The Porto Rico Journal of Public Health
and
Tropical Medicine

Official Bulletin of the Department of Health of Porto Rico and the School of Tropical Medicine of the University of Porto Rico Under the Auspices of Columbia University
Published quarterly by the Department of Health of Porto Rico.

Entered as second-class matter August 1, 1925, at the Post Office at San Juan, Porto Rico under the Act of August 12, 1912

VOL. VI SEPTEMBER, 1930 No. 1

BRUCELLA ABORTUS IN PORTO RICO

PABLO MORALES-OTERO

From the School of Tropical Medicine of the University of Porto Rico under the auspices of Columbia University.

INTRODUCTION

Investigations of the last ten years have revealed the similarity existing between the organisms causing swine and cattle abortion and that causing malta fever. Furthermore, organisms similar to those of cattle and swine abortion have been repeatedly isolated from the blood of human cases, showing signs and symptoms similar to those of malta fever. These two facts bring forth a very important public health problem, which demands prompt attention; for it is most probable that a considerable number of cases of ill health, which have been heretofore not properly diagnosed and treated, are due to the abortion bacillus.

The importance of eradicating abortion disease among cattle and hogs, must be generally recognized, not only as a problem of the industry but also as a public health problem. Milk, of course, since it is known to often contain the abortion bacillus has been suspected as the source of infection of some human cases. Others have been traced to contact with hogs or hog carcasses and some of them in the extreme south have been traced to goats infected with *B. melitensis*.

Inasmuch as the disease is communicable from various animals to man, either directly or indirectly, it presents the aspects of a public health problem, but it is not only its prevention from spreading which necessitates attention but also a great deal of work should be done to accurately determine the possible sources of contagion and define better methods of diagnosis and treatment. The wide discrepancy between the relatively high cattle infection and low incidence in man indicates that there are several factors as yet undetermined

which are responsible for this condition and which necessitate investigation.

It is not intended to present here a complete description of the disease. A brief general review of the literature with some clinical aspects, symptomatology and bacteriology of the disease will be included, but no attempt has been made to present a complete review of the entire subject. Much work, which is undoubtedly important has not been discussed and the writer has aimed chiefly to present the results of his own work with special attention to conditions existing in Porto Rico.

We now possess considerable knowledge concerning the mode of spread of the disease and how to prevent it and methods for better diagnosis and treatment are being tested. We firmly believe that there exists at present the justification to apply these facts on a larger scale through the clinical and public health laboratories that assist in diagnosis, through the public health worker who attempts preventive measures and through the clinician who carries out diagnosis and treatment.

HISTORICAL

Passages are cited from Hippocrates recounting cases of long drawn out fevers with short apyrexial intervals lasting as long as one hundred days, which may have perhaps referred to undulant fever. Certainly in the eighteenth and early part of the nineteenth centuries references were made to protracted fevers occurring in Malta.

According to Castellani [1] during the Crimean War there appears to have been a very large temporary increase of the fever incidence on the island of Malta, much of which was undoubtedly enteric, but some of it was not. It was Marston (1859) who gave an accurate account of its clinical history and post mortem appearance under the name "Mediterranean remittent" or "gastric remittent fever." Veale (1879) gave an account of the disease as seen in the invalids at Netly and so did Fazio at Naples who not only described the disease but suggested that it may be found to be of bacterial origin. Bruce in 1886 discovered the causal organism and in 1887 cultivated it on agar-agar and reproduced the disease in experimental animals. Wright and Semple (1897) showed that the serum of patients suffering from the disease had the property of agglutinating the microorganism discovered by Bruce. The British Commission [2] for the investigation of the Mediterranean Fever (1904) demonstrated that the micrococcus leaves the body mainly through the urine; that goat milk agglutinates the organism, and the germ was isolated from

such goats, although the organisms did not seem to affect injuriously such animals, even when they lived in the blood stream and were secreted in the milk by means of which the disease is conveyed to man.

The suggestion was made (Eyre) that undulant fever is primarily a disease of the goat and prophylaxis on this basis resulted in a remarkable reduction of the disease.

The abortion disease of cattle has been known as long ago as the beginning of the last century. Since then many authors have mentioned that an infection was the causative agent. The correctess of this view however was established by Frank [3], and later by Lehnert (1876) and Brauer (1880) by producing abortion experimentally through the introduction of infective material in the vagina of healthy cows.

In 1896 Bang discovered the causal organisms of contagious abortion. He named the organism *Bacillus abortus,* but had no evidence that the organism could infect human beings and no reason to suspect any relation between the organism he had discovered and that previously described by Bruce. Moreover, Bruce described his organism as a micrococcus while the organism discovered by Bang has the form of a small rod. It was not until 1917 that a comparison of the two organisms was made. Evans [4], while working on the bacterial flora of milk asceptically drawn from the udder, found rods resembling B. abortus. This finding led her to consult Dr. Eichhorn of the Bureau of Animal Industry and during the conversation the question of comparing the abortus organism with that of malta fever was discussed. Following this suggestion Miss Evans compared both organisms and found them indistinguishable, morphologically and culturally. The serum from animals inoculated with either organism, agglutinated with the other as with the homologous type and they could only be differentiated by agglutinin absorption. This work of Evans' led investigators to study the possibility of human infection with the abortus organism.

Keefer [5] in 1924 reported the first case of human infection in which Brucella abortus was isolated. Since then many investigators have reported infections in human beings in which Brucella abortus, either bovine or porcine, was held responsible for the infection.

It is only in the last few years that it has been recognized that undulant fever is fairly common in North America and in Europe and that in many instances it has no possible caprine source. Recently Moore and Carpenter [6], Huddleson [7], Beylea [8], Dickson [9], Hull and Black [10], Gilber and Coleman [11], Hardy [12], Scott and Saphire [13],

Sesinich and Giordano [14], McAlpine and Mickle [15], Biering [16] and others have reported a large number of cases occurring in North America. The cultural and serological tests made on the strains from these cases of human infections leave no question that the etiological factor in the undulant fever observed in North America is a Brucella type.

DISTRIBUTION

Formerly the disease was described as existing in endemic areas such as the coast and islands of the Mediterranean, Italy, France, Spain, North Africa and India, where it was supposed to exist endemically among goats. More recently it has been reported from many other parts of the world such as Russia, East and South Africa, Ungada, Sudan, Mauetania, China, Fiji Islands, the Philippines, North and South America and the West Indies. The disease has always been described as tropical but it also occurs in sub-tropical and temperate climates.

The infectious abortion of cattle is now well known to be widely spread in all sections in which stock farming is in vogue. (Belgium, Denmark, Germany, France, Austria, Switzerland, Hungary, England and North America.)

CLINICAL ASPECTS OF THE DISEASE

In animals: Infected goats usually manifest only very slight symptoms. According to the observations of Dubois [17] attention is directed to its presence by abortions occurring in freshly infected herds, these occur in rapid succession and affect sometimes fifty to ninety per cent of the animals. After abortion, ordinarily there are no further consequences. In some goats however disturbances of the milk secretion may occur at times, simultaneous with transitory knotty thickenings of the mammary gland tissue and a flocculent consistency of the milk. At times symptoms of lameness due to the neuritis or swelling of the joints may occur. There may be a subacute or chronic bronchitis, and finally an intensive keratitis.

In bucks, orchitis may develop as a complication. In all these cases the life of the animal is seldom threatened.

The manifestations in sheep may be similar to those occurring in goats. In cattle the main symptom is also abortion. This is usually preceded by swelling of the genitals and catarrh of the genital passages. There appears from the vagina a mucous or mucopurulent discharge which may be red or reddish-gray in color. The milk production is suddenly diminished and takes the appearance of co-

lostrum. Two or three days after the appearance of the catarrhal symptoms abortion occurs with moderate pains and mild general manifestations. A frequent occurrence in these cases is the retention of the placenta. The secretion becomes dirty brown or reddish brown, may be odorless or somewhat fetid, at times accumulates in the uterus and from time to time is discharged during straining. Gradually the discharge disappears and the animal looks apparently normal. Some of these animals do not conceive and others that do conceive abort at an earlier or later period of gestation.

Pekar [18] has also observed an arthritis which manifests itself by twitching of the extremities and frequent lying down of the animal.

SYMPTOMATOLOGY IN MAN

The onset of the disease in man is gradual and the patient continues to work though feeling ill. The temperature increases in the afternoon, being higher in the evening and lower in the morning. The third or fourth day the patient complains of severe headache, pains all over the body, limbs and rheumatic pains of severe type. These symptoms may continue for a couple of weeks at the end of which the fever may decline and the temperature may become normal for a day or two when the relapse occurs with much the same symptoms as before. This relapse subsides and another follows, relapses and intermissions recurring for months.

On physical examination one may find a coated tongue, sore throat and tender epigastrium. Later in the disease one may find bronchial catarrh, congestion of the lungs, enlargement and tenderness of the spleen. The patient gradually becomes weak and anemic. Constipation may alternate with diarrhea. The spleen may still be large and painful, the lungs may show bronchitis or even pneumonia, hemic murmurs may appear. Insomnia and hysterical emotions are not uncommon. Joints become swollen and very painful. Sudamina is a very common symptom.

The patient is anemic and prostrated. Gradually symptoms abate, the intermissions lengthen and relapses lessen in severity until the temperature remains normal and convalescence begins after a prolonged illness of months.

Two types have been described by Hughes [19], one malignant and the other intermittent.

In the malignant type the onset is sudden and the disease presents itself in an aggravated form. The symptoms may abate but the patient instead of improving grows worse. The pulse becomes

intermittent, breathing becomes laborious, vomiting becomes serious, hyperpyrexia sets in and patient dies in from five to twenty-one days.

In the intermittent type the fever continues for months with its wave-like curve, but the patient generally gets well after prolonged convalescence. A third or *ambulatory* type has been described by some authors. In this case the infected person may be unaware he is suffering from the disease and may pursue his daily work.

The clinical course of the cases of undulant fever observed in North America as described by Keefer [5], Evans [20], Huddleson [21], Gage and Gregory [22], Carpenter and Meriam [23], Scott and Saphire [13], Beylea [8], Dickson [9], Kern [24], Biering [16], Simpson and Fazier [25], and others, bears striking resemblance to the Mediterranean fever described by Bruce [26], Hughes [18], Basset-Smith [27], and Burnett [28].

Biering [16], in a study of one hundred and fifty cases, finds that one hundred and eight included farmers, farmers' wives, members of farmers' families, dairymen, stock buyers and packing-house employees. He gives a full description of the clinical characteristics of the disease as it occurs in Iowa as follows: "If special distinction is to be given to certain features of the clinical picture, emphasis should be placed on the character of the onset, the rigors, the chills with profuse sweating, the muscular and joint pains, the loss of weight and the undulant, continued and persistent character of the fever curve. The usual onset is gradual and insidious in the development of noticeable weakness with accompanying tired feeling. The patient often seems quite fresh in the morning but by the latter part of the afternoon is so fatigued as to be hardly able to get about. A headache and backache of greater or lesser severity are often features of the onset. Likewise loss of appetite, digestive distress and constipation are frequent early symptoms. After a few days or possibly several weeks, the patient becomes conscious of a hot feeling mostly in the afternoon and is usually surprised to learn that the temperature is above normal. A feeling of feverishness and light rigors and chills are often the first indication of fever. Again the onset may be ushered in abruptly, by a severe chill and rapid rise in temperature which is followed by profuse sweating and this with the general muscular pains gives the impression of a profound infection."

COMPLICATIONS

Orchitis, ulcers of the intestines, persistent vomiting and diarrhea, hyperpyrexia, bronchitis, pleural effusions, pneumonia and cardiac failure have frequently been described as complications of the disease.

Dessage, Pellerin and Vineita [29] report a case of meningitis due to *B. melitensis,* following the relapse of Malta Fever.

Baker [30] has reported a case recently presenting the clinical syndrome of intermittent hydrathrosis.

BACTERIOLOGY

Brucella melitensis is an extremely small bacterium. It is a gram negative, non-motile organism and does not form spores. In cultures it may appear singly or in short chains of two or more. The organism may be cultivated from the blood stream, urine or feces of active cases. It may also be obtained from urine or milk of infected goats. It grows best at an optimum temperature of 37°C. It may grow well aerobically and under conditions of limited anaërobiasis. Its growth is slow (three or four days) and it has been cultivated on most ordinary media. It does not liquify gelatin nor does it ferment the sugars.

Brucella melitensis abortus variety has been described by Evans as a short, slender, pleomorphic rod; sometimes so short as to appear coccoid. It is non-motile and does not form spores. It is Gram-negative. It is difficult to grow on artificial media when first isolated. In early cultures its growth is favored by partial anaërobiosis which Evans obtained by growing in a closed jar in the presence of *B. subtilis* cultures. Glycerin or serum agar are favorable media for isolation but after prolonged cultivation it grows well in ordinary media. Colonies on agar develop in forty-eight hours as very small dew drop forms. Milk is rendered slightly alkaline. It forms no acid or gas in any of the sugars. It does not liquify gelatin.

The organism may be isolated from the genital discharges of the aborted animal or from the membranes of the placenta or aborted fetus. At times it can be isolated from the milk of affected animals. In the experimental animal it is usually isolated from the spleen. In humans it has been isolated from the blood, urine and feces.

PATHOLOGY

In animals: In goats the autopsy discloses frequently a hyperemia and an acute swelling of the spleen and lymph glands. The mesenteric and inguinal glands are most frequently affected. At times there may be found a nephritis and lobular pneumonia. The udder discloses fibrinous inflammation and purulent foci. (Neri).[31]

In cattle the placenta usually shows a marked edema of a yellow

gelatinous infiltration at times localized or extending over its entire area. Its surface may be covered with muco-purulent flakes and its vessels are dilated and may be surrounded by small hemorrhages. The cotyledons are either brownish red or dirty yellow in color and may be covered with greenish yellow fibrinous or pus-like flakes. In the fetus a marked bloody serous infiltration of the subcutaneous and intramuscular connective tissue constitutes a frequent finding. Mucous and mucopurulent masses occur frequently in the stomach, especially in the abomasm, while the small intestines may disclose a hemorrhagic inflammation. The autopsy may reveal punctiform or streaked hemorrhages in the serous membranes and also in the mucous membranes of the gastro-intestinal canal as well as the urinary bladder.

The abortion strains have been studied in their relationship to their pathogenicity for guinea-pigs. Infection in guinea-pigs may occur spontaneously or through contact infection. Large doses will kill acutely. With smaller doses a subacute or chronic infection develops with enlargement of the lymph glands and spleen. The surface of the spleen may be normal or show numerous points of elevation. On section the pulp is usually soft and swollen but distinct foci are seldom seen. The liver, lungs and kidneys may show grayish foci. Microscopically these foci show epithelioid proliferations. The tissue changes closely resemble those of tuberculosis.

The testicles, bones, and joints, and less frequently the uterus may be involved.

In humans the spleen is markedly enlarged and dark red in color. It may be soft and pliable and may show marked congestion with enlarged malphigian follicles. The liver is enlarged and congested and may show cloudy swelling with round cell infiltration between the lobules.

The kidneys show congestion and may show a glomerulo-nephritis. The alimentary canal is merely congested but occasionally may show ulcers of the large intestine.

The lungs may show congestion and at times patches of consolidation.

DIAGNOSIS

The clinical diagnosis is usually based on the prominent symptoms of the disease together with laboratory findings. Prolonged undulating fevers with profuse sweating and joint symptoms are very suggestive.

Examination of the blood is very important. Blood culture can

be made in flasks of liver infusion broth. Some flasks should be incubated in a partial anaërobic chamber containing five per cent or ten per cent CO_2 and others aerobically at 37° for at least one week.

Some authors have reported positive cultures after a prolonged incubation of several weeks.

In some laboratories part of the blood for culture is inoculated intraperitoneally into a guinea-pig. The animal is autopsied after four weeks and cultures are made from the spleen and lymph glands in liver infusion agar.

The simplest method for the confirmation of the diagnosis of undulant fever and the most frequently used so far, is the agglutination test. The degree of agglutination required had been widely discussed. Some authors believe that titers of 1:20 are suggestive, while others more conservative recommend a titer of 1:160 or more. The concensus of opinion seems to be that in the presence of suggestive clinical symptoms a titer of 1:100 should be considered as positive.

Simpson [31] reports as a possible source of error in the agglutination reaction, occasional cross-agglutination of *B. abortus* and *B. tularense* in patients suffering from either undulant fever or tularemia. Francis and Evans [32] suggest that serums from suspected cases of either disease should be tested for agglutination with both *B. tularense* and *B. abortus* unless the clinical history points definitely to a recognized source of infection.

Another blood examination that may be of value is the leukocytic count which is usually normal or below normal. The differential counts usually show a decrease in polymorphonuclear neutrophiles and a corresponding increase in mononuclear cells.

The organism has been isolated from catherized urine of patients suffering from the disease. Recently Amoss and Poston [33] have described a method for the isolation of *B. abortus* from the feces. In view of the fact that some cases of undulant fever do not develop agglutinins early in the disease, various authors have made attempts to devise a test to supplement agglutination.

Burnet [34] found that the intradermal test with *B. abortus* broth filtrates was positive in fifteen to twenty per cent of human cases of undulant fever in which the agglutination reactions were negative. Shoenholz and Meyer [35] have prepared purified material for intradermal use.

In cows, hogs and goats the diagnosis is usually made by the agglutination test or by the isolation of the organism from the secretion of the genital passages. At autopsy the diagnosis is made by

the anatomical lesions already described and by culturing the organism from the viscera.

CONDITIONS IN PORTO RICO

Bovine contagious abortion did not exist in the island of Porto Rico prior to the importation of animals from the mainland to improve our stock. The importation of cattle seems to be responsible for the occurrence of the disease.

During the year 1923, while working in the Biological Laboratory of the Insular Department of Health, we were requested to assist Dr. Alfonso Rivera, who was then serving as veterinarian for the Insular Agricultural Experimental Station at Río Piedras, in finding the cause of repeated abortion in a pure bred herd near the town of Río Piedras.

Endemic abortion was unknown as a disease in the island at that time but the frequent premature expulsion of the fetus in several herds of imported cows led Dr. Rivera to suspect the existence of the disease. The systematic bacteriological examination of exudates from the placenta and fetus led to the isolation of small bacillus similar to *Brucella melitensis* (abortus) and experimental inoculation confirmed our suspicions.

The disease continued to spread from herd to herd and soon native cows of adjacent herds showed symptoms. A formidable siege of abortion developed which caused veterinarians and dairymen to consider conditions very serious. The Department of Agriculture advised immediate segregation and slaughter of affected animals but since the cases grew greater in number every day these drastic measures could not be taken without enormous losses. The segregation and vaccination of infected herds was advised. An educational campaign was undertaken by veterinarians of the Department of Agriculture. In spite of all these plausible efforts the disease continued to spread over the northern part of the island. Certain local conditions favored the spread of the infection. Herdmen, when informed by veterinarians of the serious condition of their herds, tried to hide conditions as much as possible and even denied infection in the herd. In the meantime all infected animals were segregated and sold at low prices as compared with normal prices in the market. This infected stock went to other dairies in the island where sooner or later infection appeared.

The continuous importation of cattle from the mainland has also been a factor that has contributed notably to the spread of the disease. There are no rules or regulations to prevent these from

entering from abroad and the infected stock brought from Texas or New York is scattered throughout the island, disseminating infection constantly.

At present the disease is widely scattered through the dairying regions of the north and, although conditions may not appear as serious as they were six years ago because the infection has lost some of its explosive character, it is by no means controlled and is appearing day after day in regions, formerly unaffected. When the disease was first recognized it had an explosive character and a large number of cattle aborted at one time. At present not so many abort together, but smaller numbers abort in the herd throughout the year. In regions where extensive vaccination has been carried on, abortions have been reduced to a minimum.

There are several factors which to our mind have resulted in making the disease less prevalent and less explosive in character. These are (1) the educational campaigns carried out; (2) acquired immunity of infected animals; (3) vaccination and (4) the action of sunlight upon the organisms contained in the secretions of affected animals.

The benefit of a well conducted educational campaign on any transmissible or contagious disease need not be discussed here. This is widely recognized and accepted by everybody. The acquired immunity of infected animals is also well known. It is frequently seen that a cow may have its first abortion at the sixth or seventh month of pregnancy. The next abortion may be a full-term calf and the next calving may be a perfectly normal one. It may be possible too that calves that are already infected having ingested infected milk since they were born, may have acquired a certain degree of immunity. Heiffers that were fed on infected milk and were segregated later, reacted to the agglutination test in dilutions of 1:100.

Another factor mentioned is vaccination. This has been carried on extensively in infected regions and seems to be giving very satisfactory results in stopping the symptom of abortion in heavily infected communities.

Sunlight is very intense in tropical countries like Porto Rico and has a definite and powerful germicidal action. We believe that infected secretions, when dropped in pastures or soil where they can be acted upon by sunlight are readily destroyed. Bacteria will only survive where there is dampness or protection from sunlight.

Vaccination and the action of sunlight upon infected secretions we consider of importance and these shall be the subject of further experimental work which will be published later.

SEROLOGIC SURVEY OF CATTLE SERUM *

Since endemic abortion in cattle was recognized in Porto Rico not long ago it will be of interest to know how far the disease has spread. With the cooperation of the Insular Department of Agriculture we planned a survey to find how far infection has extended in the dairies that supply milk to the city of San Juan.

San Juan is a city of over 114,000 inhabitants (Census of 1930) and consumes an average of 30,000 quarts of milk daily. This of course does not include condensed, dry powder or evaporated milk. Of the 30,000 quarts approximately 10,000 are pasteurized by two local plants. The rest of the milk is consumed either raw or boiled. We have no data available as to the percentage of milk that is boiled before consumption but since it is quite a common custom to boil milk we assume that most of it is consumed either boiled or pasteurized. Nearly all of this milk is supplied by eighty herds which are distributed among the neighboring municipalities.

METHODS

It has been imposible for us to examine the blood serum of every cow in these herds, so we decided to examine a group equal to ten per cent of the total number of animals. A group of animals equal to ten per cent of the herd was taken at random and bled. The serums were inactivated at 56° for one hour. The macroscopic agglutination test was used. The antigen was made of four strains of *B. abortus* whose agglutinability had been carefully checked. Dilutions carried were 1:40, 1:80, 1:160. The tests were incubated in a water bath at 37°C for twenty-four hours and allowed to stand in the refrigerator until the next morning when they were carefully read. All agglutinations above 1:80 were considered as positive.

The Municipality of Bayamón was the first examined. Ten herds with a total of 520 cows were visited. Fifty-two cows were examined of which twenty-three were negative and twenty-nine reacted positive giving a total percentage of infection of fifty-five per cent. Only in one herd were the samples taken all negative.

In Guaynabo eight herds were visited having a total of 550 cows. Of the fifty-five samples examined forty-four were positive giving a total percentage of eight per cent. All of these herds had reactors.

In Toa Alta five dairies were visited having 260 cows of which twenty-six blood samples were examined. Thirteen reacted positive

* This part of the work has been done in collaboration with Dr. Juan Varas of the Insular Experiment Station at Río Piedras, P. R.

and thirteen negative giving a total of fifty per cent positives. All herds had reactors.

Five herds were visited in Toa Baja with a total of 290 cows and of the twenty-nine samples examined twelve were positive giving a total percentage of positive of forty-one per cent. All herds examined had reactors.

In the municipality of Dorado eight herds were visited with 560 cows. Fifty-six samples were examined and thirty-eight were positive giving a total percentage of sixty-eight percent. All herds examined had reactors. Twenty dairies were visited at Río Piedras having 1840 cows. One hundred and eighty-four samples were examined, fifty-five negative, 129 positive, making a total of seventy percent positive. All herds visited had reactors.

In Trujillo Alto seven herds were visited with a total of 290 cows. Twenty-nine samples were examined, of which eighteen were negative and eleven positive, giving a total percentage of positive of thirty-seven per cent. One of the herds had no reactors in the sample tested and gave no history of abortion in the herd. Twelve herds were examined in Carolina with 690 cows. Sixty-nine samples of blood were taken and examined. Thirty-seven were negative and thirty-two positive, giving forty-six per cent positives. Two of the dairies had no reactors in the samples taken and there was no history of abortion in these two herds.

In Loíza six herds were visited having 280 cows. Of these twenty-eight samples were examined, twenty-four were negative and four positive, giving a percentage of sixteen per cent positive.

Three herds had no reactors among the samples taken. One had no history of abortion, premature birth or retained placenta.

The entire seventy-nine dairies supplying milk to San Juan, with a total of 5,280 cows were visited. A total of 528 samples of blood was tested taking ten per cent of the animals of each individual herd. Of these 528 samples, 216 were negative and 312 were positive, making a total of fifty-eight per cent positives.

All herds examined, except six, had reactors among the samples taken. Of these six herds, four had no history of abortion, two admitted having had premature births and retained placentas.

TABLE I

HERDS EXAMINED IN BAYAMON

No.	Owner	Municipality	Cows in herd	No. of cows exam'ned	Negative	Positive	Per cent positive	Remarks
1....	F. V....	Bayamón.....	40	4	2	2	50	
2....	F. R....	Bayamón.....	30	3	2	1	33	
3....	A. Q....	Bayamón.....	80	8	2	6	75	
4....	A. V....	Bayamón.....	30	3	2	1	33	
5....	R. V....	Bayamón.....	20	2	2	0	0	There is no history of abortion in herd.
6....	G. M....	Bayamón.....	60	6	4	2	33	
7....	C. & P.	Bayamón.....	80	8	4	4	50	
8....	A. M....	Bayamón.....	80	8	0	8	100	Vaccines have been used
9....	R. T....	Bayamón.....	60	6	3	3	50	
10....	F. F....	Bayamón.....	40	4	2	2	50	
	Totals.....		520	52	23	29	55	

TABLE II

HERDS EXAMINED IN GUAYNABO

No.	Owner	Municipality	Cows in herd	Cows tested	Negative	Positive	Per cent positive	Remarks
11....	L.....	Guaynabo.....	30	3	2	1	33	
12....	M. O....	Guaynabo.....	200	20	3	17	85	Vaccinated
13....	P. F. R..	Guaynabo.....	100	10	1	9	90	Vaccinated
14....	H.....	Guaynabo.....	80	8	3	5	52	
15....	V. D	Guaynabo.....	40	4	1	3	75	
16....	J. P.....	Guaynabo.....	30	3	0	3	100	
17....	S. C.....	Guaynabo.....	30	3	1	1	66	
18....	G. D....	Guaynabo.....	40	4	0	4	100	
	Total.....		550	55	11	44	80	

TABLE III

HERDS EXAMINED IN TOA ALTA AND TOA BAJA

No.	Owner	Municipality	Cows in herd	No. cases examined	Negative	Positive	Per cent positive	Remarks
19....	F. L. V..	Toa Alta.....	50	5	2	3	66	
20....	F. L....	Toa Alta.....	80	8	4	4	50	
21....	R. F....	Toa Alta.....	30	3	2	1	33	
22....	P. M. C.	Toa Alta.....	50	5	3	2	40	
23....	M. V....	Toa Alta.....	50	5	2	3	66	
	Total.....		260	26	13	13	50	
24....	L. E....	Toa Baja.....	30	3	2	1	33	
25....	R. I.....	Toa Baja.....	150	15	8	7	46	
26....	C. O. ..	Toa Baja.....	30	3	2	1	33	
27....	D. J. M.	Toa Baja	50	5	3	2	40	
28....	N. E. P.	Toa Baja.....	30	3	2	1	33	
	Total.....		290	29	17	12	41	

TABLE IV

HERDS EXAMINED IN DORADO

No.	Owner	Municipality	Cows in herd	Cows examined	Negative	Positive	Per cent positive	Remarks
29....	R. L. C.	Dorado.....	100	00	2	8	88	
30....	P. L. C..	Dorado.....	80	8	0		100	Vaccinated
31....	M. M...	Dorado.....	50	5	2	3	60	
32....	J. L. H..	Dorado.....	80	8	2	6	75	
33....	P. H....	Dorado.....	150	15	7	8	53	
34....	E. N. H.	Dorado.....	50	5	2	3	60	
35....	J. M....	Dorado.....	30	3	2	1	33	
36....	A. R....	Dorado.....	20	2	0	1	50	
	Total.....		560	56	18	38	68	

TABLE V

HERDS EXAMINED IN RIO PIEDRAS

No.	Owner	Municipality	Cows in Herd.	Cows examined	Positive	Negative	Per cent positive	Remarks
37....	A. M....	Río Piedras.....	50	5	3	2	40	
38....	H. M...	Río Piedras.....	100	10	4	6	60	
39....	P. L....	Río Piedras.....	50	5	1	4	80	
40....	G. F....	Río Piedras.....	100	10	4	6	60	
41....	F. S....	Río Piedras.....	50	5	2	3	60	
42....	M. R. G.	Río Piedras.....	200	20	2	18	90	
43....	E. A....	Río Piedras.....	100	10	2	8	80	
44....	M. Z....	Río Piedras.....	150	15	5	10	66	
45....	E. M. ..	Río Piedras.....	100	10	4	6	60	
46....	J. C. O..	Río Piedras.....	50	5	3	2	40	
47....	M. E. N.	Río Piedras.....	100	10	4	6	60	
48....	N. H. ..	Río Piedras.....	200	20	0	20	100	Herd was vaccinated
49....	F. C....	Río Piedras.....	100	10	4	6	60	
50....	S.J.H.C.	Río Piedras.....	50	5	0	5	100	
51....	J. L. S..	Río Piedras.....	80	8	2	6	75	
52....	M. O....	Río Piedras.....	40	4	0	4	100	
53....	L. G....	Río Piedras.....	100	10	5	5	50	
54....	J. P.....	Río Piedras.....	100	10	5	5	50	
55....	J. M. C.	Río Piedras.....	80	8	4	4	50	
56....	R. B....	Río Piedras.....	40	4	1	3	75	
	Total.....		1,840	184	55	129	70	

TABLE VI

HERDS EXAMINED IN TRUJILLO ALTO

No.	Owner	Municipality	Cows in Herd.	Cows examined	Negative	Positive	Per cent positive	Remarks
57....	A. del C.	Trujillo Alto.....	40	4	3	1	25	
58....	J. E.....	Trujillo Alto.....	60	6	0	6	100	
59....	A. P....	Trujillo Alto.....	30	3	2	1	33	
60....	E. R....	Trujillo Alto.....	30	3	2	1	33	
61....	J. E.....	Trujillo Alto.....	20	2	2	0	0	
62....	L. V. L..	Trujillo Alto.....	40	4	3	1	25	
63....	J. S.....	Trujillo Alto.....	70	7	6	1	14	
	Total.....		290	29	18	11	37	

TABLE VII

HERDS EXAMINED IN CAROLINA

No.	Owner	Municipality	Cows in Herd.	Cows tested	Negative	Positive	Per cent positive	Remarks
64....	S. B.....	Carolina.....	60	6	3	3	50	
65....	J. M.....	Carolina.....	80	8	4	4	50	
66....	M.	Carolina.....	50	5	5	0	0	No history of abortion in the herd.
67....	J. R. S..	Carolina.....	120	12	6	6	50	
68....	D. & V..	Carolina.....	80	8	2	6	75	
69....	J. P.....	Carolina.....	100	10	2	8	80	
70....	A. J. H..	Carolina.....	70	7	7	0	0	No history of abortion in the herd.
71....	F. L.....	Carolina.....	40	4	2	2	50	
72....	E. S. O..	Carolina.....	50	5	4	1	20	
73....	N. P.....	Carolina.....	40	4	2	2	50	
	Total.....		690	69	37	32	46	

TABLE VIII

HERDS EXAMINED IN LOIZA

No.	Owner	Municipality	Cows in Herd.	Cows examined	Negative	Positive	Per cent positive	Remarks
74....	J. R. C..	Loiza.....	40	4	4	0	0	
75....	R. L.....	Loiza.....	60	6	6	0	0	No history of abortion in herd.
76....	A. L. P..	Loiza.....	80	8	6	2	25	
77....	A. F.....	Loiza.....	50	5	4	1	20	
78....	P. S. B..	Loiza.....	30	3	3	0	0	
79....	C. & Q..	Loiza.....	20	2	1	1	50	
	Total.....		280	28	24	4	16	

TABLE IX

Municipality	Cows in herd	Cows examined	Negative	Positive	Per cent positive
Bayamón.....	520	52	23	29	55
Guaynabo.....	550	55	11	44	80
Toa Alta.....	260	26	13	13	50
Toa Baja.....	290	29	17	12	41
Dorado	560	56	18	38	68
Río Piedras.....	1,840	184	55	129	70
Trujillo Alto.....	290	29	18	11	37
Carolina.....	690	69	37	32	46
Loiza.....	280	28	24	4	16
Total.....	5,280	528	216	312	58

SEROLOGIC SURVEY OF HUMAN SERA

In view of the fact that abortive disease in cattle is so widespread through the dairies that supply milk to the city of San Juan, the possibility of human infection has existed for some time. It is therefore of interest to study the findings of sera obtained from people living in the community.

Due to the courtesy of the staffs of the Presbyterian Hospital, University Hospital and Municipal Hospital we were able to examine all the serums sent to these laboratories for Wassermann reactions. All serums had been previously inactivated at 56°C for thirty minutes. The macroscopic agglutination test was employed. The antigen was made of four strains of *B. abortus* whose agglutinability had been carefully checked. Tests were incubated twenty-four hours at 37°C. Readings were made when the tubes were removed from the incubator and again after twenty-four hours in the refrigerator.

The dilutions employed were 1:20, 1:40, 1:80 and 1:160. Whenever a serum showed a positive reaction it was titrated to determine its agglutination titer and the history of the patient was inquired into. Of 1,750 blood serums examined eight gave reaction, 1:80 or higher and four gave reactions 1:20 to 1:80. The histories of the cases which agglutinated 1:80 or higher are as follows:

Case No. I.

A. C., a mulatto woman, age forty-five, came to the hospital complaining of uterine hemorrhage. Had no fever, had had four children living and well, denies history of prolonged fever or joint trouble, does not complain of sweats. On examination is a well-nourished woman. Weight 155 pounds. Shows nothing abnormal except ulcer of the cervix and prolapse of the uterus.

Laboratory examinations:

Differential blood count: Polymorphonuclears sixty-eight percent. Small lymphocytes, 30. Eosinophiles two percent. Blood culture is negative. Kahn test is negative, white blood cell count 6,200 per cm. Red blood count 3,180,000. Hemoglobin seventy-two per cent (Dare). Urine has no albumen, no glucose, no casts. Feces show hookworm ova. Agglutination for *B. abortus* 1:80. Patient drinks very little milk, usually drinks it with coffee, previously boiled. She owns a cow but the cow has no history of abortion.

Case No. II.

J. C. age thirty-six. A white female came to the hospital complaining of hemorrhage from the uterus. Has a history of chills

and fevers every three weeks over a period of several months. She did not get well in spite of taking quinine which the local doctor gave her. Finally she took a bottle of a patent medicine which she believes cured her fever. Patient complains of constipation. On physical examination is a fairly well nourished woman showing nothing abnormal except enlarged lymph glands and bleeding from the uterus. She drinks very little raw milk because she cannot afford to buy it. She has no history of contact with animals.

Laboratory findings are as follows: Red blood cell count: 2,860,000 per cm. White blood cell count 3,600 per cm. Hemoglobin (Dare) fifty per cent. Polynuclears sixty per cent; Small mononuclears twenty-nine per cent. Large mononuclears six per cent. Blood for malaria: negative. Urine: Normal. Feces: Normal. Agglutination reaction for *B. abortus* 1:160.

The fact that malaria is such a common malady here and that people usually do not take enough quinine to check the attack, having the disease for several weeks, makes it very difficult to make a diagnosis on a past history such as this.

Case No. III.

M.C.P. White woman, age twenty-nine. Complains of headache, pain in the lumbar region and slight pain in the lower abdomen. Patient had a severe chill about eighteen days ago; in the afternoon she had a rise in temperature. She has had slight fever since but at present she complains of severe pain in the lumbar region, is nauseated, has pain in the lomer extremities accompanying the onset of high fever. Has had insomnia, headache and vertigo. On physical examination the patient shows nothing outstanding.

Laboratory examinations:

Red blood count: 3,010,000. White blood cell count: 4,400. Hemoglobin (Dare) forty-five per cent. Differential count: polynuclears sixty-four per cent. Small mononuclears thirty-four per cent. Eosinophiles one per cent. Blood: positive for malaria. (Estivo autumnal). Kahn: negative. Agglutination for *B. abortus:* 1:100. Urine: negative. Feces: negative for ova.

Patient was relieved by the proper administration of quinine and discharged six days later with normal temperature.

Case No. IV.

P. D. colored girl, age nineteen. Came to the out-patient clinic of one of the hospitals mentioned. Had influenza and chicken-pox

when a small child. At present has frequent headaches and is not feeling well. Has had frequent attacks of fever and pain in the joints, general malaise and dizziness. Drinks one pint of raw milk daily from one of the infected dairies. Had no contact with animals. Her serum agglutinates *Brucella melitensis* 1:160. Wassermann and Kahn are negative (no other laboratory data available).

Case No. V.

C.M. white woman, thirty-two years old. Complains of nausea and vomiting, pain in hypogastrium, pain in the lumbar region, pain over the sacrum referred to left thigh. Patient had recurrences of pain of paroxysmal type for the last month, the pain is becoming more acute. Patient has nocturia and diuria. She drinks raw cow's milk freely but the cow is owned by herself and is apparently in good health. She is having slight fever which fluctuates from 97.5 to 100°F. Physical examination is essentially negative.

Laboratory findings:

Red blood cell: 3,380,000. White blood count 5,000. Hemoglobin: Sixty per cent (Dare). Differential count: polynuclears seventy-two per cent. Small mononuclears twenty-seven. Eosinophiles one per cent. Blood agglutinates *B. abortus* in 1:160 dilution.

Case No. VI.

A. F., white male weighing 150 pounds. Is well nourished and has gained weight lately. At present feels well. About a year and a half ago he was severely ill with joint trouble and had to go to a hospital. His left knee was swollen and for three months he was incapacitated with high fever and severe sweats. It was thought that he had gonorrhea but he denied infection and urological examination revealed nothing abnormal. One day, while in bed, his shoulder joint began to swell and was very painful for some time, but was never as bad as his knee joint. In the hospital some liquid was injected into his knee joint which made it very sore for some time but finally the fever disappeared and his joint symptoms improved rapidly. When he left the hospital he was so weak that he decided to go to the United States where he entered a sanatorium in the West. He was examined and was told he was alright. At that time he had a nervous break down and a severe secondary anemia. He came back to Porto Rico and was greatly improved.

His occupation is animal husbandry. He was working in a

heavily infected herd and was drinking about three quarts of raw milk daily. He often cleans the cows that retain placenta. His blood titer for B. abortus at present is 1:640.

Case No. VII.

J. E. D. white, male, thirty-three years old. Complains of pain in the back which has persisted for two or three months. He has occasional headaches and dizziness. Catches cold very easily and is deaf in left ear. He has a slight fever in the afternoon but complains chiefly of pain in the lumbar region. Patient had G. C. infection about twelve years ago. On physical examination he presented a rigid spine, with marked muscle spasm in the lumbar region and market tenderness over this area.

The diagnosis made in this case was *arthritis*. His blood serum agglutinated B. abortus in 1:160 dilution. Upon inquiring we find that his wife has also been ill in one of the city hospitals. She has been running a fever of 103.5°F. in the evenings for the last five weeks. Feels fairly well and has a good appetite. In the afternoon she feels discomfort and slight headache and the temperature rises only to drop again in the morning. Her physical examination is essentially negative. X-Ray plates show a normal chest. Repeated blood smears examined for malaria were negative. Widal reaction and blood culture were negative for typhoid. The differential count showed slight lymphocytosis and a normal absolute count. Urine is normal. Wassermann: negative. Sputum negative for T. B. C. On the fifth week, while taking quinine, her temperature returned to normal and she left the hospital. A diagnosis of malaria was made on the basis that her temperature dropped when quinine was administered. Her blood examination for agglutination of *B. abortus* was negative. Both husband and wife gave a history of drinking raw milk but had no contact with infected animals.

The other case came to the outpatient clinic of one of the local hospitals and it has been impossible to obtain a history from him. Of the four cases that agglutinated in low dilutions, one was running a septic fever with high leucocytosis and polynucleosis. A diagnosis of perirenal abscess was made. The case was operated, the abscess drained, and the patient had a complete recovery.

The other two cases visited the outpatient clinic of one of the local hospitals. They gave a history of drinking large amounts of raw milk and of having fever and articular and muscular pains but they both have active pulmonary tuberculosis.

Of the 1,750 serums examined, twelve or about 0.68 percent,

showed agglutination in some degree. Of these twelve sera, eight agglutinated 1:80 or higher and four agglutinated in lower dilutions.

It is very hard to make a diagnosis on the past history of such as these given. A few of these cases present symptoms of undulant fever but at the same time the question of malaria arises. Others have an active tuberculosis which could very well account for the symptomatology. There are two cases however in which all other possibilities could be ruled out and the diagnosis of undulant fever seems perfectly justified.

THE EFFECTS OF SUN AND ULTRA-VIOLET LIGHT CULTURES OF B. MELITENSIS ABORTUS

We have mentioned the fact that the sun is very intense in Porto Rico and the possibility that it may exercise some influence on the secretions of infected cattle, thereby affecting to a certain extent the transmission of the disease.

In order to test experimentally the action of sunlight on cultures of B. melitensis abortus, inoculations were made of the organism (Strain 456 Hyg. Lab.) on agar slants and incubated at 37°C. for forty-eight hours. The cultures were emulsified in 5 cc. of normal saline and a bacterial count was made to determine the approximate number of bacteria contained in each cubic centimeter. Then 1 cc. portions of the bacterial suspension were pipetted off into several quartz glass test tubes (125 × 15 mm. with lip). The tubes were laid flat on an inverted ice tray, in order to keep the temperature low and the emulsion was spread out within the test tubes, the greatest depth being estimated at one-half cm. A thermometer was also placed parallel to the test tubes to record the temperature. Cultures prepared in this way were exposed to the sun at 10:30 A. M. Tubes were labeled I to VII. Tube I was not exposed to sunlight, keeping it as control. Each tube was exposed half an hour longer than the other so that Tube II was exposed thirty minutes and Tube VII three hours. After the time of exposure desired the suspensions were tested for sterility.

In making bacterial counts, dilutions were made in sterile water 1:1,000 and 1:10,000. Then one cubic centimeter was plated in agar and incubated at 37°C for forty-eight hours. Later the colonies were counted. (See table X.)

TABLE X

SUN EXPOSED CULTURES OF B. MELITENSIS ABORTUS

No. of tube	Time of exposure	Results
I.....	Not exposed.....	100,000,000 Bac per cc
II.....	From 10:30—11:00.....	5,568,000 Bac per cc
III.....	From 10:30—11:30.....	248,000 Bac per cc
IV.....	From 10:30—12:00.....	1,000 Bac per cc
V.....	From 10:30—12:30.....	2,000 Bac per cc
VI.....	From 10:30—1:00.....	Sterile Bac per cc
VII.....	From 10:30—1:30.....	Sterile Bac per cc

Agar slants inoculated from the bacterial suspensions contained in each tube resulted as follows: No growth occurred in Tubes IV, V, VI, and VII. Slight growth occurred in Tube III and normal growth in Tubes I and II. The experiment was repeated using a different technic for counting, i. e., instead of making dilutions in sterile water to plate from the dilution, the whole cubic centimeter was plated (See table XI).

TABLE XI

No. of tube	Time of Exposure	Results
I.....	Not exposed.....	Could not be counted
II.....	From 10:30—11:00...	Could not be counted
III.....	From 10:30—11:30...	Could not be counted
IV.....	From 10:30—12:00...	Could not be counted
V.....	From 10:30—12:30...	600 col. on plate
VI.....	From 10:30—1:00....	4 col. on plate
VII.....	From 10:30—1:30....	Sterile

If we compare the results of the three methods of proving sterility, by inoculation of agar slants, by dilution and plating or by plating the whole quantity we have the results as given in Table XII.

TABLE XII

No. of tube	Time of Exposure	Agar Slant	Results Dilutions and plating	Plating whole quantity
I.....	Not exposed.....	Normal growth.....	100,000,000	Could not be counted
II.....	30 min.....	Normal growth.....	5,568,000	Could not be counted
III.....	60 min.....	Slight growth	248,000	Could not be counted
IV.....	90 min.....	No growth..... ...	1,000	Could not be counted
V.....	2 hours.....	No growth.....	2,000	600 colonies per cc.
VI.....	2½ hours.....	No growth.....	Sterile....	4 colonies per cc.
VII.....	3 hours.....	No growth.....	Sterile....	Sterile

Cultures on agar slants grew well in Tubes I, II and III. This was demonstrated by the other two methods as well. Tubes IV, V,

VI and VII were sterile. This was not the case with the other methods. Tubes IV and V were not found sterile by dilution and plating and tube VI which was sterile by the first two methods was found to have four colonies per plate when the whole quantity was plated.

The dilution method has the advantage that one can estimate approximately the concentrated suspensions but errors in dilution are great and misleading since few bacteria may remain alive and are missed in the dilution when small quantities are plated. By plating the whole quantity one is unable to estimate concentrated solutions and one has no idea of the action of the germicide on the cultures. On the other hand when weaker suspensions are plated, one is confident that every particle of the exposed culture is plated and if there is any living organism it should show in the plate.

In this experiment we obtained complete sterility by all three methods in cultures of *B. melitensis abortus* which were exposed to sunlight in quartz tubes for three hours around noon time and during the month of July. The temperature recorded by the thermometer during the experiment fluctuated from 20°C. to 30°C. As a control we repeated the above experiment but protected the quartz tubes by using a filter of ground glass. The cultures were not affected by sunlight when transmitted through ground glass.

In order to compare the results we exposed cultures of *B. melitensis abortus* to the Alpine sun lamp. The previous technic was used but exposures were made as follows: The arc of the lamp was set at one foot distance from the surface of the quartz tubes. The lamp was lighted and allowed to warm for ten minutes. The tubes were labeled and set on the tray and the lamp was opened allowing the light to fall on the tubes. Tube I was not exposed. Tube II was exposed five minutes; Tube III, ten minutes and so on, every tube being exposed five minutes longer than the other so that Tube VII was exposed thirty minutes. The tubes were shaken gently every two minutes. A thermometer was set parallel to the tubes to control the temperature. (See Table XIII.)

TABLE XIII

No. of tube	Time of exposure	Agar slant	Dilution and Plating	Plating and whole quantity
I.....	.....	Growth. ...	100,000,000	Could not be counted
II.....	5 minutes..	Growth.....	5,200,000	Could not be counted
III.....	10 minutes..	Growth.....	8,000	Could not be counted
IV.....	15 minutes..	No growth.	4,000	432 colonies
V.....	20 minutes..	No growth..	1,000	72 colonies
VI.....	25 minutes..	No growth..	Sterile.....	13 colonies
VII.....	30 minutes..	No growth..	Sterile.....	2 colonies

Growth was obtained on the agar slants in tubes I, II and III. Other tubes were sterile. By dilution and plating, sterility was obtained in tubes VI and VII while by plating the whole quantity, thirteen colonies grew from tube VI and two colonies from tube VII. The result shows that the ultra-violet light of the Alpine sun lamp has a definite germicidal action on suspension of *B. melitensis abortus*. Had we exposed the suspension five minutes longer it would have probably been entirely sterile.

Organisms which survived exposure to sunlight and ultra-violet were cultured in broth and injected into mice in order to test their virulence.

Four groups of mice were used in the experiments. Group No. I was injected intraperitoneally with one cubic centimeter of a twenty-four-hour broth culture of the strain which had previously been exposed to sunlight for two hours.

Group No. III was injected similarly with a twenty-four-hour broth culture of the strain previously exposed to the Alpine sun lamp for twenty minutes. To group No. IV, 1 cc of sterile broth was given intraperitoneally.

Groups No. I and No. II died six days after injection. Group No. III died the fifth day of injection. Cultures made from the spleens of these animals revealed the presence of the organisms. Group No. IV was autopsied after thirty days and the cultures of their viscera were sterile.

Sections of viscera from mice of all groups were sent to the pathological laboratory. The virulence of the cultures of *B. melitensis abortus* is altered very little if any by exposure to ultra-violet light whether from Alpine sun lamp or direct sunlight.

In the preceding experiments we purposely eliminated the factor heat by exposing the cultures on ice. We then decided to repeat the experiment allowing the temperature to rise and recording its maximum by a thermometer placed parallel to the quartz tubes. By this method we obtained both heat and ultra-violet effect. The time of exposure was the same as in the previous experiments. Tube No. II, thirty minutes, the next tube sixty minutes and so on until the seventh tube was exposed three hours. Results were as given in table XIV.

TABLE XIV

No. of tubes	Time of exposure	Agar slant	Dilution and plating	Plating whole quantity
I.....	0	Growth. ...	100,000,000	Could not be counted
II.....	30 minutes..	Growth.....	8,000,000	Could not be counted
III.....	1 hour.....	Growth.....	240,000	Could not be counted
IV.....	1½ hour....	Slight grth..	1,000	60 colonies
V.....	2 hours.....	No growth..	1,000	10 colonies
VI.....	2½ hours...	No growth..	Sterile.....	1 colony
VII.....	3 hours.....	No growth..	Sterile.....	Sterile

The highest temperature recorded by the thermometer was 42°C. For comparison of the results obtained by exposing cultures to sunlight by both methods, see Table XV.

TABLE XV

Tube No.	Ultra violet light only		Ultra violet plus heat	
	Dilution and counting	Plating and whole quantity	Dilution and counting	Plating and whole quantity

There is undoubtedly some influence of the temperature factor in the destruction of the cultures as demonstrated in the results obtained by the whole plate method in both experiments.

Tube No. IV when exposed to ultraviolet radiation alone contained so many bacteria that they could not be counted but when exposed to the same radiation, allowing the tubes to warm up, only sixty colonies could be counted. Tube No. V which revealed the presence of 600 colonies after exposure to the ultraviolet radiation had only ten colonies when the radiation plus heat acted upon it. While Tube No. VI which showed four colonies by one method, only showed one colony by the other in both cases the cultures were completely sterile after three hours' exposure.

Organisms that survived the longest period of exposure were cultivated in broth to determine their virulence using the same method that has been previously described. Mice injected with the original cultures died in six days. Mice injected with cultures that were previously exposed and then recultered died in six days. Controls were autopsied after thirty days and found negative as we expected.

Sunlight has apparently little, if any effect on the virulence of cultures of *B. melitensis abortus* which survive its effects.

Knowing that cultures when exposed to sunlight plus heat were destroyed at a larger rate than when exposed to sunlight alone we decided to study the influence of heat alone upon the cultures. A water bath was set at 42°C. Seven tubes with one cubic centimeter

of the original emulsion of *B. melitensis abortus* were incubated in the water bath. Tube No. II was incubated for thirty minutes, No. III for one hour and so on, incubating each tube half an hour longer. No. VII then incubated for three hours. Bacterial counts were made as previously described in this article. (See Table XVI.)

TABLE XVI

No. of tube	Agar Slant	Plating by Dilution	Plating whole quantity
I.....	Growth.....	100,000,000	Could not be counted
II.....	Growth.....	108,000,000	Could not be counted
III.....	Growth.....	90,000,000	Could not be counted
IV.....	Growth.....	40,000,000	Could not be counted
V.....	Growth.....	10,480,000	Could not be counted
VI.....	Growth.....	700,000	Could not be counted
VII.....	Growth.....	308,000	Could not be counted

Suspensions of *B. melitensis abortus* when incubated in the water bath for three hours at 42°C. showed a marked diminution of viable organisms contained in the suspension. Suspensions were not sterile after three hours' incubation.

EXPERIMENTS WITH VACCINATION AGAINST BOVINE CONTAGIOUS ABORTION *

Since the recognition of Abortion disease in the island, vaccination has been used. Either living or killed cultures of *B. abortus* have been employed. The cultures have been killed by heat or by germicidal substances such as thymol, formaldehyde or phenol. Living cultures are usually administered in suspension in normal saline.

With the idea of testing both vaccines we vaccinated rabbits subcutaneously, using dead cultures in one group and living cultures in another. A third group was vaccinated with killed cultures of B. coli to be used as a control. The procedure was as follows: Forty-eight-hour agar cultures of *B. melitensis abortus* (P. R. No. I) were emulsified in normal saline to obtain an emulsion which would match approximately a No. 3—nephrometer reading. Part of this suspension was killed by heat in a water-bath at 60°C. for one hour. The remainder was used unheated. A similar suspension was prepared with cultures of B. coli and killed by heat to be used as control. Rabbits were vaccinated subcutaneously by giving three doses one every five days. They were bled eight days after the last

* This part of the work has been done in collaboration with Dr. Alfonso Rivera, Veterinary Inspector of the Department of Health of San Juan, Porto Rico.

dose. The first dose given was 0.5 of a cubic centimeter, five days later a second dose of one cubic centimeter was given and within five days a third dose of one cubic centimeter followed. They were bled from the heart eight days after the last dose and then serums tested for agglutinins to B. abortus. A group of rabbits was vaccinated with killed cultures, another group with the living suspensions and a third group with the *B. coli* suspension. Results obtained from the agglutination reactions are given in Table XVII.

S. D.	1/20	1/40	1/80	1/120	1/160	1/200	1/240	1/280	1/320	1/360	1/400	1/440	1/480	1/520	1/560	1/600	1/640	1/680
R. A.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+–
R. D.	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
R. C.	+	+–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

S. D. —Serum dilutions.
R. A.—Rabbits vaccinated with live cultures.
R. D.—Rabbits vaccinated with dead cultures.
R. C.—Control Rabbits.
(+) —Complete agglutination.
(+–)—Partial agglutination.
(–) —Negative agglutination.
The macroscopic agglutination test was used incubating at 37° C for 1 hour and left in the ice box over night. The final reading was made in the morning.

The serum of rabbits vaccinated subcutaneously with living cultures reached a titer of one to six hundred and forty (1:640) while the serum of rabbits vaccinated with heat-killed cultures reached a titer of one to one hundred and sixty (1:160). Results of these experiments indicate that living suspensions of *B. abortus* when injected subcutaneously into rabbits give a serum which is four times stronger in its agglutinative properties for *B. abortus* than the serum of rabbits similarly vaccinated with dead cultures of the organism. A similar experiment was carried out in a group of twenty (20) heifers. They were bled from the juglar vein and the agglutinating titer of their serums was determined previous to vaccination. A group of ten was vaccinated with living cultures of *B. abortus* and the rest were vaccinated with cultures killed by heating at 60°C. Emulsions were prepared by growing the organsim in agar and suspending the cultures in normal saline until the suspension matched approximately a No. 3 nephrometer reading. This emulsion was kept in 20 cc glass vials. When a dead culture was desired this same emulsion was heated in a water bath at 60° for one hour and after cooling was tested for sterility. A dose of twenty cubic centimeters was given to each animal subcutaneously. From eight to eleven days after vaccination the animals were bled again and their blood serum tested for agglutinins. (See Tables XVIII and XIX)

TABLE XVIII

SERUM TITER OF HEIFERS VACCINATED WITH DEAD CULTURES

No. of Animals	Agglu. Titer before Vaccination				After Vaccination							
	1/20	1/40	1/80	1/100	1/160	1/320	1/640	1/1280	1/2560	1/3280	1/4000	1/6240
I	+	+	−	−	+	+	+	+	−	−	−	−
II	+	+−	−	−	+	+	+	+	+−	−	−	−
III	−	−	−	−	+	+	+	−	−	−	−	−
IV	−	−	−	−	+	+	+	−	−	−	−	−
V	−	−	−	−	+	+	+	+−	−	−	−	−
VI	−	−	−	−	+	+	+	+−	−	−	−	−
VII	−	−	−	−	+	+	+	−	−	−	−	−
VIII	−	−	−	−	+	+	+	+	−	−	−	−
IX	−	−	−	−	+	+	+	+−	−	−	−	−
X	−	−	−	−	+	+	+	+	−	−	−	−

TABLE XIX

SERUM TITER OF HEIFERS VACCINATED WITH LIVING CULTURES

No. of Animals	Agglu. Titer before Vaccination				Agglu. Titer after Vaccination							
	1/20	1/40	1/80	1/100	1/160	1/320	1/640	1/1280	1/2560	1/3120	1/4000	1/6240
I	+−	−	−	−	+	+	+	+	+	+	−	−
II	+	−	−	−	+	+	+	+	+	+	+	+
III	−	−	−	−	+	+	+	+	+	−	−	−
IV	−	−	−	−	+	+	+	+	+	+	−	−
V	−	−	−	−	+	+	+	+	+	−	−	−
VI	−	−	−	−	+	+	+	+	+	+	+−	−
VII	+	+	+	+−	+	+	+	+	+	+	+	+
VIII	+−	−	−	−	+	+	+	+	+	+	−	−
IX	+−	−	−	−	+	+	+	+	+	+	+	+
X	−	−	−	−	+	+	+	+	+	+	+−	−

If we compare the agglutination titer of both sets of animals after vaccination we have Table XX.

TABLE XX

AGGLUTINATION TITER OF BOTH SETS OF ANIMALS AFTER VACCINATION

No. of Animals	Titer after Vaccination with dead cultures	Titer after Vaccination with living cultures
I	1—1280	1—3120
II	1—2560	1—6240
III	1—640	1—2560
IV	1—640	1—3120
V	1—1280	1—2560
VI	1—1280	1—4000
VII	1—640	1—6240
VIII	1—1280	1—3120
IX	1—1280	1—6240
X	1—1280	1—4000

In comparing the agglutination titers of both groups two points are readily noted. First that a great individual variation exists.

Second that similar animals with identical agglutination titers before vaccinaton, vaccinated with identical vaccines under similar conditions gave different agglutination titers. Living cultures gave as a rule higher agglutination titers than did dead cultures.

With these results in mind we planned an experiment to vaccinate on a large scale. Six herds, two in Guaynabo and four at Río Piedras, were selected to be vaccinated with living cultures. Vaccination with dead cultures was carried on in a small herd at Río Piedras and an infected herd near Río Grande was closely observed but no vaccine was given. Every cow that aborted or dropped normally was vaccinated. Non-pregnant heifers were vaccinated until eventually the whole herd was vaccinated. All herds were observed for a period of two years. The rate of abortion in these herds is shown in Table XXI.

TABLE XXI

RATE OF ABORTION IN VACCINATED HERDS

Herd No.	Location	No. of cows in the herd	Vaccinated with	No. of abortions during the period of observation	Percentage of abortion during the period of observation
I	Río Piedras	60	Living cultures	One	.6
II	Río Piedras	200	Living cultures	Six	3.0
III	Río Piedras	200	Living cultures	Seven	3.5
IV	Río Piedras	140	Living cultures	Three	2.1
V	Guaynabo	200	Living cultures	Ten	5.0
VI	Guaynabo	200	Living cultures	Eight	4.0
VII	Río Piedras	60	Dead cultures	Fifteen	25.0
VIII	Río Grande	140	No vaccine	Fifty-three	35.3

We noticed again a wide variation in the different groups, in spite of similar conditions, identical vaccine and identical methods used. Best results were obtained in herd No. I which belongs to the insular government and where a veterinary inspector is in charge. But results vary widely for herd No. V, which is one of the best kept in the island, had a rate of five per cent which is higher than the others, but we consider this very satisfactory. It may be mentioned here that these herds were heavily infected previous to vaccination and that acquired immunity may account in part for the results obtained but the same was also the case with the control herds and their rates are much higher. In all, one thousand cows were vaccinated with living cultures having an average abortion rate of 3.2 per cent during the period of observation. Unfortunately the control herd, where no vaccine was given, had an explosive outbreak

of abortion disease, having seventeen in two months, and a total of fifty-three during the period of observation, giving a total percentage of 35.3 per cent which we consider unusually high.

The abortion rate of dairies vaccinated with living cultures is much lower than abortion rates of infected dairies vaccinated with dead cultures or not vaccinated at all. To our mind there is no question that vaccination with living cultures is superior to the dead-culture injection and that it reduces the number of abortions occurring in infected herds.

The use of living cultures in vaccinating infected herds has met with considerable opposition. Some of the arguments used against the procedure are: That abortion is not eliminated or reduced to a desirable minimum, that sterility or failure to breed may occur, and that the infection is constantly kept alive in the herd. The possibility of spreading undulant fever in man from infected milk has also been brought forth. We know so far of no other agent that will reduce abortion rate in an infected herd more rapidly than living vaccines. If there is any other procedure which gives similar or better results without the danger of infection, that procedure shall be resorted to. The ideal method of isolation of healthy stock can be carried on with the idea of building a new non-infected herd but in the meantime, and before the new herd is built, vaccination is the only practical method known to us today by which abortion can be rapidly reduced to a minimum in heavily infected communities. The argument that sterility or failure to breed occurs after vaccination does not seem justified in heavily infected communities. There is no more sterility in our records among vaccinated cattle than among cattle that have suffered the disease and have never been vaccinated. Furthermore all heifers that have been vaccinated so far have conceived after copulation and dropped normally. To prove vaccination a real source of danger the percentage of cattle vaccinated that become carriers of the disease should be determined. Zeller [38] reports some experiments which indicate that an active disease is not established after subcutaneous injection of living cultures of the organism. Huddleson [39] reports satisfactory results by using avirulent strains of *B. abortus*.

The possibility of the transmission of undulant fever to man through infected milk, we consider very important. The author [40] has been able to produce experimental undulant fever in man by repeatedly feeding porcine strains of *B. melitensis abortus*. Further

experiments are being carried out attempting to produce infection through the gastro-intestinal tract with bovine strains.

It should be understood that vaccination with living cultures has only been practiced in heavily infected communities. In slightly infected herds segregation or slaughter seems justified. Non-infected herds can be kept clean by careful observation and testing.

We believe that vaccination with living vaccines, as a method for control of Bang's disease, deserves further study but it should be done under supervision of competent research specialists for the present, since this method has not passed the experimental stage.

ARE THE SPECIES OF THE GENUS BRUCELLA DIFFERENT?

Since the work of Evans [4] the differentiation of the strains of the genus Brucella, coming from different hosts has been a question of paramount importance. The problem is an interesting one. While some observers claim marked differences between the species others seem to agree in finding them identical. In view of these differences of opinion, some of the strains with which we have been working were compared.

STRAINS USED

The strains used for comparison are as follows:

Brucella melitensis (P & S) human source; collection of the School of Tropical Medicine.

Brucella melitensis (21) abortus variety—Bovine source; collection of the School of Tropical Medicine.

Brucella melitensis (428 A) human source; collection of the School of Tropical Medicine.

Brucella melitensis (E. 420) abortus variety—equine source; courtesy of Dr. F. Huddleson.

Brucella melitensis (456) abortus variety—bovine source; courtesy of the Hygienic Laboratory.

Brucella melitensis (483) abortus variety—porcine source; courtesy of the Hygienic Laboratory.

Brucella melitensis (P. R. I.) abortus variety—bovine source; isolated in Porto Rico.

Brucella melitensis (P. R. II) abortus variety—bovine source; isolated in Porto Rico.

MORPHOLOGY

Opinions seem to agree that there is very little morphological difference between the species, yet some authors (Duncan)[41] mention

CHART I

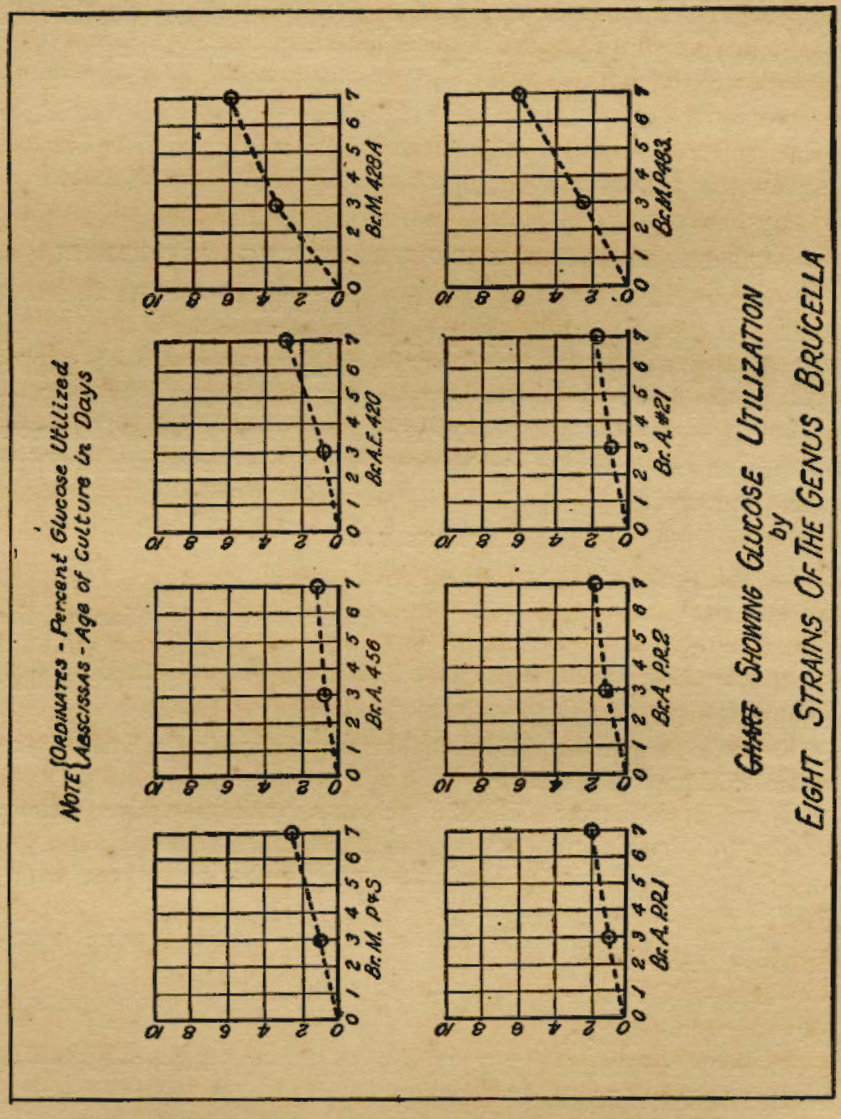


Chart Showing Glucose Utilization by Eight Strains Of The Genus Brucella
Note (Ordinates - Percent Glucose Utilized
(Abscissas - Age of Culture in Days
Br.M. 428A
Br.M.P.483
Br.A.E.420
Br.A.#21
Br.A.456
Br.A.P.82
Br.M. P.45
Br.A.PRJ

the fact that Bruce described his organism as a coccus while Bang described his as a bacillus which might attain a length of 3 micra. These authors report no marked morphological difference of the species when examined in smears from cultures on agar or glucose agar, but when the organisms were cultivated in peptic-digest-blood-agar the *B. abortus* strain frequently develops a long bacillary form. On transferring the cultures back to glucose agar the *B. abortus* strains reverted to the coccal type.

Alice Evans [42] says "The general statement may be made that on the average the length of the bacterial cells is somewhat greater in strains from bovine and porcine sources than in strains from human and caprine sources, although the morphologies are otherwise identical."

The strains we are working with show very little if any difference in morphology. The difference is so negligible that we can not identify one strain from the other by its morphological characteristics.

METABOLISM

Studies on the metabolism of the abortus melitensis group have shown some differences in the behavior of the different species. McAlpine and Slanetz [43] have shown that the group can be divided into two well marked subgroups by means of their action on glucose. The abortus variety of Bovine origin shows inability to utilize the available glucose in the medium, whereas B. abortus of porcine and human origin can do so.

We have studied the glucose metabolism of the strains with which we are working. The methods used are the same as those described by McAlpine and Slanetz but the glucose determinations were made by Somogy's modification of the Shaffer-Hartman method only.

More recently the same authors [44] have studied the glucose metabolism of strains that have grown for several generations on plain Fairchild peptone agar and they have found that these organisms tend to lose their ability to utilize glucose. Transfer in liver infusion broth with continued incubation at 37°C for two weeks, caused the strain to develop a mucoid form in which the glucose utilizing power was restored. The mucoid forms of bovine strains tested did not utilize glucose.

Huddleson and Hasley and Torrey [45] have shown that the liberation of hydrogen sulphide by different species of abortus growing on

special media containing organic sulphur, may serve to differentiate the varieties. According to the rate of hydrogen sulphide production they were able to separate the strains into three groups, namely, those which produced a considerable amount over two days and those that failed to produce a detectable amount of gas. In the first group fell all strains of the porcine type, in the second all the bovine and in the third the melitensis and para-melitensis. Studying further the sulphur metabolism of this group they have found that the production of hydrogen sulphide depends upon the amount of available sulphur, in sulphur compounds present in the medium. When there is an excess of this substance, the melitensis group will also produce small amounts of hydrogen sulphide during the first twenty-four hours.

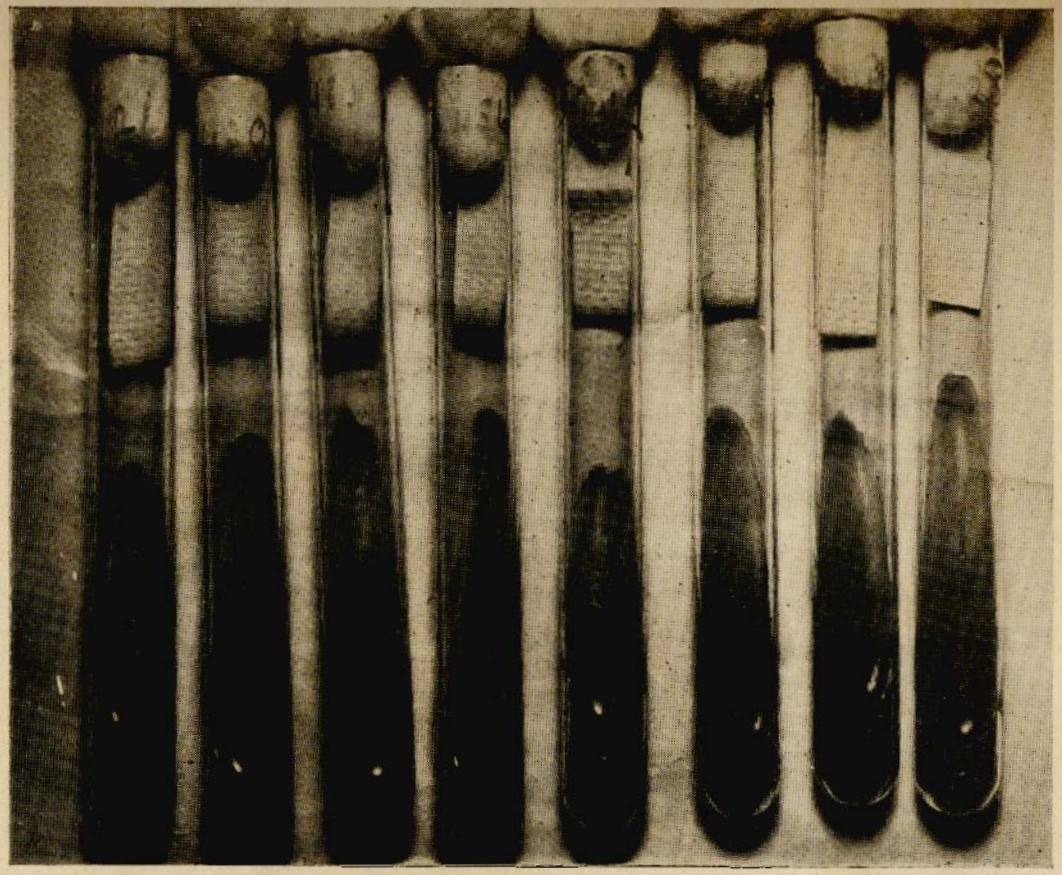
We have had some difficulty in repeating Huddleson's experiments. Our strains were growth in plain agar pH 7.4 and when transplanted to liver infusion agar pH 6.6 they did not grow at all. Later it was found to be a matter of adaptation, by gradually passing the strains through liver infusion broth and lowering the pH gradually, the organism grew in the liver infusion agar pH 6.6.

Inoculations were made of all strains in liver infusion agar pH 6.6, using the lead acetate paper as an indicator. At the same time controls were made on plain agar, pH 7.4, using the original strains. More controls were carried consisting of uninoculated agar, pH 7.4, and uninoculated liver infusion, pH 6.6 All of these were incubated the same day at 37°C. in the incubator. In the first twenty-four hours the formation of sulphide was marked on strains P. 483, B. 456, P. R. I., P. R. II., and E. 420. Strain B. M. P. & S. and B. 21 formed very little sulphide in twenty-four hours, and B. M. 428 A did not form any at all. In the next forty-eight hours an increase in the sulphide production occurred in all strains, except strain BM 428A which produced none.

The strains that produced the largest amount were B 456, B. P. R. I., B. P. R. II., and P. 483. B. P. & S. and B. 21 came next.

The controls planted in plain agar, pH 7.4, also produced a sulphide in the first twenty-four hours that was recorded by the lead acetate paper. The controls of uninoculated media were absolutely negative.

It is claimed that *B. abortus* will not grow in primary culture or in early subculture at the carbon dioxide tension of air, but that it requires an increased concentration of this gas. The optimum con-



PRODUCTION OF A SULPHIDE ON PLAIN AGAR pH 7.4

centration according to Huddleson [46] has been found to be from five to ten percent. McAlpine and Slanetz [47] have shown that strains of *B. abortus* of bovine origin require an addition of five to ten percent CO_2 for the promotion of luxurious growth, whereas porcine and human strains are inhibited by low concentrations of this gas. The effect of CO_2 is not due to any alterations in the reaction of the medium.

Huddleson [48] studying the selective action of dyes in mediums towards the growth of Brucella found that he could separate the organism into three distinct groups. He used methyl violet (certified) in a dilution 1:100,000 in beef liver agar, pH 6.6, basic fuchsin (certified) in a dilution 1:50,000 and thionin (certified) dilution 1:50,000 used under the same conditions. Methyl violet and Basic fuchsin completely inhibits the growth of porcine strains but they have little, if any, effect on bovine abortus or melitensis. In thionin the abortus species fail to grow while the growth of porcine or melitensis species is little, if at all, affected by the presence of the dye in the medium. Our strains have been tested by this method and the results are given in Table XXII.

TABLE XXII

GROWTH OF EIGHT STRAINS OF BRUCELLA IN HUDDLESON'S DYE MEDIUMS

Strain No.	Basic Fuchsin				Methyl Violet				Thionin				Control			
	24	48	72	96 hrs.	24	48	72	96 hrs.	24	48	72	96 hrs.	24	48	72	96 hrs.
P 483	−	−	−	−	−	−	−	−	+	+	+	+	−	+	+	+
A 456	−	+	+	+	−	+	+	+	−	−	−	−	−	+	+	+
HP and S	+	+	+	+	−	+	+	+	−	−	−	−	+	+	+	+
E 420	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+
M 428 A	−	+	+	+	−	+	+	+	−	+	+	+	−	−	+	+
A 21	+	+	+	+	+	+	+	+	−	−	−	−	+	+	+	+
PR I	−	−	+	+	−	−	+	+	−	−	−	−	−	+	+	+
PR II	−	−	+	+	−	−	+	+		−	−	−	−	+	+	+

The number and variety of strains tested is not enough to warrant any conclusion but the writer believes that the method represents an effort at the approach of a practical and accurate method of determining the type of species of a given strain of the genus Brucella and should be further tested and its use encouraged.

Working on a serological basis different opinions have been published. It is generally admitted that the porcine or bovine strains cannot be differentiated by agglutinin absorption. Doyle and Spra [49] state that bovine and swine strains are alike serologically. Hayes [50], in similar studies, seems to find no differences. Cotton [51] studied six

strains of swine and concluded that they are identical with the bovine strains. Orcutt [52], working with six strains, finds no evidence of serological distinction between the six cultures studied which included two human strains.

Evans [42] describes eight serological groups. Skariac [53] found he could differentiate abortus from human strains by agglutinin absorption. Khaled [54], by a comparative study of thirty strains of B. abortus and B. melitensis, found that his melitensis serum agglutinated both sets of cultures alike but the abortion antiserum agglutinated the abortion cultures to a slightly higher titer than the melitensis.

Burnet [55] states that melitensis serum agglutinates B. abortus and B. melitensis alike or B. abortus to a higher titer. B. abortus antisera however always agglutinates B. abortus to a higher titer than B. melitensis. It seemed desirable for us to examine the serological relations of the strains with which we are working. Complete series of agglutinins and cross-agglutinins were carried out and each serum was absorbed by all cultures and tested for agglutinins against each strain. The method of Feusier and Meyer was followed. The immune serum was diluted 1:10 with normal saline. The culture was grown in agar and suspended in normal saline. Equal volumes of serum dilution and the suspension were mixed and incubated in a water bath at 37°C for two hours, then allowed to stand in the refrigerator overnight. The mixture was centrifuged the next day and the clear supernatant fluid was tested with the absorbing strain for complete absorption. If agglutinins still remained for the absorbing strain more culture was added until absorption was complete. Then the supernatant fluid was tested against the other strains.

TABLE XXIII

AGGLUTINATIONS BEFORE ABSORPTION

Strain	PR I	PR II	A 456	P 483	HP and S	E 420	A 21	M 428 A
PR I	+	+	+	+	+	+	+	+
PR II	+	+	+	+	+	+	+	+
A 456	+	+	+	+	+	+	+	+
P 483	+	+	+	+	+	+	+	+
HP and S	+	+	+	+	+	+	+	+
E 420	+	+	+	+	+	+	+	+
A 21	+	+	+	+	+	+	+	+
M 428 A	+	+	+	+	+	+	+	+

Note: Dilutions were carried as high as 1:640. All strains agglutinated with homologous and heterologous serum.

TABLE XXIV

RESULTS OBTAINED AFTER ABSORPTION WITH HOMOLOGOUS SERUMS

Strain	PR I	PR II	A 456	P 483	HP and S	E 420	A 21	M 428 A
PR I.....	—	—	—	—	—	—	—	—
PR II.....	—	—	—	—	—	—	—	—
A 456.....	—	—	—	—	—	—	—	—
P 483.....	—	—	—	—	—	—	—	—
HP and S....	—	—	—	—	—	—	—	—
E 420.....	—	—	—	—	—	—	—	—
A 21.....	—	—	—	—	—	—	—	—
M 428 A.....	—	—	—	—	—	—	—	—

All the cultures absorbed all agglutinins of the homologous serum, leaving none for the homologous or heterologous strains.

TABLE XXV

RESULTS OBTAINED AFTER ABSORPTION WITH HETEROLOGOUS SERUMS

Strain	PR I	PR II	A 456	P 483	HP and S	E 420	A 21	M 428
PR I.....	—	—	—	—	—	—	—	—
PR II.....	—	—	—	—	—	—	—	—
B 456.....	—	—	—	—	—	—	—	—
P 483.....	—	—	—	—	—	—	—	—
HP and S....	—	—	—	—	+	—	—	—
E 420.....	—	—	—	—	—	—	—	—
B 21.....	—	—	—	—	—	—	—	—
M 428 A.....	—	—	—	—	—	—	—	+

When absorption was carried out with heterologous serum all cultures absorbed all agglutinins of the heterologous serum except serum M 428 A and serum H P & S which after absorption with heterologous strains still agglutinated its homologous culture in low dilutions. These results indicate that strain M 428 A and strain H P & S are the only strains of this group that we are working with that can be differentiated by agglutinin absorption by the method described. The rest of the strains could not be separated by agglutination or agglutinin absorption.

Experimental inoculations in animals have shown that the porcine and human strains are usually more virulent than the bovine type.

Smith [56] points out that by infecting guinea pigs with minute doses the porcine variety can be distinguished from the bovine by the character of the lesions resulting in the spleen and lymph nodes.

He also points out striking similarity in the lesions produced in guinea pigs by porcine and human strains.

In a group of mice inoculated by us with large doses in order to test the virulence of our cultures the acute lesions produced were histologically studied. There seem to be no appreciable difference in the acute lesions produced in mice by any of the strains.

Pathogenicity for human beings has been mentioned as a point of difference between the different species. Some authors claimed that the abortus variety is not pathogenic for man. The author [40] has demonstrated that the porcine strain when ingested in milk causes a disease similar to undulant fever. Bovine strains when ingested in similar doses did not cause any symptoms. This does not mean that if ingested in larger doses of higher virulence in more susceptible individuals they would produce the disease, but it certainly demonstrates that certain porcine strains are more virulent for man than certain bovine strains. Most authors admit that the melitensis strains are as a rule more virulent for human beings than the abortus strains and that the disease produced by the second is generally of a milder type than that produced by the melitensis. Another argument frequently used in favor of the higher virulence of the melitensis species is the frequent infection of laboratory workers with *Brucella melitensis* while laboratory infection with strains of *Brucella abortus* is very rare.

Judging from our work, and the work of other authors, there seems to be a striking similarity between some of the strains isolated from human beings and some of the porcine strains in their action towards CO_2, their dextrose metabolism and the pathological lesions produced by them in guinea pigs.

TOXIC PRODUCTS OF BRUCELLA ABORTUS

Some of the signs and symptoms of undulant fever suggest the production of toxins, but these have never been demonstrated.

Several experiments were planned to demonstrate exogenous or endogenous toxic products in filtrates from cultures of *B. abortus*. *B. abortus* (Porcine 483) was grown in 100 cc. of 2 percent peptone broth, pH 7.2, for three weeks at 37°C. The cultures were then filtered through an N. Berkefeld filter. Mice were injected with filtrates intraperitoneally. Simultaneously, groups of mice were inoculated with different doses of the broth culture and of sterile broth.

The mice inoculated with the broth culture died and showed the

typical lesions in the viscera. The mice injected with normal broth were autopsied after thirty days of the injection and showed normal viscera. Mice inoculated with the filtrate of the N. filter died from ten to twenty-five days after inoculation. Autopsy findings revealed the typical lesions of *B. abortus*.

In culturing the filtrates used in these inoculations we found that they were not sterile and that some organisms had passed through the filter.

The experiment was repeated using a Berkefeld W. filter. All mice injected survived and when autopsied the animals did not show lesions.

A new series of experiments was planned allowing the cultures of *B. abortus* 483 to grow in broth, pH 7.2, for three weeks, then the cultures were ground with sterile sand and allowed to stand for another three weeks. These cultures were then filtered through a Berkefeld W. filter and the filtrates were inoculated intraperitoneally into mice, using various doses. Controls were inoculated with normal broth. Both sets of animals survived and when autopsied showed no demonstrable lesions.

Apparently *B. abortus* is capable of going through an N. Berkefeld filter. Sterile filtrates of a broth culture of B. abortus when injected into mice, did not affect them and produced no appreciable pathologic lesions in their viscera.

EXPERIMENTS ON TRANSMISSION

The peculiar conditions existing in Porto Rico, where we have a high infection of endemic abortion in cattle and apparently no human case morbidity has interested us greatly. It is very difficult to determine without careful study of the factors responsible for this condition.

The general custom of boiling milk may account for a low incidence in many diseases spread through milk. This would not eliminate, however, any number of cases that could be infected through skin contact.

Reviewing the literature on the possible modes of transmission and possible etiology of this disease we find wide differences of opinion as to the causative agent of undulant fever in North America and the mode or modes of transmission. King and Caldwell [57] are of the opinion that raw milk infected with *Brucella abortus* produces agglutinins and causes undulant fever in man. Carpenter and King [58] state "our study of the disease shows clearly that *B. abortus* is only slightly pathogenic for man and it must be that only most

virulent strains in milk are of danger to him", and further, "from the data submitted we believe that milk is the logical source of *B. abortus* infection in man. Whether the origin of the strains that are pathogenic for man are from cattle or swine, we are unable to state at this time."

McAlpine and Mickle [59] comment "therefore it seems probable that ordinary bovine strains have very little if any pathogenicity for man but that cows may become infected with strains which can give rise to human infections."

Orr and Huddleson [60] in studying the epidemiolgical aspect of the problem in Michigan say: "This study reveals the fact that in a group of five hundred individuals, equally divided into males and females, of all age groups, constantly exposed to the abortus organism through an infected milk supply, only 1.4 per cent showed evidence of active infection. This result would indicate that among the human population susceptibility to infection to *B. abortus*, bovine type is very low, and that human infection is determined by some factors as yet undetermined. The low human susceptibility is, no doubt, responsible for the relatively low incidence of undulant fever."

Zinsser [61] quotes as follows, referring to the Brucella group: "As far as the two organisms are concerned (*B. melitensis* and *B. abortus*) their habitat and pathogenicity are so much alike, that they may be justly regarded as varieties of the same species. Malta fever, getting into man through goats' milk and *B. abortus* having been as we shall see, shown to cause a disease not unlike malta fever in individuals who obtain it from cows' milk."

Kristensen and Holm [62] reporting 500 cases observed in Denmark during the period of April 1st and December 1st, 1928, isolated the organism *Brucella abortus* in twenty-three cases, in twenty-one instances from the blood, once from an ovarian abscess and once from the placenta in a case of human abortion. After studying the organisms isolated they concluded that the human strains isolated in Denmark are identical with *Brucella abortus* of bovine origin, but that they can be differentiated from *B. melitensis* and from strains of *B. abortus* of porcine origin.

On the other hand other investigators, such as Bastai [63], think that B. abortus is pathogenic for cattle and not for man. He remarks that laboratory infection at the Tunis Pasteur Institute is very common among laboratory workers with *B. melitensis*, so much so that vaccination is compulsory, but in the veterinary laboratory where foetuses and membranes of infected cattle are examined daily, infection is unknown and veterinarians who vaccinate with virulent

bacilli are never attacked." The areas of distribution of melitensis and abortus infection do not correspond" says the author, "indeed in districts where abortus is more common, melitensis is absent."

Smith [56] writes, "Statements based on comparative bacteriological studies have been made showing that *B. abortus* and *B. melitensis* are identical and that a disease similar to malta fever, namely undulant fever, may be produced in man by the bovine type of organism. The well defined geographical distribution of the disease caused by caprine species, its relation to goats' milk, the high degree of infectiousness of *B. melitensis* for laboratory workers, wide diffusion of infectious abortion all over the United States and Europe with a very low incidence of human disease, all militate against this identification. In the meantime the evidence that *B. abortus* producing disease of the placenta in cattle may produce a disease in man, simulating malta fever, must be regarded as inadequate in establishing any such relationship."

Varcellana [64], after doing several experiments with monkeys, cows, and goats, concludes that *B. abortus,* after passage through goats, does not acquire increased virulence for the monkey and that B. melitensis, after passage through cows, does not suffer any loss of virulence for the animal (m. sinicuss). Tests with human beings were not tried but the author maintains that by analogy the question of the pathogenicity of Bang's bacillus for man is still unproved.

Burnet [65] inoculated men under the skin with doses of 200,000,000 *B. abortus* and they did not develop any fever and had no symptoms whatever although they were resistant to subsequent melitensis injection whereas control volunteers contracted the infection. This author concludes that B. abortus is only slightly pathogenic, or rarely pathogenic for man, or else there are special races of B. abortus, which are capable of producing abortion in cattle and fever in man.

The wide discrepancies of opinions between different authors, in North America as well as in Europe, makes the subject extremely interesting and leaves it open to further investigation.

Extensive studies have been made in the transmission of undulant fever to man. Numerous experiments have also been made in the transmission of contagious abortion in cattle.

Shaw, Eyre [66] and others report experiments in which large doses were commonly followed by infection. Similar results have been reported by Huddleson,[67] Sims and Miller,[68] Rettger, White [69], Flesheimer,[70] Cotton,[71] and Sanderson and Rettger [72].

Horrocks [73], Shaw [66], and others have demonstrated that the

mucous membrane of the nose, or conjunctiva may be the portal of entry in animals infected by exposure to contaminated dust.

Morelli,[74], Shaw [66], Shoeder and Cotton [75] obtained infection in some instances by instillation of broth cultures into the eyes of experimental animals.

Frank, Lenbert and Brauer [3], Bang [76] and others have proven the possibility of infection in animals through the mucous membranes of the genital tract.

Eyre reports infection of a goat with infected milk through the abraded skin. Burnett [77] reports typical infection following application of the organism to the shaved skin of guinea-pigs.

Hardy, Hudson and Jordan [78] have recently published experiments in which guinea pigs were easily infected through normal or abraded skin with cultures of *B. melitensis abortus* obtained from human cases, one strain being classified as bovine and the other as porcine.

The subject as it stands today gives rise to several possibilities.

1st. Infection may take place through the alimentary tract or by way of the skin, or by both.

The question of skin infection suggests the possibility of infection through abraded or normal skin.

2nd. The etiological factor should be exactly determined.

(*a*) *B. Melitensis abortus* (bovine) may be the cause of undulant fever.
(*b*) The porcine strain may infect cattle and then cause human infections.
(*c*) The possibility of some melitensis strains infecting cattle must be considered.

It is well known that Carpenter [58] succeeded in infecting cattle with porcine strains. So did Varcellana [64]. Evans [79] succeeded in infecting cattle with *B melitensis*. So there are several possibilities one must have in mind in determining the etiology of these cases. So far epidemiological studies points out the milk supply as the possible main source of infection, but in some States such as Iowa, Hardy[80] has demonstrated that skin infection is an important factor.

As in every case of infection we must consider several factors, such as resistance of the host to the infection, number of organisms ingested and virulence of the organism. It is extremely difficult to evaluate accurately all of these factors in natural infection. In order to have an accurate control of all factors concerning this particular infection we have planned experiments in which human

volunteers were either fed cultures of known virulence in pasteurized milk or were inoculated through the abraded or normal skin.

METHODS

Volunteers who were physically fit were taken to the hospital where temperature, pulse, respiration and general observations could be made daily. Several cultures of the strains to be tested were made in broth and plain agar tubes. From the broth cultures 1 cc. of a twenty-four-hour growth was injected intraperitoneally into a mouse which was isolated and closely observed. Another twenty-four hour broth culture of the same organism was poured into a half pint of pasteurized milk and vigorously shaken. The milk was taken to the hospital and fed to the patient in our presence.

The cultures in plain agar were incubated for forty-eight hours. The day previous to the inoculation, a group of guinea pigs had small areas on their ventral surface denuded of hair by depilation. The next day they were inoculated with a platinum loop of the creamy growth of the organisms. The loop was moved over the surface of the denuded area until the culture was well disseminated.

Another group of guinea pigs was inoculated by shaving roughly a small area on the ventral surface so as to purposely produce abrasions. On this abraded area a loop full of the creamy forty-eight hour growth was rubbed in with the wire. The same day a group of volunteers who had been examined previously were inoculated by producing a small abrasion on the skin of the arm over the region of the deltoid muscle. The procedure was identical with that used in vaccinating against smallpox. Then a loopful of the creamy growth of the forty-eight hour agar culture was rubbed gently over the abraded area.

In another group of volunteers the same operation was performed over the normal skin in the same region.

The experimental animals were closely observed and autopsied on the fourth week after inoculation. Their viscera were studied bacteriologically and pathologically for abortus infection.

The patients were closely observed over a period of three months. Observations on temperature, pulse, respiration, physical signs and laboratory findings could be made daily.

RESULTS

Strain Brucella melitensis abortus 456 (Bovine source). (Courtesy of the Hygienic Laboratory.) Date of isolation Sept. 1917.

Case No. I.

A. L. mulatto, twenty-eight years old weighs 132 pounds. W. B. C. 4,500; R. B. C. 3,500,000; homoglobin seventy per cent (Talquist); Wasserman, negative. Differential count; Polymorphonuclears seventy per cent. Large lymphocytes four per cent. Small lymphocytes twenty-four per cent. Eosinophiles two per cent. Widal reaction: negative. Agglutination for *B. abortus* negative in 1:10. 1:20, 1:40 and 1:80. Feces positive for hookworm.

A mouse was injected on July 19. Patient drank infected milk on July 19 at 10 A. M. On the morning of July 25 (6 days after inoculation) the mouse died. Autopsy of the animal revealed an intense congestion of the lungs, a congested mushy liver in which minute white areas could be seen, and an enlarged spleen with small whitish nodule-like structures scattered throughout. Cultures were made and the organs were sent to the pathology laboratory for microscopic examination. Cultures made from the spleen were positive for *B. melitensis abortus.*

An agglutination test on the patient's blood (6 days after ingestion of *B. abortus*) was negative in dilutions of 1:10, 1:20, 1:40 and 1:80. August 2nd (eight days later) the patient was reexamined and showed no abnormal symptoms. He had had no fever and had a good appetite and slept well. Agglutination test on his blood for *B. abortus* was again negative in 1:10, 1:20, 1:40 and 1:80. The patient was discharged August 15 (twenty-seven days after ingestion of *B. abortus*). The patient ran no fever during his stay in the hospital. Agglutination test on this date was negative for *B. abortus* in dilutions 1:10, 1:20, 1:40 and 1:80. Blood culture taken August 15 was also negative.

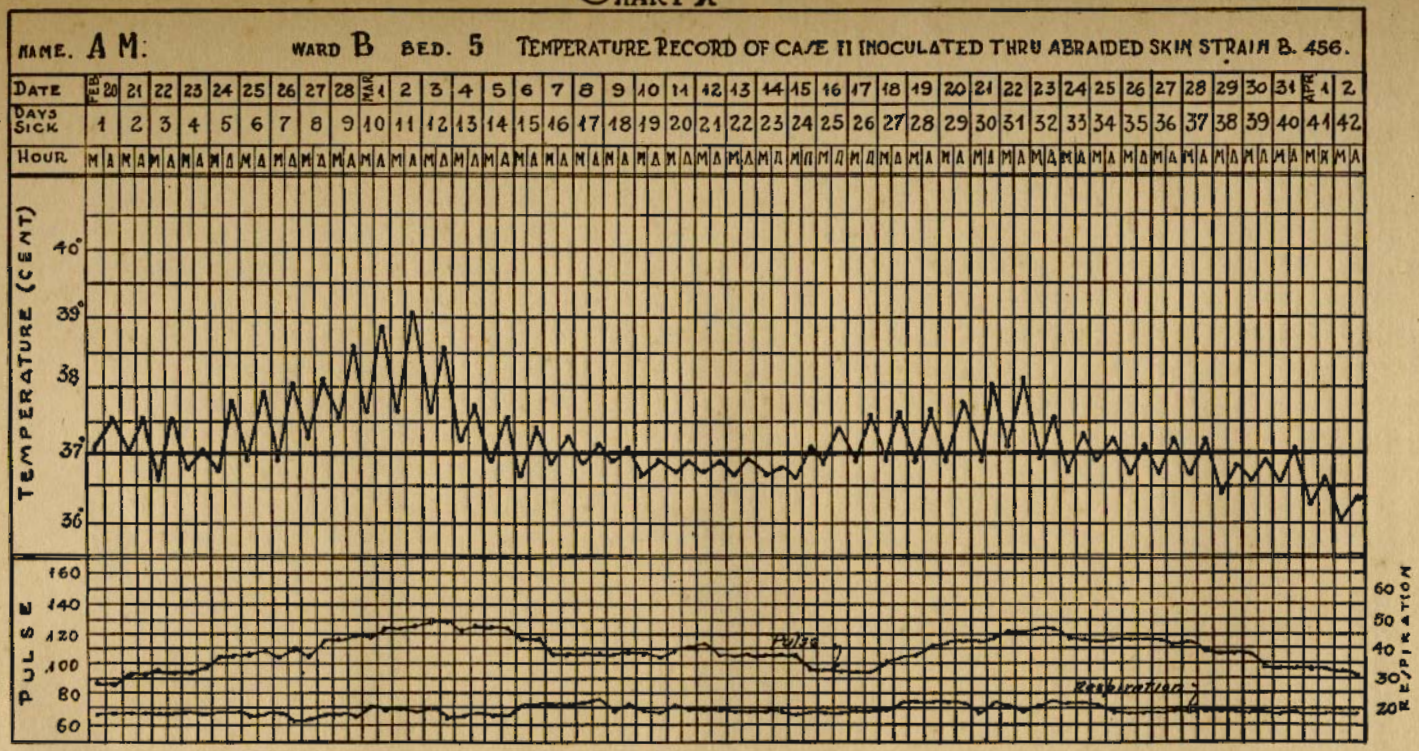
On February 10th, A. M. was inoculated with the same strain (456) through the abraded skin and P. L. H. through the normal skin. The same day two guinea-pigs were inoculated with the same strain, one through abraded skin and the other through unabraded skin.

The guinea-pigs were autopsied after four weeks and histological sections of their viscera were made and examined. A positive culture was obtained from the pig inoculated through abraded skin. Cultures from the spleen and lymph glands of the guinea-pig inoculated through unabraded skin were negative.

Case No. II.

Patient, A. M. is a white man, age 22. Weighs 116 pounds Laboratory findings before inoculation were as follows: W. B. C.

CHART X


NAME. A M. WARD B BED. 5 TEMPERATURE RECORD OF CASE II INOCULATED THRU ABRADED SKIN STRAIN B. 456.
DATE
DAYS SICK
HOUR
TEMPERATURE (CENT)
PULSE
RESPIRATION
Pulse
Respiration

4,700; R. B. C., 4,500,000; Hemoglobin, eighty per cent. Differential count: Polymorphonuclears sixty-six per cent; large lymphocytes two per cent; small lymphocytes twenty per cent; eosinophiles twelve per cent; Kahn reaction, negative. Widal reaction, negative; agglutination for *B. abortus* negative 1:20, 1:40 and 1:80. Feces: positive for hookworm. Analysis: No albumen, no sugar, no casts. Was inoculated by skin abrasion February 10. The patient continued to feel well until February 20th (10 days after inoculation) when he complained of general malaise, headache and dizziness. His temperature was 37.5 on the evening of this day but he was able to be up. Next day his temperature was normal in the morning and slightly higher in the afternoon but he said he felt better. The day after the temperature was subnormal in the morning and again slightly raised in the afternoon. The patient remained this way for about four or five days when he began to feel worse and was confined to bed. His main symptoms were headache, fever, pain over the joints and severe sweats, especially at night.

His W. B. C. count at this time was 3,500 per cm. Blood was negative for malaria. Widal: negative. Differential count: polymorphonuclears forty; large lymphocytes six per cent and small lymphocytes forty-four per cent. Eosinophiles ten per cent. His blood culture was positive on the 11th day of the disease and the agglutination test was still negative (April 7).

Case No. III.

P. L. H. who was inoculated through normal skin is a white man, 22 years old, weighing 126 pounds. Laboratory findings before inoculation were as follows: W. B. C. 4,500; R. B. C., 4,500,000. Hemoglobin eighty-five per cent. Differential count: Polymorphonuclears fifty-nine per cent. Small lymphocytes thirty-two per cent. Large lymphocytes one per cent. Eosinophiles eight per cent. Widal reaction: Negative. Malaria: negative; Agglutination for B. abortus: Negative, 1:80. Wasserman and Kahn reactions: negative. Feces: positive for hookworm. Urine: Normal. This patient had been entirely well. His temperature had always been normal. His blood culture and agglutination test for *B. abortus* were negative on April the 9th. These three cases were inoculated with same strain. The case inoculated through abraded skin is the only one that developed the infection. The other two cases remained perfectly normal.

Strain *B. abortus* No. 21. (Bovine source.) Collection of the School of Tropical Medicine.) Date of isolation—unknown F. G. age 29, white weighed 144 lbs. Physical examination showed no

abnormalities except a small healed ulcer of left leg over the tibia, and some enlarged lymph glands.

Laboratory findings: W. B. C. 7,600. R. B. C. 4,500,000. Hemoglobin: eighty per cent (Talquist). Differential count: Polymorphonuclears sixty-three per cent. Small lymphocytes twenty-six per cent. Large lymphocytes four per cent. Eosinophiles seven per cent. Wassermann reaction, positive (+++). Widal reaction: Negative. Agglutination for B. abortus, negative in dilutions 1:10, 1:20, 1:40 and 1:80. Feces, positive for uncinariasis and trichuriasis. Urine: No albumen, no glucose, no casts.

On June 25, the atient was fed a twenty-four hour culture of *B. melitensis abortus* (Strain No. 21 School of Tropical Medicine) in pasteurized milk. At the same time a mouse was injected intraperitoneally with 1 cc. of a twenty-four hour culture. On July 9 (15 days after injection) the patient had developed no symptoms. On July 14 (19 days after) the patient felt perfectly well. His blood culture at this time was negative and the agglutination test for *B. abortus* in dilutions of 1:20, 1:40 and 1:80 were negative.

On July 23 (twenty-eight days after ingestion of the culture) the patient was again fed a forty-eight hour broth culture of the same organism in milk. On July 24 (twenty hours after second feeding) patient complained of a severe pain in the abdomen, was nauseated, had diarrhea all night, passing eight stools in the last four hours. Patient also complained of severe griping pains with tenesmus. His skin was moist and cold and he had severe perspiration with a temperature of 37.2°C. On July 24 (second day after the second feeding of culture) symptoms subsided noticeably. The patient felt better, had no fever and no headache but complained of dizziness and general weakness. He had no desire for food. On the third day the patient felt much better and most of the gastro-intestinal symptoms had subsided. On July 26 the blood was examined. Blood culture and agglutination test for *B. abortus* were both negative.

The patient continued to feel well on the 8th of August (fifteen days after second feeding of culture and forty-three days after the first). His blood was again tested for B. melitensis abortus and both blood-culture and agglutination test were negative. On August 10th the patient left the hospital unexpectedly and never returned.

It is difficult to determine whether the gastro-intestinal symptoms this patient had were due to the forty-eight hour culture that he drank or to some other food taken. Upon inquiry we found that all patients in the ward had eaten for lunch, rice and beans, meat,

macaroni and sweet potatoes. For supper they had vegetable soup, mashed potatoes, rice, beans and coffee. None of the other patients showed any abnormal symptoms suggestive of food poisoning. The injected mouse was killed and autopsied on July 25th (a month after injection). It presented a large wall of cysts in the liver very similar to cysts caused by tenia in rats. Organs and pus from the cysts were cultured in liver infusion agar and organs were sent to the pathology laboratory for histological study. The cultures made from spleen and pus from the cysts in the liver were all negative for B. melitensis abortus.

On February 10, two cases were inoculated with the same strain, one through unabraded and the other through abraded skin. At the same time two guinea-pigs were inoculated and autopsied after four weeks. Cultures made from the spleen and lymph glands of both guinea pigs were negative.

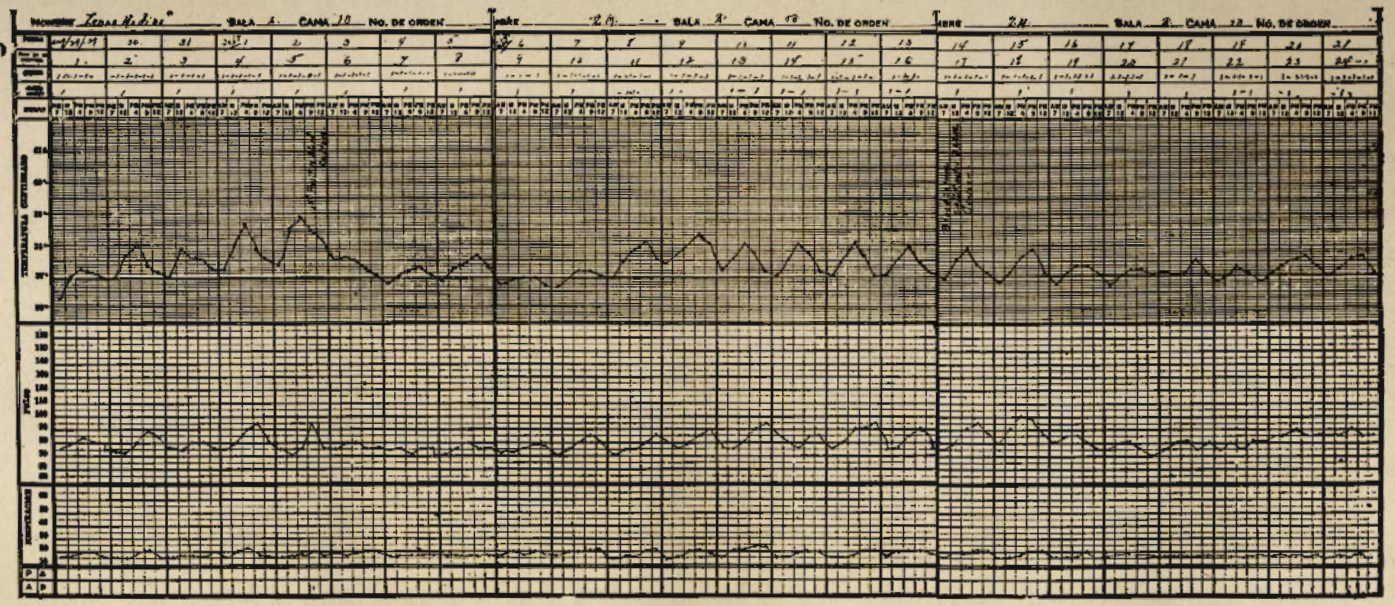
Case No. V.

P. A., mulatto, age 31, weighs 187 pounds. Was inoculated through abraded skin February 10. The laboratory findings before inoculation were as follows: W. B. C. 6,600. R. B. C. 5,000,000. Hemoglobin eighty-five per cent. Differential count: polymorphonuclears seventy-five per cent. Large lymphocytes three per cent; Small lymphocytes twenty-two per cent. Widal reaction: negative. Malaria: negative. Wassermann reaction: negative. Agglutination for *B. abortus:* Negative 1:80 dilution. Feces: Negative for parasites. Urine: Normal. This patient was closely observed for a period of two months. His temperature was never over 36.9 during the period of observation. He felt well and complained of no symptoms whatsoever. His blood culture and agglutination test were negative for *B. abortus* on April 15th.

Case No. VI.

M. C., mulatto, age 22 years, weighs 128 pounds. Was inoculated through normal skin on February 10th. The laboratory findings before inoculation were as follows: W. B. C. 5,600. R. B. C. 4,800,000. Hemoglobin eighty per cent. Differential count: polymorphonuclears seventy-two per cent. Large lymphocytes four per cent. Small lymphocytes twenty-two per cent. Eosinophiles two per cent. Widal reaction: negative. Malaria: negative. Agglutination for B. abortus in 1:80, negative. Kahn: positive (+++). Feces, negative for parasites. Urine: normal. The patient did not develop any abnormal symptoms during the period of observation. His temperature and pulse were normal throughout.

CHART II



TEMPERATURE RECORD OF CASE VII INOCULATED WITH STRAIN P. 483 BY MOUTH

As seen by these reports there is nothing indicative of Brucella infection. These findings confirm the findings of other authors that *Brucella abortus* loses its virulence when kept for a long period on artificial media. This strain had been kept for several years in the collection of the School of Tropical Medicine.

Strain *Brucella melitensis abortus* variety (Strain 483 Porcine source). (Courtesy of the Hygienic Laboratory.) Date of isolation February 1922.

Case No. VII.

Z. M., age 36, male mulatto, weighs 140 pounds. On physical examination appears to be normal and in good health. Laboratory findings: W. B. C. 6,800. R. B. C. 3,500,000. Hemoglobin seventy per cent, (Talquist). Wassermann Reaction: Negative. Differential count: polymorphonuclears, seventy percent. Large lymphocytes, two per cent. Small lymphocytes, twenty-four per cent. Eosinophiles, four per cent. Agglutination test for *B. melitensis abortus*, negative in 1:20, 1:40 and 1:80. Feces: Positive for hookworm. Urine: No albumen, no glucose or casts. On July 23, the patient was fed a twenty-four hour broth culture of *B. melitensis abortus* (Porcine Hyg. Lab. 483) in a half pint of pasteurized milk. The same day a mouse was injected intraperitoneally with 1 cc. of a twenty-four hour broth culture of the same organism. On the afternoon of the 25th the mouse showed complete paralysis of both hind legs. On the morning of the 26th the mouse died. The autopsy revealed a congested lung, a dark congested liver and slightly enlarged spleen. Cultures were made of the spleen in liver infusion agar and were positive after forty-eight hours' incubation. The organs were sent to the pathology laboratory for histologic examination.

The patient was closely observed from the day of inoculation to August 5th (thirteen days after inoculation) and did not complain of any symptoms nor show anything abnormal. Blood culture and agglutination test made on this date were negative for *B. melitensis abortus*. On August 9th (seventeen days after the first inoculation) we decided to give this man a second culture. He was fed a twenty-four hour broth culture of the same organism (*B. abortus* Porcine Hyg. Lab. 483) in pasteurized milk. On August 21 (twenty-eight days after first feeding and eleven days after the second), the patient showed nothing abnormal and had no complaint other than being tired of confinement in the hospital. He was bled on the 22nd and blood culture and agglutination test made for *B. melitensis abortus* were negative. On August 29th (19 days after the second

feeding) the patient did not feel well and had a slight fever of 37.6°C. He admitted that he had not been feeling well for the last two days (since the 27th). He had general malaise and a marked weakness in both knees; he also had severe headaches and a marked tenderness over the region of the spleen. On examination the patient revealed a cold, moist skin, a temperature of 37.6°C, pulse of 85, respiration 20. The spleen was slightly palpable. The next day the patient felt worse. He had developed a severe pain in the left knee joint and had higher fever. Blood examination for malaria was negative. Blood culture was negative. Differential blood count was: Polymorphonuclears sixty-three per cent, large lymphocytes thirty-three per cent. W. B. C. 3,000.

The patient continued to have a fever of 38.5°C. in the afternoons which subsided in the mornings. On September 2nd his blood culture was positive for *B. abortus* after forty-eight hours' incubation. On September 6th the pain he had in his left knee subsided and he began to complain of severe pain in the left shoulder joint. On September 6th and 7th the fever had subsided and the patient felt much better but on September 8th the temperature rose again and remained up for eight or nine days when it began to subside again.

The agglutination test on this patient had been negative since the beginning until September 14 (eighteen days after the onset of the disease) when his serum agglutinated *B. abortus* in dilution 1:100. His blood culture was also still positive. On this date he complained of severe pain in his right knee.

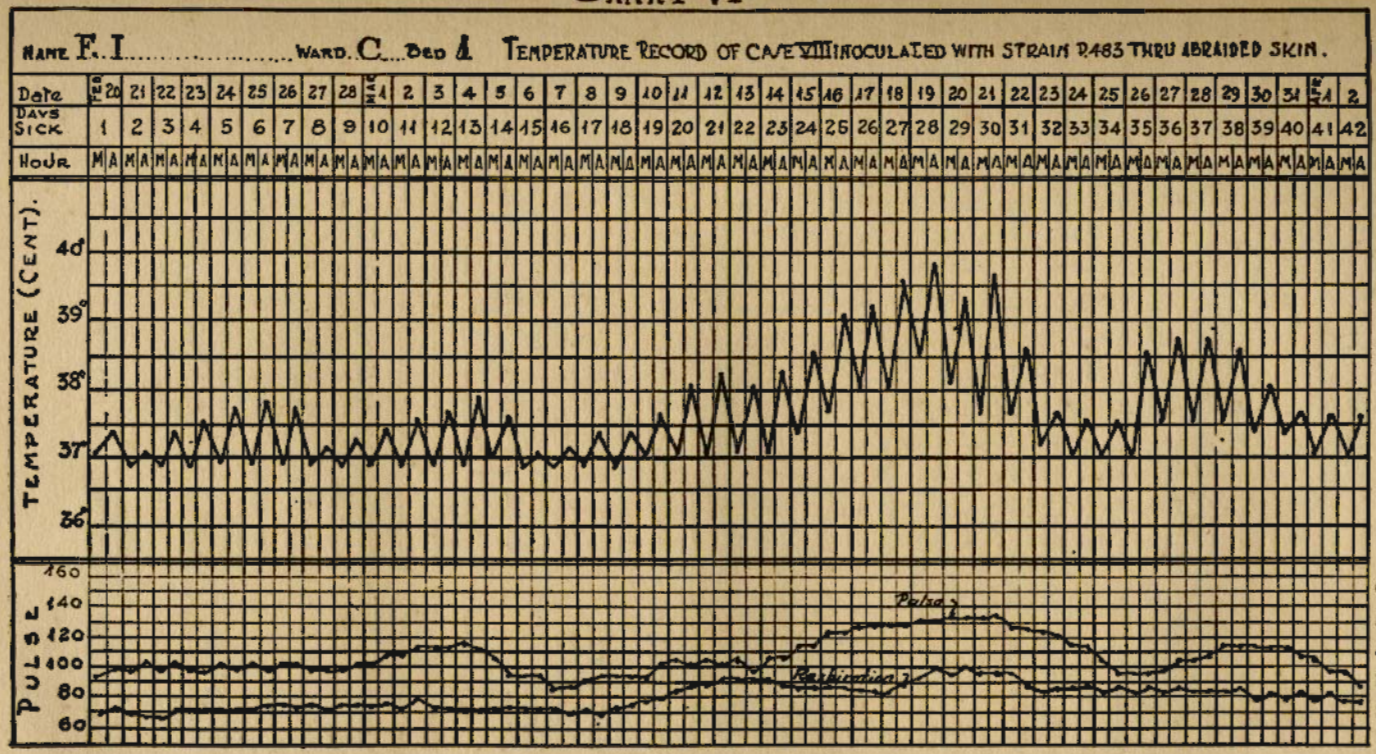
His condition continued very much the same, having febrile periods alternating with fever-free periods of short duration. His serum agglutinated *B. abortus* on February 2nd at 1:640 dilution.

On February 10th, two cases were inoculated with the same strain, one through normal and the other through abraded skin. Two guinea pigs were similarly inoculated and observed for four weeks and then autopsied. Cultures made from the organs of the guinea-pig inoculated through normal skin were negative. Culture made from the organs of the guinea-pig inoculated through abraded skin were positive for *B. abortus.*

Case No. VIII.

F. I., white, 20 years old, weighing 140 pounds was inoculated through abraded skin on February 10th. The laboratory findings before inoculation are as follows: W. B. C. 4,600. R. B. C. 4,000,000. Hemoglobin seventy-five per cent. Differential count; polymorphonuclears seventy per cent. Small lymphocytes twenty per cent. Large

CHART VI


NAME F. I. WARD. C. BED 1. TEMPERATURE RECORD OF CASE VIII INOCULATED WITH STRAIN R483 THRU ABRADED SKIN.
Date
Days Sick
Hour
TEMPERATURE (CENT).
PULSE
RESPIRATION.
Pulse
Respiration

lymphocytes two per cent. Eosinophiles eight per cent. Malaria: negative. Widal reaction, negative. Agglutination test for *Brucella abortus* negative in dilutions 1:20; 1:40; 1:80. Wassermann reaction: negative. Feces: Trichuris, hookworm and ascaris. Urine: Normal.

The patient continued to feel well until February 20th, (ten days after inoculation) when he complained of slight headache and no desire for food, mental apathy and marked physical depression. His temperature was 37.3°C in the afternoon and subnormal on the following morning. His condition continued more or less the same nearly eighteen days. He felt apparently well in the morning, but in the afternoon had a slight rise in temperature which never went beyond 38°C. At this period the patient began to feel worse, his temperature went up to 39.8°C in the afternoon and 38°C in the morning. He was very dull mentally and had lost weight. He had no desire for food and his chief complaint was the severe pain over his joints and profuse cold sweats at night. His blood culture was positive on February 28th after forty-eight hours' incubation. The agglutination titer on March 15 was 1:320. His W. B. C. count was 2,080. Polymorphonuclears, forty per cent. Large lymphocytes, twelve per cent. Small mononuclears, forty-eight per cent. The patient continued with high remittent fever, articular pains and severe sweats at night, was constipated and on April 15th his serum titer for *B. abortus* was 1:160.

Case No. IX.

E. J., 21 years old. Negro, weighs 127 pounds. Was inoculated February 10th on normal skin. The laboratory findings before inoculation were: W. B. C. 7,000. R. B. C. 3,500,000. Hemoglobin sixty per cent. Differential count: Polymorphonuclears sixty-eight per cent. Small lymphocytes twenty-six per cent. Large lymphocytes two per cent. Eosinophiles, four per cent. Wassermann reaction: negative. Widal reaction: negative. Malaria: negative. Agglutination test for *Brucella abortus* negative 1:20; 1:40; 1:80. Feces, negative for intestinal parasites. Urine, normal.

This patient was normal through the period of observation. He gained six pounds in weight. His temperature was normal or subnormal. Agglutination test and culture for Brucella abortus were negative.

Strain *Brucella abortus* P. R. I (Bovine source) Isolated in Porto Rico. February—1929.

Case No. X.

J. R. G., Age 23. White (male) weighs 124 pounds. A physical examination revealed a fairly well nourished young man. Except for gonorrhea infection the patient was apparently normal.

Laboratory findings: W. B. C. 9,500. R. B. C. 4,420,000. Hemoglobin eighty per cent (alquist). Kahn: Positive (++). Differential count: Polymorphonuclears seventy-five per cent. Large lymphocytes two per cent. Small lymphocytes nineteen per cent. Eosinophiles four per cent. Feces: Negative for intestinal parasites. Urine: slight traces of albumen. Glucose, negative. Sediment, loaded with pus cells.

On August 9, patient was fed a twenty-four hour broth culture of *B. melitensis abortus* (Strain isolated in Porto Rico—Porto Rico No. I) in pasteurized milk. At the same time a mouse was injected intraperitoneally with 1 cc. of a twenty-four hour broth culture of the same organism. The mouse died August 21, (twelve days after injection). At autopsy the animal revealed more or less the same lesions as described previously. Cultures made from the spleen were positive for *B. melitensis abortus.*

The patient was observed closely and showed no symptoms. Blood culture and agglutination test made August 25th were negative for *B. melitensis abortus.*

On September 15th (thirty-seven days after feeding of culture) this patient was reexamined. He complained of nothing and showed no signs or symptoms that were suspicious of any infection. His blood culture was negative and the agglutination test for *B. melitensis abortus* was also negative. The patient was discharged.

Two guinea-pigs were inoculated through abraded and normal skin with Strain P. R. I. After four weeks they were autopsied. Cultures made from the organs of the guinea-pig inoculated through normal skin were negative. Those made from the organs of the guinea-pig inoculated through abraded skin were positive for *B. abortus.*

Case No. XI.

L. M., mulatto, age 23, weighed 134 pounds. Was inoculated through abraded skin on February 10th. The laboratory findings before inoculation were: W. B. C. 6,500. R. B. C., 4,000,000. Hemoglobin seventy-five per cent. Differential count: Polymorphonuclears seventy per cent. Large lymphocytes one per cent. Small lymphocytes twenty-two per cent. Eosinophiles six per cent. Basophiles one per cent. Malaria: Negative. Widal reaction: negative. Agglutination for B. abortus: Negative, 1:20, 1:40, 1:80 dilutions.

Wassermann reaction: positive (+ + +) Feces: positive for hookworm.

On February 25, (fifteen days after inoculation) the patient began to have a slight rise in temperature in the afternoon. The patient was not confined to bed. His temperature was slightly raised to 37.3°C. in the evenings and normal or subnormal in the mornings. He made no complaint other than that he felt slightly depressed in the evenings. On March 4, after running fever for seven days his temperature was normal and stayed so for three days when it started to rise again slightly in the evenings and came down again in the mornings to 30°C in the afternoons which lasted for eight days when his temperature was again normal for two days only to rise again as before.

The patient had very little if any constitutional symptoms until March 30 when he complained of severe cramps in the abdomen and had diarrhea. This condition continued for eight days with nausea pain over the abdomen and severe diarrhea with mucous.

Examination of the stools several times revealed only the presence of *B. coli, B. proteus* and *B. abortus.* In spite of the slight fever, the patient began to weaken markedly, due to his gastro-intestinal symptoms. To relieve him bismuth and paregoric were used liberally, and finally we decided to administer acriflavine intravenously. The patient was given three intravenous injections of 5 cc of a two percent solution of acriflavine. The injections were given every other day. After these the temperature came down. The patient had apparent relief.

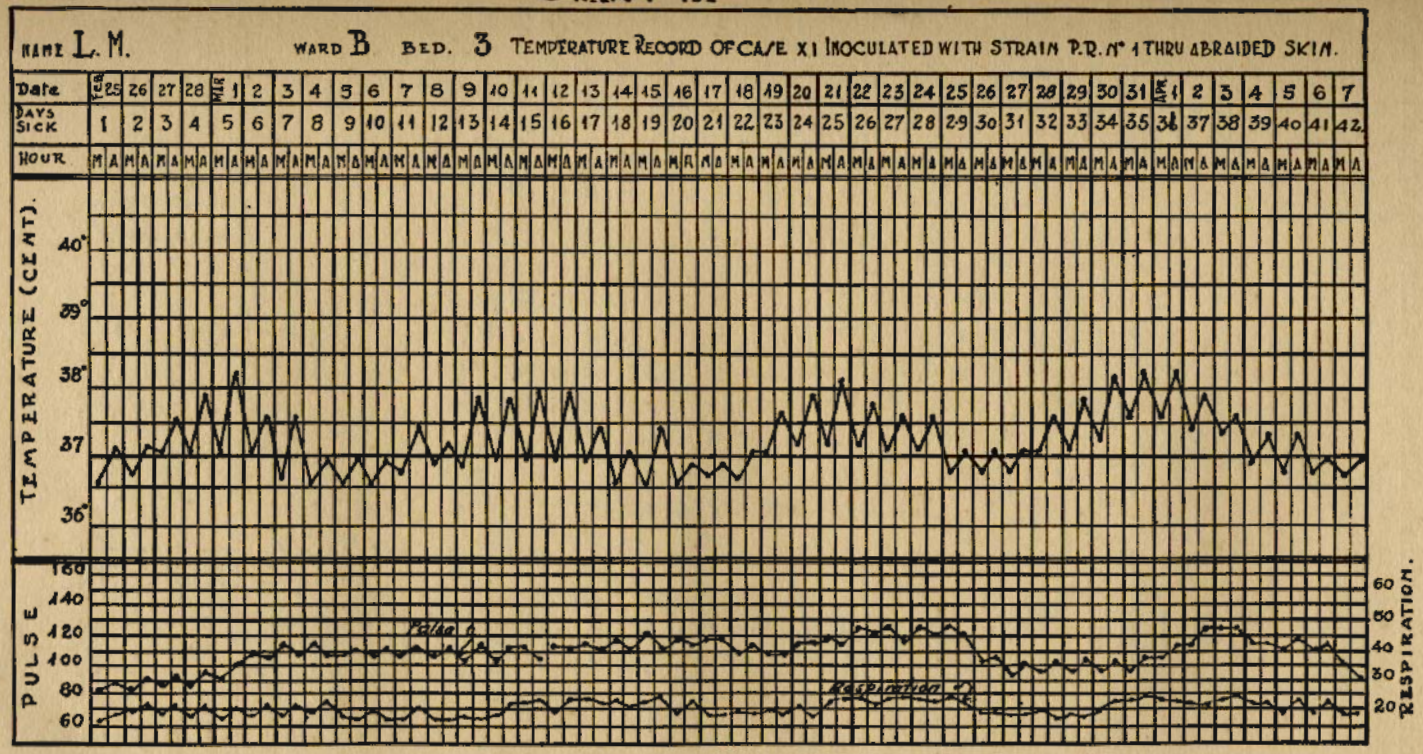
All blood cultures made on this case were negative for *B. abortus.* His serum agglutinated *B. abortus* in a dilution 1:320 on the third week of the disease.

Case No. XII.

On the same date (February 10th), A. .R, Mulatto, 22 years old, weighing 127 pounds, was inoculated through the normal skin. The laboratory findings before inoculation were: W. B. C. 7,000. R. B. C. 4,500,000. Hemoglobin eighty per cent. Differential count: Polymorphonuclears seventy-two per cent. Small lymphocytes twenty-seven per cent. Large lymphocytes one per cent. Malaria: negative. Widal reaction: negative. Agglutination for *B. abortus:* negative. Wassermann reaction: negative. Feces: negative for parasites. Urine. normal.

The patient went through the period of observation with no symptoms suggestive of infection. His temperature was always

CHART IX


NAME L. M.
WARD B BED. 3 TEMPERATURE RECORD OF CASE XI INOCULATED WITH STRAIN P.R. N° 1 THRU ABRADED SKIN.
Date
Days Sick
Hour
TEMPERATURE (CENT).
40°
39°
38°
37
36°
PULSE
160
140
120
100
80
60
Pulse
Respiration
RESPIRATION.
60
50
40
30
20

normal or subnormal. The blood culture and agglutination test for *B. abortus* were negative on April 14.

Strain *B. melitensis* 428 A. (human source). Collection of the School of Tropical Medicine. Date of isolation unknown.

Case No. XIII.

A. R. P., age 26, white (male) weighed 134 pounds. Physical condition apparently normal.

Laboratory findings: W. B. C. 6,000. R. B. C. 4,500,000. Hemoglobin seventy-five per cent. Wassermann reaction negative. Differential count: Polymorphonuclears, seventy-two per cent. Large lymphocytes two per cent. Small lymphocytes, twenty-three per cent. Eosinophiles, one per cent. Transitionals, two per cent. Agglutination test for *B. melitensis abortus:* Negative. Feces: Negative for intestinal parasites. Urine: No albumen, no glucose, no casts. On August 9 the patient was fed a twenty-four hour broth culture of *B. melitensis abortus* (428 A) and at the same time a mouse was injected intraperitoneally with 1 cc of a twenty-four hour culture of the same strain. The mouse died the 19th (ten days after injection). At autopsy the animal revealed similar lesions to those previously described. The organism was isolated from the viscera.

The patient was observed very closely and showed no symptoms suggestive of undulant fever. His blood culture and agglutination reaction for *B. melitensis abortus* were both negative on August 31 (twenty-two days after inoculation).

Examination of the patient on September 15 showed nothing abnormal. The blood culture and agglutination test were negative for *B. abortus.* The patient was discharged from the hospital.

Two guinea-pigs were inoculated, one through abraded skin and the other through normal skin. After four weeks they were autopsied. The organism could not be isolated from their viscera.

Case No. XIV.

C. B., white, 27 years old, weighed 145 pounds. Was inoculated through abraded skin with strain *Brucella melitensis* 428 A, on February 10.

Laboratory findings before inoculation were as follows: W. B. C. 6,500. R. B. C. 4,000,000. Hemoglobin eighty per cent. Differential count: Polymorphonuclears sixty-eight per cent. Large lymphocytes two per cent. Small lymphocytes twenty-six per cent. Eosinophiles four per cent. Malaria: negative. Widal reaction: negative. Wassermann: negative. Agglutination for *B. abortus:* negative. Feces: negative for parasites. Urine: Normal.

Patient continued to feel well throughout the period of observation. Had no symptoms or signs suspicious of infection. His temperature has been normal throughout and his blood culture and agglutination test were negative for *Brucella abortus* on April 15.

Case No. XV.

F. A. L., colored, 24 years old, weighs 146 pounds.

Laboratory findings: W. B. C. 6,300. R. B. C. 4,200,000. Hemoglobin seventy-five per cent. Differential count: polymorphonuclears sixty-eight per cent. Small lymphocytes twenty-nine per cent. Large lymphocytes two per cent. Eosinophiles one per cent. Wassermann: negative. Malaria: negative. Widal reaction: negative. Agglutination test for *Brucella abortus*, negative in 1:20, 1:40, 1:80. Feces, negative for parasites. Urine, normal.

The patient was inoculated through normal skin on February 10. He was closely observed throughout the period of observation and had no symptoms or signs suggestive of infection. The blood culture and agglutination test were negative for Brucella abortus on April 10. These three cases could not be infected with a known human strain that had been cultured on artificial media for many years. The guinea-pigs did not contract infection. Mice injected intraperitoneally died and showed lesions after ten days.

Strain *Brucella abortus* P. R. II (Bovine source) Isolated in Porto Rico. August 1929.

Case No. XVI.

J. D., colored, 23 years old, weighed 157 pounds, apparently normal and in good health.

Laboratory findings: W. B. C. 7,200. R. B. C. 5,000,000. Hemoglobin ninety per cent. Differential count: polymorphonuclears seventy-three per cent. Large lymphocytes seven per cent. Small lymphocytes twenty per cent. Wassermann: negative. Malaria: negative. Widal reaction: positive in 1:80 dilutions (gives a history of vaccination). Feces, negative for parasites. Urine, normal.

On February 17 the patient was fed a twenty-four hour broth culture of *Brucella melitensis abortus* strain PR II in half a pint of pasteurized milk. The same day a mouse was injected intraperitoneally with 1 cc. of a twenty-four hour broth culture of the same organism. The mouse died six days after inoculation. Cultures made from the spleen on liver infusion agar were found positive for *B. abortus*.

The patient was closely observed and showed no symptoms. The blood culture and agglutination test were negative for *Brucella abortus* on April 22.

Two guinea-pigs were inoculated on February 10, one through normal skin and the other through abraded skin. They were autopsied after four weeks. *B. abortus* was cultured from the lymph glands of the guinea-pig inoculated through abraded skin after prolonged incubation.

Case No. XVII.

R. M., colored, 22 years old was inoculated through abraded skin with strain P R II, on February 10. The laboratory findings before inoculation were: W. B. C. 5,600. R. B. C. 3,750,000. Hemoglobin eighty per cent. Differential count: Polymorphonuclears sixty-five per cent. Large lymphocytes four per cent. Small lymphocytes twenty-three per cent. Eosinophiles eight per cent. Widal reaction: negative. Malaria: negative. Agglutination test for *B. abortus:* negative 1:40–1:80. Wassermann reaction: positive (++++). Feces, positive for hookworm. Urine, normal.

The patient was closely observed. He had been complaining of general malaise, mild headache and little if any desire for food. On February 20, (ten days after inoculation) his temperature went up to 38°C in the evening. The next day his temperature went up to 38°C and every day rose slightly higher reaching on the 7th day to 39.7°C. Fever persisted for ten days, when it declined. The patient was afebrile for three days and again the temperature began to rise in the evenings, this time persisting for sixteen days when it was normal again for six days, only to rise again as before. His blood culture and agglutination test were positive for *B. abortus.*

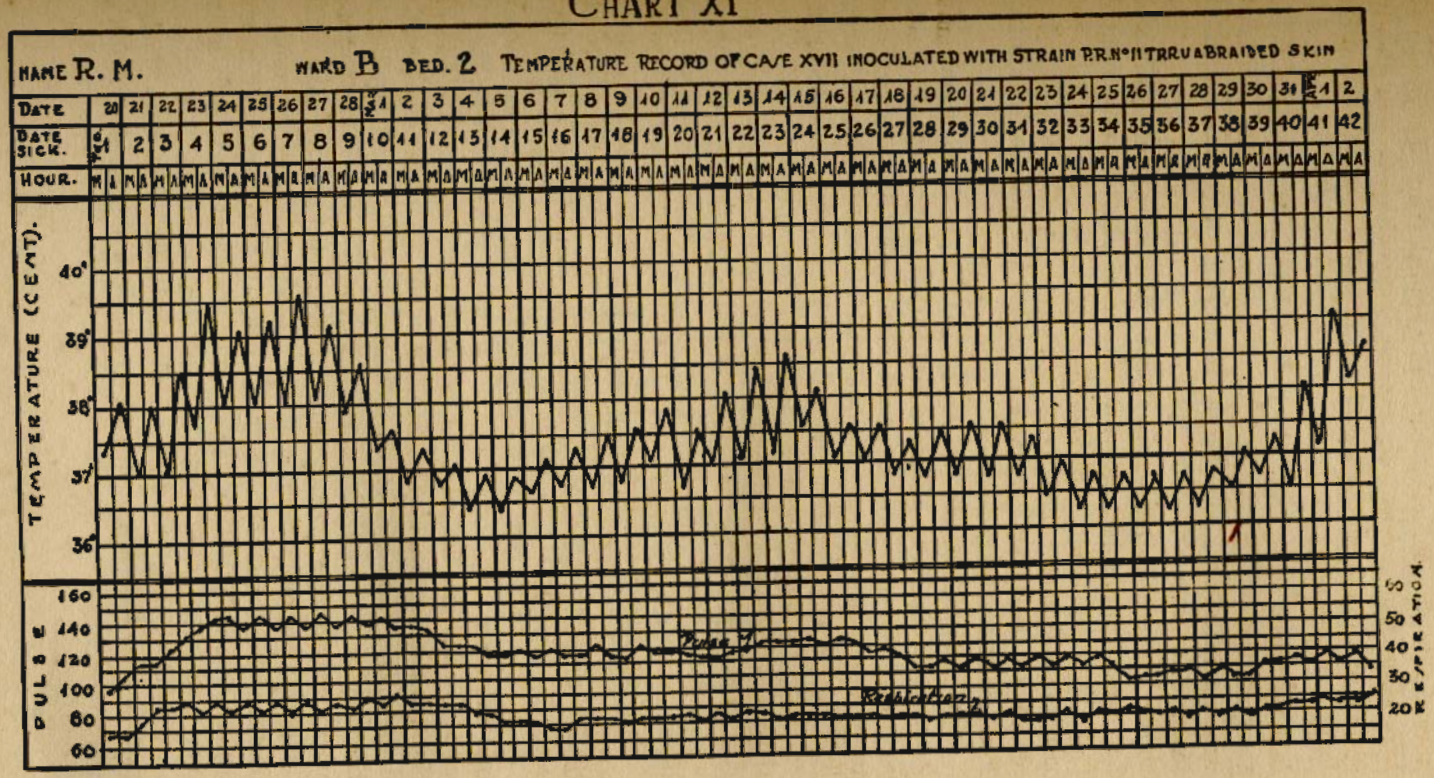
On physical examination the patient showed nothing abnormal except a very mild bronchitis. He complained through his illness of severe frontal headache, intense polyarticular pain and severe cold sweats at night.

Case No. XVIII.

R. S., mulatto, 23 years old, weighed 157 pounds. Was inoculated through normal skin on February 10. The laboratory findings before inoculation were as follows: W. B. C. 6,520. R. B. C. 4,200,000. Hemoglobin seventy percent. Differential count: Polymorphonuclears sixty-five per cent. Large lymphocytes one per cent. Small lymphocytes twenty-eight per cent. Eosinophiles six per cent. Malaria: negative; widal reaction, negative; Wassermann reaction, negative. Agglutination for *B. abortus,* negative. Feces, negative for parasites. Urine, normal.

On March 3rd, (21 days after inoculation) this man became ill, with severe chills and high fever. He had nausea and vomiting at

CHART XI


NAME R. M.
WARD B BED. 2 TEMPERATURE RECORD OF CASE XVII INOCULATED WITH STRAIN P.R.N°11 TRRU ABRAIDED SKIN
DATE
DATE SICK.
HOUR.
TEMPERATURE (CENT).
40°
39°
38°
37°
36°
PULSE
160
140
120
100
80
60
Pulse
Respiratory
RESPIRATION
50
40
30
20

the onset of his illness, complained of pain in the back, especially over the lumbar region. The next day his temperature was normal but on March 5 he again had a severe chill and a temperature of 39.5°C. Blood examination for malaria was positive for *P. vivax*. Upon administration of quinine the patient became well. He was closely observed thereafter and had no symptoms or signs indicative of Brucella infection. The blood culture and agglutination test were negative for *B. abortus* on April 22.

Strain Brucella melitensis P & S (Human source) Collection of the School of Tropical Medicine. Date of isolation unknown.

Case No. XIX.

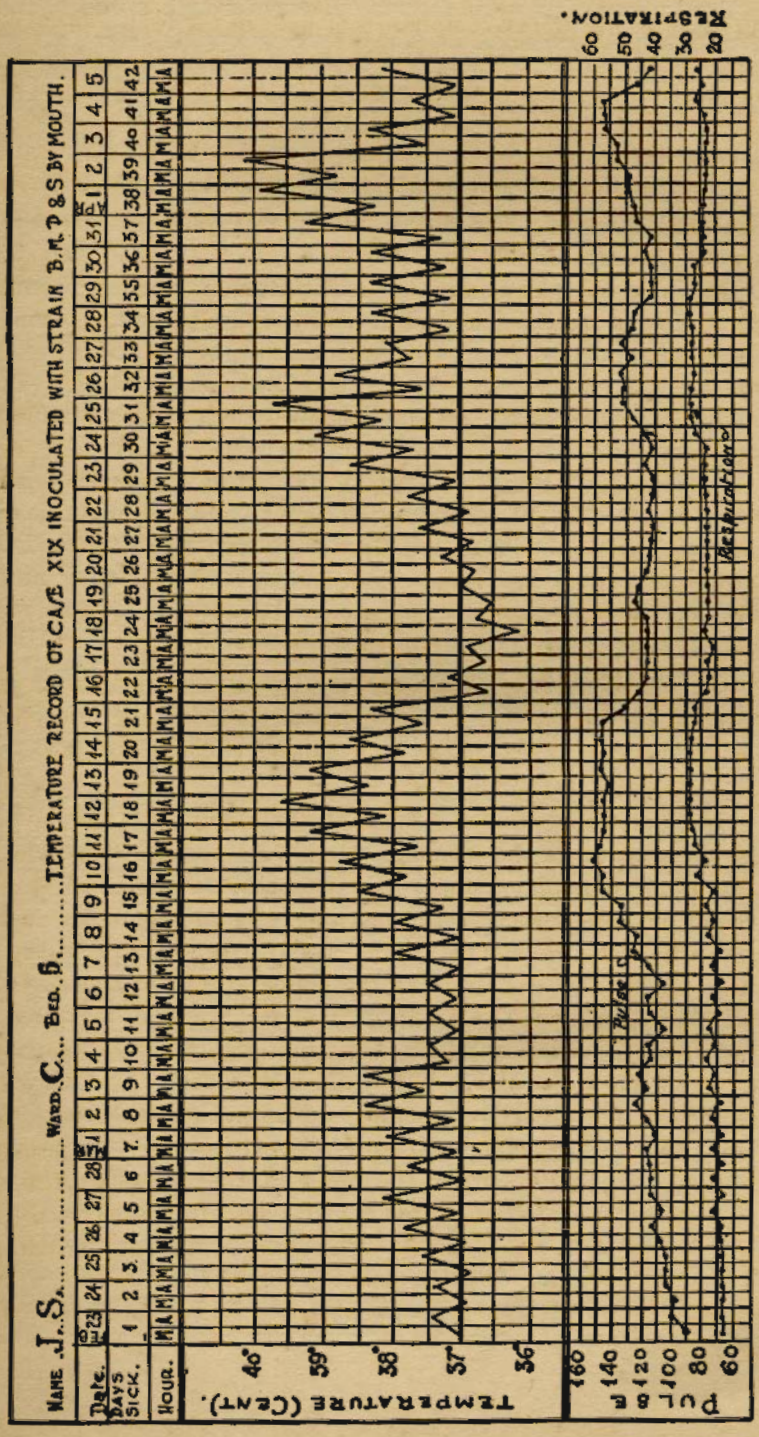
J. S. C., mulatto, 23 years old, weighed 106 pounds; in apparently good health.

Laboratory findings: W. B. C. 6,700. R. B. C. 4,500,000. Hemoglobin eighty percent. Differential count: polymorphonuclears seventy-two per cent. Small lymphocytes twenty-two per cent. Large lymphocytes four per cent. Eosinophiles two per cent. Malaria: negative. Widal reaction: negative. Wassermann reaction, negative. Feces: negative for parasites. Urine, normal.

On February 17 this man was fed a twenty-four hour broth culture of strain B. M. P & S. A mouse was injected intraperitoneally with 1 cc of broth of a twenty-four hour culture. The mouse died twenty-five days after injection.

The patient continued well until February 23 (ten days after inoculation) when he complained of an intense headache, and generalized muscular pains. His temperature was 37.4°C in the evening and subnormal in the morning. The temperature increased gradually each day and on March 2 and 3 it was 38.4 in the evenings. On the 9th day of fever the temperature came down to 37°C and was slightly increased (37.5°C) in the evenings for three days, at the end of which it began increasing slightly every evening and within six days (on March 13) it reached 39.2°C. The temperature again came down by lysis, remained subnormal for three days (March 17th) only to rise again as before. Blood culture was positive on February 27 (5 days after the onset of the disease). His serum agglutinated in 1:100 on March 4 (ten days after onset). On April 2 his serum titer was 1:2560. The blood count on this date was: W. B. C. 2,680. Differential count: Polymorphonuclears forty-six per cent. Large lymphocytes fourteen per cent: Small lymphocytes forty per cent.

CHART V.


NAME J.S. WARD C. BED 6 TEMPERATURE RECORD OF CASE XIX INOCULATED WITH STRAIN B.M.P & S BY MOUTH.
Date.
Days sick.
Hour.
TEMPERATURE (CENT.)
PULSE
RESPIRATION.
Re-inoculation

Two guinea-pigs were inoculated, one through abraded and the other through normal skin. They were autopsied after four weeks. Cultures made on liver infusion agar from the the spleen and lymph glands of both experimental animals were positive for *B. melitensis.*

Case No. XX.

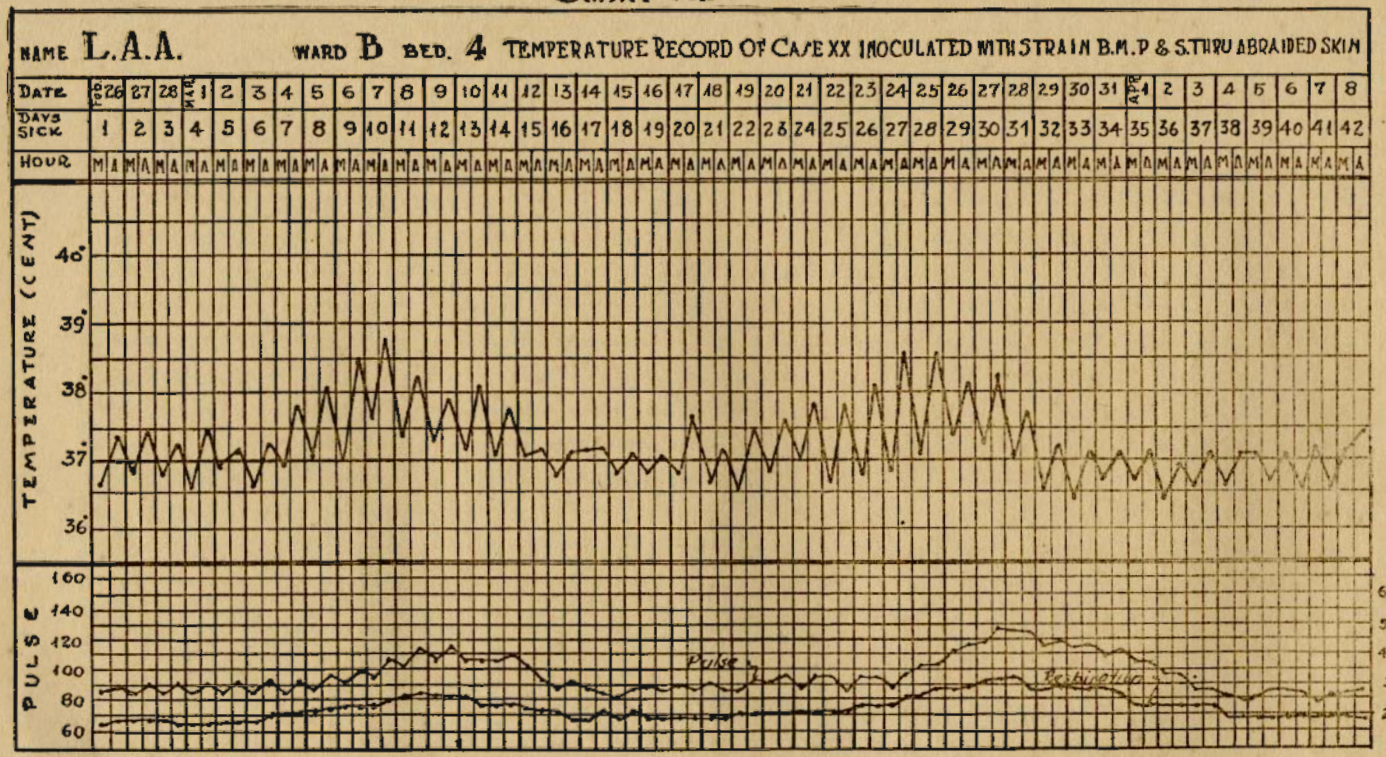
L. A. A., colored, 20 years old and weighing 138 pounds. Was inoculated through abraded skin on February 10. Laboratory findings before inoculation: W. B. C. 7,300. R. B. C. 4,500,000. Hemoglobin eighty-five per cent. Differential count: Polymorphonuclears seventy-two per cent: Large lymphocytes, one per cent. Smal lymphocytes twenty-seven per cent. Malaria, negative. Widal reaction: negative. Wassermann reaction, negative. Agglutination for *B. abortus,* negative, 1:20, 1:40 and 1:80. Feces: negative for parasite. Urine, normal.

On February 11th (one day after inoculation) the site of inoculation was slightly inflamed and the next day the axillary lymph glands were painful, especially on palpation. These symptoms subsided and no local lesion could be recognized. The patient progressed normally until February 26 (sixteen days after inoculation) when he complained of severe headache and had no desire for food. His temperature on this date was 37.5°C. in the evening. He remained the same for seven or eight days, slightly ill in the evening and much better in the morning. His headache became worse at night. His temperature during these days fluctuated from 36.5°C. to 37.5 or 37.8°C. From March 5 on his temperature increased gradually in the evenings reaching 38.7°C. on the afternoon of March 7. From then on it gradually declined and from March 12 to 16 his temperature was about normal. On the 17th it started to rise gradually as before. His blood culture was positive on the 10th day of the disease (March 7). The agglutination test in this case had been negative in dilutions of 1:20, 1:40, 1:80 and 1:160 several times. Since this seemed to be a typical case of infection we reported agglutination tests several times in these dilutions but always with negative results. On March 24 (twenty-seven days after onset) his serum titrated to a high dilution. The serum was negative in dilutions from 1:40 to 1:320 but was strongly positive in dilutions 1:640 and 1:1280.

Case No. XXI.

J. R. D., white, 33 years old, weighing 150 pounds was inoculated on the normal skin on February 10 with strain B. M. P & S. The laboratory findings before inoculation were as follows: W. B. C.,

CHART VIII.


NAME L.A.A. WARD B BED. 4 TEMPERATURE RECORD OF CASE XX INOCULATED WITH STRAIN B.M.P & S.THRU ABRAIDED SKIN
DATE
DAYS SICK
HOUR
TEMPERATURE (CENT)
PULSE
RESPIRATION
Pulse
Respiration

6,000. R. B. C., 5,000,000. Hemoglobin, ninety per cent. Differential count: polymorphonuclears, sixty-two per cent. Large lymphocytes six per cent. Small lymphocytes thirty-two per cent. Malaria: negative. Widal reaction, negative. Wassermann reaction: negative. Agglutination test for B. abortus; negative 1:40–1:80 dilutions. Feces: Negative, for parasites. Urine, normal.

This patient went through the period of observation and showed no signs or symptoms suggestive of infection. On April 7 his blood culture and agglutination test were negative for *B. abortus.*

Strain *Brucella Melitensis* (420) Equine Source. (Courtesy of Dr. F. Huddleson.) Date of isolation 1921.

This strain has been classified as a porcine strain. A mouse was injected with 1 cc of a twenty-four hour broth culture intraperitoneally. The mouse died in six days. Cultures made from the spleen and lymph gland were positive for *B. abortus.*

Case No. XXII.

On February 17, a twenty-four hour broth culture of the organism was fed to S. O., white male, 22 years old, weighing 165 pounds. The laboratory findings before inoculation were: W. B. C. 7,200. R. B. C. 4,500,000. Hemoglobin eighty per cent. Differential count: Polymorphonuclears seventy per cent. Small lymphocytes twenty-six per cent. Large lymphocytes four per cent. Malaria, negative. Widal reaction, negative. Wassermann reaction, negative. Agglutination test for *B. abortus,* negative 1:40–1:80 dilutions. Feces, negative for parasites. Urine, normal.

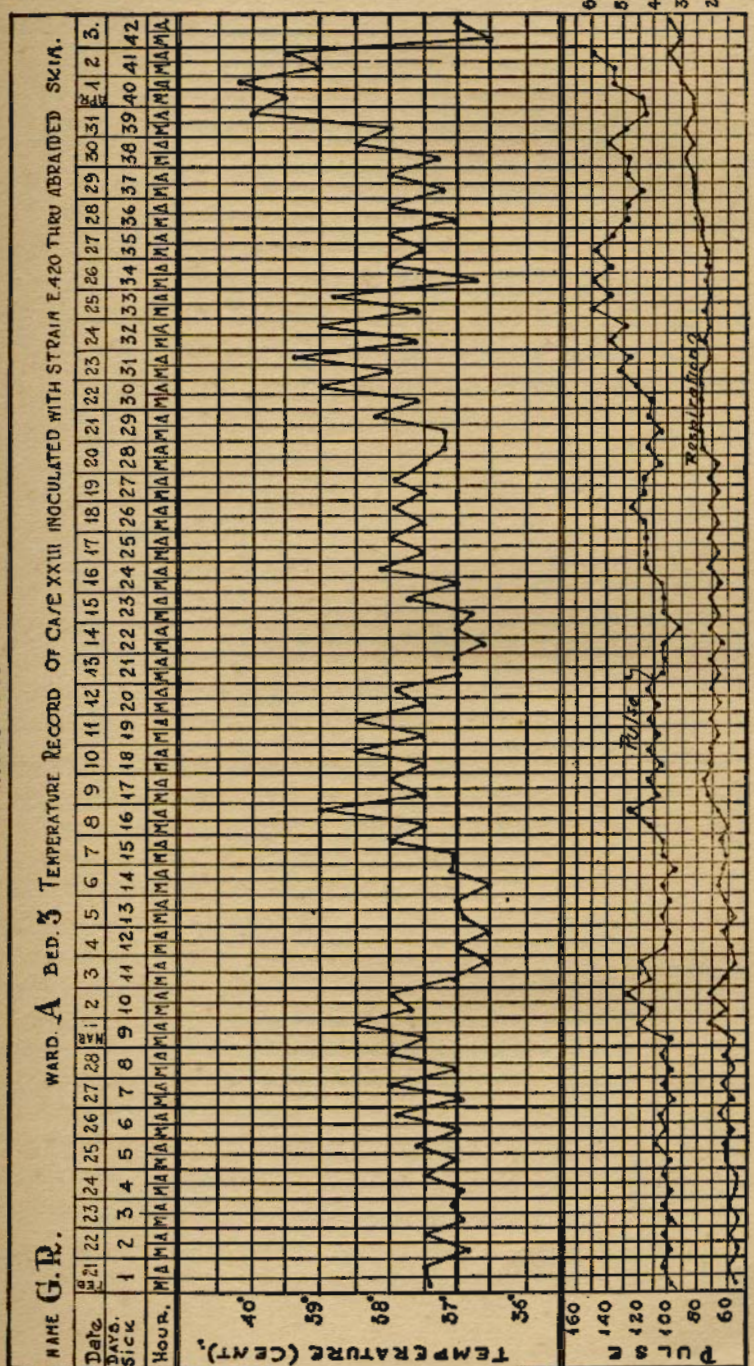
This man had no complaint during the period of observation. He felt well, ate and slept well, was able to go about and do his work. He had a slight temperature in the evening fluctuating from .2 to .5 of a degree C. from March 6 (seventeen days after feeding). His temperature was very irregular, having several days without fever and then having some days with a slight evening rise. He had no other symptom suggestive of infection. His physical findings were essentially negative. His blood culture was negative several times. His blood serum agglutinated 1:40 dilutions on April 10.

Two guinea-pigs were inoculated, one on the normal skin and the other on the abraded skin. One guinea-pig died one week after inoculation. The other, three weeks after inoculation. Cultures made from the spleen were positive in both cases.

Case No. XXIII.

G. R., mulatto, 22 years old, weighed 117 pounds. Was inoculated on the abraded skin February 10. Laboratory findings are as fol-

CHART VII


NAME G.R. WARD A BED 3 TEMPERATURE RECORD OF CASE XXIII INOCULATED WITH STRAIN E.420 THRU ABRADED SKIN.
TEMPERATURE (CENT).
PULSE
RESPIRATION.
Pulse
Respiration

lows: W. B. C. 4,600. R. B. C. 4,500,000. Hemoglobin eighty per-cent. Differential count Polymorphonuclears sixty-five per cent. Small lymphocytes twenty-five per cent. Large lymphocytes two per cent. Eosinophiles six per cent. Transitionals two per cent. Malaria, negative. Widal reaction, negative. Wassermann reaction, negative. Agglutination test for *B. abortus, negative.* Feces, positive for hookworm. Urine, normal.

February 21 (eleven days after inoculation) this man became ill. He had chills, generalized muscular pains and a slight temperature of 37.5°C. His temperature was normal in the morning and slightly increased in the afternoons, increasing gradually, reaching its maximum on March 1st (38.5°C) when it began to decline. It was normal or subnormal for four days then it gradually rose again forming several wave-like undulating curves.

On March 31 (thirty-nine days after onset) the patient was very ill. His temperature was 40°C. He had generalized muscular and articular pains, severe cold sweats at night. He had lost weight progressively and was markedly emaciated and weak. His pulse (145) was weak and feeble. We decided to give him 5 cc of a two percent acriflavine solution intravenously. His temperature was 39.5°C. the next morning but in the afternoon it reached 40.2°C. On the next day another injection was given. His temperature rose to 39.5°C. The day following this injection his temperature dropped to normal. Two more injections were given. The patient had a normal temperature for fifteen days and gained five pounds in weight.

The blood culture of this patient was positive on February 27 (seven days after onset). His serum agglutinated on the second week in 1:160 and on the sixth week in 1:2560.

Case No. XXIV.

L. V., Age 23, mulatto, weighs 123 pounds. Was inoculated on the normal skin February 10. His laboratory findings before inoculation were: W. B. C. 9,000. R. B. C. 5,000,000. Hemoglobine ninety percent. Differential Count: Polymorphonuclears seventy-six per-cent. Large lymphocytes two per cent. Small lymphocytes eighteen per cent. Eosinophiles fourteen per cent. Widal Reaction: Negative. Malaria: Negative. Wassermann Reaction: Positive (+++). Agglutination for B. abortus: Negative 1:40, 1:80, 1:160. Feces: Positive for trichuris, ascaris and ankylostoma. Urine: Normal. This patient had no signs or symptoms indicative of infection.

As seen by the results published so far it has been impossible for us to obtain infection by feeding bovine strains. We decided to

obtain further data by feeding freshly isolated strains from the mainland. Through the courtesy of Dr. C. M. Carpenter and Dr. H. V. Hardy we were able to obtain freshly isolated bovine strains from New York and Iowa. Since it is hard to get volunteers for the work we omitted inoculations through abraded skin. Previous inoculations have proven the possibility of this method of infection. Patients were fed cultures by mouth and inoculated on the normal skin with these new strains.

Strain *B. abortus* 508 (bovine) isolated from milk. Courtesy of Dr. C. M. Carpenter.

Case No. XXV.

M. R., white, 52 years old and weighing 111 pounds, was fed a twenty-four hour broth culture of *B. abortus* 508 in pasteurized milk. The laboratory findings before feeding were: W. B. C. 7,500. R. B C. 4,000,000. Hemoglobin eighty per cent. Differential Count: Polymorphonuclears sixty-two per cent. Large lymphocytes six per cent. Small lymphocytes twenty-two per cent. Eosinophiles ten per cent. Malaria: Negative. Wassermann: Negative. Agglutination test for *B. abortus:* Negative 1:40, 1:80 and 1:160. Feces positive for hookworm and bilharzia. Urine: Normal.

A mouse was injected intraperitoneally with 1 cc of broth of a twenty-four hour broth culture. The mouse died four days after inoculation. The organism was recovered from the viscera.

The patient was closely observed from the date of feeding, May 28, and developed no signs or symptoms indicative of infection (June 15). The serum agglutination and blood culture were negative.

Case No. XXVI.

J. R. colored, 23 years old and weighing 106 pounds, was inoculated on the normal skin with strain Brucella abortus 508. The laboratory findings before inoculation were as follows: W. B. C. 3,600. R. B. C. 3,500,000. Hemoglobin seventy per cent. Differential count: Polymorphonuclears seventy per cent. Small lymphocytes twenty-five per cent. Eosinophiles 1.5 per cent. Basophiles five per cent. Wassermann Reaction: Positive (++++). Agglutination test for *B. abortus:* Negative: 1:40, 1:80, 1:160. Widal Positive (probably vaccination). Feces: Negative for parasites.

The guinea pig inoculated through unabraded skin was autopsied four weeks after inoculation. Cultures made from the spleen were positive for B. abortus. The patient was carefully observed through the period of observation and had no signs or symptoms of undulant

fever. His serum agglutination and blood culture were negative on June 15–30 (45 days after inoculation).

Strain *Brucella abortus* 646 (bovine) isolated from cows' milk. Courtesy of Dr. C. M. Carpenter.

Case No. XXVII.

On April 28, M. C., white, 22 years old and weighing 122 pounds, was fed a twenty-four hour broth culture of strain Brucella abortus 646 in pasteurized milk At the same time a mouse was injected as a control as in other cases. The laboratory findings of the patient were: W. B. C. 6,650. R. B. C. 3,500,000. Hemoglobin sixty-five per cent. Differential Count: Polymorphonuclears sixty per cent. Small lymphocytes thirty-two per cent. Large lymphocytes six per cent Eosinophiles two per cent. Malaria: Negative. Widal: Negative. Wassermann (+ + + +) Agglutination test for B. abortus: Negative in 1/40, 1/80, 1/160 dilutions. Feces: Positive for trichuris and ankylostoma. Urine: Normal.

The mouse died six days after injection. Cultures made from the spleen were positive for B. abortus.

The patient was closely observed and was perfectly normal throughout the period of observation. The blood culture and agglutination were negative for *B. abortus* on June 15 (48 days after inoculation).

Case No. XXVIII.

E. F., white, male 18 years old, weighing 119 pounds, was inoculated on the normal skin with strain B. abortus 646, at the same time a guinea-pig was inoculated with the same strain on the normal skin. The laboratory findings of this patient were: W. B. C. 3,700. R. B. C. 4,750,000. Hemoglobin eighty-five. Differential Count: Polymorphonuclears seventy-four percent; small lymphocytes thirty-three percent. Large Lymphocytes three percent. Malaria: Negative. Widal Reaction: Negative. Wassermann: Negative. Agglutination test for B. abortus: Negative 1:40, 1:80 and 1:160. Feces: Negative for parasites. Urine: Normal.

The guinea-pig was autopsied four weeks after inoculation. Cultures made from the spleen were negative.

The patient passed through the period of observation and on June 15 (45 days after inoculation) the blood culture and agglutination test for *B. abortus* were negative.

Strain Brucella abortus 649 (Bovine isolated from cows' milk) Courtesy of Dr. C. M. Carpenter.

Case No. XXIX.

E. L., colored, 23 years old, weighing 133 pounds, was fed a twenty-four hour broth culture of strain B. abortus 649 in pasteurized milk on April 28.

A mouse was injected as a control as in other cases.

Laboratory findings of the patient before inoculation were: W. B. C. 7,800. R. B. C. 5,000,000 Hemoglobin ninety per cent. Differential Count: Polymorphonuclears seventy per cent. Small lymphocytes twenty-two per cent. Large lymphocytes three per cent. Malaria: Negative. Widal Reaction: Negative. Wassermann Reaction: (+++). Aggutination test for B. abortus: Negative: 1:40, 1:80, 1:160. Feces: Negative for parasites. Urine: Normal.

The mouse inoculated died after two days. Cultures made from the spleen and lymph glands were positive for B. abortus.

The patient was carefully observed and developed no signs or symptoms indicative of infection. The blood culture and agglutination test were negative for B. abortus on June 15, 1930.

Case No. XXX.

L. T., white male, age 25 years, weighing 138 pounds, was inoculated on the normal skin with strain *B. abortus* 649. The same day a guinea pig was inoculated on the normal skin with the same strain and in the usual manner. The patient's laboratory findings before inoculation were as follows: W. B. C. 6,500. R. B. C. 4,200,000. Hemoglobin eighty-five per cent. Differential Count: Polymorphonuclears sixty per cent. Small lymphocytes thirty per cent. Large lymphocytes 6.5 per cent. Eosinophiles 1.5 per cent. Transitionals two per cent. Wassermann: Negative. Malaria: Negative. Widal: Negative. Agglutination for *B. abortus* negative: 1:40, 1:80, 1:160. Feces: Negative for parasites. Urine: Normal.

The guinea-pig was autopsied four weeks after inoculation. Cultures from the spleen were positive for B. abortus.

The patient was closely observed and developed no signs or symptoms of the disease. The blood culture and agglutination test were negative on June 15, 1930.

Strain No. 58 Busic (Bovine) isolated from the cream of the Busic family cow. (Courtesy of Dr. A. V. Hardy.)

History of the strain:

No. 58 Busic. Organism isolated from the cream of the Busic family cow. Mrs. Busic one year ago contracted tularemia which ran a typical course. Blood for agglutination test taken October 23, 1929, which resulted in the agglutination of Brucella abortus in dilutions 1:640 and bacterium tularense in 1:160. Dr. Hardy investi-

gated the case finding a typical case of undulant fever. The specific organism was not found in blood cultures.

Willis Busic about the same time developed a typical clinical case of undulant fever, the patient agglutinated Brucella abortus in dilutions 1:2560, but the specific organism was not obtained from blood cultures.

One cc. of cream from the family cow was inoculated into each groin of two guinea pigs both of which developed high titres. Autopsy of the first pig revealed only an enlarged spleen. Cultures on liver infusion agar from the spleen after four days under ten per cent CO_2 tension revealed typical colonies which agglutinated specific immune sera. The organism fails to grow in ordinary incubator but grows well in ten per cent Co_2.

Case No. XXXI.

M. C., white male, 30 years old, weighing 124 pounds ingested a twenty-four hour broth culture of strain No. 58 in pasteurized milk on April 28. At the same time a mouse was injected as a control. The patient's laboratory findings before the inoculation were: W. B. C. 3,300. R. B. C. 3,850,000. Hemoglobin seventy-five per cent. Differential count: Polymorphonuclears seventy per cent. Large lymphocytes four per cent. Small lymphocytes twenty-two per cent. Eosinophiles four per cent. Basophiles one per cent. Transitionals one per cent. Wassermann Reaction: Negative. Widal Reaction: Negative. Malaria: Negative. Agglutination reaction for *B. abortus:* Negative 1:40 1:80, 1:160. Feces: Negative for parasites. Urine: Normal.

The mouse died twenty-four hours after inoculation. Cultures made from the spleen were positive for B. abortus.

The patient was normal throughout the period of observation and on June 17 (50 days after inoculation) the blood culture and agglutination test for *B. abortus* were negative.

Case No. XXXII.

V. R. mulatto, age 22 years, weighing 129 pounds was inoculated on the normal skin with strain No. 58 on April the 28th. At the same time a guinea-pig was inoculated as a control on the normal skin. The laboratory findings of this patient before inoculation were: W. B. C. 6,200. R. B. C. 5,000,000. Hemoglobin ninety-five per cent. Differential count: Polymorphonuclears sixty per cent. Small lymphocytes thirty-three per cent. Large lymphocytes four per cent. Eosinophiles one per cent. Transitionals two per cent.

Malaria: Negative. Widal Reaction: Negative. Wassermann: Positive (++). Agglutination: for *B. abortus*. Negative in 1:40, 1:80, 1:160 dilutions. Feces: Negative for intestinal parasites. Urine: Normal.

The guinea-pig was autopsied four weeks after inoculation. Cultures made from the spleen were positive for B. abortus.

The patient was closely observed throughout the period of observation and was always found normal. On June 15th the blood culture and agglutination test for *B. abortus* were negative. Stain 263 Oakdale Pig (Bovine). (Courtesy of Dr. Hardy.) History of the strain:

No. 263 Oakdale pig: Eight cows to the herd developed demonstrable agglutinations in the blood. Cream taken from each of these cows was inoculated into two pigs. Pigs from seven of these cows gave high titres ranging from 1:160 to 1:10,240.

No. 263 was bled four weeks after inoculation and manifested a high titre. At six weeks the pig was autopsied and the blood titred 1:10,240. The spleen was six times normal size, congested and irregularly roughened but no abscesses were demonstrable. The liver contained hundreds of pin points to pin-head-sized abscesses. A few small glands were found in the iliac region and groin. Blood culture revealed a bovine organism.

Case No. XXXIII.

R. R., white, 22 years old and weighing 123 pounds was fed a twenty-four hour broth culture of strain No. 263 in pasteurized milk. The same day a mouse was injected. The laboratory findings before inoculation were: W. B. C. 7,300. R. B. C. 4,500,000. Hemoglobin eighty-five per cent. Differential Count: Polymorphonuclears sixty-six per cent. Small lymphocytes thirty per cent. Large lymphocytes four percent. Malaria: Negative. Widal Reaction: Negative: 1:40, 1:80, 1:160. Feces: Negative for parasites. Urine: Normal.

The mouse died eighteen hours after inoculation. Cultures made from the spleen and liver were positive for B. abortus.

The patient was normal throughout the period of observation and the blood culture and agglutination test were negative on June 15.

Case No. XXXIV.

J. S., mulatto, 20 years old and weighing 137 pounds was inoculated on the normal skin with strain 263 on April 28. At the same time a guinea-pig was inoculated. Laboratory findings of the patient

before inoculation: W. B. C. 9,200. R. B. C. 3,500,000. Hemoglobin seventy per cent. Differential Count: Polymorphonuclears seventy-six per cent. Small lymphocytes twenty per cent. Large lymphocytes two per cent. Eosinophiles two per cent. Malaria: Negative. Widal Reaction: Negative. Wassermann Reaction: Positive (+++). Urethral exudate G. C. positive. Feces: Negative for parasites. Urine: Traces of albumen, no sugar, numerous pus cells.

The guinea pig was autopsied four weeks after inoculation. Cultures made from the spleen were positive for B. abortus.

The patient developed orchitis from his gonohrrea. Upon adequate treatment the patient improved. His blood culture and agglutination test for B. abortus were negative on June 15, 1930. Strain D. 304 Lerch (Bovine) Courtesy of Dr. Hardy. History of Strain:

No. D–304 Lerch: Mr. Lerch developed a typical clinical case of undulent fever. His blood showed agglutinins on high titre and a bovine organism was isolated from blood culture.

Cream from his four cows was inoculated into guinea-pigs and six of the eight developed titres.

Autopsy of pig D–304: glands in the groin were split-pea sized while those of the iliac group were navy-bean sized. The spleen was twice normal size but no abscesses were found. The liver contained numerous pin-point abscesses. Blood taken at autopsy agglutinates in dilutions of 1:1,280. Organisms were isolated from spleen, liver and lymph nodes. The organism was agglutinated by specific immune serum.

Case No. XXXV.

J. C., colored, 19 years old and weighing 148 pounds was fed a twenty-four hour broth culture of B. abortus D 304 in pasteurized milk. Simultaneously a mouse was injected. The laboratory findings of the patient before inoculation were: W. B. C. 6,500. R. B. C. 4,000,000. Hemoglobin 80 per cent. Differential count: Polymorphonuclears sixty per cent. Large lymphocytes two per cent. Small lymphocytes thirty per cent. Eosinophiles eight per cent. Malaria: Negative. Widal Reaction: Negative. Wassermann Reaction: Negative. Agglutination test for B. abortus 1:40, 1:80, 1:160. Feces: Positive for hookworm. Urine: Normal.

The mouse died twenty-four hours after inoculation. Cultures made from the lymph glands were positive for B. abortus.

The patient never showed any signs or symptoms of infection and was normal throughout the period of observation. The blood

culture and agglutination test were negative for *B. abortus* on July 15, 1930.

Case No. XXXVI.

I. S., white male, 22 years old weighing 120 pounds was inoculated on the normal skin with strain D–304 on April 28. A guinea-pig was also inoculated with the same strain on the same date.

Laboratory date of the patient before innoculation were: W. B. C. 4,000. R. B. C. 4,000,000. Hemoglobin eighty per cent. Differential count: Polymorphonuclears seventy per cent. Large lymphocytes twenty-two per cent. Small lymphocytes eight per cent. Malaria: Negative. Widal Reaction: Negative. Wassermann Reaction: Negative. Feces: Negative for parasites.

The guinea-pig was autopsied four weeks after inoculation. Cultures made from the organs were positive for B. abortus.

The patient was normal throughout the period of observation and his blood cultures and agglutination test were negative on June 15, 1930.

REPEATED INOCULATIONS

In view of the results of previous experiments we planned new ones repeating the dosage until the organism could be demonstrated in the stool.

Four patients were fed twenty-four hour broth cultures of the organism in pasteurized milk every twenty-four hours for several days.

Case No. XXXVII.

E. G. R., 23 years, white, weight 145 pounds. Had elephantiasis of left leg, otherwise normal.

Laboratory findings: W. B. C. 7,620. R. B. C. 3,500,000. Hemoglobin seventy-four per cent. Differential Count: Polymorphonuclears seventy-four per cent. Large lymphocytes four per cent. Small lymphocytes twenty-two per cent. Eosinophiles two per cent. Wassermann: Negative. Malaria: Negative. Agglutination test for B. abortus Negative. 1:40, 1:80, 1:160. Feces: Negative for parasites. Urine: Negative, albumen, glucose, negative. Sediment, no casts.

The patient was fed a twenty-four hour broth culture of *B. abortus* (Bovine), (Hyg. Lab. 456), in a half pint of pasteurized milk on August 26th, 27th, 28th, 29th and 30th (five cultures in all). The feces of this patient were examined daily. Seventy-two

hours after the first feeding Brucella abortus was isolated in the feces. The patient continued under observation and up to the present time has had no signs or symptoms of undulant fever. His blood culture and agglutination test for *B. abortus* were negative up to October 30, 1929. Patient was discharged.

Case No. XXXVIII.

L. R., white, age 22 years, weight 126 pounds. Had eruption resembling yaws over the body, otherwise apparently in good health. Laboratory findings: W. B. C. 6,250. R. B. C. 4,000,000. Hemoglobin seventy-five per cent. Differential Court: Polymorphonu-seventy-six per cent. Large Lymphocytes: two per cent. Small Lymphocytes: eighteen per cent. Eosinophiles: four per cent. Wassermann: positive (++++). Widal: Negative. Malaria: Negative. Agglutination for B. abortus: Negative. Feces: Negative for hookworm. Urine: Normal.

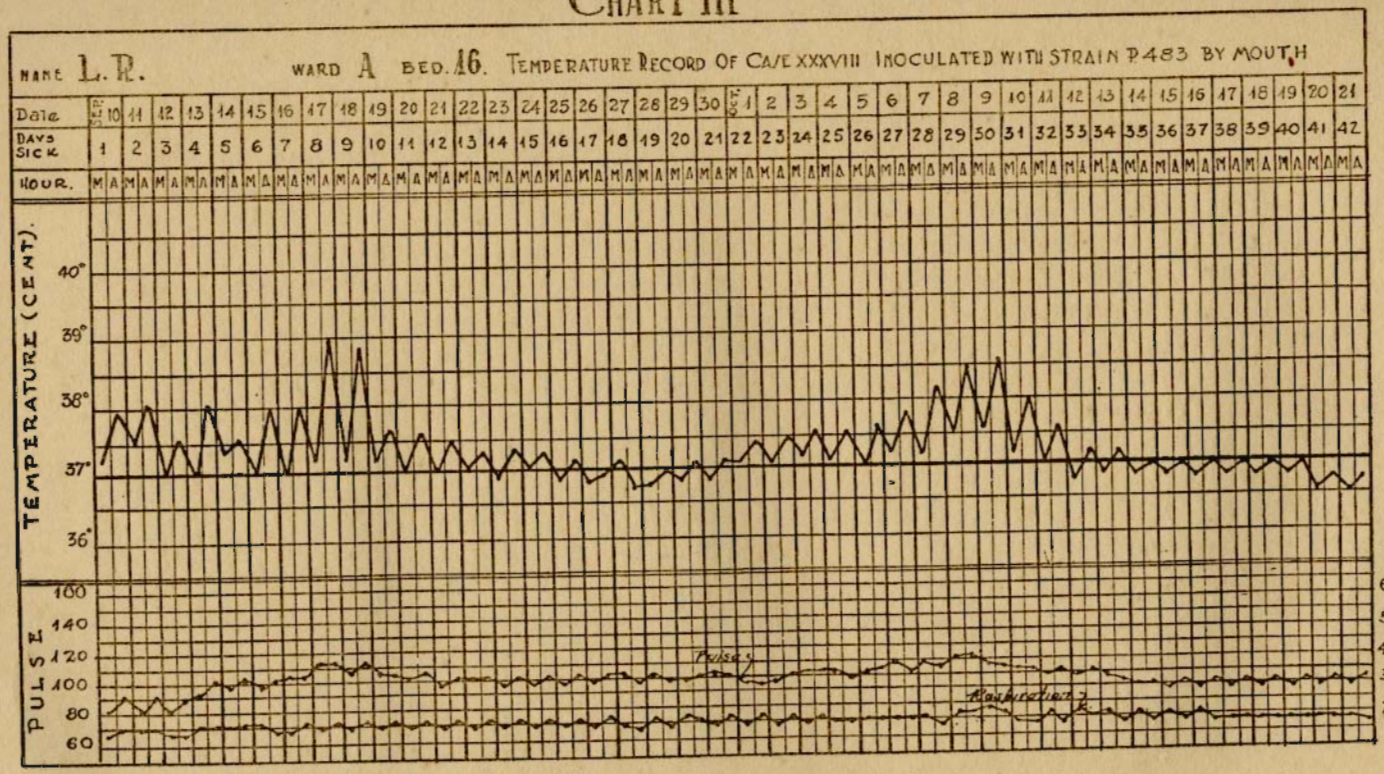
The patient was fed a twenty-four hour culture of B. abortus (Porcine) (Hyg. Lab. 483) in milk on August the 26th, 27th, 28th, 29th and 30th. Ninety-six hours after the first feeding the organism was isolated from the feces of the patient. Patient felt well until September 7, when he complained of severe headache. He had no fever at that time and a purgative was ordered. There was apparent relief from headache after purgation but be continued to have lassitude and general weakness and the 10th (eleven days after the last feeding and fifteen days after the first) began to have fever and a slight pain over the region of the spleen. Fever continued for a period of eleven days. The temperature then began to decline. He had no other symptoms suggestive of the disease. His blood culture was positive on September 15 (five days after the fever started) but his serum was negative for agglutination at this time.

His temperature was normal or subnormal for a period of six days and then it rose gradually being slightly higher every day and reaching 38.7°C., on October 9. At this time his serum agglutinated 1:320. On October 12 his temperature dropped to normal and was normal or subnormal for several days when another relapse began. His serum titer before leaving the hospital was 1:160.

Case No. XXXIX.

E. P., colored, 56 years and weighing 159 pounds. Was fed a twenty-four hour broth culture of *B. abortus* No. 508 in half pint of pasteurized milk on May 1st, 2nd, 3rd, 4th and 5th (five cultures in all). Laboratory findings: W. B. C. 7,800. R. B. C. 4,000,000. Hemoglobin seventy-five per cent. Differential Count: Polymorphonuclears

CHART III


NAME L. R. WARD A BED. 16. TEMPERATURE RECORD OF CASE XXXVIII INOCULATED WITH STRAIN P 483 BY MOUTH
Date
Days Sick
Hour.
TEMPERATURE (CENT).
40°
39°
38°
37°
36°
PULSE
160
140
120
100
80
60
Pulse
Respiration
RESPIRATION.
60
50
40
30
20

clears seventy-six per cent. Large lymphocytes four per cent. Small Lymphocytes twenty per cent. Widal: Negative. Malaria: Negative. Agglutination for B. abortus: Negative. Feces: Negative for parasites. Urine: Normal.

The patient has been under observation and up to the present time presents nothing abnormal. His blood culture and agglutination test were negative for B. abortus on June 25, 1930.

Case No. XL.

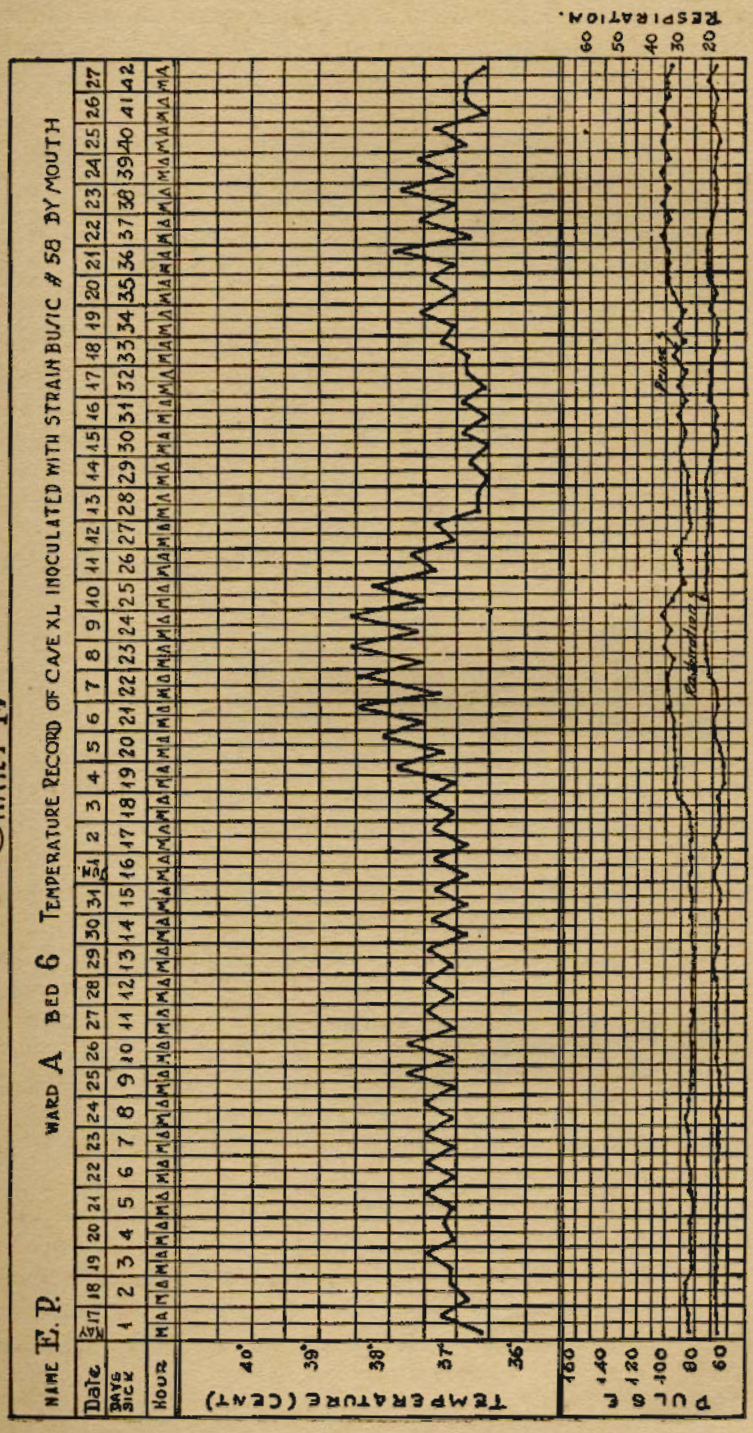
J. J. S., colored, age 23 years. Weight 140 pounds. Was fed a twenty-four-hour broth culture of *B. abortus* Busic No. 58 in half a pint of pasteurized milk on May 1st, 2nd, 3rd, 4th, 5th, 6th and 7th.

The laboratory findings of this patient before feeding were as follows: W. B. C. 6,000. R. B. C. 4,000,000. Hemoglobin: eighty per cent. Differential Count: Polymorphonuclears: sixty-two per cent. Large lymphocytes: four per cent. Small lymphocytes: thirty per cent. Eosinophiles: four per cent. Widal: Negative. Wassermann: Negative. Malaria: Negative. Agglutination test for *B. abortus* Negative: 1:40, 1:80, 1:160. Feces: Positive for hookworm. Urine: Normal.

The patient felt perfectly well until May 17th (17 days after first feeding and 10 days of the last). Then he began to feel general weakness, slight frontal headache and had no desire for food. On physical examination he revealed nothing abnormal except for slight tenderness in the epigastrium and a temperature of 37.2°C. The patient continued more or less the same for eighteen days. His temperature was normal or subnormal in the mornings running from 0.2 to 0.8 of a degree C in the afternoons. On the 4th and 5th of June his temperature went up to 38°C coming down to 37.5 or 37.6 in the mornings and up to 38.1 or 38.2°C in the afternoons. The patient was confined to bed. At this time he complained of intense headache, slight polyarticular pain and severe sweats. He had a very tender epigastrium and his spleen was slightly palpable. Temperature declined on June 12th and remained down for several days only to go up again. His blood culture was positive for B. abortus after repeated cultures on May 25 (9th day of the disease). His serum agglutinated on June 5th 1:320 and on June 18, 1:640. His blood count at this time was: W. B. C. 3,280. Polymorphonuclears fifty percent. Large lymphocytes ten percent. Small lymphocytes: forty percent.

This patient was able to be up and about with a slight fever for

CHART IV


NAME E. P.
WARD A BED 6
TEMPERATURE RECORD OF CAVE XL INOCULATED WITH STRAIN BUVIC # 58 BY MOUTH
Date
Days Sick
Hour
TEMPERATURE (CENT)
PULSE
RESPIRATION.

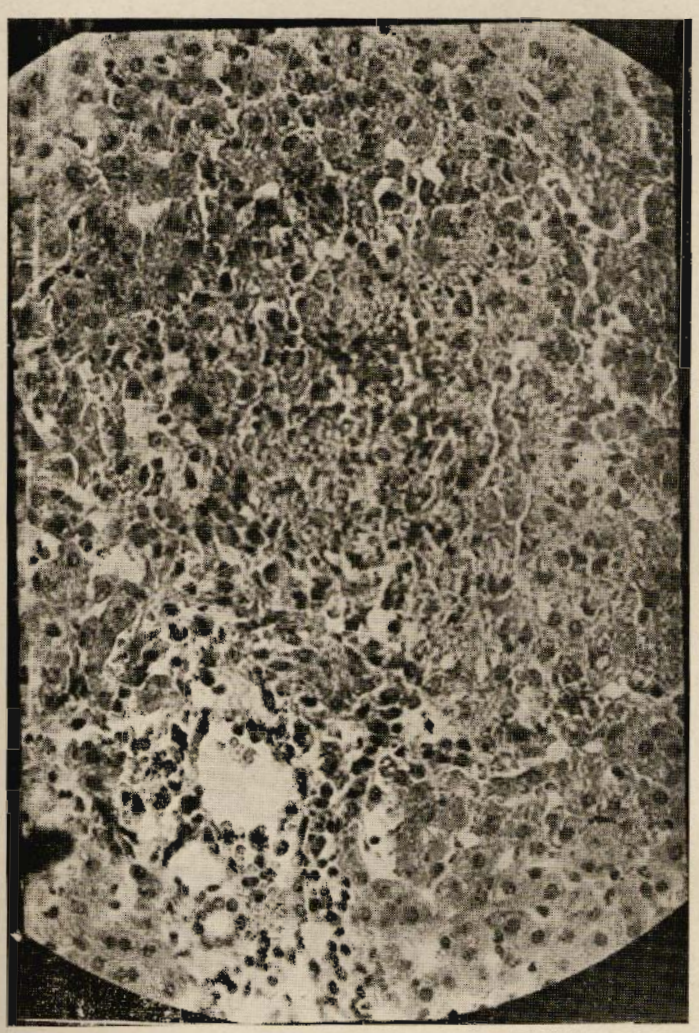
the first eighteen days of his illness. He was confined to bed for five days when his temperature was highest. He then remained up and walked around the ward for the rest of his stay in the hospital.

PATHOLOGY OF MOUSE AND GUINEA-PIG ORGANS REMOVED AT AUTOPSY

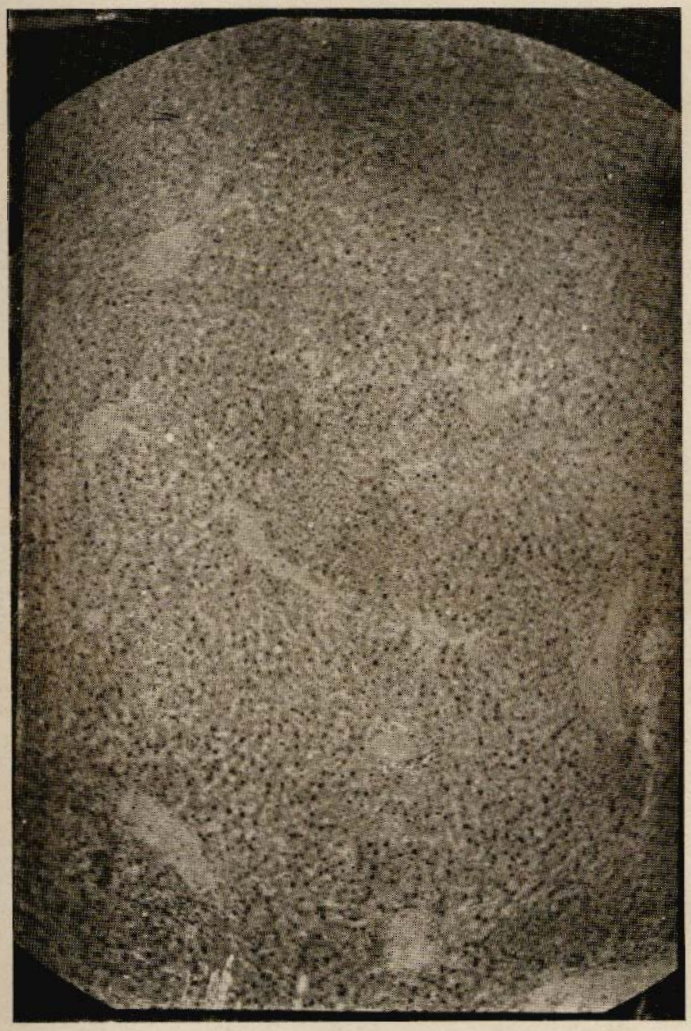
In order to control the virulence of the various cultures with which we have worked each have been tested in mice and guinea-pigs. In mice the cultures were administered by the intraperitoneal route while in guinea-pigs we have attempted to infect through the abraded as well as the unabraded skin. All fourteen cultures were tested in mice and all fourteen were tested in guinea-pigs by inoculation through the unabraded skin. Only the first eight of the following cultures tested were given to guinea-pigs through the abraded skin (1) B. Abortus (Hyg. Lab. 456), (2) B. Melitensis No. 21, S. T. M., (3) B. Abortus (Porcine) Hyg. Lab. 483, (4) B. Abortus (P. R. No. I), (5) B. Melitensis (428) A, (6) B. Abortus (P. R. No. II), (7) B. Melitensis (P&S), (8) B. Abortus (Equine) E–420, (9) B. Abortus (Bovine) 508, (10) B. Abortus (Bovine) 646, (11) B. Abortus (Bovine) 649, (12) B. Abortus (Busic), (13) B. Abortus (Bovine) 263 and (14) B. Abortus (Bovine) D–304.

The pathological changes produced in mice following the injection of these various cultures are fairly uniform. Complete studies of these animals were not made in every case but the chief changes are found in the liver and spleen though the lungs and the kidneys in some animals showed definite pathology. The changes found in the lungs consisted of congestion of the vessels, thickening of the alveolar walls and infiltration of polymorphonuclear cells in the walls. The kidneys frequently showed some degeneration with congestion of the tubules, at times hyaline casts and granular changes. In the livers of several animals one noted amyloid formation in the neighborhood of the periportal vessels and elsewhere. The liver cells and cords were compressed, there was infiltration of polymorphonuclear cells, lymphocytes and mononuclear cells in the portal areas and in the sinusoids. Several of the spleens also showed deposits of amyloid. There was also congestion of the splenic pulp, leucocytic infiltration, in some animals hemorrhages in the splenic pulp and in some megalokaryocytes were found in the pulp of this organ. Gram stains of the spleen and liver sections frequently showed the presence of Gram-negative bacilli, presumably Br. *melitensis*. From several of the mice Br. *melitensis* was cultured from these organs.

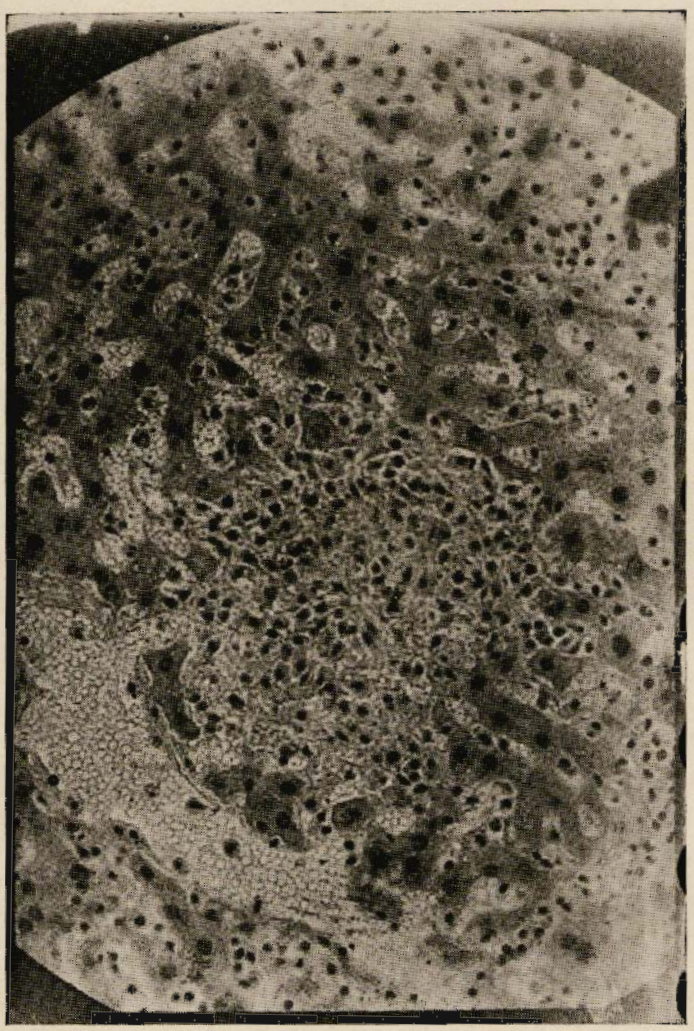
LIVER OF A GUINEA PIG SHOWING TYPICAL LESIONS (LOW POWER)



LESIONS IN THE GUINEA PIG (HIGH DRY)



LIVER OF MOUSE SHOWING FOCAL NECROSIS



A NECROTIC AREA (MOUSE LIVER)

It is not believed that the formation of amyloid in the spleens and livers of a few of these animals indicates anything specific, for control healthy mice from this stock have been found to have amyloid in these organs. The other organs (testes, adrenald, etc.,) showed no special pathology which could be related to the infection under investigation.

Six of the guinea-pigs which were inoculated with cultures (456, No. 21, 483, No. 1, 428 A, No. II) on the normal or unabraded skin showed no pathology. Changes noted in the organs of the other eight animals were as follows: The lungs of four animals showed some broadening of the septa between the alveoli and infiltration with epithelioid cells, plasma cells, lymphocytes and an occasional polymorphonuclear leucocyte, or definite confluent nodules of epithelioid cells. Most of the livers showed numerous tubercle-like structures composed of epithelioid cells and areas of necrosis scattered throughout the tissue. Lymphocytes, plasma cells and occasional polymorphonuclear cells were also noted. Similar tubercle-like structures were also noted in the spleens with occasional rudimental giant cells in the center. Some of the nodules were confluent showing necrosis in the center. All stains for tubercle bacilli were negative. The kidneys showed little change except for occasional areas of infiltration by lymphocytes and plasma cells or slight necrotic changes in the glomeruli. The other organs exhibited no changes of special note or importance.

Eight of the cultures (483, No. II, 428 A, No. I, No. 21, 456, P&S and E–420), were inoculated into guinea-pigs through the abraded skin. Guinea-pigs which received strains No. 21 and 428 A showed little pathology, if any. The livers and spleens of the other six animals all showed the tubercle-like structures described above. In one animal (receiving strain 483) tubercle-like structures were also found in the periosteum while in three animals (receiving strains P&S, 456 and No. II) these structures were noted in lymph glands. Various degrees of cellular infiltration in the livers and spleens as noted above were found. The kidneys, adrenals and other organs were not remarkable. Stains for tubercle bacilli were uniformly negative. The infecting organism was frequently recovered from the liver, spleen or lymph glands. In summary one may state that the characteristic pathological change in the organs of these animals experimentally infected with various strains of B. abortus melitensis is the presence of very definite tubercle-like structures from which material no tubercle bacilli are discernible in stained sections.

DISCUSSION

From these experiments it seems that infection can take place more readily through abraded skin than through the gastro-intestinal tract. The question of dosage is a very important one. Relatively large single doses produced infection through abraded skin with organisms that had been isolated for some time. These same organisms, when fed repeatedly in large doses, did not produce infection.

It required seven twenty-four-hour broth cultures of a virulent bovine strain to produce a mild infection.

Porcine strains in our experiences are more virulent than bovine strains and can produce infection through the gastro-intestinal tract when fed in smaller doses than the former. We have confirmed Hardy's findings that the unabraded skin is a ready portal of entry for infection in guinea-pigs.

We were unable to produce infection through unabraded skin in man but we feel that larger doses at repeated intervals should be tried before infection through unabraded skin can be denied. Out of forty cases inoculated by various methods with various strains only ten contracted the infection. Of these, six were infected through abraded skin and four through the gastro-intestinal canal. Of the six infected through abraded skin, three were inoculated with bovine strains, one with a melitensis strain and two with porcine strains. A single dose was enough to produce infection in these cases. Of the four cases inoculated through the gastro-intestinal tract two were inoculated with porcine strains, one with a melitensis strain and one with a bovine strain. A single dose only produced infection with the melitensis strain. Two or more doses were required with the porcine strain and seven consecutive doses were required to produce a mild case with the bovine strain.

If we take into consideration these findings we would expect that among rural population, men would be affected more than women and children by having greater exposure through their occupation. It should also be noted that gastro-intestinal infection should be more common in rural districts where milk is used directly from the family cow which is infected, than among the urban population where there is a reasonable amount of dilution of the infective agent in milk.

The low incidence among urban population can be explained, not only on the basis of virulence of the organism and natural susceptibility of the individual, but also by taking into consideration that large repeated doses of the bovine strains are necessary to produce infection through the gastro-intestinal tract.

Two hundred individuals may be consuming milk from an infected dairy for a certain period of time without evidence of infection. It is the individual that, by chance, gets repeated doses of virulent organisms, large enough to overcome his natural resistance, who will develop infection. The others will continue to consume milk until similar conditions exist to bring about infection in them. Available records indicate that cows excrete from 45 to 450 organisms per cc. of milk. It is also well known that not all infected cattle secret organisms in their milk. Uninfected milk added to infected milk dilutes the infectious agent. This dilution is imperfect and certain quantities of milk will eventually have more virulent organisms than others. Undoubtedly milk from the same cow does not go to the same individual every day nor are the same number of organisms ingested by the same person every day. So several factors have to be carefully considered. Virulence of the organisms, host resistance to infection, doses ingested, frequency with which doses are ingested, abrasion of the gastro-intestinal canal, etc. We are under the impression that only the most virulent bovine strains, when ingested in sufficient doses, are of danger to man.

CLINICAL ANALYSIS

The incubation period of the experimental cases varied from ten to seventeen days.

The onset was insidious in all cases except in one that was taken ill suddenly with a relatively high temperature. Headache, slight fever, general malaise and anorexia were common symptoms at the onset. Definite chills were absent but chilliness or chilly sensations were sometimes recorded. Epigastric tenderness was frequently found. Muscular pains and polyarticular pains of severe type were constantly present.

Severe sweats were very common. Physical findings were frequently absent. In some cases a coated tongue, epigastric tenderness and slightly enlarged spleen were found. Another case presented slight bronchitis. Pain in the neck, in the back, in the hips in the knees, elbow and ankle joints were recorded. Gastro-intestinal symptoms were infrequent but in two cases diahrrea with blood and mucous and griping pains were noticed.

The undulant type of fever curve which characterizes the disease was frequent in some cases. Others, however, presented a long continued course, usually intermittent with occasional peeks, or waves, and afebrile periods. The afebrile periods varied from two to several days. Blood: Blood cultures were positive in all but one

case. Some cases were positive as early as the fifth day and in others on the eleventh day.

The agglutination test was positive in all but one case. The earliest agglutination recorded was on the tenth day. Others did not agglutinate until twenty-three days after the onset. In one of the cases recorded agglutination ocurred only in higher dilutions. Lower dilutions were negative.

White blood cell counts fluctuated from 2,080 in some cases to 3,500 in others. The differential counts varied from forty to sixty-three per cent. Polymorphonuclears to from thirty-three to forty-eight lymphocytes.

SUMMARY AND CONCLUSIONS

Endemic abortion among cattle was recognized for the first time in Porto Rico during the year 1923. In spite of the measures taken to control the infection it has spread very rapidly. The northern district, where the herds that supply milk to the city of San Juan are located is heavily infected.

Sunlight has a definite deleterious effect on cultures of Brucella abortus. Cultures exposed to sunlight were sterile in three hours. Vaccination with living vaccines in cattle have proved superior to the dead culture injection. We believe that vaccination with living vaccines as a method for control of Bang's disease deserves further study, carefully supervised under competent personnel.

In spite of the high cattle infection which we have in Porto Rico human infection is very low. Only twelve serums agglutinated out of 1,750 examined. Of these only two cases showed definitely confirmed symptoms and signs of undulant fever. This low morbidity may be due to the fact that most of the milk is consumed either boiled or pasteurized.

Experimental infection with Brucella abortus in man suggests that the porcine strains are more virulent for man than are the bovine varieties.

Only the most virulent bovine strains can infect man through the gastro-intestinal tract. Smaller doses are necessary to produce infection through abraded skin than through the gastro-intestinal canal.

ACKNOWLEDGMENTS

We wish to thank Miss Evans, Drs. Hardy, Carpenter, Huddleson and Roses for their courtesy in sending us their cultures. We are under special obligation to Dr. Smetana and Dr. Kesten of the Department of Pathology and Drs. Rivera D. V. S. and Varas D. V. S.

who collaborated with us in this work and to Mr. Hernández and Miss Monge for efficient aid in the laboratory.

BIBLIOGRAPHY

1. Castelani and Chalmers: (1919) Manual of Tropical Medicine 3rd. Ed. Univ. Series.
2. (1905–07) Reports of the Commission for the Investigation of Mediterranean Fever Reports 1 to 7.
3. Hutyra and Marek: (1922) Pathology and Therapeutics of the Diseases of Domestic. **1**: 780.
4. Evans: (1918) Jour. Infect. Dis. **22**: 580.
5. Keefer: (1924) Johns Hopkins Bul. **36**: 6.
6. Moore and Carpenter: (1929) Am. Jour Med. Sc., **178**: 115.
7. Huddleson: (1926) Report Vet. Div. Mich State Coll. p. 27.
8. Beylea: (1927) Jour. Am. Med. Asso. **88**: 182.
9. Dickeson: Soc. of Clinical Invest. cited by Evans.
10. Hull and Black: (1927) Jour. Am. Med. Asso. **88**: 463.
11. Gilbert and Coleman: (1928) Jour. Bact. **15**: 40.
12. Hardy: (1928) U. S. P. H. Reports **43**: 2459.
13. Scott and Saphire: (1928) Am. Jour. Med. Sc., **175**: 66.
14. Sensewich and Giordano: (1927) Jour. Am. Med. Asso. **90**: 1782.
15. McAlpine and Mickle: (1928) Am. Jour Pub. Health **18**: 609.
16. Biering: (1929) Jour. Am. Med. Asso. **93**: 897.
17. Dubois cited from Hutyra and Marek.
18. Pekar cited from Hutyra and Marek.
19. Hughes: (1897) Mediterranean Fever London.
20. Evans: (1927) Jour. Am. Med. Asso. **88**: 630.
21. Huddleson: (1926) Jour. Am. Med. Asso. **86**: 943.
22. Gage and Gregory: (1926) Jour. Am. Med. Asso. **87**: 848.
23. Carpenter and Merian: (1926) Jour. Am. Med. Asso. **87**: 1269.
24. Kern: (1928) Am. Jour. Med. Sc., **176**: 405.
25. Simpson and Fraizer: (1929) Jour. Am. Med. Asso. **93**: 1958.
26. Bruce: (1887) Practitioner **39**: 161.
27. Bassett and Smith: (1922) Undulant Fever in the Tropics Editor Byam and Archibald.
28. Brunett: (1816) Practical accounts of Mediterranean Fever London.
29. Dessage, Pellerin and Vinerta: (1926) Los Progresos de la Clínica **34** (4): 452.
30. Baker: (1929) Arch. Inter. Med. **44**: 128.
31. Neri: quoted from Hutyra and Marek.
32. Simpson W. M.: (1928) Ann. Inter. Med. **1**: 1007.
33. Francis Evans: (1926) Pub. Health Reports **41**: 1273.
34. Amoss and Poston: (1929) Jour. Am. Med. Asso. **93**: 170.
35. Burnet: (1922) Arch. Inst. Pasteur de L'Afrique Du Nord **2**: 187.
36. Schoenholz and Meyer: (1927) Jour. Infect. Dis. **40**: 453.
37. Morales-Otero: (1925) P. R. Health Review **1**: 31.
38. Teller: (1923) Arch. f. Tierch Bd. **49**: 65.

39. Huddleson: (1929) Michigan Agr. Exper. St. Tech. Bul. No. 88.
40. Morales-Otero: (1929) P. R. Jour. Pub. Health & Trop. Med. **5**: 144.
41. Duncan: (1928–29) Trans. Roy. Soc. of Trop. Med. & Hyg. **22**: 269.
42. Evans: (1925) Hyg. Lab. Bul. 143.
43. McAlpine and Slanetz: (1928) Jour. Infect. Dis. **43**: 232.
44. McAlpine, Wayne and Bringham: (1929) **45**: 485.
45. Huddleson, Hasley and Torrey: (1927) Jour. Infect. Dis. **40**: 352.
46. Huddleson: (1921) Cornell Veterinarian **9**: 210.
47. McAlphine and Slanetz: (1928) Jour. Infect. Dis. **43**: 233.
48. Huddleson: (1929) Symposium Am. P. H. Assoc.
49. Doyle and Spray: (1920) Jour. Infect. Dis. **27**: 165.
50. Hayes: (1921) Jour. Am. Vet. Assoc. **40**: 435.
51. Cotton: (1922) Jour. Am. Vet. Assoc. **42**: 179.
52. Orcutt: (1926) Jour. Exper. Med. **43**: 225.
53. Skariac: (1922) Hyg. u. Infectious Krankn **95**: 358.
54. Khaled: (1921) Jour. Hyg. **20**: 319.
55. Burnet: (1921) Arch. Inst. Pasteur de L'Afrique du Nord **3**: 45.
56. Smith: (1926) Jour. Exper. Med. **43**: 317 and 207.
57. King and Caldwell: (1929) Am. Jour. Med. Sc., **178**: 115.
58. Carpenter and King: (1929) Symposium on Undulant Fever Am. Pub. Health Assoc.
59. McAlpine and Migle: (1929) Symposium on Undulant Fever Am. Pub. Health Assoc.
60. Orr and Huddleson: (1929) Symposium on Undulant Fever Am. Pub. Health Assoc.
61. Zinsser: (1928) A Text Book in Bacteriology. (D. Appleton & Co.
62. Histensen and Holm: (1929) Tet. & Bakt. **12**: 281.
63. Bastai: (1927) Munch Med. Woch. **74**: 2141.
64. Varcellana Gior. de Clin. Med.: (1929) **10**: 634.
65. Burnet Et.: (1928) C. R. Acad. Sci. **187**: 545.
66. Shaw, Eyre and others: (1906–7) Report of Med. Com. Pt. 4 & 5.
67. Huddleson: (1910) Tech. Bul. No. 32 Mich. Agr. Col. Exp. St. I.
68. Simons and Miller: (1921) Jour. Am. Vet. Asso. **58**: 532.
69. Rettger and White: (1921) Stors Exper. St. Bul. No. 108.
70. Fleishermier et al. Jour. Infect. Dis. **29**: 663.
71. Cotton: (1922) Jour. Am. Vet. Asso. **62**: 179.
72. Sanderson and Rettger: (1923) Jour. Infect. Dis. **32**: 181.
73. Horrocks: (1906–7) Report of Med. Fever. Comm. Part **1**: 46.
74. Morelli: (1926) Amm. di Ottomologia **54**: 1206.
75. Shroeder and Cotton: (1924) J. Am. M. Vet. A. **65**: 211.
76. Bang.: (1897) J. Compt. Patho. & Ther. **10**: 125.
77. Burnett: (1921) Arch. D'Inst. Pasteur d l' Afrique du Nord p. 165.
78. Hardy, Hudson and Jordan: (1929) Jour. Infect. Dis. **45**: 271.
79. Evans: (1925) Hygienic Lab. Bull No. **143**: 33.
80. Hardy: (1929) Jour. Am. Med. Asso. **93**: 891.

ENDEMIC ABORTION IN PORTO RICO (1930)

Less than 50% infection
50% to 70% "
70% to 80% "
Less than 10% "

ENDEMIC ABORTION IN PORTO RICO (1923)