,000	General lymphadenitis; spleen 3.5 by 2; nodular, enclosed in inflammatory tissue from mesentery; testes atrophic; other organs negative.	Spleen cultures positive	470	

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