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STUDIES IN VACCINAL IMMUNITY TOWARDS DISEASE OF THE
BOVINE PLACENTA DUE TO BACILLUS ABORTUS
(INFECTIOUS ABORTION).

By

THEOBALD SMITH, M.D., AND RALPH B. LITTLE, V.M.D.



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INTRODUCTION.

When the laboratories of this Department were opened for active research early in 1917, infectious abortion was then regarded as the most serious menace to the dairy industry. Although much work had been done both in this and other countries, there appeared to be many gaps to be filled. The opportunity to investigate it in a large dairy herd at close range was at hand and a study of actual cases of the disease was begun at once and maintained up to the present. This material furnished the basis for a study of the pathology and bacteriology of the diseased placenta and of the fetus. The udder as a locus of continued infection with the bacillus was drawn into the investigation. During this period of more than 5 years, about 40 cases of abortion came to our notice in which *Bacillus abortus* could not be demonstrated. The presence of vibrios or spirilla and the production of disease of the placenta with pure cultures established a second type of placental disease, easily confused with the first.

The regular employment of specific agglutination has shown a very close relation between a high agglutinin titer and the presence of *Bacillus abortus*. A low titer after expulsion of the fetus was always found in the absence of *Bacillus abortus*. In several cases of disease due to *Bacillus abortus* a low titer was present before expulsion of the fetus and a high titer soon after.

Experiments to determine the efficacy of some method of active immunization, or vaccination as we shall call the process, had been made by others on a small scale. Larger undertakings were under way but not yet published. It seemed, therefore, desirable to get some first hand information on this important subject and in 1918 experiments were started at first on a small group and later on larger groups. The data brought together are not at all extensive and figures based on them hardly conclusive. What has been accomplished, however, is a fairly thorough study of the effect of living cultures of *Bacillus abortus* on the individual animal. These have

been followed in the first large experiment through two pregnancies. A large amount of equally detailed work was done upon groups of control heifers without which the results obtained from the vaccinated groups could not have been interpreted: Cases of abortion occurring among cows in the second or higher pregnancies were studied at the same time. These are not included since vaccination of cows, with the exception of a small group described on page 74, was not attempted in this experiment.

Since vaccination is merely an attempt to anticipate and circumvent nature with her own methods, the results of vaccination can be interpreted only in the light obtained by a study of the natural disease. The application of vaccines raises many questions whose answer presupposes a comprehensive, as well as detailed knowledge of the relation between *Bacillus abortus* and the organism of the cow. In fact, without such information any interpretation of the value of vaccinal treatment is likely to remain unsatisfactory. In the attempt to fill certain gaps, it has been necessary in the course of this investigation to give considerable time to the study of spontaneous cases, the pathology of the placental disease, the effect of the disease on the fetus, the distribution of the bacillus in the fetal membranes and the fetus, and the biology of the bacillus itself. The results of most of these special inquiries have been published. Some are still under investigation. Some of this material has been incorporated briefly in the following report.

During the course of this investigation, assistance has been given at different times in the routine bacteriological examination of fetuses by Miss Marian S. Taylor, Drs. G. Martinaglia and W. A. Hagan, Miss Marion L. Orcutt, and Miss Helena A. M. Tibbetts. Miss Orcutt has also given material assistance in testing the agglutinins of blood and milk.

THE NATURE AND CAUSES OF PLACENTAL DISEASE.

For an understanding of the mode of operation of vaccination and the results to be expected, a brief statement of our knowledge of the nature of the disease as developed through investigation carried on from 1897 to the present will be necessary. Cows infected with

Bacillus abortus do not show any recognizable disturbance of health until shortly before expulsion of the fetus. Then there may be changes in the external genitalia and the udder similar to those preceding normal birth. These changes naturally depend for their extent on the age of the fetus at the time of abortion. In the series of cases comprising this report the aborting animals were usually over 5 months pregnant. From a compilation of 915 cases by the German Imperial Health Office, over 85 per cent of the cows were in the 6th or later months of pregnancy. A certain number of cows whose placenta is diseased may give birth to full term, normal calves. When the blood serum of such cows is titrated against a culture of *Bacillus abortus* a relatively high agglutinin titer is observed.

Following the discharge of the fetus the fetal membranes are either wholly or partially retained. A prompt invasion of the uterine cavity by streptococci, *Bacillus pyogenes*, and anaerobic bacilli takes place and the membranes are liquefied and gradually discharged. A continuing discharge from the patent cervical canal, often very offensive, is an indication of the process going on. In practice veterinary surgery takes cognizance of this condition and the decomposing placenta is removed manually and the uterine cavity irrigated. Abortion with adherent placenta has been charged with producing sterility. Reference to protocols and tables in this report shows that most aborters subsequently conceived and carried the fetus to full term.

The premature expulsion of the fetus is due to a diseased condition of the placenta. The placenta of the cow belongs to the class of adeciduate placentas. The placental tissue is brought together in the form of circular islands or cotyledons spread over the entire chorion. These islands are dense groups of vascular finger-like projections or villi which fit into corresponding pits or depressions of the uterine mucosa. These depressions are similarly gathered together into compact masses or caruncles. The union between fetal and maternal tissue is such that with a certain amount of force the fetal cotyledons can be separated from the maternal caruncles, the villi coming out of the latter as a finger out of a glove. When the fetus is expelled prematurely, the changes going on at the termination of a normal pregnancy to disengage the fetal tissue have not

taken place and the placenta remains adherent except where necrosis of placental tissue has brought about a premature separation.

The chorion when diseased presents a variety of gross appearances depending on the intensity and age of the pathological process. The normally smooth, translucent chorion on which the cotyledons are implanted is discolored and injected, or else thickened and opaque. In the early stages a soft, mucoid exudate in the form of small flakes lies upon it. The affected cotyledons are either wholly or partly necrotic. The disease process probably invades them from the periphery and in the early stages a ring of necrotic villi encloses the normal ones. Later individual villi within the cotyledon become yellowish, necrotic, and finally the entire cotyledon undergoes this change. The disease process is frequently found limited in area and localized in one or two foci. More rarely the entire placenta is involved. Associated with these changes is marked edema of the subchorionic tissue. The amnion is not affected. The result of the placental disease is a slow or rapid contraction of the fetal circulation which largely accounts for the changes found in the fetus itself.

When the fetus is expelled after the 6th month it is frequently normal in gross appearance and alive, because after this period slight changes may set the mechanism of expulsion in motion. When the fetus is younger the changes are well marked, because the fetus is retained a longer time. These changes vary from case to case. In some fetuses subcutaneous and intramuscular edema is pronounced. In others either the pleural or the peritoneal cavity or both may be filled with a transudate stained with hemoglobin. Autolysis following death of the fetus may be marked. The organs are as a rule free from lesions, focal or diffuse, except the lungs which may be firm and interlarded with gray and whitish foci. A frequent result of the disease is a discharge of meconium into the amniotic fluid. This is in part deposited on the fetus, and in part swallowed by the fetus. The stomach fluids then become opaque, yellowish, owing to the suspended fecal matter.

The etiological factor in this type of placental disease is a minute bacillus discovered by B. Bang (1) in Copenhagen in 1897. The bacillus is a very small, short rod, non-motile, and Gram-negative.

It is in active Brownian motion when seen in fluids under the microscope. It fails to ferment carbohydrates and is a strictly aerobic organism growing only in the open bulb of the fermentation tube. Nevertheless when taken from the diseased tissues it fails to grow in open culture tubes. Nowack introduced the ingenious procedure of placing in a closed jar with the inoculated tubes a culture of *Bacillus subtilis*. Since 1912 we have simply sealed the tubes with sealing wax. The bacillus develops then on ordinary nutrient agar.

Histological studies of the diseased placenta have shown that the primary lesion is a destruction of the chorionic epithelium by *Bacillus abortus*. This organism in some way gains entrance into the epithelial cell and eventually fills it completely (2). The diseased cells become detached and, together with mucus and fibrin, form the mucoid, flocculent exudate. The chorion deprived of epithelium becomes filled with immigrant cells, which die and form layers of nuclear débris at the surface of the denuded epithelium. To the naked eye this infiltration appears in the form of white plaques, very firm, and embedded so that vigorous scraping fails to dislodge them. The diseased chorion finally becomes leathery and wrinkled. From the chorion the epithelium of the peripheral villi of the cotyledons is invaded and filled with bacilli and necrosis follows.

The fetus is almost regularly invaded by *Bacillus abortus* from the diseased chorion. Very rarely the tissues are sterile. The bacilli are found only in the digestive and respiratory tracts of some cases. In others, in addition the spleen, liver, and other viscera contain them.

The mode of entrance of the bacillus has been the subject of more or less discussion and experiment. The bacilli are discharged in enormous numbers when cows abort and when the placenta is diseased even though the calf is full term. The udders of a certain percentage of heifers and cows become the seat of multiplication and *Bacillus abortus* is discharged continuously or intermittently when the udder is active. There is therefore ample opportunity given for taking the infectious agent in with the food. Some authorities regard this mode as the chief mode of infection. In a few instances disease of the generative organs of the bull have been found and therefore coitus is looked upon by others as the chief mode of infec-

tion. It is probable that as in tuberculosis the virus gains entrance in several different ways and that local conditions determine more or less the successful mode of exposure and infection.

A third means for transmitting the infectious agent is from mother to fetus *in utero*. The survival of the virus in the calf to maturity has not been actually traced in any case. Two facts make such transmission probable. In a few cases among the many autopsied during the past 5 years foci of collapse in the lungs associated with *Bacillus abortus* have been encountered in young calves. This fact harmonizes with the almost constant presence of *Bacillus abortus* in the lungs of aborted fetuses. The second fact is the occasional presence of *Bacillus abortus* in the udder of virgin heifers. Neither of these facts proves the direct transmission from generation to generation, since the young calf may get rid of the virus in the lungs and the virgin heifer may ingest or acquire the virus in some other way.

Like other infectious diseases, this one presents many variations from what might be considered a normal type. No two cases are precisely alike. A series of gradations may be constructed ranging from the most acute to the most chronic, from the severest to the mildest type, but it may not be possible to observe all types in one herd or in one locality owing to different types of virulence and different degrees of inherited and acquired immunity. In the present work three types have stood out: (1) the premature expulsion of the fetus, (2) the delivery of a full term calf but associated with diseased placenta, and (3) normal calving and apparently normal placenta with some existing focus of infection in the placenta or in the udder.

DISEASE OF THE PLACENTA ASSOCIATED WITH AGENTS OTHER THAN *BACILLUS ABORTUS*.

In placing an estimate on the value of any method of artificial immunization, it is of essential importance that all cases of disease due to agents other than the one used as a vaccine be recognized. Premature expulsion of the fetus in recent years has been quite universally ascribed to *Bacillus abortus* exclusively. In 1917, there was encountered in the herd in which the present work was done a disease of the placenta not due to *Bacillus abortus*. The gross appearance of the placenta or portions thereof obtainable and that of the fetus did not present characters sufficiently unique to make a diagnosis possible. With the aid of films, cultures, guinea pig inoculation, and agglutination, *Bacillus abortus* was excluded and a vibrio was recognized as the agent (3, 4, 5). Up to 1923 about 40 cases from the same herd have come under observation. Inoculation of pure cultures of the vibrio produced disease of the placenta and expulsion of the fetus prematurely. With one exception, *Bacillus abortus* was absent, although it might have been expected that mixed infections would be numerous. Cases of vibrionic disease occurred chiefly among cows in their second or higher pregnancies. A few however involved heifers.

We have at present no information concerning the prevalence of vibrionic abortion among cows in this country, and so far no one has reported the finding of *Vibrio fetus*. Recently it has been identified as a cause of abortion in Denmark (6) and Germany (7). Only a thorough study of a series of consecutive cases in any large herd will bring it to light if it is present, since the naked eye inspection of fetus and placenta, if obtainable, is of no diagnostic value. Especially valuable is the study of the pregnant uterus should this be obtainable after slaughter of the cow, since the diagnosis of vibrionic infection is difficult unless the vibrio has invaded the fetus, from which pure cultures are then obtainable. Pure cultures are regularly obtainable from the uterochorionic space of the intact pregnant uterus.

Disease of the placenta with premature expulsion of the fetus is occasionally associated with *Bacillus pyogenes*. Such cases when thoroughly studied usually reveal either *Bacillus abortus* or *Vibrio fetus* as the underlying cause. Very rarely neither of these can be traced; nevertheless the evidence in general places *Bacillus pyogenes* in the group of secondary agents.

Disease of the placenta is now and then secondary to some infectious disease of the dam, such as tuberculosis, and perhaps other imperfectly studied bovine diseases. The virus gains access to the placental circulation from some focus in the body of the dam through the circulation. Miscellaneous infectious processes will probably be revealed in the future with a more intensive and extensive study of the pregnant uterus of the cow. A case of placental disease due to a mould (*mucor*) was described by one of the authors in 1920 (8). Whether the placental disease was primary, or secondary to disease of some other organ of the dam could not be determined since only the pregnant uterus was obtainable.

Abortion not associated with disease of the placenta may occur through injury, such as blows or falls, or through the ingestion of toxic substances in the food. All such cases must be studied from various angles to ensure an accurate diagnosis of the underlying cause.

IMMUNITY NATURALLY ACQUIRED IN AN INFECTED HERD.

Disease of the placenta due to *Bacillus abortus* follows the laws that govern other infectious diseases. Numerous observers, both those immediately concerned in managing herds of dairy cattle and those who have watched the disease for the purpose of discovering its operations and devising remedies and preventives, agree in maintaining that in an infected herd receiving few or no accessions from without there is a gradual rise in resistance observable. The rate of this increase depends on a number of factors, such as the opportunity for taking in doses of virus and the rate at which generations follow one another. The chances for infection in turn depend on the management of the disease and the promptness with which the susceptible animals are removed from the herd.

The progress of the disease in a recently exposed herd with no earlier history of abortion would be as follows: With few exceptions, nearly all pregnant animals exposed would abort during the first and probably the second pregnancy. Certain animals may be so susceptible as to abort a third time. They would probably be eliminated from the herd by most owners at this stage. In any case, they produce few viable offspring and thus eliminate themselves. Later generations will continue to abort in the first pregnancy, or failing satisfactory exposure, in the second. How long this condition may last if the herd should be left without interference could only be answered after actual trial. Data obtained from the herd concerned in this report indicate that fewer and fewer heifers will abort and that full term calves may be born with or without diseased placenta. There will be so called carriers in which the udder may be the site of multiplication of the bacillus over an indefinite period. The occasionally infected placenta may be the source of infectious material at the time of expulsion of the full term fetus. Sporadic abortions may be looked for even in relatively immune herds, when the resistance has been lowered by various agents such as exposure, injury, defective or toxic food, exciting and driving animals over long distances, or any agency depressing vitality and causing a generalization of virus present in some organ such as the udder. The relative immunity of a herd subject to the virus of infectious abortion through a number of generations and in which little or no open disease manifests itself may show itself when such animals are transferred to herds in which the disease does not yet exist in the endemic form. Then we may expect disease in the new herd.

The condition of an immune herd in which abortion occurs only now and then may not, however, remain stationary. Increased resistance is followed by a decrease in the virus produced and disseminated. Animals instead of acquiring small protective doses fail to receive these. Gradually animals grow up without protection. The resistance representing the inherited quota that is due to natural selection rather than acquired specific resistance will become diluted by the appearance of non-resistant variants. Sooner or later another epidemic wave may break out and follow the course pursued by the first but on a lower plane of activity because of a certain residual

amount of resistance in the herd. Epidemic waves may thus be looked for from time to time with intervals in which cases occur sporadically. The length of such intervals and the height or amplitude of the successive waves are resultants of numerous interacting factors which depend on management and other environmental conditions. They will therefore not be alike in any two herds.

A detailed study of unrestricted infectious diseases among domestic animals would be so costly as to be unjustifiable. Epidemiological laws must be worked out by interpolation of fragmentary data derived from various infected foci before means of suppression have become available. In the case of bovine infectious abortion two such methods present themselves. The infection may be eradicated by a destruction of all aborters and carriers or the resistance of the animals may be increased artificially by vaccination. It is towards the second problem that the following experiments have been directed. No attempt is made in this report to discuss the first problem and therefore no attitude is taken for or against either method of suppression. The second problem was chosen because it was the one that was open to attack under the conditions prevailing. The first method, that of elimination, is relatively simple in view of the high degree of accuracy of the agglutination test, but it is likely to prove very costly.

FIRST EXPERIMENT: VACCINATION WITH LIVING CULTURES.

Earlier Work.

Following the discovery of the infectious agent in placental disease of cows, the hope of devising methods for the production of increased specific resistance or immunity stimulated experiments in vaccination in different countries. The experiments were of two kinds according to the method for testing resistance. In one group, the vaccinated animals were artificially infected usually by inducing them to eat diseased tissues containing *Bacillus abortus*. The other method consisted in vaccinating a certain percentage of a herd and awaiting the results of spontaneous infection. The first method is of restricted value because of the danger of overstraining the acquired resistance with large doses, not accessible to the cow under natural conditions. The results of the second method are subject to misinterpretation unless the numbers in the experiment are large. On the other hand, the larger the numbers the more variable the material since it is impossible under prevailing conditions to treat large numbers simultaneously. All statistical material, therefore, unless the differences in results between treated and untreated animals are wide and uniformly so in different hands, is of little value in the formulation of conclusions. In the following summary only those earlier and contemporary experiments are referred to which dealt with fairly large numbers and in which natural exposure served as the test of resistance.

The earliest field experiments on a large scale with both living and dead bacilli were made in England by Stewart Stockman (9). His experiments extended over a number of years beginning with 1908. His material represented herds, large and small, not under his immediate supervision. The results were gathered by the owners and reported as pregnancies terminating normally or prematurely. Suspensions of living bacilli were used on non-pregnant heifers and bacilli killed by heat on pregnant cows. The bacilli were cultured in potato broth for a month before being used as a vaccine. His results are best presented in the form of tables taken from his report. It will be noted that the number of abortions in the vac-

cinated groups was much smaller than in the untreated control group. In the final summary as given in the third table, the number of control animals is much lower than the sum of the controls in the two groups tabulated separately. We are unable to account for this discrepancy and have added our interpretation in parentheses.

TABLE I.
Results of Immunisation on Herds of Group I.

	No. of herds.	Heifers.	Cows.	Total animals.	Less those which died, were barren, or were sold.	Aborted.	Per cent.
Immunisation with live bacilli.	11	69	158	227	201	10	4.9
Controls.	11	51	211	262	243	49	20.1

TABLE II.
Results of Immunisation on Herds of Group II.

Immunisation with live bacilli.				367	292	22	7.5
Controls.				210	189	52	27.5

TABLE III.
Summary of Results of Immunisation on Herds of Groups I and II.

Immunisation with live bacilli.				493	32	6.5
Controls.				265 (432)	101	38.0 (23.3)

In a compilation of data on various methods of immunization practiced by the Imperial Health Office (10) on different herds throughout Germany, there were employed living cultures, killed cultures, living cultures with immune serum, as well as killed cultures with serum. The vaccines were applied in 80 herds. Of the 3,006 head, 1,356 were selected as controls and 1,650 were treated with the different vaccines listed above. The total number of abortions among the vaccinated fell from 25.21 per cent to 15.15 per cent. During the same period the number of abortions among the controls rose from 16.31 per cent to 22.65 per cent. The cases selected for treatment differed essentially from ours in that cows which had aborted once or oftener and pregnant animals were chosen for treatment. Animals from 2 to 11 years of age were included. The amount of living cultures used con-

sisted for each animal of two agar tubes, incubated 8 days and suspended in 10 cc. of physiological salt solution.

The various vaccines shared in this result as follows:

TABLE IV.

Immunizing material employed.	Abortion before treatment.	Abortion after treatment.	Increase or decrease.
	<i>per cent</i>	<i>per cent</i>	
Dead organisms, two doses, with 4 to 8 wks. interval.	18.51	13.20	64 to 117
“ “ with serum.....	21.76	13.60	32 “ 20
Living “ one dose.....	29.09	6.36	128 “ 28
“ “ with serum.....	16.36	5.45	9 “ 3
Controls (plain broth).....	16.31	22.65	175 “ 245

Owing to the miscellaneous material vaccinated, it is difficult to estimate the value of the final figures presented especially since the condition among the controls was essentially different from that of the herd under our observation. Thus our records show a relatively low percentage of repeated abortions among controls. Among the animals dealt with by the German workers, of 175 cows having aborted once, 113, or 64.56 per cent, aborted a second time. Among 910 head of controls which had not aborted, only 132, or 14.50 per cent, aborted during the observation period. In these two classes after vaccination the results were better among those which had aborted once before. Thus of 333 animals which had aborted once before, only 15.91 per cent aborted a second time after vaccination as compared with 64.56 per cent abortions among controls. Of 1,195 which had not aborted, 8.16 per cent aborted after vaccination as compared with 14.50 per cent abortions among the control group.

In Wisconsin vaccination of heifers, pregnant and non-pregnant cows was carried on in 1919 and 1920 by F. B. Hadley and associates (11). In the following table only that material is reproduced which bears on the work of this Department. It includes heifers and cows not pregnant.

TABLE V.

Description of animals.	No. of animals less those which died, were barren, or were sold.	Aborted.	Per cent.
Heifers vaccinated.....	127	28	22.0
Controls (unvaccinated).....	24	8	33.3
Cows that have not aborted vaccinated.....	207	17	8.2
“ “ “ “ “ left as controls.....	18	10	55.6

The figures for heifers, treated and untreated, do not differ essentially from our own. Those of non-pregnant cows kept as controls are very high.

E. C. Schroeder (12), without giving details, refers to a large herd in which 911 were under observation one year and 453 a second year. 617 animals were treated with living vaccine the first year and 311 in the second. The control animals numbered respectively 294 and 142. The abortion rate of treated and untreated cattle during the first year was 13.12 and 17.7 per cent respectively; that during the second year was 10.29 and 14.09 per cent.

C. O. Jensen (13) describes experiments with living bacilli on non-pregnant heifers. They received two or three doses, the last about 8 weeks before conception. There were in all 447 vaccinated and 424 control animals. The results of the experiment were not particularly encouraging; of the vaccinated animals 23.7 per cent, and of the control animals 36.8 per cent aborted. However, the proportion partly within the single herd and partly in the different years has been quite variable. The summarized results for 1916-18 show 28.2 per cent abortions among the vaccinated animals against 34.5 per cent among the control animals. The corresponding numbers for the preceding year's trial are on the other hand 14.3 per cent against 40 per cent. What the causes of this conspicuous difference were could not be ascertained, but the difference cautions prudence in judging the results of individual experimental series. The author and his collaborators saw in their results such uncertainty in regard to the achievements with vaccination with living cultures that it seemed to them probable that they were not on the way to a method applicable in practice. Jensen admits however the possibility that his results were vitiated by error arising from faulty coöperation on the part of owners of the cattle.

Schermer and Ehrlich (14) treated 203 cows in 14 herds with living cultures and 213 with heated cultures. Before vaccination, 79, or 19 per cent, aborted; after vaccination, 22, or 5½ per cent, aborted. Of these 22 aborters, 5 had received living and 17 dead cultures.

Craig and Kehoe (15) recently tried living cultures on a dairy herd of 28 cows in which infectious abortion was prevailing. All cows and heifers were vaccinated. Although the authors had no control animals with which to compare the vaccinated they conclude that the vaccination was of material value in reducing abortion.

Material and Methods.

The higher susceptibility of the young to infectious diseases makes it probable that in infectious abortion the heifer is more predisposed than the older cow. This is evident in the frequent abortions during the first pregnancy, which grow fewer with the age of the cows. Hence the best test of any vaccination is upon the virgin heifer. If abortions in this class can be reduced by vaccination, the procedure

is likely to succeed with older cows. The animals drawn into the experiment to be described, with the exception of one small group of cows, were heifers vaccinated some time before conception.

The Vaccine.—In deciding upon the material and method for producing immunity, it seemed best to confine this experiment to one kind of vaccine. The study of the disease by others had already indicated that the only kind of vaccine which promised any appreciable success was the living culture. This was injected but once, since observations had shown that a high agglutinin titer following one dose persists in most cases during and after pregnancy. In the use of living virus as a vaccine two factors play a predominant rôle; one is the relative virulence of the strain of bacilli used as a vaccine, the other is the degree of resistance attained by the herd under the influence of the disease itself.

If the virulence of the culture is too high, we may expect actual placental disease as a result of vaccination in a certain per cent of the herd. If it is too low, the protective value may fall below the point at which invasion of the pregnant uterus by the natural virus is prevented. If the herd has been recently invaded by the disease, a vaccine which is nearly harmless to a herd in which the disease has prevailed for years may still be virulent enough to produce injury. In actual practice there is more or less change in the individuals of the herd and therefore more or less variation in resistance. The new animals may be more or less immune than the older ones, according to the earlier contact of them or their ancestors with the disease. There is furthermore no accurate measure of resistance. Perhaps the number of successive abortions of the same cow may give some information. Herds in which only the heifers abort, after due exposure, may be regarded as fairly resistant. Highly susceptible types are naturally eliminated by the death of the offspring.

In view of the difficulty of measuring resistance and of the varying immunity of the individual animals, it is inadvisable to use cultures freshly isolated from cases of the disease.

Two strains of *Bacillus abortus* were used as vaccines. One (No. 281) was only 32 days under cultivation when injected into the first cases. The same strain was continued in use on later groups. The last group was injected when the strain had been under cultivation

574 days. A second strain was used on a small group when 22 days old. Both strains were isolated from the same herd. Serological differences were not observed.

A better method would have been to inject the successive groups with cultures of the same age. This would have necessitated obtaining fresh cultures at definite times, a condition not always to be met. There was probably some attenuation of the first strain from first to last. When 2 years and 7 months under cultivation, the first strain was used as a vaccine on a small group of cows. The assembled data on page 74 show that the culture produced a high agglutinin titer in all injected cows within the usual time.

After the vaccinations had been completed, the oldest of the two cultures (No. 281) was subjected to a virulence test on guinea pigs. The strain had been isolated October 4, 1918. On September 22, 1922, nearly 4 years later, three guinea pigs received into the peritoneal cavity a suspension of bacilli from this strain prepared as follows: A suspension in bouillon about as dense as a 24 hour bouillon culture of the typhoid bacillus was diluted 1,000 times and $\frac{1}{2}$ cc. of this dilution injected. Computations made in this laboratory indicate that about 5 million bacilli were injected. At the same time a precisely parallel test was made with a fresh strain isolated from a fetus 11 days before.

The disease following the inoculation of these two strains was much the same. *Bacillus abortus* was recovered from the spleen of the six animals. Two from each set had been kept for 10 weeks before they were chloroformed. This test indicates that a considerable degree of virulence persisted in the culture chiefly used as a vaccine. Just what relation this guinea pig virulence bears to the original bovine virulence cannot be stated.

The dose injected as a vaccine was from 2.5 to 5 cc. of a suspension equivalent in density to a 24 hour bouillon culture of the typhoid bacillus. This density is roughly equivalent to one 3 times the density of a suspension measuring 2.4 with the Gates instrument. It is also approximately equivalent to one agar slant containing 6 cc. of agar. 1 cc. of the suspension contains roughly 1 billion bacilli.

In several early cases, the vaccine was injected into a jugular vein; in the rest, under the skin of the neck. Only one injection was given.

Methods of Studying the Vaccinated Animals.—The course of the investigation following the injection of the living culture consisted in testing samples of blood for agglutinins from time to time and the occasional testing of the secretions of the inactive udder. At the time of birth the placenta was examined, when not destroyed by the dam, and one or more guinea pigs inoculated with a salt solution suspension of uterine fluid obtained on a swab inserted into the uterus as soon after discharge of the fetus as possible. The examination of films from the swab frequently made an immediate diagnosis possible. When a heifer aborted, the fetus was autopsied and cultures made from various regions of the digestive tract, the lungs, liver, kidney, and spleen. Unless the diagnosis was definitely made with the microscope, guinea pigs were inoculated with suspensions of uterine fluid, portions of placenta if obtainable, and contents of the digestive tract and lung suspensions from the fetus. The colostrum and the milk were tested for agglutinins in some cases. The placenta, unless entirely retained, was sectioned and the presence of *Bacillus abortus* in the chorionic epithelium looked for.

Whenever possible the heifers were followed through a second and a third pregnancy and the same procedure was used to determine the presence of *Bacillus abortus*.

In the event that vibrios were found associated with the fetal membranes or the fetus or both, the same procedures were followed to determine whether *Bacillus abortus* could be demonstrated.

The agglutination test was carried out substantially as given in an earlier publication (16). A series of dilutions beginning with 1:10 or 1:20 and extending in a series with a common factor, 2, to 1:1,280 or higher, was used in all cases to obtain a complete picture of the reaction and to control errors. The figures quoted in the protocols give the dilution in which some clumping is still recognizable, usually as an increase in the deposit when compared with control tubes. This agglutination limit is removed about two steps from complete clumping and clearing of the suspending fluid. Thus if clumping is complete at 1:320, the limit is usually at 1:1,280, rarely at 1:640.

The milk and colostrum were first treated to effect the removal of fat and casein. The fat was removed after sedimentation or centrifugation, the casein with the use of rennet (17).

Culture Methods.—Throughout, the slanted agar tube sealed with sealing wax was used. Usually bits of tissue or fluid were transferred from fetus or inoculated animal, rubbed over the surface of the agar, and deposited in the condensation water. This simple method has not given rise to any doubt in its reliability after 5 years.

When material such as placental tissue was presented guinea pigs were inoculated as a rule. In some cases when the putrefactive changes had not begun, *Bacillus abortus* has been recovered by first washing the tissue in sterile salt solution or a pure deep well water repeatedly and then transferring particles of tissue to inclined agar. From this a loopful of condensation water is rubbed over a second and a third tube. Or the tissue may be rubbed over the surface of an agar plate and the Petri dish sealed to a flat piece of glass or to the cover with paraffin.

The guinea pig served as a means for demonstrating *Bacillus abortus* when culture methods became ineffective. The tissue to be inoculated was rubbed with sterile sand and suspended in salt solution. The contents of the digestive tract were treated in the same way to facilitate injection. When the material was fresh, intraperitoneal injection was practiced. Guinea pigs very rarely succumbed to peritonitis when even somewhat decomposed placental tissue was repeatedly washed before it was ground and suspended in salt solution for injection. The inoculated guinea pigs were kept variable periods of time before they were chloroformed. Positive cultures are obtainable at any time from a week to 3 months after injection. In the guinea pig kept 3 to 4 weeks, the lesions are as yet slightly developed, whereas in the guinea pig kept 2 to 3 months, they are conspicuous and striking. On the other hand, the number of colonies developing on the agar surface are fewer with the age of the disease. When bits of spleen as large as peas or even larger are transferred, colonies appear even in the long standing cases. Cultures were always prepared from the guinea pig even when the gross lesions were characteristic. It always sufficed to transfer bits of spleen to several tubes and seal them.

Of the greatest value in making a diagnosis of the type of abortion was the inoculation of guinea pigs with suspensions of uterine swabs obtained as soon as possible after expulsion of the fetus. The rapid

invasion of the uterine cavity with streptococci and septic anaerobes when it contains portions of adherent placenta makes haste necessary. The not infrequent absence of *Bacillus abortus* from the tissues of the fetus makes it undesirable to rely too exclusively on cultures from the fetus. In a fair number of cases in this report only the swab inoculation brought *Bacillus abortus* to light.

Vibronic abortion in the herd under observation became a matter of considerable importance in so far as a neglect of this type of placental disease would have interfered with the relative accuracy of the work. Thus in one control group of 72 heifers, there were 24 abortions. Of these, 3 were demonstrated cases of vibronic disease and 2 were doubtful in that the agglutinin titer towards *Bacillus abortus* was low and the latter organism was not recovered. These 5 cases represent 20 per cent of the actual abortions. Whenever a case was suspected as being associated with vibrios, the procedure outlined for the detection of *Bacillus abortus* was followed. *Vibrio fetus* develops in cultures under conditions suitable for *Bacillus abortus*. It fails to maintain itself in guinea pigs after injection. Hence it does not interfere with the detection of *Bacillus abortus* by this means.

The Effect of Injecting Living Cultures of Bacillus abortus.

Subcutaneous injection produces a local swelling whose size depends on the dose introduced. This swelling appears on the 2nd or 3rd day, reaches in the case of the dosage used in the present work about the size of the palm of the hand and a thickness of $\frac{1}{2}$ inch, more or less. It subsides within 1 or 2 weeks and completely disappears. Suppuration does not occur. The temperature begins to rise on the day following the injection and on the 2nd day may be 2° or 3°C. above normal. It falls on the 3rd and is usually normal on the 4th day. The weight of the heifer may drop or remain stationary, so that at the end of a month there may be a maximum loss of 50 or 60 pounds as compared with a control. Eventually the loss is made up. None of the vaccinated animals showed any poor condition later on traceable to the vaccination.

When the same dose is injected into a jugular vein, the immediate effects are more pronounced. The animal within $\frac{1}{2}$ to 1 hour begins to breathe rapidly and irregularly and remains in this condition for several hours. The temperature begins to rise on the same day and reaches a maximum on the next day. The increase is from 2° to 3°C. Normal conditions are reestablished within 3 or 4 days. The intravenous injection is a more severe procedure than the subcutaneous injection. There seems to be no difference in the immunizing value of the two and the subcutaneous method was used exclusively in later vaccinations. Beyond the effects noted, nothing occurred in the vaccinated animals to justify the inference that any harm had been done.

The Appearance of Agglutinins.

Investigators have employed both agglutination and complement fixation tests in the study of infectious abortion. In earlier work done in this Department, the agglutinin titer corresponded so closely with the presence or absence of *Bacillus abortus* that it was chosen as the serological reaction in the vaccination experiments. The method has already been described in former publications. The interpretation of the test is given on page 19.

The normal agglutinin limit of cow's blood fluctuates between 1:5 and 1:80. That is to say, in the large numbers of cases studied since 1917, the maximum, 1:80, was not found associated with *Bacillus abortus* infection excepting in rare cases. It may occur for instance that 1:80 represents a point in a rising or a falling curve. In the former case the infectious process is in its beginnings, in the latter it is subsiding. The limiting titer of 1:80 may be somewhat high for herds wholly free from this infectious agent. For these, 1:40 may be nearer the maximum. In the herd under investigation the presence of the disease for some years may have tended to increase the density of the infectious agent so that some of the animals may have taken up minute doses from time to time without developing any recognizable foci of disease.

Following the injection of living cultures, the agglutinin titer of the blood begins to rise a few days later and may be at its maximum in 10 days (Tables X to XII). The level then reached is usually

1:640 or 1:1,280. This high level may be maintained with fluctuations until termination of the first pregnancy and even on through the second and third. In other cases the high level first attained may gradually give way to a medium titer of 1:320 and this level may be continued with fluctuations above or below it. Finally, in a third smaller group of animals the agglutinin titer may gradually drop to a level which may be regarded as falling within the normal zone of 1:80 or lower.

In general the maintenance of a continued high titer through pregnancy signifies one of three conditions: (1) infection of the placenta which will terminate in premature expulsion of the fetus; (2) infection of the placenta with normal calving; (3) normal calving and normal placenta with infection of the udder.

In the two large groups of cases to be discussed later, the vaccinated and the controls, the agglutinin curve is not the same. In the vaccinated heifers it begins high as a result of the vaccination and takes one of the three courses outlined above. The control heifers begin with a low titer and maintain this throughout unless infection takes place. This rarely begins before pregnancy. Among the 53 heifers vaccinated, 3 had a high agglutinin titer before vaccination, the rest were normal. The titer may rise at any time during pregnancy when infection occurs or when multiplication takes place either in the udder, the placenta, or in both places.

An agglutinin titer from 1:20 to 1:80 associated with abortion, or adherent placenta and normal parturition, is as a rule suggestive of some cause other than *Bacillus abortus*. Abortion may now and then be due to injury inflicted by falls or blows, or to faulty food or poisons, but the proof that bacteria are not responsible is difficult to furnish. The largest number of such cases have been associated with vibronic disease of the placenta, as stated previously.

The effect of living cultures of *Bacillus abortus* injected at least 2 months before conception on the course of pregnancy may be conceived of as protective against the invasion of the natural virus, as failing to protect, *i.e.* as indifferent, or as actually causing disease of the placenta and abortion. The third possibility is of great importance but not demonstrable at present. In order to ascribe abortion in a vaccinated cow or heifer to the vaccination we must

prove that the bacilli isolated from the placenta and fetus are the progeny of the injected culture. Attempts have been made to devise methods by which the vaccinating bacillus might be distinguished from the natural virus in cases of abortion following vaccination. No definite results have been obtained. The species, *Bacillus abortus*, is very homogeneous in its characters. The changes undergone by the bacillus, used as vaccine, during artificial culture are slight and they might conceivably be wiped out and the original characters restored in the tissues of the cow.

DETAILED ACCOUNT OF THE VACCINATED AND CONTROL GROUPS OF HEIFERS.

It is generally agreed that infectious abortion after its introduction into a herd soon develops into epidemic proportions and after a certain number of years declines to a sporadic affection subject to environmental conditions. When artificial methods of immunization are applied, the controls or untreated animals should belong to the same group, if possible, if the interpretation of occurrences after vaccination is to be fairly accurate. In the present undertaking two groups of control heifers have been studied. The first or earlier group antedates slightly and overlaps the vaccinated group, while the second overlaps and extends beyond this group. It was considered important to keep these groups distinct to show to what if any degree the curve of spontaneous disease was falling. In presenting the data, the first group of controls therefore naturally precedes the group of vaccinated heifers.

Summary of the First Group of Control Heifers.

72 cases were studied. Of these, 3 animals aborted as a result of vibronic disease. A fourth aborted a living fetus with retained placenta but *Bacillus abortus* was not demonstrated and the agglutinin titer was low. This case and the 3 undoubted cases of vibronic disease are excluded from the statistical summary.

Among the 68 remaining heifers, 20 expelled the fetus prematurely as a result of disease due to *Bacillus abortus*, demonstrated through cultures, guinea pig inoculations, and sections of the diseased placenta. In 4 additional cases, the full time fetus was expelled normally but the placenta was infected and locally diseased. If we consider these cases normal, we have 20 out of 68 aborting, or 29.4 per cent. If we include the placental infection, the percentage rises to 35.3.

Following the first pregnancy 19 cows were eliminated from the herd leaving 53. Of these, 38 became pregnant a second time.

30 gave birth to full term fetuses, with one diseased placenta, and 8 aborted. Including these we get 21.0 per cent as the abortion rate in the second pregnancy. Of the 8 aborters, 4 had aborted in the first pregnancy. Up to the time of completing this report, 17 of this group had given birth to third calves, all of which were normal. One of these cows had aborted in the second pregnancy, 2 in the first, and 2 in both first and second.

In the first group of heifers the mucoid fluid from the quiescent udder of 9 cases during the first pregnancy was tested on guinea pigs. Of these, 4 were found to harbor *Bacillus abortus*. After the discharge of the calf or fetus, the milk of 23 was tested on guinea pigs and 10 were infected. These 14 cases of udder infection were associated with 8 normal births, 4 abortions, and 2 with infected placenta.

In Table VI are given various facts concerning this control group. The first column states the result of the first pregnancy, the second the condition of the placenta when obtainable. In the third column the presence or absence of *Bacillus abortus* in the udder fluid (before calving), the milk, and the fetus is recorded. In the fourth column the agglutinin titer is given as low, medium, or high. *l.* signifies up to 1:80, *m.* to 1:320, and *h.* above this. The successive letters indicate the order in which the titer changed. The fifth column gives information of the second calving or abortion with the period elapsing since the first. The remaining columns are self-explanatory. In all cases, the sign + indicates the isolation of *Bacillus abortus*, the sign - failure to recover it. The absence of + or - indicates that no attempt was made to isolate it.

Notes on Individual Animals of the First Control Group.

In the following pages the important facts in the history of the individual heifers of the first control group are given in some detail, together with statements of autopsies on fetuses, cultures obtained, etc. The limiting agglutinin titers with dates are also included.

No. 405.—Black and white, born Feb. 7, 1917. Agglutinin titers: Feb. 12, Apr. 1, and May 13, 1919, 1:320; July 8, 1:640. On July 15, heifer calved normally.

TABLE VI.
First Experiment. First Group of Controls.

Case No.	First pregnancy.	Placenta, condition of.	<i>B. abortus</i> isolated.*	Agglutinin titer of blood.	Second pregnancy, terminated after first.	Placenta, condition of.	<i>B. abortus</i> isolated.*	Agglutinin titer of blood.	Remarks.
405	Normal.	Normal, -	m +	m., h.	Normal, 10 mos. 26 days. (Sold.)	Retained.			
423	Aborted (8-9 mos.).	Diseased, +	f +, m -	m., l.	(Killed.)				Vibronic disease.
433	Aborted.		(Vibrio.)	l.	(Died.)				
438	"	Retained.	m -	l., l.	(Sold.)				
449	"	Diseased, -	(Vibrio.)	l.	"				
453	"	Retained, +	f +	m., h.	"				
456	(near full term). Aborted (7-8 mos.).	" +	f +, m +	l., m.	"				
457	Aborted (6 mos.).	"	f +, m -	m., h.	"				
458	"	" +	f +, m +	l., h.	"				
460	(near full term). Aborted (6-7 mos.).	" +	f +, m -	l., h.	Aborted (6-7 mos.).	Retained, +	f +	h.	
461	Aborted (6 mos.).	Diseased, +	f +, m -	m.	Normal, 18 mos. (Sold.)	Discharged, lost.		l.	
463	"	Retained.		l., l.					
466	(fetus living). Normal.	Normal, -	u -, m +	m., m.	Normal, 1 yr.	Retained, diseased, + Normal, -		m.	
473	Aborted (near full term).	Retained, +	m -	l., m., h.	" 14 mos. 22 days.			h.	3rd calf normal. Blood titer 1:320.
474	Full term.	Diseased, +	u -	m., h.	Normal, 10 mos. (Sold.)	" -		h.	
476	Aborted (near full term).	Retained, +	u +, f +	l., m., h.					
482	Aborted (near full term).	"	f +, m -	l., h.	Normal, 11 mos. 27 days.	Discharged, lost.		l.	
483	Aborted (near full term).	Diseased, +	u -	l., m., h.	Aborted (near full term), 1 yr. 13 days. (Sold.)	Diseased, +		h.	3rd calf normal. Blood titer high.
486	Aborted (6 mos.).	Retained, +	f +	l., h.	"				
487	Normal.	Normal.		l., m., l.					
488	"	Diseased, +	m +	l., m., h.	Normal, 11 mos. 11 days. (Died.)	Normal.		h.	3rd calf normal.
494	Aborted (near full term).	Retained, +	u +	l., h.					
495	Normal.	Normal.		l.	Normal, 15 mos. 18 days. Aborted.	Discharged, lost.		l.	
503	Aborted (living fetus).	Partly retained, diseased, +	f +	l., h.		Retained.	(Fetus lost.)	h.	3rd calf normal. Blood titer low.

* f = fetus, m = milk, u = udder fluid.

TABLE VI.
First Experiment. First Group of Controls.

Case No.	First pregnancy.	Placenta, condition of.	<i>B. abortus</i> isolated.*	Agglutinin titer of blood.	Second pregnancy, terminated after first.	Placenta, condition of.	<i>B. abortus</i> isolated.*	Agglutinin titer of blood.	Remarks.
405	Normal.	Normal, —	m +	m., h.	Normal, 10 mos. 26 days. (Sold.)	Retained.			Vibrionic disease.
423	Aborted (8-9 mos.).	Diseased, +	f +, m —	m., l.					
433	Aborted.		(Vibrio.)	l.	(Killed.)				
438	"	Retained.	m —	l., l.	(Died.)				
449	"	Diseased, —	(Vibrio.)	l.	(Sold.)				
453	"	Retained, +	f +	m., h.	"				
	(near full term).								
456	Aborted (7-8 mos.).	" +	f +, m +	l., m.	"				
457	Aborted (6 mos.).	"	f +, m —	m., h.	"				
458	"	" +	f +, m +	l., h.	"				
	(near full term).								3rd calf normal. Blood titer 1:320.
460	Aborted (6-7 mos.).	" +	f +, m —	l., h.	Aborted (6-7 mos.).	Retained, +	f +	h.	
461	Aborted (6 mos.).	Diseased, +	f +, m —	m.	Normal, 18 mos.	Discharged, lost.		l.	
463	"	Retained.		l., l.	(Sold.)				
	(fetus living).								
466	Normal.	Normal, —	u —, m +	m., m.	Normal, 1 yr.	Retained, diseased, +		m.	
473	Aborted (near full term).	Retained, +	m —	l., m., h.	" 14 mos. 22 days.	Normal, —		h.	
474	Full term.	Diseased, +	u —	m., h.	Normal, 10 mos.	" —		h.	
476	Aborted (near full term).	Retained, +	u +, f +	l., m., h.	(Sold.)				
482	Aborted (near full term).	"	f +, m —	l., h.	Normal, 11 mos. 27 days.	Discharged, lost.		l.	3rd calf normal. Blood titer high.
483	Aborted (near full term).	Diseased, +	u —	l., m., h.	Aborted (near full term), 1 yr. 13 days.	Diseased, +		h.	
					(Sold.)				
486	Aborted (6 mos.).	Retained, +	f +	l., h.	"				
487	Normal.	Normal.		l., m., l.					
488	"	Diseased, +	m +	l., m., h.	Normal, 11 mos. 11 days.	Normal.		h.	
					(Died.)				
494	Aborted (near full term).	Retained, +	u +	l., h.					
495	Normal.	Normal.		l.	Normal, 15 mos. 18 days.	Discharged, lost.		l.	
503	Aborted (living fetus).	Partly retained, diseased, +	f +	l., h.	Aborted.	Retained.	(Fetus lost.)	h.	3rd calf normal. Blood titer low.

* f = fetus, m = milk, u = udder fluid.

TABLE VI—Continued.

Case No.	First pregnancy.	Placenta, condition of.	<i>B. abortus</i> isolated.*	Agglutinin titer of blood.	Second pregnancy, terminated after first.	Placenta, condition of.	<i>B. abortus</i> isolated.*	Agglutinin titer of blood.	Remarks.
505	Normal.	Normal.		l., m.	Normal, 14 mos. 5 days.	Discharged, lost.		l.	3rd calf normal. Blood titer 1:160.
522	"	"	m +	l., h.	Normal, 1 yr. 7 days.	" "			3rd calf normal. Blood titer high.
547	"	Discharged, lost.		l., h.	Aborted (living fetus), 1 yr. 10 days.	Retained, +	m +	h.	
551	"	" "		l., h.	Aborted. (near full term), 13 mos. 11 days.		f +, m -	h.	3rd calf normal. Blood titer high.
564	"	" "	m -	l., h.	Normal, 1 yr. 16 days.	Normal.		h.	3rd calf born 14 mos. 5 days after 2nd. Placenta normal. Blood titer high.
586	"	" "	m +	m., m.	Normal, 14 mos. 22 days.	Retained, -		m.	
642	"	Retained.	u +	l., h.	Normal, 13 mos. 7 days.	Discharged, lost.	m +	h.	3rd calf normal. Blood titer 1:160.
775	"	Normal.		l.	Normal, 14 mos. 10 days.	" "			
776	"	Discharged, lost.		l.	(Sold.)				
777	"	Normal.		l., h.	"				
778	"	Discharged, lost.		l.	"				
779	"	" "		l., l.	Normal, 10 mos. 21 days.	Discharged, lost.		l.	
780	"	" "	m +	l., m., h.	Normal, 16 mos. 4 days.	" "		h.	3rd calf normal. Blood titer high.
781	"	" "	m -	l.	Normal, 18 mos. 10 days.	Retained.		l.	3rd calf normal. Blood titer low.
783	"	" "	u +, m -	m., h.	(Sold.)				
784	"	Normal.		l., m.	Normal, 11 mos. 6 days.	Discharged, lost.		h.	3rd calf normal. Blood titer medium.
785	Aborted.	Retained.	(Fetus lost.)	h.	(Sold.)				
786	"	"	" "	h.	Premature, 13 mos.	Retained.		h.	
787	Normal.	Discharged, lost.		l.	Normal, 1 yr. 14 days.	Discharged, lost.		l.	3rd calf normal. Blood titer low.
788	Aborted.	Retained.	(Fetus lost.)	l.	Normal, 1 yr. 5 days.	" "		l.	3rd calf normal. Blood titer 1:320
789	Normal.	Discharged, lost.		l., h., l.	Normal, 1 yr. 9 days.	" "		l.	
790	"	" "	m -	l., m.	11 mos. 19 days.	" "		l.	

* f = fetus, m = milk, u = udder fluid.

TABLE VI—*Concluded.*

Case No.	First pregnancy.	Placenta, condition of.	<i>B. abortus</i> isolated.*	Agglutinin titer of blood.	Second pregnancy. terminated after first.	Placenta, condition of.	<i>B. abortus</i> isolated.*	Agglutinin titer of blood.	Remarks.
791	Normal.	Normal.		l., l.	(Sold.)				
793	"	Discharged, lost.		l., l.	Normal, 11 mos. 19 days.	Discharged, lost.		l.	3rd calf normal. Blood titer low.
794	"	" "		l., l.	(Sold.)				
795	"	" "		l., l.	"				
796	"	Normal.	m —	l., h.	"				
797	"	Discharged, lost.	m +	l., m.	"				
798	Aborted.		(Fetus lost.)	m., h.	(Died, metritis.)				
799	Normal.	Discharged, lost.	m +	m., h.	Slightly premature, 2 yrs. 5½ mos.	Retained, +		h.	3rd calf nearly full term, dead. Swab +. Placenta retained.
800	"	" "		l.	(Sold.)				
801	"	" "		l.	Normal, 14 mos. 11 days.	Discharged, lost.			
802	"	" "		l.	Normal, 13 mos. 28 days.	" "			
803	"	Retained, +	u —	m., h.	(Sold.)				
804	"	Discharged, lost.		l.	Normal, 15 mos. 14 days.	Normal.			3rd calf normal. Blood titer low.
805	"	Normal.		l., h.	(Sold.)				
806	"	Discharged, lost.		l., m.	Normal, 2 yrs. less 21 days.				3rd calf normal. Blood titer 1:320.
807	"	" "		l.	(Sold.)				
808	"	" "		l., h.	Aborted.	Diseased.	(Fetus lost.)		
809	"	Normal.		l.	Normal, 15 mos. 26 days.	Discharged, lost.		l.	3rd calf normal. Blood titer 1:40.
810	"	Discharged, lost.		l.	Normal, 15 mos. 9 days.	" "		l.	3rd calf normal. Blood titer low.
811	"	" "	u —, m —	l., m.	(Sold.)				
813	Aborted.	Retained.	(Fetus lost.)	h.	"				
814	Normal.	"		m., l.	"				
815	"	Normal.		l.	Normal, 1 yr. 23 days.	Normal.		l.	Aborted, 3rd pregnancy. Blood titer 1:320.
816	"	Discharged, lost.			Normal, 14 mos.	Discharged, lost.			
817	"	" "		l.	(Sold.)				
819	Aborted.	Retained.	(Fetus lost.)	h.	"				

* f = fetus, m = milk, u = udder fluid.

The placenta was expelled and appeared normal. One guinea pig inoculated with amniotic fluid and another with suspension of placental tissue were negative. July 19, of three guinea pigs receiving milk into abdomen, two yielded cultures of *B. abortus*. Agglutinin titers: July 25, 1:640; Oct. 21, above 1:320; Jan. 29, 1920, 1:1,280. On June 10, cow gave birth to full term calf. The placenta was retained. Sold.

No. 423.—Fawn, born July 4, 1917. Sept. 30, 1919, heifer aborted a living fetus which was killed soon after expulsion and autopsied. Weight 32 lbs. Autopsy shows in general normal conditions of digestive tract and absence of transudates from serous cavities. Meconium still unexpelled. Miscellaneous bacteria had spread down digestive tract as far as colon. Cultures from rectal contents yield pure *B. abortus*. Cultures from other portions of digestive tract contain several species. Spleen cultures sterile. Streptococci in liver cultures.

About one-third to one-half of total placenta obtainable. In this the chorion is extensively involved. Whitish plaques make the chorion opaque in spots. The small adventitious cotyledons and the periphery of the large ones are yellowish, cheesy. Films show small groups of *B. abortus*. Epithelial cells filled with them rare. Guinea pigs inoculated respectively with scrapings of placenta, lung, and stomach fluid of fetus develop large spleens containing *B. abortus*.

Oct. 1, titer of blood 1:320. Oct. 9, milk with agglutinin titer of 1:1,280 was tested on three guinea pigs with negative results. Apr. 15, 1920, titer of mixed milk 1:320. Oct. 13, titer of blood 1:80. Sold.

No. 433.—Black and white, born Apr. 18, 1917. Titer of blood, Oct. 22, 1919, 1:20. Oct. 24, heifer aborted. Disease due to *Vibrio fetus*. For a detailed account of this case see *Journal of Experimental Medicine*, 1920, xxxii, 683. Sterile, slaughtered.

No. 438.—Black and white, born July 7, 1917. Nov. 9, 1919, heifer aborted. The placenta was retained. Disease due to *Vibrio fetus*. For more data on this case see *Journal of Experimental Medicine*, 1920, xxxii, 683. Nov. 13, titer of blood 1:40. Nov. 18, milk with titer less than 1:20 was tested on three guinea pigs with negative results. Apr. 15, 1920, titer of blood 1:80; titer of mixed milk less than 1:20. Died.

No. 449.—Fawn, born Oct. 10, 1918. Agglutinin titers: Feb. 12 and Apr. 1, 1919, 1:80; Nov. 25, 1:20. Dec. 2, heifer aborted. Disease of chorion due to *Vibrio fetus*. For a detailed account of this case see *Journal of Experimental Medicine*, 1920, xxxii, 683. Agglutinins for *B. abortus* low. Sold.

No. 453.—Black and white, born Aug. 11, 1917. Nov. 25, 1919, titer of blood 1:320. Dec. 9, heifer aborted. The placenta was retained. Fetus, male, weighs 43 lbs. and is 90 cm. long. Coat of hair complete. Caudal extremity including rectum eaten away by some animal. Organs in general of normal appearance and transudates absent. Contents of stomachs normal. Lungs without any inflated regions. *B. abortus* was isolated in pure culture from fourth stomach,

colon, spleen, kidney, liver, and lungs, and through guinea pigs from fourth stomach and colon contents, lung, and uterine swab. Dec. 10, colostrum with titer of 1:1,280 was tested on three guinea pigs with positive results in each case; titer of blood 1:1,280. Sold.

No. 456.—Solid fawn, born June 12, 1917. Nov. 25, 1919, titer of blood 1:80. Dec. 22, heifer gives birth to a living fetus weighing 33 lbs. and 82 cm. long. The autopsy showed nothing noteworthy. *B. abortus* was isolated only from the spleen. Cultures from the fourth stomach and lungs were contaminated by bacteria invading the digestive tract from the mouth. Those from colon and rectum remained sterile. Guinea pigs were inoculated with material from the lungs, rectum, and fourth stomach with negative outcome. *B. abortus* was, however, isolated from a suspension of uterine exudate (swab) through a guinea pig. The placenta was retained. Dec. 26, milk with titer of 1:80 was tested on guinea pigs with positive results. Dec. 30, titer of blood 1:320. Sold.

No. 457.—Fawn and white, born Sept. 9, 1917. Dec. 10, 1919, titer of blood 1:160. Dec. 23, heifer aborted a hairless fetus, 61 cm. long. Marked subcutaneous edema; transudate colored with hemoglobin. 160 cc. of dark red fluid in abdomen. Stomach fluid contains a large amount of swallowed meconium. Countless colonies of *B. abortus* develop in agar slants of contents of fourth stomach and colon, and of lung tissue. Placenta retained. Dec. 26, milk with titer of 1:160 was tested on three guinea pigs with negative results. Dec. 30, titer of blood 1:1,280. Sold.

No. 458.—Fawn and white, born Nov. 13, 1917. Nov. 25, 1919, titer of blood 1:40. Dec. 26, heifer aborted. The placenta was retained. Fetus is 90 cm. long with full coat of hair. Autopsy shows partial inflation of lungs with air. There are no transudates. Edemas absent. A slight amount of fecal matter in stomachs. *B. abortus* was isolated in cultures from lungs, liver, colon, and rectum. Guinea pigs inoculated with suspension from uterine swab and with suspension of lung tissue were positive for *B. abortus*. Dec. 30, milk with titer of 1:320 was tested on three guinea pigs with positive results in two; titer of blood 1:640. Sold.

No. 460.—Black and white, born Mar. 15, 1917. Nov. 25, 1919, titer of blood 1:20. Dec. 30, heifer aborted. Placenta retained. Fetus weighs 18 lbs. and is 69 cm. long. Without coat of hair. Considerable subcutaneous and intermuscular edema. 200 cc. of blood-colored fluid in abdominal cavity and 160 cc. in pleural cavity. Stomachs contain much fecal matter. Organs in general soft and pulpy. *B. abortus* was isolated from contents of fourth stomach, colon, and rectum, from bits of tissue of liver, spleen, kidneys, and lungs. Guinea pigs inoculated at the same time with material from a uterine swab, fourth stomach, meconium, and lungs proved positive for *B. abortus*. Jan. 2, 1920, titer of blood 1:640. Jan. 5, milk with titer of 1:640 was tested on three guinea pigs with negative results.

Oct. 13, titer of blood above 1:320. Oct. 14, cow aborted a second time. The placenta was retained. Fetus weighs 26 lbs. and is 78 cm. long. Hair starting to grow around mouth, hoofs, etc. There was some fecal matter (meconium) in stomachs. Otherwise there was nothing noteworthy. *B. abortus* was isolated in cultures from fourth stomach, colon, rectum, spleen, and lungs. Cultures from kidneys and liver remain sterile. Guinea pigs inoculated with contents of fourth stomach, lung tissue, and uterine swab suspension were positive for *B. abortus*. Sold.

No. 461.—Black and white, born June 4, 1917. Dec. 2, 1919, titer of blood 1:320. Jan. 2, 1920, heifer aborted. Only a small piece of placenta obtained. The cotyledons have resting on them small bits of yellowish, soft, mucoid masses, made up of cells, among which are large epithelial elements, filled with *B. abortus*. Cultures on agar from such bits gave a majority of *B. abortus* colonies.

The fetus weighs 13½ lbs. and measures 59 cm. Coat of hair not developed. Peritoneal cavity contains about 200 cc. of blood-colored fluid. The stomach fluids contain more or less meconium. Liver cells contain much fat. No other noteworthy changes. *B. abortus* was isolated from fourth stomach, colon, rectum, liver, kidneys, spleen, and lungs.

Three guinea pigs were inoculated with milk drawn 3 days after the abortion. None developed the typical lesions and cultures from the spleens failed to develop. Titer of milk 1:160. Titer of blood: Jan. 2, 1:320; Oct. 13, 1:80; Mar. 29, 1921, 1:160.

July 2, cow calved normally. Placenta expelled and about half obtained, which appeared normal. Swab tested on a guinea pig with negative result. Titer of blood 1:80; titer of the colostrum 1:320. Sold.

No. 463.—Solid fawn, born Sept. 29, 1917. Titer of blood: Dec. 2, 1919, 1:10; Jan. 6, 1920, 1:20. Jan. 9, heifer aborted. Placenta retained. Fetus alive when born. Killed. Weighed 25 lbs., measured 33 inches, and had a full coat of hair. The autopsy showed fresh hemorrhage into the peritoneal cavity. The rectum was markedly distended with fecal matter. No edema or transudates. *B. abortus* was not isolated from this case, either directly or through guinea pigs, nor was it obtained through a guinea pig from the uterine swab. Jan. 13, milk with titer less than 1:10 was tested on three guinea pigs with negative result; titer of blood 1:80. Sold.

No. 466.—Black and white, born Apr. 24, 1917. Titer of blood: Jan. 6, 1920, 1:160; Jan. 29, 1:320. Jan. 13, udder fluid tested on guinea pigs with negative result. Feb. 13, heifer calved normally. Placenta was expelled and appeared normal. Swab tested on three guinea pigs with negative result. Feb. 18, milk with titer of 1:320 was tested on three guinea pigs with positive result in each case. Feb. 25, titer of blood 1:320.

Feb. 13, 1921, cow gave birth to a normal calf. The placenta was retained. A swab tested on guinea pigs gave positive results. The placenta was removed 2

days after the calf was born. The portion obtained was putrefactive. The chorion exhibited thickened whitish areas. The cotyledons were in part necrotic.

Feb. 13, titer of blood 1:320; titer of colostrum 1:2,560. Titer of milk: Feb. 23, 1:640; Mar. 3, 1:320. Sold.

No. 473.—Black and white, born June 25, 1917. Titer of blood: Nov. 25, 1919, 1:10; Jan. 6, 1920, 1:40; Jan. 30, 1:320. Feb. 11, heifer calved. Calf lived only a short time. Placenta retained. Films prepared from swabs from uterine cavity show many abortion bacilli, some intracellular. Feb. 25, milk with titer of 1:320 was tested on three guinea pigs with negative results. Titer of blood: Feb. 25, 1:1,280; Oct. 13, above 1:320; Mar. 29, 1921, 1:640.

May 3, 1921, cow discharged full term calf. Placenta expelled. Suspensions from uterine swab injected into a guinea pig with negative result. The placenta is normal with exception of two cotyledons in which the bulk of the villi appear as short grayish nodules. Titer of blood 1:1,280; titer of the colostrum 1:2,560.

May 18, 1922, cow calved normally. Placenta was expelled but lost. June 8, titer of blood 1:320.

No. 474.—Black and white, born Mar. 22, 1917. Titer of blood: Dec. 2, 1919, 1:160; Jan. 6 and 30, 1920, 1:320. Jan. 30, udder fluid tested on four guinea pigs with negative results. Apr. 21, heifer gave birth to full term calf. The placenta was expelled 3 hours later. The unoccupied horn was diseased. Several cotyledons were completely necrotic, the remainder showed marginal necrosis. Patches of exudate, 1 to 2 mm. in diameter, attached to chorion. Minute mucoid masses lying loosely on the membrane. Films of the exudates show immense numbers of minute bacilli, resembling *B. abortus*. Sections of hardened material show the characteristic groups of infected epithelium still *in situ*. Apr. 22, titer of blood 1:320. Titer of the colostrum on day of parturition 1:1,280. July 13, titer of mixed milk less than 1:20. Oct. 13, titer of blood above 1:320.

Feb. 21, 1921, cow calved normally. The placenta was expelled but lost. A guinea pig was inoculated with a salt solution suspension of uterine swab with negative result. Titer of colostrum 1:640. Titer of blood the following day 1:640. Mar. 3, titer of mixed milk 1:20. Sold.

No. 476.—Black and white, born May 29, 1917. Titer of blood: Nov. 25, 1919, 1:10; Jan. 6, 1920, 1:80; Jan. 30, 1:320. Jan. 20, udder fluid tested on two guinea pigs with positive results. Mar. 1, heifer aborted a living fetus. The placenta was retained and removed after the 4th day. The fetus weighed 57 lbs. and was 97 cm. long, nearly full term. The autopsy presented nothing noteworthy. *B. abortus* was isolated in pure culture from fourth stomach, colon, rectum, spleen, liver, kidneys, and lungs. Films from a uterine swab showed epithelial cells filled with *B. abortus* and a guinea pig inoculated with a salt solution suspension of it developed a large spleen from which *B. abortus* was cultured. Mar. 10, titer of blood 1:1,280. Sold.

No. 482.—Black and white, born Aug. 20, 1917. Titer of blood: Dec. 2, 1919, 1:20; Jan. 6, 1920, 1:20. Feb. 17, heifer aborted a living fetus which died soon

after. Weight of fetus 52 lbs., length 92 cm. The autopsy showed nothing noteworthy. *B. abortus* was isolated from the contents of the fourth stomach through a guinea pig. Guinea pigs inoculated with rectal contents and suspension of lung tissue were negative. The placenta was retained. Feb. 25, milk with titer of 1:80 was tested on three guinea pigs with negative results. Titer of blood 1:1,280.

Feb. 13, 1921, cow calved normally. The placenta was expelled but lost. Titer of blood, Feb. 14 and May 1, 1:80; titer of mixed milk, Feb. 16, 1:40. Sold.

No. 483.—Black and white, born May 1, 1917. Titer of blood: Nov. 25, 1919, 1:20; Jan. 6, 1920, 1:80; Jan. 30, 1:320. Jan. 20, udder fluid tested on three guinea pigs with negative results. Feb. 20, heifer aborted. The placenta was expelled after 4 to 5 hours. The chorion was diseased both as to the cotyledons and the membrane itself. The peripheral zone of the former is yellowish, necrotic. The chorion is in part whitish, opaque, thickened; in part covered with soft, loose, yellowish masses in which are large epithelial cells, filled with minute bacteria like *B. abortus*.

The fetus, alive when born, was killed and autopsied. Weight 49 lbs., 95 cm. long. Transudates absent. There were no noteworthy abnormalities. Cultures from colon, rectum, liver, and spleen remain sterile. One from fourth stomach develops moulds. The other remains sterile. Diagnosis based on the presence of *B. abortus* in films from uterine exudate and from sections of placenta in which much of the epithelium was still *in situ* and densely filled with fine bacilli. Titer of blood, Feb. 25, 1:640; Oct. 13, above 1:320.

Mar. 5, 1921, cow calved prematurely. Still giving milk at this time. The calf was weak and undersized. The placenta was diseased, but only about one-half obtainable. The chorion was thickened in spots and films from exudate show *B. abortus* within large cells. Mar. 5, titer of blood 1:1,280; titer of milk, slightly resembling colostrum, 1:2,560. Mar. 9, titer of mixed milk 1:1,280.

Mar. 16, 1922, cow calved normally. The placenta was expelled but lost. Apr. 7, titer of blood 1:1,280.

No. 486.—Fawn, born Mar. 2, 1918. Titer of blood: Dec. 2, 1919, less than 1:20; Jan. 6, 1920, 1:40; Feb. 25, 1:1,280. Feb. 29, heifer aborted. The placenta was retained. Fetus weighs 8,675 gm. and measures 64 cm. in length. Hair beginning to grow on the ears, mouth, etc. Blood-colored edema of subcutis and muscles and transudates of the same tint in peritoneal, pleural, and pericardial cavities. Delicate layer of fibrin on pleura. Considerable fecal matter mixed with mucoid fluid of stomachs.

B. abortus isolated in cultures from fourth stomach, colon, spleen, liver, kidneys, and lungs. Positive results obtained by inoculating guinea pigs with contents of fourth stomach, meconium, and lungs. *B. abortus* was obtained from uterine swab through a guinea pig. Sold.

No. 487.—Black and white, born June 6, 1917. Titer of blood: Nov. 25, 1919, 1:10; Jan. 6, 1920, 1:20; Jan. 30 and Feb. 25, 1:80. On Mar. 30, heifer calved

normally. The placenta was expelled and appeared normal. Titer of colostrum 1:320. Mar. 31, titer of blood 1:160. Apr. 1 and July 13, titer of milk less than 1:20. Oct. 22, titer of blood 1:40; of mixed milk less than 1:20.

No. 488.—Black and white, born Aug. 14, 1917. Titer of blood: Nov. 25, 1919, less than 1:20; Jan. 6 and 30, 1920, 1:40; Feb. 25, 1:160. Mar. 8, heifer gave birth to full term calf. The placenta was discharged within 4 hours. Disease was demonstrated by the yellowish necrotic margin of most cotyledons and necrotic villi within. Small, mucoid flakes resting on the cotyledons. In the central portion of the fetal sac the chorion is opaque, whitish, and somewhat thickened. Films prepared from the uterine swab and sections of the placenta show the characteristic infection of the chorion epithelium with *B. abortus*. Colostrum, with titer of 1:640, was tested on three guinea pigs with positive result in each case. Mar. 10, titer of blood 1:640. July 13, mixed milk, with titer less than 1:20, was tested on guinea pigs with negative results. Oct. 13, titer of blood above 1:320.

Feb. 18, 1921, cow calved normally. The placenta was expelled and appeared normal. Titer of blood 1:640; titer of colostrum 1:10,240. The calf, though weak and undersized, was brought to the Institute. On Mar. 24, it received subcutaneously a dose of *B. actinoides*. It was killed Apr. 8 and *B. abortus* was isolated from the lungs. Mar. 3, titer of mixed milk 1:160. Nov. 18, titer of blood above 1:320.

Mar. 12, 1922, cow calved normally. The placenta was expelled but lost. Sold.

No. 494.—Black and white, born Aug. 13, 1917. Titer of blood: Dec. 2, 1919, 1:20; Jan. 6, 1920, 1:80; Feb. 25, 1:1,280. Udder fluid collected Mar. 4 and 17 and each sample tested on a guinea pig with positive result. Apr. 3, heifer aborted a living fetus with complete coat of hair. 88 cm. long. Fetus was killed and autopsied. Nothing noteworthy found. The meconium in rectum was dry and dark, like normal meconium. Cultures from spleen, liver, and kidneys negative for *B. abortus*. One guinea pig inoculated with a suspension of meconium negative for *B. abortus*.

The placenta was retained. Films made from a uterine swab showed characteristic groups of *B. abortus* and a guinea pig inoculated with a suspension from the same developed a large spleen from which *B. abortus* was isolated.

Apr. 5, titer of blood 1:2,560; titer of colostrum 1:1,280. Died.

No. 495.—Black and white, born Aug. 24, 1917. Titer of blood: Dec. 2, 1919; 1:20; Jan. 6 and Mar. 15, 1920, 1:40. Apr. 15, heifer calved normally. The placenta was expelled and appeared normal. Apr. 21, titer of blood 1:20. Mar. 29, 1921, titer of blood 1:20. Aug. 2, cow calved normally. The placenta was expelled but lost. Titer of blood 1:20; of colostrum 1:40.

No. 503.—Black and white, born Dec. 20, 1917. Titer of blood: Dec. 10, 1919, 1:40; Jan. 6, 1920, 1:20; Feb. 25, 1:80; Apr. 6, 1:640. June 15, heifer aborted. The placenta from the non-gravid horn was expelled, remainder retained. One

cotyledon completely necrosed and the chorion surrounding it beset with yellowish-white plaques 6 to 7 mm. in diameter. In the other cotyledons there are necrotic villi scattered through them and on periphery. Films of placental scrapings show epithelial cells filled with *B. abortus*. Sections of hardened tissue show patches of epithelium still *in situ*, filled with *B. abortus*. The fetus was alive when born but died 7 hours later. It weighed 57 lbs. and measured 100 cm. in length. The autopsy showed nothing noteworthy. *B. abortus* was isolated from the contents of the colon and rectum in pure culture. Other cultures contained miscellaneous bacteria. Titer of colostrum 1:1,280. June 16, titer of blood 1:1,280.

Mar. 14, 1921, cow aborted. The placenta was retained. Fetus not obtained. Nov. 10, titer of blood 1:1,280. Jan. 24, 1922, titer of colostrum 1:1,280.

Feb. 5, cow gave birth to full term twin calves. Most of the placenta was expelled. A small part examined appeared normal. Feb. 9, three guinea pigs inoculated with suspensions from uterine swab were negative as to *B. abortus*. Feb. 7, titer of blood 1:80.

No. 505.—Black and white, born Apr. 15, 1917. Titer of blood: Nov. 25, 1919, less than 1:10; Jan. 6, 1920, less than 1:20. Jan. 19, heifer calved normally. The placenta was expelled and appeared normal. Titer of blood: Jan. 26, less than 1:20; Mar. 31, less than 1:160; Apr. 15, less than 1:320.

Mar. 24, 1921, cow calved normally. The placenta was expelled but lost. Dec. 21, titer of blood 1:80.

Feb. 24, 1922, cow calved normally. The placenta was expelled but lost. Mar. 10, titer of blood 1:160.

No. 522.—Black and white, born Feb. 8, 1917. Titer of blood: Dec. 2, 1919, 1:20; Jan. 6, 1920, 1:20; Feb. 25, 1:160; Apr. 6, 1:640.

Oct. 18, heifer calved normally. The placenta was expelled and appeared normal. Oct. 19, titer of blood 1:640; of colostrum 1:160. Sediment from milk tested on three guinea pigs with positive result in one case only. Whole milk injected into abdomen of two guinea pigs in doses of 7 and 5 cc. respectively with negative result.

Jan. 21, 1921, titer of mixed milk 1:80.

Oct. 25, cow calved normally. Placenta expelled but lost. Titer of colostrum 1:640. Nov. 10, titer of blood 1:640. Third calf born on pasture during the summer of 1922. Blood titer, Dec. 15, is 1:640.

No. 547.—Fawn and white, born Sept. 5, 1917. Nov. 13, 1919, titer of blood less than 1:20. Nov. 27, heifer calved normally. The placenta was expelled but lost. Titer of blood: Dec. 10, 1:40; Mar. 31, 1920, 1:640; Apr. 15, 1:1,280; Oct. 13, above 1:320. Dec. 7, cow aborted a living fetus which was killed and autopsied. Weight of fetus 35 lbs., length 86 cm. It had a complete coat of hair. The organs showed nothing noteworthy. *B. abortus* was isolated through a guinea pig from a suspension made from a uterine swab. Guinea pigs inoculated with contents of stomach, rectum, and lung tissue were negative. Colostrum

with titer of 1:1,280 was tested on three guinea pigs with positive results. Titer of blood 1:1,280. Sold.

No. 551.—Fawn and white, born June 24, 1917. Oct. 22, 1919, titer of the blood less than 1:20. Nov. 4, heifer calved normally. The placenta was expelled but lost. Titer of blood: Dec. 10, 1:10; Mar. 31, 1920, and Apr. 15, 1:1,280.

Dec. 15, cow aborted a living fetus which was killed and autopsied. Weight 44 lbs., length 84 cm. About 50 cc. of fluid, serum-like, in peritoneal cavity. No other noteworthy features. *B. abortus* was isolated from the colon. Cultures from the other segments of the digestive tract contained large Gram-positive rods. Guinea pigs inoculated with material from the fourth stomach, rectum, and lungs proved negative. Three guinea pigs inoculated with milk were negative for *B. abortus*. Dec. 17, titer of milk 1:1,280. Nov. 10, 1921, titer of blood 1:1,280.

Apr. 25, 1922, cow calved normally. The placenta was expelled but lost. Apr. 27, suspension from uterine swab inoculated into a guinea pig with negative result. June 8, titer of blood 1:1,280.

No. 564.—Black and white, born May 2, 1917. Titer of blood: Dec. 2, 1919, 1:40; Jan. 6, 1920, 1:160. Jan. 10, heifer calved normally. The placenta was expelled but lost. Jan. 13, titer of blood 1:640. Jan. 20, milk with titer of 1:160 was tested on three guinea pigs with negative results. Oct. 13, titer of blood 1:320.

Jan. 26, 1921, cow calved normally. The placenta was expelled and appeared normal, except a few villi in cotyledons of the unoccupied horn. These appeared as if inorganic salts had been deposited in them. Sections indicate necrosis of certain villi. Titer of blood 1:640; of colostrum 1:2,560. Feb. 3, titer of mixed milk 1:320.

Mar. 31, 1922, cow calved normally. The placenta was expelled and appeared normal. Apr. 7, titer of blood 1:640.

A fourth, normal calf born Apr. 23, 1923. Placenta normal.

No. 586.—Black and white, born June 2, 1917. Oct. 7, 1919, titer of blood 1:160. Oct. 26, heifer calved normally. The placenta was expelled but lost. Dec. 3, milk with titer of 1:160 was tested on three guinea pigs with positive results. Titer of blood: Nov. 14, 1:320; Oct. 13, 1920, above 1:320.

Jan. 17, 1921, cow calved normally. The placenta was retained. Two guinea pigs were inoculated with a suspension of uterine fluid (swab) with negative results. Sold.

No. 642.—Black and white, born Aug. 9, 1917. Titer of blood: Dec. 2, 1919, 1:10; Jan. 2 and 30, 1920, 1:80; Feb. 25, 1:1,280; Mar. 10, 1:640. Jan. 30, udder fluid tested on two guinea pigs with positive result in each case. Mar. 24, heifer gave birth to full term calf. The placenta was retained for over a day. Titer of blood: Apr. 1, 1:2,560; Mar. 29, 1921, 1:1,280.

May 1, cow calved normally. The placenta was expelled but lost. June 22, titer of blood above 1:640. July 20, milk with titer of 1:160 was tested on three guinea pigs with negative results. Direct cultures positive.

Apr. 5, 1922, cow calved normally. The placenta was expelled but lost. Apr. 7, titer of blood 1:160.

No. 775.—Red and white, born Feb. 21, 1917. Titer of blood: Feb. 12 and Apr. 1, 1919, 1:20. May 10, heifer calved normally. The placenta was expelled and appeared normal. Titer of blood: June 4, 1:40; Sept. 22, 1:20; Jan. 29, 1920, 1:40; Apr. 1, 1:80. July 20, cow calved normally. The placenta was expelled but lost. Sold.

No. 776.—Red and white, born Feb. 26, 1917. Titer of blood: Feb. 12, 1919, less than 1:10; Apr. 1, 1:20. May 15, heifer calved normally. The placenta was expelled but lost. June 4, titer of blood less than 1:10. Sold.

No. 777.—Red and white, born Mar. 5, 1917. Titer of blood: Feb. 12, 1919, 1:10; Apr. 1 and May 12, 1:20. June 23, heifer calved normally. The placenta was expelled and appeared normal. Titer of blood: Aug. 11, 1:640; Sept. 22, 1:320; Jan. 29, 1920, 1:1,280. Sold.

No. 778.—Red and white, born Mar. 11, 1917. Titer of blood: Feb. 12, 1919, less than 1:10; Apr. 1 and May 12, 1:20. June 22, heifer calved normally. The placenta was expelled but lost. Titer of blood: Aug. 1, 1:40; Oct. 21, less than 1:10; Jan. 29, 1920, 1:40. Sold.

No. 779.—Fawn, born Mar. 19, 1917. Titer of blood: Feb. 12 and May 12, 1919, 1:20. July 26, heifer calved normally. The placenta was expelled but lost. Titer of blood: Aug. 1, 1:40; Sept. 24, 1:20; Jan. 29 and Mar. 31, 1920, 1:80. June 16, cow calved normally. The placenta was expelled but lost. May 1, 1921, titer of blood 1:80.

No. 780.—Black and white, born Apr. 21, 1917. Titer of blood: Nov. 25, 1919, 1:40; Dec. 30, 1:320. Jan. 18, 1920, heifer calved normally. The placenta was expelled but lost. Jan. 20, milk with titer of 1:160 was tested on three guinea pigs with positive results. Jan. 26, titer of blood 1:1,280. May 22, 1921, cow calved normally. The placenta was expelled but lost. Nov. 10, titer of blood 1:1,280. July 2, 1922, cow gave birth to a third calf. The placenta was expelled but lost. Aug. 23, titer of blood 1:1,280.

No. 781.—Fawn, born Apr. 21, 1917. Titer of blood: Nov. 25, 1919, less than 1:10; Dec. 30, 1:20. Jan. 7, 1920, heifer calved normally. The placenta was expelled but lost. Jan. 29, milk with titer less than 1:10 was tested on three guinea pigs with negative results. Titer of blood: Jan. 26, 1:160; Mar. 31, 1:320; Oct. 13, 1:80. July 17, 1921, cow gave birth to a full term calf. Placenta retained. July 18, the placenta was removed but not examined. Dec. 21, titer of blood 1:80. Sept. 23, 1922, heifer calved normally. Placenta normal. Blood titer 1:80.

No. 783.—Fawn, born May 12, 1917. Titer of blood: Dec. 2, 1919, 1:320; Dec. 30, 1:160. Jan. 14, 1920, udder fluid tested on three guinea pigs with one positive result. Jan. 19, heifer calved normally. The placenta was expelled but lost. Milk with titer of 1:160 was tested on three guinea pigs with negative results. Jan. 26, titer of blood 1:640. Sold.

No. 784.—Black and white, born May 12, 1917. Titer of blood: Nov. 25, 1919, less than 1:10; Dec. 30, 1:20. Jan. 22, 1920, heifer calved normally. The placenta was expelled and appeared normal. Titer of blood: Jan. 26 and Mar. 31, 1:40; Oct. 13, above 1:320. Dec. 28, cow calved normally. The placenta was expelled but lost. Nov. 10, 1921, titer of blood above 1:320. Dec. 27, cow calved normally. The placenta was expelled but lost. June 8, 1922, titer of blood 1:320. Third calf born Oct. 28, 1922. Placenta discharged but lost. Feb. 2, 1923, titer of blood 1:640.

No. 785.—Black and white, born May 13, 1917. Titer of blood, Oct. 7, 1919, 1:1,280. Oct. 19, heifer aborted on pasture. Placenta retained. Fetus not obtained. Oct. 21, titer of blood 1:1,280. Sold.

No. 786.—Fawn and white, born May 17, 1917. Sept. 17, 1919, heifer aborted. The placenta was retained. Fetus was lost. Sept. 26, titer of blood 1:1,280. Oct. 17, 1920, cow calved. The placenta was retained. The calf was weak and below normal. Oct. 19, titer of blood 1:640. Sold.

No. 787.—Black and white, born May 24, 1917. Nov. 13, 1919, titer of blood less than 1:10. Dec. 2, heifer calved normally. The placenta was expelled but lost. Titer of blood: Dec. 30, 1:20; Mar. 31, 1920, 1:40; Oct. 13, 1:20. Dec. 16, cow calved normally. The placenta was expelled but lost. Dec. 17, titer of blood 1:40; titer of colostrum 1:80. Nov. 11, 1921, cow calved normally. The placenta was expelled but lost. Dec. 21, titer of blood 1:40.

No. 788.—Black and white, born May 24, 1917. Sept. 23, 1919, heifer aborted on pasture. Fetus lost. Placenta retained. Sept. 26, titer of blood 1:20. Sept. 28, 1920, cow calved normally. The placenta was expelled but lost. Oct. 13, titer of blood 1:20. Aug. 9, 1921, cow calved normally. The placenta was expelled but lost. Dec. 21, titer of blood 1:320. July 2, 1922, cow calved normally. The placenta was expelled and appeared normal except for some large and small cotyledons which may have been prematurely detached. Guinea pigs inoculated with bits of ground-up placental tissue all died of septicemia. Aug. 23, titer of blood 1:320.

No. 789.—Black and white, born June 2, 1917. Titer of blood: Nov. 25, 1919, 1:10; Jan. 2, 1920, 1:20. Jan. 21, heifer calved normally. The placenta was expelled but lost. Titer of blood: Jan. 26, 1:40; Mar. 31, 1:320; Apr. 15, 1:640; Oct. 13, 1:80. Jan. 30, 1921, cow calved normally. The placenta was expelled but lost. Swab tested on guinea pigs with negative results. Titer of blood 1:40; titer of colostrum 1:80. Feb. 2, titer of mixed milk less than 1:20. Sold.

No. 790.—Black and white, born June 10, 1917. Titer of blood: Dec. 2, 1919, 1:40; Jan. 6, 1920, 1:20; Jan. 30, 1:40. Feb. 11, heifer calved normally. The placenta was expelled but lost. Feb. 25, titer of blood 1:320. Mar. 1, milk with titer less than 1:20 was tested on guinea pigs with negative results. Mar. 10, titer of blood 1:320. Jan. 30, 1921, cow calved normally. The placenta was expelled but lost. Nov. 10, titer of blood 1:80. Sold.

No. 791.—Fawn, born June 12, 1917. Titer of blood: Nov. 21, 1919, less than 1:10; Dec. 30, 1:20. Jan. 20, 1920, heifer calved normally. The placenta was expelled. It appeared normal except for one cotyledon of which a portion of the margin was necrotic. Titer of blood: Jan. 26, 1:20; Mar. 31, 1:40. Sold.

No. 793.—Black and white, born June 18, 1917. Oct. 22, 1919, titer of blood 1:20. Oct. 23, heifer calved normally. The placenta was expelled but lost. Titer of blood: Nov. 14, 1:40; Mar. 31, 1920, 1:40. Oct. 12, cow calved normally. The placenta was expelled but lost. Swab tested on guinea pigs with negative results. Oct. 13, titer of blood 1:80. Oct. 11, 1921, cow gave birth to full term calf. Probably some of the placenta was retained for she had a uterine discharge for a number of days after parturition. Jan. 25, 1922, titer of blood 1:40. Sold.

No. 794.—Red and white, born June 18, 1917. Nov. 21, 1919, titer of blood less than 1:20. Nov. 27, heifer calved normally. The placenta was expelled but lost. Titer of blood: Dec. 10, 1:10; Mar. 31, 1920, 1:20; Oct. 13, less than 1:10. Sold.

No. 795.—Red and white, born June 18, 1917. Nov. 21, 1919, titer of blood 1:20. Dec. 6, heifer calved normally. The placenta was expelled but lost. Dec. 10, titer of blood 1:40. Sold.

No. 796.—Red and white, born June 22, 1917. Titer of blood: Nov. 25, 1919, 1:20; Dec. 30, 1:40. Jan. 17, 1920, heifer calved normally. The placenta was expelled and appeared normal. Jan. 29, milk with titer less than 1:20 was tested on guinea pigs with negative results. Titer of blood: Jan. 26, 1:320; Mar. 31, 1:1,280; Oct. 13, above 1:320. Sold.

No. 797.—Fawn and white, born June 23, 1917. Nov. 25, 1919, titer of blood 1:10. Dec. 18, heifer calved normally. The placenta was expelled but lost. Jan. 20, 1920, milk with a titer of 1:160 was tested on three guinea pigs with positive results. Titer of blood: Dec. 30, 1919, 1:320; Oct. 13, 1920, above 1:320. Sold.

No. 798.—Red and white, born June 25, 1917. Titer of blood: Nov. 25, 1919, 1:160; Dec. 30, 1:640. Jan. 3, 1920, heifer aborted and fetus was lost. Cow died of septic metritis.

No. 799.—Fawn, born June 25, 1917. Titer of blood: Oct. 7 and Dec. 30, 1919, 1:320. Jan. 19, 1920, heifer calved normally. The placenta was expelled but lost. Swab tested on a guinea pig with negative result. Jan. 20, colostrum with titer of 1:1,280 was tested on three guinea pigs with positive result in each case. Jan. 26, titer of blood 1:1,280. Oct. 13, titer of blood above 1:320. June 30, 1922, cow gave birth to a premature calf. The placenta was retained. Guinea pigs inoculated with suspensions of placental tissue and uterine swab developed the characteristic *B. abortus* lesions.

June 6, 1923, dead calf born. Placenta retained. *B. abortus* present. June 8, titer of blood 1:2,560.

No. 800.—Fawn, born June 25, 1917. Oct. 7, 1919, titer of blood less than 1:20. Nov. 5, heifer calved normally. The placenta was expelled but lost. Nov. 14, titer of blood 1:20. Sold.

No. 801.—Red and white, born July 10, 1917. Nov. 11, 1919, heifer calved normally. The placenta was expelled but lost. Titer of blood: Nov. 13, 1:40; Mar. 31 and Apr. 15, 1920, 1:80; Oct. 13, 1:40. Jan. 22, 1921, cow calved normally. The placenta was expelled but lost. Sold.

No. 802.—Red and white, born July 12, 1917. Nov. 13, 1919, titer of blood less than 1:20. Dec. 3, heifer calved normally. The placenta was expelled but lost. Titer of blood: Dec. 10, 1:40; Mar. 31, 1920, 1:40; Oct. 13, 1:20. Jan. 31, 1921, cow calved normally. The placenta was expelled but lost. Titer of blood 1:40; of colostrum 1:40. Titer of blood: May 1, 1:20; May 17, 1:40. Sold.

No. 803.—Black and white, born July 14, 1917. Titer of blood: Dec. 2, 1919, 1:320; Jan. 6, 1920, 1:640. Jan. 20, udder fluid tested on guinea pigs with negative results. Jan. 25, heifer gave birth to a full term calf. The placenta was retained. Jan. 26, swabs tested on two guinea pigs with positive results for *B. abortus* in both cases. Titer of blood: Jan. 29, 1:640; Oct. 13, above 1:320; Nov. 10, 1921, 1:1,280. Sold.

No. 804.—Red and white, born July 16, 1917. Oct. 22, 1919, titer of blood 1:10. Oct. 27, heifer calved normally. The placenta was expelled but lost. Titer of blood: Nov. 14, 1:20; Mar. 31 and Apr. 15, 1920, 1:160; Oct. 13, 1:40. Feb. 10, 1921, cow calved normally. The placenta from gravid horn was expelled and appeared normal. Titer of blood 1:80; of colostrum 1:160. Feb. 16, titer of milk 1:80. Mar. 10, 1922, cow calved normally. The placenta was expelled but lost. Apr. 7, titer of blood 1:40. Mar. 8, 1923, fourth normal parturition terminated. June 8, titer of blood 1:40.

No. 805.—Fawn, born July 17, 1917. Dec. 2, 1919, heifer calved normally. The placenta was expelled and appeared normal. Titer of blood: Dec. 10, 1:80; Mar. 31, 1920, 1:320; Oct. 13, above 1:320. Sold.

No. 806.—Black and white, born July 22, 1917. Titer of blood: Nov. 25, 1919, less than 1:20; Jan. 6 and 30, 1920, 1:20; Mar. 10 and Apr. 1, 1:40. Apr. 3, heifer calved normally. The placenta was expelled but lost. Apr. 19, titer of mixed milk less than 1:20. Titer of blood: Apr. 15 and 19, 1:320; Oct. 13, above 1:320; Nov. 10, 1921, above 1:320. Second normal calf born Mar. 13, 1922. Third normal calf born Apr. 22, 1923. Placenta normal. May 25, titer of blood 1:320.

No. 807.—Fawn, born July 26, 1917. Nov. 13, 1919, titer of blood 1:20. Nov. 24, heifer calved normally. The placenta was expelled but lost. Titer of blood: Dec. 10, 1:20; Mar. 31, 1920, 1:40. Sold.

No. 808.—Black and white, born Aug. 1, 1917. Nov. 25, 1919, titer of blood: 1:20. Dec. 25, heifer calved normally. The placenta was expelled but lost. Titer of blood: Dec. 30, 1:20; Mar. 31 and Apr. 15, 1920, 1:1,280; Oct. 13, above 1:320. May 31, 1921, cow aborted. Most of the placenta was retained. A small piece obtained was found diseased. No bacteriological diagnosis was made. Sold.

No. 809.—White, born Aug. 10, 1917. Titer of blood: Dec. 2, 1919, 1:10; Jan. 6, 1920, 1:20; Jan. 30, 1:40. Feb. 20, heifer calved normally. The placenta was expelled and found normal. Titer of blood: Feb. 25, 1:80; Apr. 15, 1:40; Oct. 13, 1:20. June 15, 1921, cow calved normally. The placenta was expelled and lost. June 16, titer of blood 1:40. Third calf born Aug. 16, 1922. About two-thirds of placenta obtained. This is normal. A guinea pig inoculated with swab suspension is negative as to *B. abortus*. Aug. 23, blood titer 1:40.

No. 810.—Red and white, born Sept. 6, 1917. Oct. 7, 1919, titer of blood less than 1:20. Nov. 28, heifer calved normally. The placenta was expelled but lost. Titer of blood: Dec. 30, 1:40; Mar. 31 and Oct. 13, 1920, 1:20. Mar. 7, 1921, cow calved normally. The placenta was expelled but lost. Titer of blood 1:40; titer of colostrum 1:160. May 1, titer of blood 1:20.

Jan. 23, 1922, cow calved. The placenta was retained and later removed. There was evidence of localized lesions of some cotyledons but neither *B. abortus* nor *Vibrio fetus* was found in films. Jan. 25, titer of blood 1:40. Sold.

No. 811.—Red, born Sept. 27, 1917. Titer of blood: Nov. 25, 1919, 1:80; Jan. 2, 1920, 1:80. Dec. 26, 1919, udder fluid tested on a guinea pig with negative result. Jan. 5, 1920, heifer calved normally. The placenta was expelled but lost. Jan. 6, titer of blood 1:160. Jan. 8, a milk sample with titer of 1:20 was tested on three guinea pigs with negative results. Jan. 20, titer of blood 1:320. Sold.

No. 813.—Light fawn, born Nov. 4, 1917. Oct. 21, 1919, heifer aborted. The fetus was lost. The placenta was retained. Oct. 22, titer of blood 1:1,280. Sold.

No. 814.—Black, born Dec. 18, 1917. Titer of blood: Dec. 2, 1919, 1:160; Jan. 6 and Feb. 25, 1920, 1:160. July 16, heifer gave birth to full term calf. The placenta was retained. No tests on guinea pigs were made. Oct. 13, titer of blood 1:20. Sold.

No. 815.—Black and white, born Dec. 22, 1917. Titer of blood: Dec. 2, 1919, less than 1:20; Jan. 6, 1920, 1:20; Feb. 25, 1:40; Apr. 6, 1:20; Oct. 13, 1:40. Oct. 18, heifer calved normally. The placenta was expelled and appeared normal. Nov. 10, 1921, cow calved normally. The placenta was expelled and appeared normal. Titer of blood 1:40. Heifer aborted her third calf Mar. 30, 1923. No material was obtained for examination. The placenta was retained. Agglutinin titer of blood 1:320.

No. 816.—Solid fawn, born Sept. 12, 1917. On Nov. 20, 1919, heifer calved normally. The placenta was expelled but lost. Jan. 20, 1921, cow calved normally. The placenta was expelled but lost. Titer of blood: Dec. 21, 1:80; Apr. 22, 1922, 1:160. Cow used in another experiment.

No. 817.—Light fawn, born Oct. 10, 1917. Nov. 23, 1919, titer of blood 1:80. Dec. 16, heifer calved normally. The placenta was expelled but lost. Sold.

No. 819.—Fawn, born Nov. 1, 1917. Nov. 21, 1919, titer of blood 1:1,280. Nov. 20, heifer aborted. The placenta was retained. The fetus was lost. Sold.

SUMMARY OF THE FIRST GROUP OF VACCINATED HEIFERS.

53 heifers were vaccinated. 4 of these were disposed of before birth of a calf and are ruled out. 2 were pregnant when vaccinated. One of these aborted $2\frac{1}{2}$ months later. On the assumption that the abortion was due to the vaccination, owing to pregnancy, this case is also ruled out.

Of the remaining 48 heifers, 34 gave birth normally and without indications of placental disease. 6 calved normally but *Bacillus abortus* was demonstrated in the placenta or the uterine cavity at birth. One heifer (No. 622) had a diseased placenta which was probably due to vibrios. This one is regarded as normal. 8 heifers aborted. *Bacillus abortus* was demonstrated in all but one (No. 484). This is however included, although the evidence points to vibronic disease. There were therefore 40 full term, live fetuses born in this group.

The second pregnancies have been followed to the end in 38 of these heifers. 34 have delivered normal calves. In some, 2 of which had twins, the placentas were adherent, but the tests for *Bacillus abortus* were positive in but one of these. It is probable that vibrios played a rôle here. The 4 heifers that aborted are of interest in that 3 aborted twice (Nos. 485, 612, 760); a fourth (No. 638), whose first calf was normal but the placenta diseased, aborted the second time. In No. 632 both calves were full time, but the placenta was diseased each time. It is of interest to point out that of all the vaccinated heifers which dropped full time calves at the end of the first pregnancy, only one aborted in the second.

9 cows have been followed through the third pregnancy. 8 gave birth to normal calves; the ninth aborted and vibrios were isolated from the fetus. The udder secretion before parturition was tested in 14 cases. In 6, it contained *Bacillus abortus*.

Table VII gives a synopsis of the events in each case as far as they have been followed. The dose injected is given in cc. of a suspension of *Bacillus abortus* from agar slants equal in density to a 24

TABLE VII.

First Experiment. Vaccinated Group of Heifers.

Case No.	Dose in-jected.	Total age of culture.	Calf or fetus discharged (after vaccination).	Agglutinin titer of blood.	Approximate age of fetus or calf.	<i>B. abortus</i> isolated.*	Placenta, condition of.	Second pregnancy, terminated after first.	Placenta, condition of.	Agglu-tinin titer of blood.	<i>B. abortus</i> isolated.*	Remarks.
293 (p. 41)	5	32 days.	1 yr. 7 days.	h., m.	Full term.	m —	Normal dis-charge, lost.	Normal, 11 mos. 13 days.		h.		3rd calf normal; placenta normal. Agglutinin titer high; milk +.
294 (p. 42)	5	32 "	1 " 3 mos. less 1 day.	h., m.	" "	m —, u —	Normal dis-charge, lost.	Normal, 11 mos. 29 days.	Normal.	m.		
303 (p. 43)	2.5	81 "	1 " 28 days.	h.	" "	m +, u ++	Retained, +	(Killed.)				
304 (p. 44)	2.5	81 "	18 mos. 11 "	h., m.	" "	m —, u —	Normal dis-charge, lost.	Normal, 11 mos. 9 days.	Normal.	m.		
336 (p. 45)	2.5	153 "	11 " 22 "	h.	" "	m +, u —	Normal.	Normal, 14 mos. 18 days.	"	h.		
337 (p. 45)	2.5	153 "	11 " 19 "	h., m.	" "	u —	"	Normal, 15 mos. 3 days.	"	m.		Pregnant when vaccinated. 3rd normal calf born 10 mos. 19 days after 2nd. Blood titer high. 4th normal calf born 14 mos. after 3rd. Blood titer high.
411 (p. 47)	2.5	153 "	4 " 20 "	h.	" "	m +	" —	Normal, 16 mos. 6 days.	Discharged, lost.	h.	—, m +	
454 (p. 46)	2.5	153 "		m.		u +		(Killed.)				
479 (p. 47)	2.5	153 "	11 mos. 20 days.	h.	Full term.	u —, m +		Normal, 10 mos. 21 days.	Discharged, lost.	h.	m —	
480 (p. 48)	2.5	153 "	11 " 17 "	m.	" "	m —, u —	Normal.	Normal, 16 mos. 24 days.	Retained.	m.		
484 (p. 46)	2.5	153 "	11 " 18 "	l., m.	Aborted (fe-tus lost).	m —, u —	Retained, —	Normal (twins), 1 yr. 28 days.	Normal dis-charge, lost.	m.	—	3rd normal calf born 1 yr. 22 days after 2nd. Titer high.
489 (p. 48)	2.5	153 "	11 " 27 "	h., m.	Full term.	m —	Normal, —	Normal, 15 mos. 16 days.	Discharged, lost.	h.	—	
496 (p. 49)	4	220 "	13 " 29 "	m., h.	" "	u +	" —	Normal, 13 mos. 11 days.	Normal.	h.	—	
504 (p. 49)	4	220 "	1 yr. 28 "	m.	" "	u —	"	Normal, 1 yr. 27 days.	" —	m.		
524 (p. 50)	4	220 "		l., m.				(Killed.)				
527 (p. 48)	2.5	153 "	19 mos. 29 days.	h.	Full term.	u +	Normal.	Normal (twins), 1 yr. 2 days.	Retained.	h.		3rd normal calf born 1 yr. less 5 days after 2nd. Placenta normal. Blood titer high.
612 (p. 53)	2.5	1 yr. 208 "	1 yr. 20 "	h., h.	Aborted (7-8 mos.).	f +	+	Aborted (8-9 mos.), 11 mos.		h.	f +, m +	

TABLE VII—Continued.

Case No.	Dose injected.	Total age of culture.	Calf or fetus discharged (after vaccination).	Agglutinin titer of blood.	Approximate age of fetus or calf.	<i>B. abortus</i> isolated.*	Placenta, condition of.	Second pregnancy, terminated after first.	Placenta, condition of.	Agglutinin titer of blood.	<i>B. abortus</i> isolated.*	Remarks.
619 (p. 52)	2.5	1 yr. 208 days.	13 mos. 9 days.	h.	Full term.		Discharged, —	(Died.)				
622 (p. 56)	2.5	1 " 208 "	13 " 9 "	m., m.	Premature(?).		Diseased, —	Normal, 1 yr. 6 mos. 2 days.	Normal.	l.	—	Vibronic disease in first pregnancy (?).
625 (p. 53)	2.5	1 " 208 "	14 " 3 "	h.	Full term.		" +	Normal, 15 mos. 4 days.	Discharged.	h.		3rd normal calf born 10 mos. and 25 days after 2nd. Placenta lost.
626 (p. 55)	2.5	1 " 208 "	14 " 7 "	h.	" "		Normal, —	"	"	h.		3rd normal calf born 11 mos. 5 days after 2nd.
627 (p. 52)	2.5	1 " 208 "	14 " 7 "	m.	" "		" —	10 mos. 14 days. Normal, 1 yr. less 1 day.	lost, — Adherent, —	m.		
629 (p. 56)	2.5	1 " 208 "	14 " 3 "	m., h.	" "		Discharged, lost, —	Normal, 11 mos. 2 days.	Discharged, lost.	m.		3rd normal calf born 11 mos. 6 days after 2nd.
630 (p. 56)	2.5	1 " 208 "	14 " 5 "	m., m.	" "		Normal, —	Normal (twins), 10 mos. 12 days.	Retained, —	l.		
631 (p. 55)	2.5	1 " 208 "	14 " 5 "	m., m.	" "		Discharged, lost, —	(Sold.)				
632 (p. 54)	2.5	1 " 208 "	14 " 12 "	h., h.	" "		Diseased, +	Full term, 18 mos. 23 days.	Retained, +	h.		
633 (p. 55)	2.5	1 " 208 "	14 " 12 "	m., m.	" "		Normal, —	Normal, 21 mos. 3 days.	Discharged, lost, —			
634 (p. 54)	2.5	1 " 208 "	14 " 12 "	m.	" "		" —	Normal, 1 yr. 1 mo. 18 days.	—			
636 (p. 50)	4	1 " 163 "	15 " 29 "	h., m., l.	" "		" —	(Killed.)				
638 (p. 55)	2.5	1 " 208 "	14 " 12 "	h., h.	" "		Discharged, +	Aborted (6-7 mos.), 1 yr. 3 mos. 25 days.	+	h.		
641 (p. 49)	4	220 "	18 " 3 "	h.	" "	m —, u +	Retained (?).	Normal, 17 mos. 6 days.	Discharged, lost.	m.		3rd normal calf born 14 mos. and 16 days after 2nd. Blood titer high.
646 (p. 53)	2.5	1 yr. 208 "	15 " 8 "	m., m.	" "		Normal, —	Normal, 16 mos. 7 days.	Normal.	l.		
650 (p. 51)	2.5	1 " 208 "	16 " 2 "	m., h.	" "		Discharged, lost, +	(Sold.)				
652 (p. 51)	2.5	1 " 208 "	16 " 9 "	m., h.	Aborted (7-8 mos.).	f +	Retained, +	"				
656 (p. 54)	2.5	1 " 208 "	16 " 15 "	h., h.	Aborted (6 mos.).	f +	Diseased, +	Normal, 11 mos. 8 days.	Discharged, lost, —	h.		3rd, normal calf born 1 yr. and 6 days after 2nd. Placenta lost.
657 (p. 53)	2.5	1 " 208 "	16 " 16 "	h.	Full term.		Normal, —	(Sold.)				
669 (p. 56)	2.5	1 " 208 "	17 " 13 "	m., m.	" "		" +	(Sterile.)				

TABLE VII—*Concluded.*

Case No.	Dose injected.	Total age of culture.	Calf or fetus discharged (after vaccination).	Agglutinin titer of blood.	Approximate age of fetus or calf.	<i>B. abortus</i> isolated.*	Placenta, condition of.	Second pregnancy, terminated after first.	Placenta, condition of.	Agglutinin titer of blood.	<i>B. abortus</i> isolated.*	Remarks.
690 (p. 52)	2.5	1 yr. 208 days.	18 mos. 20 days.	m.	Full term.		Discharged, lost, —	Normal, 1 yr. 26 days.	Normal.	m.		
760 (p. 51)	2.5	1 " 208 "	7 " 26 "	m., h.	Aborted.		Decomposed.	Aborted, 13 mos. 3 days.	Diseased, +	h.		3rd, normal calf born 17 mos. and 7 days after 2nd. Placenta normal.
762 (p. 49)	4	220 "		h.		u +	(Killed, not pregnant.)					
765 (p. 50)	4	220 "	16 mos. 16 days.	h.	Full term.		Discharged, lost.	Normal, 15 mos. 10 days.	Discharged, lost.	h.		
768 (p. 50)	4	1 yr. 163 "	14 " 12 "	m., h., m.	" "		Normal.	Normal, 10 mos. 14 days.	" local disease.	l.		3rd normal calf born 15 mos. and 11 days after 2nd. Placenta lost. Agglutinin titer above normal before vaccination.
770 (p. 51)	4	1 " 163 "	13 " 13 "	m., h., m.	" " (dystocia).		"	(Killed.)				Agglutinin titer above normal before vaccination.
772 (p. 52)	2.5	1 " 208 "	2 yrs. less 20 days.	m., h.	Full term.		"	Normal, 11 mos. 26 days.	Discharged, lost.		Swab —	
773 (p. 54)	2.5	1 " 208 "		h., m.		(Sterile, killed.)						
774 (p. 55)	2.5	1 " 208 "	17 mos. 29 days.	l., l.	Full term.		Discharged, lost.	Normal, 1 yr. less 15 days.	Discharged, lost.	l.		
<i>Group Vaccinated with Another Strain of B. abortus (No. 420).</i>												
485 (p. 58)	5	22 days.	2 mos. 17 days.	h.	Aborted (6 mos.).	f +	Retained.	Aborted (fetus lost), 10 mos. 1 day.	+	h.		Pregnant when vaccinated. 3rd, normal calf born 15 mos. after 2nd. Agglutinin titer high. Placenta normal.
540 (p. 58)	5	22 "	11 " 8 "	h.	Aborted (8–9 mos.).	f +	"	(Killed.)				Infected when vaccinated.
553 (p. 59)	5	22 "	1 yr. 20 "	h., m.	Full term.		Normal.	" traumatic pericarditis.)				
556 (p. 58)	5	22 "	1 " 25 "	h., m., h.	Aborted.	f +, m +	Retained.	Full term, 17 mos. 2 days.	Retained, —	h.		
560 (p. 59)	5	22 "	13 mos. 11 "	h., m., l.	Full term.		Normal.	Full term, 14 mos. 14 days.	Normal.	l.		3rd normal calf born 15 mos. and 20 days after 2nd. Placenta discharged, lost.
566 (p. 59)	5	22 "	1 yr. 24 "	h., m., l.	" "	m —	"	Full term, 13 mos. 10 days.	Discharged, lost, —	m.		Aborted in 3rd pregnancy. Vib- rionic disease.
614 (p. 59)	5	22 "	13 mos. 26 "	h., m., l.	" "		Discharged, lost, —	Full term, 14 mos. 24 days.	Normal.	l.		3rd pregnancy normal.

hour typhoid culture. The total period of artificial cultivation of the strain used is given in days. In the third column the time elapsing between the vaccination and birth or abortion is given. It will be noticed that in a considerable number of cases this period exceeded the expected by from 1 to 6 months. It is impossible to determine how this delay in breeding affected the vaccinal immunity on account of the small number of animals treated. For the other signs, the reader is referred to a description of Table VI, page 26. The page number under each case refers to additional information on the case.

Notes on Individual Vaccinated Heifers.

In the following pages a brief history is given of each case and such details as could not be tabulated. All agglutinin titers with dates are included, as well as autopsy notes on fetuses, placentas, and the results of cultures and tests on guinea pigs.

No. 293.—Fawn and white heifer. Weight 540 lbs. Born July 26, 1917. Received into jugular vein, Nov. 5, 1918, 5 cc. of a suspension of *B. abortus* equal to a 24 hour bouillon culture of typhoid bacilli in density.

The vaccine was prepared as follows: The source of *B. abortus* was a colony from an agar culture of stomach contents of a fetus (No. 281) prepared Oct. 4. Slant agar tubes were inoculated from this colony Oct. 30 and incubated 6 days. The growth was removed with salt solution and the resulting suspension reduced to the density of a 24 hour bouillon culture of typhoid bacilli.

Between 1 and 2 hours after the injection the respiration was rapid and irregular for nearly 4 hours. The temperature rose from a normal average of 38.4°C. ascertained before injection to a maximum of 40.4°C. for 48 hours. It fell on the 3rd day and on the 4th it was normal and remained so. There was a loss in weight 2 weeks after the injection of 60 lbs. The original weight was regained after 4 weeks.

The agglutination titer before and after the injection of *B. abortus* is given in the following table, which is reproduced to illustrate the range of test.

TABLE VIII.

Agglutination Titer of Blood Serum of Heifer 293 before and after Vaccination.

Date.	Serum dilutions.								Control.
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	
Nov. 4	—	—	—	—	—	—	—	—	—
Dec. 11	C.	C.	C.	C.	C.	++ ++	++	—	—

Nov. 12, 1919. The heifer dropped a full term calf. The placenta was destroyed by the dam.

Nov. 13. Agglutination titer of blood serum 1:320.

Nov. 19. Agglutination titer of milk 1:80. Milk withdrawn from udder, sedimented over night to remove cream, centrifuged, and the concentrated fluid injected into the peritoneal cavity of three guinea pigs, 5 to 6 cc. into each animal. The animals remained well. 51 days after inoculation they were chloroformed and cultures made from bits of spleen tissue. All tubes remained free from growth.

Oct. 13, 1920. Agglutination titer of blood serum 1:320.

Oct. 25. Dam dropped a second full term calf. Agglutination titer 1:1,280. (The second calf was used in certain experiments, kept under observation for 2½ months, and then killed and autopsied.)

Jan. 24, 1921. Titer of blood 1:640.

Oct. 1. Gives birth to a third full term calf. Placenta discharged promptly. Titer of colostrum 1:1,280. The colostrum was concentrated with centrifuge and three guinea pigs injected with deposit. From two of these *B. abortus* was subsequently recovered.

Oct. 3. Titer of blood 1:640.

No. 294.—Fawn heifer, born July 27, 1917.

Nov. 5, 1918. Treated precisely like her companion, No. 293, with the same lot of vaccine.

The temperature reaction following the intravenous injection was much the same as for No. 293, but the maximum temperature was 41.5°C., a degree higher than for No. 293. There was a drop in weight from 555 to 520 lbs. in 3 weeks. Thereafter the weight rose again and reached 600 lbs. 7 weeks after vaccination.

TABLE IX.

Agglutination Titer of Blood Serum of Heifer 294 before and after Vaccination.

Date.	Serum dilutions.								Control.
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	
Nov. 4	±	—	—	—	—	—	—	—	—
Dec. 11	C.	C.	C.	C.	C.	++ +	+	—	—

Nov. 16. Slight vaginal discharge appears. Inoculated into two guinea pigs. Result negative as to *B. abortus* after 7 weeks.

Jan. 8, 1920. Milky fluid drawn from udder injected into three guinea pigs. Result after 7 weeks negative as to *B. abortus*.

Jan. 26. Titer of blood serum 1:160.

Feb. 4. Heifer drops a normal calf. Placenta probably destroyed by heifer during the night.

Feb. 25. Titer of blood serum 1:160.

Apr. 1. Titer of milk (right fore quarter) 1:640.

July 13. Milk drawn from four quarters near the end of milking and concentrated by centrifuging, after removing the cream. Injected into three guinea pigs. Result negative as to *B. abortus*.

Feb. 2, 1921. Gives birth to a normal calf. Titer of blood 1:160; of colostrum 1:640. A small piece of placenta recovered was normal.

Cow reacted to tuberculin subsequently and was slaughtered.

No. 303.—Fawn heifer, born Sept. 25, 1917.

Dec. 24, 1918. Weight 510 lbs. On this day heifer receives subcutaneously, left side of neck, the following vaccine. The strain used was the same as that for Nos. 293 and 294. 4 day agar slants were washed off with normal salt solution and the turbidity brought to that of a 24 hour bouillon culture of typhoid bacilli. 2½ cc. was injected. A swelling appeared within a few days and lasted about 3 weeks. It was about 2 inches in diameter at the base and ½ inch thick.

After the injection, the temperature was slightly above normal next day. The day after it registered from 40° to 41.1°C. during the day. After that it fell to above 39°C. On Dec. 30, it was normal. The weight dropped 30 lbs. in 2 weeks. In another week it was only 10 lbs. below the original weight. The agglutination titer had not been determined before injection. On Jan. 16, it was slightly above 1:1,280.

Jan. 6, 1920. Heifer in advanced stage of pregnancy. Titer of blood serum 1:640.

Jan. 14. A little mucoid fluid pressed from the udder inoculated into two guinea pigs. *B. abortus* isolated from spleen of one of these after 7 weeks.

Jan. 20. A second sample, drawn yesterday, tested in the same way. *B. abortus* recovered from both guinea pigs.

Jan. 21. Gave birth to a normal calf. Placenta retained.

Jan. 22. Stained films prepared from a swab inserted in the uterus show typical masses of *B. abortus*. Portions of the retained placenta removed show no macroscopic lesions of chorion or cotyledons. The diseased portion may have been retained.

Jan. 30. Titer of blood serum 1:1,280.

July 1. Agglutination titer of milk 1:640.

July 8. To determine the persistence of infection in the udder discovered before calving, milk collected today was concentrated and injected into three guinea pigs. From every one *B. abortus* was recovered in spleen cultures after 6 weeks.

Oct. 13. Titer of blood serum probably 1:1,280 (complete at 1:320).

This cow was killed soon after so that further study of this case was interrupted.

No. 304.—Black and white heifer, born June 17, 1917.

Dec. 24, 1918. Weight 520 lbs. This heifer was treated with No. 303 and received the same dose, but intravenously. As a result rapid respirations were observed about 20 minutes after the injection and lasted several hours. The temperature rose about 0.5°C. on the same day. On the 3rd, it rose from 40.3° to 41.4°C. at 4 p.m. For the next 3 days it fluctuated between 39° and 40°C. On Dec. 30, 6 days after injection, it fell to normal and remained there until the animal was returned to the herd on Jan. 17.

Jan. 16, 1919. The agglutination titer had not been taken before injection. Today it was slightly above 1:1,280.

Dec. 2. Heifer pregnant. Titer of blood serum 1:320.

Jan. 6, 1920. Titer of blood serum 1:320.

Jan. 22. Slightly milky fluid withdrawn from udder and injected into two guinea pigs. The cultures from these were both negative for *B. abortus*.

June 22. Titer of blood serum 1:320.

July 5. Heifer gave birth to a full time normal calf. Placenta destroyed by heifer.

July 7. Milk collected from four quarters near end of milking and tested; agglutination titer 1:20. Three guinea pigs injected with concentrated milk were negative as to *B. abortus*.

Oct. 13. Titer of blood serum 1:320.

June 14, 1921. Second full term calf born. Placenta discharged normally. A suspension of uterine fluid from a swab was injected into a guinea pig with negative result. Titer of blood on this day 1:160.

Nine heifers received on March 6, 1919, subcutaneously, a dose of *Bacillus abortus* equivalent to $2\frac{1}{2}$ cc. of a salt solution suspension of the

density of a 24 hour bouillon culture of typhoid bacilli. Two of these (Nos. 336 and 337) were kept under observation as to temperature, weight, and general condition for 4 weeks. The rest were injected in the field and not seen except as noted in the following protocols.

No. 336.—Holstein heifer, born Sept. 4, 1917.

Mar. 6, 1919. Weight 800 lbs. Injected subcutaneously at 2.30 p.m. a dose of *B. abortus*. The temperature did not rise until next day. Then, taken at intervals of 2 hours it fluctuated between 40° and 40.8°C. On the 3rd day, it fluctuated between 40.5° and 41.3°C. It was normal Mar. 10, 4 days after injection. There was no fall in weight.

The agglutination titer before and after vaccination is shown in the following table.

TABLE X.

Agglutination Titer of Blood Serum of Heifer 336 before and after Vaccination.

Date.	Serum dilutions.								Control.
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	
Mar. 6	C.	+	—	—	—	—	—	—	—
" 24	C.	C.	C.	C.	C.	++ ++	++	—	—

Nov. 25, 1919. Heifer pregnant. Agglutination titer of blood serum 1:640.

Dec. 26. About 3 cc. of a viscid, turbid fluid drawn from udder and injected into three guinea pigs. Result negative as to *B. abortus*.

Jan. 10, 1920. Similar test made on three guinea pigs with fluid withdrawn today. Result negative.

Jan. 29. Agglutination titer 1:640.

Feb. 28. Heifer gave birth to normal calf. Placenta discharged within 9 hours. Found normal. Microscopic examination of uterine swab negative.

Mar. 10. Titer of blood serum 1:640.

July 8. Titer of milk 1:20. A sample of mixed milk near end of milking inoculated into three guinea pigs after removing cream and centrifuging. *B. abortus* recovered from the spleen of one of these. Titer of blood: Oct. 13, 1:320; Mar. 29, 1921, 1:640.

May 18. A normal calf born today. Placenta discharged.

June 22. Titer of blood 1:640.

No. 337.—Black and white heifer, born Sept. 4, 1917.

Mar. 6, 1919. Weight 690 lbs. Injected this day same as No. 336. The temperature reaction was similar to that of No. 336. It lasted 4 days. During

the 3rd and 4th days, it was above 40°C., rising to a maximum of 40.6°C. There was no fall in weight.

The titer of the blood serum before and after the injection is given in the following table.

TABLE XI.

Agglutination Titer of Blood Serum of Heifer 337 before and after Vaccination.

Date.	Serum dilutions.								Control.
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	
Mar. 6	C.	++ ++	+	—	—	—	—	—	—
" 24	C.	C.	C.	C.	C.	C.	++ ++	++	—

Dec. 2, 1919. Titer of blood serum 1:320.

Jan. 6, 1920. Titer of blood serum 1:320.

Jan. 20. A small amount of watery, mucoid fluid withdrawn from udder and injected into a guinea pig. Result negative.

Feb. 25. Heifer gave birth to a normal calf. The placenta discharged *in toto* was carefully examined but lesions referable to *B. abortus* were not detected. In about a dozen cotyledons, a small number of villi in each cotyledon are whitish, not easily crushed, opaque in transmitted light. Probably calcified, for addition of 5 per cent HCl gives rise to gas bubbles, originating within the core of the villus. Titer of blood serum 1:640.

July 13. Titer of milk less than 1:20.

Oct. 13. Titer of blood serum 1:320.

May 28, 1921. Full term calf born today. A small piece of placenta recovered was found normal. Titer of blood and colostrum 1:320.

No. 454.—Fawn and white heifer born Nov. 25, 1917. Mar. 6, 1919. Vaccinated. May 29. Reacted positively to tuberculin. Aug. 11. Titer of blood serum 1:160. Oct. 10. A small amount of milky fluid drawn from the udder and injected into three guinea pigs. *B. abortus* isolated subsequently from each pig. Oct. 30. Slaughtered by butcher. Not pregnant.

No. 484.—Black and white heifer, born Sept. 5, 1917. Mar. 6, 1919. Vaccinated. Nov. 25. Titer of blood serum: complete agglutination at 1:40. Jan. 6, 1920. Titer 1:80. A swab introduced into the vagina was washed off in salt solution and the resulting suspension injected into two guinea pigs. Result negative as to *B. abortus*. Microscopic examination of films from the swab failed to show *B. abortus* but several *vibrios* were detected. Jan. 20. Several cc. of fluid drawn from udder and injected into one guinea pig. It was

killed after 2 months. Cultures negative. Feb. 24. Aborted. Placenta retained and fetus could not be found. Titer of blood 1:320. Mar. 2. Milk drawn from udder was treated as heretofore and 5 cc. injected into the peritoneal cavity of each one of three guinea pigs. Result negative as to *B. abortus*. Titer of milk at the time of injection 1:40. Oct. 13. Titer of blood 1:80.

Mar. 23, 1921. Gives birth to twins. Placenta lost. Blood titer 1:160. Two guinea pigs inoculated with a suspension of uterine fluid were negative for *B. abortus*.

No. 479.—Black and white heifer, born Sept. 5, 1917. Mar. 6, 1919. Vaccinated. Dec. 2. Titer of blood serum nearly complete at 1:320. Jan. 6, 1920. Titer 1:640. Jan. 20. Pregnant. Fluid withdrawn from udder and injected into three guinea pigs. Result negative as to *B. abortus*. Feb. 26. Gives birth to a normal calf. Films made from uterine swab negative. Titer of blood 1:640. July 13. Milk, with titer of 1:160, tested on three guinea pigs with positive result in each case. Oct. 13. Titer of blood above 1:320. Jan. 16, 1921. Normal calf born. Jan. 19. Titer of blood 1:1,280. July 25. Titer of mixed milk 1:640. Three guinea pigs inoculated with this milk negative for *B. abortus*.

No. 411.—Black and white heifer, born Sept. 10, 1917. Mar. 6, 1919. Vaccinated. This heifer was pregnant at the time of vaccination, but this was not known. When this was discovered it was thought best to keep the animal under closer observation and she was brought to the Institute stables Mar. 11. The temperature was taken twice daily until Apr. 22 and then discontinued. During this time it was normal. Subsequently it was found that she had been bred Oct. 15, 1918.

May 13. Titer of blood serum 1:1,280. May 27. Weight 800 lbs. July 8. Titer of serum 1:1,280. July 26. Heifer gives birth to a weak calf. In 5 days it had become normal and was returned with the dam to the herd. The placenta was discharged within 5 hours and found normal. July 28. Two guinea pigs were injected with amniotic fluid and suspension of placental tissue. One died from some other disease. Cultures from the other were negative as to *B. abortus*. Aug. 1. Titer of serum above 1:1,280. Aug. 3. Milk drawn from the udder towards the end of milking was skimmed, centrifuged, and the resulting fluid injected into three guinea pigs. One died prematurely. Cultures from the second were negative, from the third positive as to *B. abortus*. Jan. 29, 1920. Serum titer 1:1,280. Oct. 13. Serum titer complete at 1:320.

Dec. 2. Cow gives birth to a second calf, apparently normal. The placenta was discharged and destroyed by the dam. Titer of heifer's blood 1:1,280. A guinea pig inoculated with a suspension of uterine fluid on a swab was negative for *B. abortus*. Dec. 4. Three guinea pigs inoculated with milk all develop *B. abortus* lesions.

Oct. 21, 1921. A third calf born at full term. Titer of blood 1:640; of colostrum above 1:2,560.

Dec. 23, 1922. A fourth calf born. Placenta normal. Titer of blood, 3 days later, 1:1,280.

No. 480.—Black and white heifer, born Sept. 12, 1917. Vaccinated Mar. 6, 1919. Nov. 25. Titer of blood serum 1:160. Jan. 6, 1920. Titer of blood serum 1:160. Feb. 17. Fluid drawn from udder injected into two guinea pigs. One died in 18 days, probably of scurvy. The second was chloroformed after 2 months. Cultures from spleen negative. Feb. 23. Heifer gives birth to a normal calf. Placenta discharged soon after. Feb. 25. Serum titer 1:640. July 8. Milk drawn from the udder, with a titer of less than 1:20, prepared as heretofore and injected into the peritoneal cavity of three guinea pigs. Two died within a month as a result of abscesses in the abdominal cavity, containing staphylococci. The third was killed after 6 weeks. Cultures from the spleen negative. Oct. 13. Titer of blood serum 1:320.

July 17, 1921. Gives birth to full term calf. Placenta retained. July 26. Titer of blood 1:320. On Nov. 10, it was 1:160.

No. 489.—Black and white heifer, born Sept. 20, 1917. Vaccinated Mar. 6, 1919. Nov. 25. Titer of blood serum 1:640. Jan. 6, 1920. Titer of blood serum 1:640. Mar. 4. Calved normally. Placenta discharged soon after and found normal. Mar. 5. Guinea pig inoculated with a suspension in salt solution from swab introduced into uterus. Result negative. Mar. 8. Milk from four quarters near end of milking skimmed, centrifuged, and injected into three guinea pigs. Cultures from these negative. Titer of this sample of milk 1:320. Mar. 10. Serum titer 1:320. Apr. 1. Titer of milk from right fore quarter 1:80. Oct. 13. Serum titer above 1:320. June 16, 1921. Titer above 1:320.

June 20. Full term calf born. Placenta lost. A guinea pig inoculated with uterine fluid collected on a swab negative. Nov. 10. Titer of blood above 1:320.

July 12, 1922. Calved normally. Placenta expelled and lost. Blood titer 1:1,280.

No. 527.—Fawn heifer, born Dec. 21, 1917. Vaccinated Mar. 6, 1919. Dec. 2. Titer of blood serum above 1:640. Jan. 6, 1920. Titer of blood serum 1:1,280. Feb. 25. Titer of blood serum 1:1,280. Mar. 17. A small quantity of fluid expressed from the udder and injected into a guinea pig. Cultures positive for *B. abortus*. Nov. 4. Serum titer 1:1,280. Calved normally. Placenta discharged and found normal.

Nov. 6, 1921. Gives birth to twin calves, probably 20 days premature. Both able to stand, but both died in 3 days. The placenta was retained. Nov. 10. Titer of blood 1:1,280.

Nov. 1, 1922. Calved normally. Placenta normal. On Dec. 15, blood titer 1:1,280.

Another lot of six heifers was vaccinated May 12, 1919, in much the same way as the preceding lot. The strain of *Bacillus abortus* used

was the same. The suspension of bacilli was so diluted that the individual dose was equivalent to 4 cc. and roughly corresponded to the growth on one agar slant and to the dose injected into the preceding lot.

No. 762.—Black and white heifer, born Nov. 1, 1917. Vaccinated May 12, 1919. Dec. 2. Titer of blood serum above 1:320. Jan. 6, 1920. Titer of blood serum 1:640. Feb. 25. Titer of blood serum 1:1,280. Mar. 4. Some fluid drawn from the udder injected into a guinea pig. Cultures positive for *B. abortus*. Dec. 14. Killed for beef, because non-pregnant.

No. 504.—Black and white heifer, born Nov. 4, 1917. Vaccinated May 12, 1919. Dec. 2. Titer of blood serum above 1:160. Jan. 6, 1920. Titer of blood serum 1:160. Feb. 25. Titer of blood serum 1:320. Mar. 17. A small amount of fluid expressed from udder and injected into a guinea pig. Result negative. June 10. Calved normally. Discharged placenta found normal. June 16. Titer of blood serum 1:320. July 9. Titer of milk less than 1:20. Titer of blood, Oct. 13 and Mar. 29, 1921, 1:320.

July 7. Calved normally. The placenta was promptly discharged and found normal. A guinea pig test of uterine swab negative. July 25. Titer of blood 1:320.

July 5, 1922. Cow calved normally (third calf). Placenta expelled but lost. Uterine fluid suspension injected into two guinea pigs with negative result. Aug. 23. Blood titer 1:320.

No. 641.—Black and white heifer, born Nov. 7, 1917. Vaccinated May 12, 1919. Dec. 2, Jan. 6, 1920, and Feb. 25. Titer of blood serum 1:1,280. Mar. 5. Some fluid expressed from udder and injected into a guinea pig. Cultures positive for *B. abortus*. Nov. 15. Difficult parturition. Calf full term, normal. Retention of placenta and metritis. Nov. 22. Titer of blood serum 1:1,280. July 21, 1921. A sample of mixed milk with an agglutinin titer of 1:320 was concentrated by centrifugation and tested on three guinea pigs, and by direct cultures. Both negative for *B. abortus*.

Apr. 21, 1922. Calved normally. Placenta expelled but lost. Blood titer, on June 8, 1:320. Third normal calf born July 7, 1923. Placenta lost. Agglutinin titer of blood 1:640, 4 days later.

No. 496.—Black and white heifer, born Nov. 10, 1917. Vaccinated May 12, 1919. Nov. 25. Titer of blood serum complete at 1:160. Jan. 6, 1920. Titer of blood serum 1:320. Feb. 18. Fluid expressed from udder and injected into two guinea pigs. Cultures from both positive for *B. abortus*. Feb. 25. Titer of blood serum 1:640. June 30. Titer of blood serum 1:1,280.

July 11. Gives birth to full term calf. Posterior presentation. Placenta discharged within 18 hours. Titer of blood serum 1:1,280. Oct. 13. Titer of blood serum above 1:320. June 22, 1921. Titer of blood 1:640.

Aug. 22. Calved normally. Tests on three guinea pigs with suspensions from suspicious areas of placenta and uterine swab, all negative. Nov. 10. Titer of blood 1:640.

No. 524.—Black and white heifer, born Nov. 11, 1917. Vaccinated May 12, 1919. Dec. 2 and Jan. 6, 1920. Titer of blood serum 1:80. Feb. 25 and Oct. 19. Titer of blood serum 1:320. Oct. 24. Slaughtered because reacting positively to tuberculin. Diagnosis of tuberculosis confirmed. Uterus contains a fetus 28 inches long, coat of hair showing only on legs, muzzle, ears, etc. The membranes and fetus were normal with the following exceptions: There is a small amount of clear yellowish serum in thorax and abdomen. Slight interlobular edema of lungs. The rumen contains a few fecal pellets but the mucoid fluid contents are water-clear. Tubes receiving some fluid of amnion and allantois remain sterile.

No. 765.—Fawn and white heifer, born Jan. 11, 1918. Vaccinated May 12, 1919. Dec. 2. Titer of blood serum above 1:640. Jan. 6, 1920, and Feb. 25. Titer of blood serum 1:1,280.

Sept. 28. Calved normally on pasture. Placenta not recovered. Microscopic examination of uterine swabs negative. Oct. 13. Titer of blood serum complete at 1:320.

Jan. 7, 1922. Cow calved normally. Placenta expelled but not recovered. Titer of blood 4 days later above 1:1,280.

March 16, 1920. Three heifers (Nos. 768, 636, and 770) receive subcutaneously, each, the 3 days' growth on one agar slant of *Bacillus abortus* No. 281, suspended in salt solution.

No. 768.—Black and white, born July 29, 1918. Vaccinated Mar. 16, 1920. Agglutination titer: Mar. 4 (before vaccination), 1:320; Apr. 6, 1:1,280; Nov. 22, 1:320; Jan. 17, 1921, 1:320; Apr. 19, 1:160.

Calved normally May 28. Placenta discharged and found normal. The agglutinin titer on the day of calving was for the blood 1:320, colostrum 1:640.

Cow calved normally Apr. 11, 1922. The discharged placenta showed cotyledons in unoccupied horn with islands of grayish, gelatinous villi. Sections of fixed tissue contain necrotic villi, densely filled with cell debris. Several vibrios detected in films but attempts to isolate them through guinea pigs failed. The blood titer of the cow on June 8 was 1:80. Third normal calf born July 22, 1923. Placenta lost.

No. 636.—Black and white, born Aug. 25, 1918. Vaccinated Mar. 16, 1920. The agglutinin titers were: Mar. 4 (before treatment), 1:40 +; Apr. 6, 1:2,560; Nov. 22, 1:160; Apr. 11, 1921, 1:160. Calved normally July 15. The placenta was discharged and partly destroyed by the dam. The remainder normal. Agglutinin titer, July 28, 1:80. A guinea pig inoculated from uterine swab

obtained soon after parturition failed to show *B. abortus* in the spleen. Cow killed as reactor.

No. 770.—Black and white heifer, born Aug. 26, 1918. Vaccinated Mar. 16, 1920. The agglutinin titers were: Mar. 4 (before vaccination), 1:320; Apr. 9, 1:2,560; Nov. 22 and Jan. 17, 1921, 1:320; Feb. 14 and Apr. 19, 1:160. Calved Apr. 29. The calf was very large and had to be removed mechanically. Placenta discharged; normal. Agglutinin titer, May 4, 1:160. Killed in October as reactor.

In these three cases, two heifers had a preliminary titer of 1:320 which indicates spontaneous infection. It was thought best however to vaccinate them. In each one the pregnancy was normal.

April 30, 1920. Vaccine prepared for 24 heifers from agar slants of Strain (calf) 281, 5 days in incubator. The growth was washed off, suspended in salt solution, and the density adjusted by further dilution to that of a 24 hour bouillon culture of typhoid bacilli. Each heifer was to receive $2\frac{1}{2}$ cc. of this suspension corresponding to about one-half of an agar slant. This dose, before injection, was diluted with salt solution so that 5 cc. was injected into each animal.

Eighteen animals were vaccinated April 30, and 6, May 5. The suspension used was kept at about 40°C. in the meantime.

No. 652.—Fawn and white, born Oct. 25, 1918. Vaccinated Apr. 30, 1920. Agglutinin titers: Mar. 22 and Apr. 21 (before vaccination), 1:40; Nov. 22, above 1:320; Jan. 11 and Apr. 10, 1921, 1:640.

Heifer aborted a living fetus Sept. 9, weighing 32 lbs., which lived 3 days. *B. abortus* was recovered from spleen and liver in cultures and from the lungs through guinea pigs subsequently. Placenta retained and removed on the 3rd day. Two guinea pigs inoculated with suspension of uterine swab were both positive for *B. abortus*. Sept 21. Blood titer 1:1,280. Sold.

No. 650.—Red and white, born Nov. 6, 1918. Vaccinated Apr. 30, 1920. Bred successfully Oct. 23. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:40; Nov. 22, above 1:320; Jan. 11, 1921, and Apr. 11, 1:1,280.

Calved normally Sept. 2, 1921. Placenta destroyed by dam. Agglutinin titer on this day 1:1,280. Two guinea pigs injected with a suspension of uterine exudate obtained with a swab developed the usual lesions and cultures from the spleens were positive for *B. abortus*. Sold.

No. 760.—White and red, born Nov. 7, 1918. Vaccinated Apr. 30, 1920, when pregnant. Date of breeding unknown. Agglutinin titers as follows. Mar. 22 and Apr. 21 (before vaccination), 1:20 to 1:40; Nov. 22, 1:320+.

Aborted Dec. 26. Fetus and placenta when obtained were decomposed. No bacteriological examination made. The agglutinin titer taken the day after abortion was 1:1,280 and about the same level Apr. 19, 1921.

Cow aborted again Jan. 29, 1922. The placenta was adherent. From a uterine swab *B. abortus* was isolated directly in cultures and through a guinea pig. The placenta was removed later. Both chorion and the cotyledons show the various stages of disease due to *B. abortus*. The latter demonstrated within cells in films. Sections show infected epithelial cells and necrotic villi. The fetus was not autopsied. Blood agglutinins 1:1,280. Third, normal calf born July 6, 1923. Placenta normal.

No. 619.—Black and white, born Nov. 7, 1918. Vaccinated Apr. 30, 1920. Bred Sept. 2. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:40 to 1:80; Nov. 22, Jan. 17, 1921, Feb. 14, and Apr. 19, complete at 1:320 (i.e. probably 1:1,280).

Calved June 9. Placenta discharged and found normal with exception of one cotyledon in which most of the villi are short, nodular, and dull brownish in color. Sections of fixed and hardened tissue indicate localized inflammation of some villi in this cotyledon. Two guinea pigs inoculated with suspensions of uterine fluid (swab) were negative. Cow died July 24 as a result of a small tear in the uterus.

No. 690.—Red and white, born Nov. 12, 1918. Vaccinated Apr. 30, 1920. Breeding date not known. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:40 to 1:80; Nov. 22, Jan. 11, 1921, and Apr. 19, 1:320.

Calved normally Nov. 20, 1921. Placenta lost or destroyed. Blood titer next day 1:1,280. A uterine swab was tested on two guinea pigs with negative result.

Dec. 16, 1922. Calved normally. Blood titer 1:320.

No. 772.—Red heifer, born Nov. 14, 1918. Vaccinated Apr. 30, 1920. Repeatedly bred during 1921. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:20 to 1:40; Nov. 22, 1:320++; Jan. 11, 1921, and Oct. 31, 1:1,280. Heifer calved normally Apr. 10, 1922. Placenta was normal. Blood titer, June 8, 1:640. A second, full term calf born Apr. 5, 1923. The placenta was destroyed by the dam. A fetid discharge from vagina Apr. 9. A guinea pig inoculated with a suspension from a uterine swab was negative for *B. abortus*. May 25. Blood titer 1:640.

No. 627.—Black and white, born Nov. 16, 1918. Vaccinated Apr. 30, 1920. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:20 to 1:40; Nov. 22 and Apr. 11, 1921, 1:160. Calved normally July 7. Titer of blood on this day 1:160, of colostrum 1:320. Placenta was discharged and found normal. One guinea pig inoculated with suspension of uterine fluid (swab) negative.

July 6, 1922. Calved normally. Placenta retained and removed July 8. Two guinea pigs inoculated with suspension of uterine swab. One died of septi-

cemia, the other was negative as to *B. abortus*. Aug. 23. Blood titer 1:320. Sold.

No. 612.—Red heifer, born Nov. 19, 1918. Vaccinated Apr. 30, 1920. Pasture bred in September. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:40; Nov. 22, Jan. 11, 1921, and Apr. 11, 1:1,280.

Aborted May 20. Fetus weighs 26 lbs. and is 76 cm. long. Lungs not inflated. *B. abortus* isolated directly in pure culture from fourth stomach and lungs, and also through guinea pigs. Guinea pig inoculated with contents of rectum negative. Agglutinin titer, Nov. 10, 1:1,280.

Cow aborted a second time, Apr. 20, 1922. Fetus fully developed. Transudate of 200 cc. of fluid in pleural cavity. Meconium soft, indicating earlier discharge. *B. abortus* was diagnosed in films from uterine swab and isolated in pure culture from fourth stomach, large intestine, rectum, and liver of fetus; and from fourth stomach, lungs, rectum, and uterine swab through guinea pigs. Blood titer, June 8, 1:1,280. Milk test positive for *B. abortus*, July 3.

No. 625.—Red and white, born Nov. 26, 1918. Vaccinated Apr. 30, 1920. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:40; Nov. 22, complete at 1:320; Apr. 11, 1921, 1:1,280.

July 3. Gives birth to a full term calf. Titer of blood 1:1,280; of colostrum 1:5,120. Placenta discharged within 4 hours. Parts preserved in Zenker's fluid indicate a thickening of the chorion. Films showed large numbers of minute bacteria within epithelial cells. Sections of the placenta show the characteristic infection of the chorionic epithelium with *B. abortus*. Of two guinea pigs inoculated with suspensions of the placenta, one died within 24 hours. The other was negative for *B. abortus* when examined several months later.

Oct. 7, 1922. Gives birth to a normal calf. Placenta normal. Blood titer 1:1,280. Third normal calf born Sept. 1, 1923. Placenta lost.

No. 646.—Black and white, born Nov. 29, 1918. Vaccinated Apr. 30, 1920. Date of breeding unknown. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:40 to 1:80; Nov. 22, 1:320; Jan. 11, 1921, 1:160.

Calved normally Aug. 8, 1921. Blood titer 1:160. Placenta discharged and found with some yellowish villi in some of the cotyledons. Of three guinea pigs inoculated with a suspension of ground-up placental tissue, two died soon after of septic infection. The third survived and proved negative as to *B. abortus*. Dec. 15, 1922. Calved normally. Placenta normal. Blood titer 1:80.

No. 657.—Black and white, born Dec. 1, 1918. Vaccinated Apr. 30, 1920. Breeding date not known. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:40 to 1:80; Nov. 22, complete at 1:320; Jan. 11, 1921, 1:1,280; Apr. 11, 1:640.

Calved normally Sept. 16, 1921. Placenta normal. Agglutinin titers on day of calving were: blood 1:1,280, colostrum the same. A guinea pig inoculated with a suspension of uterine fluid (swab) proved negative for *B. abortus*. Sold.

No. 634.—Black and white, born Dec. 2, 1918. Vaccinated Apr. 30, 1920. Breeding date unknown. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:40; Nov. 22, 1:320; Apr. 11, 1921, 1:160.

Calved normally July 12, 1921. Placenta discharged within 6 hours, normal. Agglutinin titers on this day were: blood 1:320, colostrum 1:640. Two guinea pigs inoculated with suspension of uterine fluid (swab) were negative as to *B. abortus*.

Aug. 30, 1922. Calved normally. Two guinea pigs inoculated with suspension from uterine swab negative.

No. 773.—Red heifer, born Dec. 5, 1918. Vaccinated Apr. 30, 1920. Bred repeatedly. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:20 to 1:40; Nov. 22, considerably above 1:320; Jan. 11, 1921, 1:1,280; Apr. 19, above 1:320.

This heifer was slaughtered because sterile.

No. 656.—Fawn and white heifer, born Dec. 9, 1918. Vaccinated Apr. 30, 1920. Bred three times, the last date being Mar. 14, 1921. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:40; Nov. 22, Jan. 17, 1921, and Feb. 14, above 1:320, probably 1:1,280.

Heifer aborted Sept. 15. The titer of both blood and colostrum on this day was above 1:1,280.

The fetus was 22 inches long. Coat of hair beginning to show around hoofs, nose, etc. The lesions of usual type. *B. abortus* was isolated from fourth stomach, rectum, lung, and kidney, but not from spleen and liver.

A piece of placenta, about one-fourth to one-third of the whole hanging from vagina was cut off and examined. The chorion and cotyledons were diseased, the former thickened, roughened, and nodular. No characteristic epithelial-cell infections were noted in films, but *B. abortus* was seen in smaller endothelial-cell phagocytes.

A number of guinea pigs were inoculated with material from the fetus and the placenta. *B. abortus* was isolated through them from the placenta and from the fourth stomach, meconium, and lung of fetus. A guinea pig inoculated with a suspension from the uterine swab was negative Nov. 10. Agglutinin titer somewhat above 1:1,280.

Aug. 23, 1922. Cow calved normally. Placenta probably expelled but not recovered. Two guinea pigs inoculated with suspension of uterine fluid were negative. Blood titer 1:1,280. Aug. 25. Blood titer 1:1,280; colostrum 1:2,560. Third, normal calf born Aug. 29, 1923. Placenta lost.

No. 632.—Fawn and white heifer, born Dec. 11, 1918. Vaccinated Apr. 30, 1920. Agglutinin titer as follows: Mar. 22 and Apr. 21 (before vaccination), 1:20; Nov. 22, above 1:320; Jan. 11, 1921, and Apr. 11, 1:1,280.

Heifer was delivered of a dead calf, July 12, owing to malposition of fetus. The placenta was discharged *in toto* but was slightly diseased. Films from this show many bacilli recognized as *B. abortus*. Three guinea pigs, two inoculated with material from placenta and one with material from uterine swab, all developed the characteristic lesions due to *B. abortus* and cultures were positive.

Agglutinin titer of blood 13 days after calving 1:1,280, that of mixed milk a trifle lower.

Feb. 4, 1923. A full term calf born. Placenta retained but a small portion was obtained showing the typical exudate. Numerous cells filled with *B. abortus* found in it. Guinea pig test positive for *B. abortus*. Agglutinin titer, Mar. 9, 1:1,280.

No. 626.—Black and white, born Dec. 14, 1918. Vaccinated Apr. 30, 1920. Agglutinin titer as follows: Mar. 22 and Apr. 21 (before vaccination), 1:20; Nov. 22, above 1:320; Jan. 11, 1921, and Apr. 11, 1:1,280.

Heifer calved normally during night of July 7. Placenta normal. A guinea pig inoculated with suspension of uterine fluid (swab) proved negative. Titer of blood on day of calving 1:1,280, of colostrum 1:2,560.

Second normal calf born May 21, 1922. Guinea pig tests negative for *B. abortus*. The placenta was discharged but lost. Agglutinin titer 1:2,560. Calved normally a third time on Apr. 26, 1923. The placenta was normal. May 25. Blood titer 1:2,560.

No. 633.—Red heifer, born Dec. 16, 1918. Vaccinated Apr. 30, 1920. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:40; Nov. 22, 1:160; Jan. 11, 1921, 1:80; Apr. 11, 1:160. Heifer calved normally July 12. Placenta normal. Guinea pig inoculated from suspension of uterine exudate (swab) negative. Agglutinin titer of blood 13 days after calving 1:160; milk less than 1:20. A second normal calf born Apr. 15, 1923. The placenta was expelled but lost. A guinea pig test of uterine swab negative.

No. 774.—Black and white, born Dec. 29, 1918. Vaccinated Apr. 30, 1920. Agglutinin titers as follows: Mar. 22 and Apr. 21 (before vaccination), 1:20 to 1:40; Nov. 22, 1:80; Jan. 11, 1921, and Apr. 11, 1:40. Heifer calved normally Oct. 29. Placenta discharged and destroyed by dam. Titer of blood 12 days later 1:20+.

Oct. 14, 1922. Calved normally. Placenta discharged and lost. Blood titer 1:80.

No. 631.—Black and white heifer, born Jan. 2, 1919. Vaccinated May 5, 1920. Agglutinin titers as follows: Mar. 25 and Apr. 21 (before vaccination), 1:40; Nov. 22, Jan. 11 and Apr. 19, 1921, 1:320. Heifer calved, July 10, normally. Placenta discharged but not recovered. Two guinea pigs inoculated with suspension from uterine swab negative. Titer of blood 15 days after calving 1:320, milk 1:40. Sold.

No. 638.—Black and white heifer, born Jan. 2, 1919. Vaccinated May 5, 1920. Agglutinin titers as follows: Mar. 25 and Apr. 21 (before vaccination), 1:20 to 1:80; Nov. 22, well above 1:320; Jan. 11, 1921, and Apr. 11, 1:1,280.

Heifer calved normally July 17. Placenta discharged entire, covered with dirt. Although lesions not recognized, films proved positive for *B. abortus*, as well as a guinea pig inoculated with a suspension of uterine fluid (swab). On the day of calving the agglutinin titer of blood was 1:1,280, of colostrum 1:2,560.

Heifer aborted her second calf Nov. 11, 1922. Fetus between 6 and 7 months. Lungs show that the fetus had breathed. Much meconium in stomach fluids.

Otherwise the fetus appears normal. *B. abortus* isolated from lungs, fourth stomach, cecum, and rectum in pure culture and subsequently through guinea pigs from fourth stomach, rectum, and contents of uterine swab. The placenta was typically diseased. Nov. 18. Blood titer 1:1,280.

No. 630.—Black and white heifer, born Jan. 13, 1919. Vaccinated May 5, 1920. Agglutinin titers as follows: Mar. 25 and Apr. 21 (before vaccination), 1:40; Nov. 22, 1:320; Jan. 11, 1921, 1:320; Apr. 11, 1:160.

Heifer calved normally July 10. Only small portions of placenta recovered in the yard where the calf was dropped. These appeared normal. Two guinea pigs inoculated with a suspension of uterine fluid (swab) were negative. Agglutinin titer of blood 15 days later 1:160.

Cow gave birth to full term twins May 22, 1922. The placenta was adherent and later removed in small pieces but not completely. Two guinea pigs inoculated with suspension from uterine swab were negative as to *B. abortus*. Blood titer of cow, June 8, less than 1:160.

No. 669.—Black and white, born Jan. 9, 1919. Vaccinated May 5, 1920. Agglutinin titers: Mar. 25 and Apr. 21 (before vaccination), 1:80; Nov. 22, 1:320+; Jan. 11, 1921, 1:640; Apr. 11, 1:320. Heifer calved normally Oct. 18. Only a small piece of placenta recovered for examination. This was normal. A guinea pig inoculated with suspension of uterine fluid (swab) was positive for *B. abortus*. The agglutinin titers on day of calving were: blood 1:320, colostrum 1:2,560. Heifer regarded as sterile subsequently.

No. 629.—Black and white, born Jan. 12, 1919. Vaccinated May 5, 1920. Agglutinin titers: Mar. 25 and Apr. 21 (before vaccination), 1:80; Nov. 22, Jan. 11, 1921, and Apr. 11, 1:320.

Heifer calved normally July 8. Placenta not obtained for examination. Two guinea pigs inoculated with suspension of uterine fluid (swab) were negative. Agglutinin titer, July 25, 1:640.

Cow calved a second time June 10, 1922. The placenta was discharged but not recovered. Calf full term, normal. Blood titer 1:320. A third calf was born May 16, 1923. The placenta was normal. May 25. Blood titer 1:160.

No. 622.—Black and white, born Jan. 31, 1919. Vaccinated May 5, 1920. Agglutinin titers: Mar. 25 and Apr. 21 (before vaccination), 1:80; Nov. 22, Jan. 11, 1921, and Apr. 11, 1:320.

Heifer calved June 14. Calf is small and possibly somewhat premature. It was however able to suckle and was kept until over 4 months of age.

The placenta was retained and removed next day. About one-half of the total area of the chorion is thickened and leathery; in the remainder the thickening and ulceration is restricted to numerous small foci. In the first half the cotyledons are entirely necrotic; in the remainder only individual villi or groups are involved. Sections of fixed and hardened tissue show complete loss of chorionic epithelium with subjacent cellular infiltration. Two guinea pigs inoculated with suspensions of uterine fluid (swab) were negative for *B. abortus*. The agglutinin titer for *B. abortus* on day of calving was 1:320. The diagnosis of *B. abortus* disease

remains unconfirmed and leaves open the possibility of *Vibrio fetus* being the etiological factor.

Dec. 16, 1922. Calved normally. Placenta normal. A guinea pig inoculated with suspension of uterine swab negative. Agglutinin titer, Feb. 2, 1923, 1:80.

December 6, 1919. The following seven heifers were vaccinated with a culture of *Bacillus abortus* isolated through a guinea pig from the lungs of Fetus 420. The guinea pig was chloroformed November 14. The culture was therefore a relatively fresh culture of which the second transfer from the guinea pig was used as the vaccine. The slanted, sealed agar cultures were 4 days old when the growth was washed off and adjusted to the opacity of a 24 hour bouillon culture of the typhoid bacillus. Each heifer received subcutaneously 5 cc. equivalent to 8/9 of a slanted agar tube.

The agglutinin titers of these seven animals 4 days before and 31 days after the injection of the vaccine are given in the following table.

TABLE XII.

Agglutinin Titer of Heifers.

Blood Samples for First Test Collected 4 Days before Vaccination, for Second Test, 31 Days after Vaccination.

Case No.	Test No.	Serum dilutions.									Control.
		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	
485	1st	C.	++	—	—	—	—	—	—	—	—
	2nd	C.	C.	C.	C.	C.	++++	++	—	—	—
540	1st	C.	C.	C.	C.	C.	++++	+	—	—	—
	2nd	C.	C.	C.	C.	C.	++++	++	—	—	—
553	1st	+++	—	—	—	—	—	—	—	—	—
	2nd	C.	C.	C.	C.	C.	C.	++++	+	—	—
556	1st	++	—	—	—	—	—	—	—	—	—
	2nd	C.	C.	C.	C.	C.	C.	++++	++	—	—
560	1st	C.	+	—	—	—	—	—	—	—	—
	2nd	C.	C.	C.	C.	C.	C.	++++	—	—	—
566	1st	+++	+	—	—	—	—	—	—	—	—
	2nd	C.	C.	C.	C.	C.	++++	++	—	—	—
614	1st	+++	—	—	—	—	—	—	—	—	—
	2nd	C.	C.	C.	C.	C.	++	+	—	—	—

It will be noted that one heifer had been infected as indicated by the high titer. It was nevertheless included and received the vaccine with the rest. One heifer (No. 485) was found to be pregnant, unknown to the attendant, after the vaccine had been given.

Of these seven cases, four gave birth to normal, full term calves; the one with an initial high agglutinin titer, the one pregnant at the time of vaccination, and one other aborted. The more detailed notes of these cases follow.

No. 485.—Fawn heifer, born July 8, 1918. Vaccinated Dec. 6, 1919. Date of breeding unknown. A fetus was discharged Feb. 23, 1920. Length 60 cm., weight 2,280 gm. The placenta was retained. The autopsy of the fetus showed the usual pathological conditions. Cultures from the digestive tract and organs showed a variety of bacteria, but *B. abortus* was recovered from the fourth stomach contents through a guinea pig. Agglutinin titer 2 days after abortion 1:1,280. Oct. 13. Titer above 1:320.

Heifer aborted a second time Dec. 24. The fetus could not be found. A uterine swab showed the presence of *B. abortus*. On Nov. 10, 1921, the agglutinin titer was high, 1:1,280. Cow calved normally Mar. 24, 1922. The placenta was normal and a guinea pig inoculated with suspension of uterine swab was negative as to *B. abortus*. The blood titer was 1:1,280 and the milk titer the same 4 days later.

No. 540.—Fawn and white heifer, vaccinated Dec. 6, 1919. Agglutinins to *B. abortus* in the blood high at the time (see Table XII). Apr. 6, 1920. Titer 1:2,560.

Fetus discharged Nov. 14, 1920. Placenta retained. The autopsy of fetus showed the lungs partially inflated. Pathological changes very slight. *B. abortus* recovered in pure culture from colon and rectum. This heifer had been driven some distance shortly before abortion took place and had also been tested with tuberculin. The heifer was killed Sept. 27, 1921. Uterus found normal.

No. 556.—Black and white heifer, born Mar. 21, 1918. Vaccinated Dec. 6, 1919. Agglutinin titer: Apr. 6, 1920, 1:1,280; Nov. 22, above 1:320.

Fetus found by attendant Dec. 31, 1920. Placenta retained. Autopsy of fetus showed the usual changes. Lungs airless, heavy, liver-like, dark red. Interlobular edema. *B. abortus* was recovered in cultures from fourth stomach, colon, and rectum.

The colostrum milk, with a titer of 1:2,560, was collected Jan. 1, 1921, and one liter centrifuged down to obtain the sediment in concentration for injection into guinea pigs. One out of three became infected and *B. abortus* was obtained from it in pure culture. Agglutinin titer on day of abortion 1:1,280; May 1, 1921, above 1:320. Cow calved normally June 2, 1922, but the placenta was retained. Three guinea pigs inoculated with a suspension from a uterine swab failed to

show *B. abortus* disease later. The blood titer the day after parturition was 1:640.

No. 553.—Black and white heifer, vaccinated Dec. 6, 1919. Calf born Dec. 26, 1920. Placenta found normal. Agglutinin titers as follows: Apr. 6, 1920, 1:640; Nov. 22, 1:320; Dec. 27 (day after calving), 1:160; May 1 and 7, 1921, 1:80. Heifer killed Nov. 7. Wire found penetrating pericardium.

No. 566.—Black and white heifer, vaccinated Dec. 6, 1919. Calf born Dec. 30, 1920. Placenta discharged soon after and found normal after a careful examination. Agglutinin titers as follows: Apr. 6, 1920, 1:160; Nov. 22, 1:80; Dec. 30 (day of calving), 1:160; May 1, 1921, 1:80. One-half liter of the colostrum collected on the day of birth was concentrated by centrifugation and the sediment injected into three guinea pigs. Cultures from these, killed 54 days after inoculation, remained sterile.

Cow calved a second time Feb. 10, 1922. The placenta was expelled but not recovered. A guinea pig inoculated with a suspension of a uterine swab was negative as to *B. abortus*. Blood titer of cow at birth of calf 1:160.

Jan. 26, 1923. Cow dropped a fetus, 11 inches long. Vibrios and staphylococci isolated from fetus. A guinea pig inoculated with a suspension of a uterine swab was negative for *B. abortus*.

No. 614.—Black and white heifer, vaccinated Dec. 6, 1919. Calved normally Feb. 1, 1921. Placenta discharged during the night and destroyed by the dam. Uterine swab collected next day was stirred up in normal saline and the suspension injected into a guinea pig. Result negative. Agglutinin titers of this heifer ran as follows: Jan. 6, 1920, 1:640; Apr. 6, 1:320; Nov. 22, 1:160; Jan. 17, 1921, 1:80; Feb. 1 (day of calving), 1:160; May 1, 1:80.

Cow calved a second time Apr. 25, 1922. The placenta was normal. Titer of blood, June 8, 1:40. June 1, 1923. Cow calved normally. Blood titer 1:80.

No. 560.—Black and white heifer, vaccinated Dec. 6, 1919. Normal calf born Jan. 17, 1921. Placenta discharged 2 hours later was normal. The later agglutinin titers were as follows: Apr. 6 and Nov. 6, 1:160; Mar. 29, 1922, and May 1, 1:80.

Cow calved normally Mar. 31, 1922. The placenta was discharged and found normal. Blood titer Apr. 7, 1:80. Third normal calf born July 20, 1923. Placenta expelled but lost.

Summary of the Second Group of Control Heifers.

The births or abortions in this group cover the period of 1921, 1922, and the first quarter of 1923. It thus overlaps and extends beyond the period covered by the births of the vaccinated group.

62 heifers were under observation during the first pregnancy. One of these (No. 851) was slaughtered and a partially mummified fetus about 4 inches long found in the uterus. This heifer had a low

agglutinin titer, and is, therefore, excluded as being probably a case of vibronic abortion. 2 others showed signs of having aborted (Nos. 839, 840), but the fetus could not be found. One of these had a low, the other a medium titer. The latter is included in the statistical summary, the former not. A fourth heifer (No. 592) dropped a small, partially macerated fetus. The guinea pig test of uterine fluid was negative for *Bacillus abortus* and the agglutinin titer was low throughout. This case is also excluded as probably vibronic. Of the 59 remaining heifers, 12 aborted, or 20.3 per cent. In addition, there were 4 cases of normal birth but with diseased placenta in which the presence of *Bacillus abortus* was demonstrated.

40 became pregnant again, and of these 7 aborted. Of these, only 2 had aborted before, and one had had an infected placenta with normal calving. 3 of the full term second pregnancies had infected placentas. Of these, one had passed through a normal first pregnancy, one had an infected placenta, and the third had aborted her first calf.

The following table summarizes the more important facts. The reader is referred to page 26 for an explanation of the abbreviations and signs used.

TABLE XIII.
First Experiment. Second Control Group.

Case No.	First pregnancy.	Placenta, condition of.	<i>B. abortus</i> isolated.*	Agglutinin titer of blood.	Second pregnancy, terminated after first.	Placenta, condition of.	<i>B. abortus</i> isolated.*	Agglutinin titer of blood.
565	Aborted (8 mos. \pm).	Retained, +	f +	m., h.	(Sold.)			
592	Aborted (small, macerated fetus).	Discharged, -		l., l.	Normal, 1 yr. less 2 days.	Discharged, lost.		
595	Aborted (5-6 mos.).	Retained, +	f -	h., h.	(Sold.)			
635	Normal.	Normal, -		l., m.	Normal, 15 mos. 22 days.	Discharged, lost.		l.
637	"	" -		l., l.	(Sold.)			
639	"	"		l., l.				
645	"	"		l., m.				
648	"	"		l.	(Sold.)			
649	Aborted (fetus lost).	Retained, +		l., m.	"			
659	Aborted (5-6 mos.).	Partly retained, +		l., h., h.	Full term, 14 mos. 19 days.	Diseased, +		h.
660	Normal.	Discharged, lost, -		l., m., h.	Full term, 16 mos. 3 days.		Swab -	l.
664	"	Normal, -		h., l.	Normal, 13 mos. 23 days.	Discharged.	" -	l.
667	Aborted (6-7 mos.).	Retained, +	f -	h., h.	(Sold.)			
668	Aborted (near full term).	Partly retained, diseased, +	f +	l., m.	Aborted, 8 mos. 7 days.	Retained.	f +	

TABLE XIII—Continued.

Case No.	First pregnancy.	Placenta, condition of.	<i>B. abortus</i> isolated.*	Agglutinin titer of blood.	Second pregnancy, terminated after first.	Placenta, condition of.	<i>B. abortus</i> isolated.	Agglutinin titer of blood.
671	Aborted (near full term).	Retained, +	f +	l, h.	Aborted, 18 mos. 20 days.		f +	h.
680	Normal.	Discharged, lost, +		l, m.	Normal, 17 mos. 12 days.		Swab +	h.
686	" (calf dead).	Normal.		l.	Normal, 15 mos. 22 days.	Discharged, lost.		
688	Premature.	Retained, +		l, h.	Normal, 11 mos.	Normal.		h.
689	Normal.	Normal, —		l, h.	" 13 mos. 10 days. (Sold.)	Discharged, lost.	Swab —	h.
692	" (calf dead).	Discharged (?), lost, —		l, l.				
693	Aborted (fetus alive).	Retained (?), +		l, h.	Normal, 18 mos. 7 days.	Normal.		
695	Normal.	Normal, —		l, l.	Normal, 17 mos. 1 day.	Discharged, lost.		
696	"	Discharged, lost, —		l, h.				
701	"	Normal, —		l, m.	Aborted, 8 mos. 27 days.	Retained, +	f +	h.
706	Aborted (fetus alive).	Diseased, +		l, m.				
707	Normal.	Retained, —		l, l.	Normal, 13 mos. 7 days.	Normal, —		l.
708	"	Discharged, lost, —		l, l.	Normal, 13 mos. 21 days.	"		l.
710	"	" " +		l, h.				
713	"	" " —		l, l.	Normal, 1 yr. less 1 day.	Normal.		l.
714	"	" " —		l, l.	Normal, 15 mos. 28 days.	Discharged, lost.	Swab —	l.

756	Normal.	Discharged, diseased, +	1., h.	Normal,	Normal.		h.
820	"	Normal.	1., m.	11 mos. 16 days. Aborted, 11 mos. less 2 days.	Retained.	f +, swab +	h.
821	"	Discharged, lost.	1., l.	Normal,	Discharged, lost.		m.
822	"	" "	1., l.	11 mos. less 7 days. Normal,	Retained.	Swab -	l.
823	"	" "	1., l.	10 mos. 13 days. Aborted,	" +	f +	h.
824	"	Slightly diseased, +	1., m.	10 mos. 3 days. Aborted,	" +	f +, swab +	
825	"	Normal.	1., l.	1 yr. 22 days. (Sold.)			
826	"	Discharged, lost.	1., l.	Normal,	Normal.		l.
827	"	" "	1., l.	15 mos. 2 days. Normal,	"		l.
828	"	Normal.	1., l.	11 mos. 17 days. Normal,	Discharged, lost.		l.
829	"	Discharged, lost.	1., l.	13 mos. 1 day. Normal,	" "		l.
830	"	Normal.	1., l.	13 mos. 17 days. Normal,	Normal.		l.
831	"	Discharged, lost.	1., l.	13 mos. 9 days. Normal,	Discharged, lost.		l.
832	"	" "	l.	13 mos. 28 days. (Sold.)			
833	"	Retained.	l., h.	"			
834	"	Normal.	1., l.	Normal,	Discharged, lost.	Swab +	l.
835	"	Discharged, lost.	1., l.	10 mos. 28 days. Normal,	" "		
				16 mos. 18 days.			

TABLE XIII—*Concluded.*

Case No.	First pregnancy.	Placenta, condition of.	<i>B. abortus</i> isolated.*	Agglutinin titer of blood.	Second pregnancy, terminated after first.	Placenta, condition of.	<i>B. abortus</i> isolated*	Agglutinin titer of blood.
836	Normal.	Normal.		l., l.	Normal, 15 mos.	Normal, —		l.
837	Full term	"		l., l.	"	"		
	(dystocia).				16 mos. 7 days.			
839	Aborted (?).	(Discharge from uterus.)		l., l.	(Sold.)			
840	" (?)	(Swollen udder, discharge from vagina.)		m., m.	"			
841	Normal.	Normal.		l., l.	Normal, 1 yr. less 16 days.	Discharged, lost.		l.
842	"	Discharged, lost.		l., l.	(Sold.)			
843	"	" "		l., l.	Aborted, 1 yr. 12 days.			h.
846	"	Retained.		l., l.				
847	"	Normal.		h., m.	Normal, 10 mos. 21 days.	Discharged, lost.		m.
848	"	Discharged, lost.		l., l.	Normal, 15 mos. 8 days.	" " —		l.
849	"	" "		l., l.	(Sold.)			
850	"	Normal.		l., l.	Normal, 1 yr. 17 days.	Discharged, lost.		l.
851	(Dead, partly mummified fetus, about 4 in. long found at slaughter.)		f —	l.				
950	Aborted.	Retained.	f +		(Sold.)			
1027	Full term (dystocia).	Discharged, lost.		l., h.	"			

Notes on Individual Animals of the Second Control Group.

No. 565.—Fawn and white, born Apr. 24, 1919. Titer of blood: Mar. 15, 1920, 1:320; Dec. 10, 1:640; Jan. 17 and Apr. 19, 1921, above 1:320. Oct. 8, heifer aborted. Fetus with coat of hair, weighing 33 lbs., and measuring 82 cm. in length. Some meconium on the body and protruding from rectum. Stomachs contain much fecal matter. Lungs not inflated. Transudates absent. The placenta was retained. Films prepared from a uterine swab showed *B. abortus* free and in epithelial cells. *B. abortus* was isolated in pure culture from fourth stomach, colon, rectum, and lungs of fetus. Cultures from spleen, liver, and kidneys negative. *B. abortus* was obtained from contents of fourth stomach, rectum, lungs, and uterine swab through guinea pigs. Oct. 8, titer of blood 1:1,280. Sold.

No. 592.—Black and white, born May 25, 1919. Titer of blood: Mar. 15 and Dec. 10, 1920, 1:40; Jan. 27, 1921, 1:40. Apr. 13, heifer aborted. Fetus very small and disintegrated within the membranes. Nothing noteworthy about these. A guinea pig inoculated with a suspension of material collected on a swab from the uterus remained normal and cultures were negative. Apr. 21, titer of blood 1:40. Second pregnancy terminated normally Apr. 11, 1922. The placenta was normal. June 8, blood titer 1:40.

A third, full time calf was born Mar. 11, 1923. The placenta was normal.

No. 595.—Fawn and white, born Dec. 24, 1918. Titer of blood: Mar. 22 and Apr. 21, 1920, 1:640; Nov. 22, titer above 1:320; Feb. 14, 1921, titer above 1:320. Apr. 18, heifer aborted. Fetus hairless. Weight 19 lbs., length 56 cm. No transudates. Digestive tract normal. Cultures from digestive tract and lung, liver, and spleen remained sterile. One culture from kidney contained staphylococci. Guinea pigs were inoculated with material from fourth stomach, rectum, and lung tissue. These remained normal and spleen cultures were negative. One guinea pig inoculated with suspension from a uterine swab obtained after expulsion of fetus developed the usual lesions and *B. abortus* was isolated from it in pure culture. Apr. 19, titer of blood above 1:320. Sold.

No. 635.—Fawn and white, born Nov. 7, 1918. Titer of blood: Mar. 22, 1920, 1:80; Apr. 21 and Nov. 22, and Jan. 11, 1921, 1:160; Apr. 11, 1:80. July 13, heifer calved normally. The placenta was expelled. A small part obtained appeared normal. July 13, swab tested on guinea pigs with negative results. July 14, titer of colostrum 1:20. July 28, titer of blood 1:80. Nov. 4, 1922, calved normally. The placenta was expelled but lost. Mar. 9, 1923, blood titer 1:80.

No. 637.—Black and white, born Nov. 24, 1918. Titer of blood: Mar. 22, 1920, 1:40; Apr. 21 and Nov. 22, 1:80; Jan. 11, 1921, 1:40; Apr. 11 and Apr. 21, 1:80. July 18, heifer calved normally. The placenta was expelled and appeared normal. Swab tested on guinea pigs with negative results. July 25, titer of mixed milk 1:20. Sold.

No. 639.—Fawn, born Nov. 23, 1918. Titer of blood: Mar. 22 and Apr. 21, 1920, 1:80; Nov. 22, 1:40; Jan. 11 and Apr. 11, 1921, 1:80. July 17, heifer calved normally. The placenta was expelled and appeared normal. Titer of blood 1:80; of colostrum 1:40. July 28, titer of blood 1:40.

No. 645.—Fawn and white, born Dec. 31, 1918. Titer of blood: Mar. 25, Apr. 21, and Nov. 22, 1920, 1:80; Apr. 11, 1921, 1:80. July 25, heifer calved normally. The placenta was expelled and appeared normal. Titer of blood 1:160; of colostrum 1:320.

No. 648.—Fawn, born Oct. 5, 1919. Titer of blood: Mar. 25 and Apr. 21, 1920, 1:40. Aug. 23, 1921, heifer calved normally. The placenta was expelled and appeared normal. Sold.

No. 649.—Fawn and white, born July 28, 1919. Titer of blood: Mar. 15 and Nov. 22, 1920, 1:40; Apr. 19, 1921, 1:80. Sept. 1, heifer aborted. The placenta was retained. The fetus was lost. Swab tested on guinea pigs with result positive for *B. abortus*. Dec. 21, titer of blood 1:320. Sold.

No. 659.—Fawn and white, born Apr. 7, 1919. Titer of blood: Mar. 15, 1920, 1:40; Dec. 14, above 1:320; Apr. 14, 1921, above 1:320. Sept. 20, heifer aborted. The fetus was not yet with coat of hair. About one-half of the placenta obtained and *B. abortus* detected in films of this. The lesions of the chorion involve both cotyledons and chorion. The latter is firm and leathery in portions, otherwise beset with minute grayish patches. Subchorionic tissue at these places markedly edematous. Chorionic epithelium filled with *B. abortus*. The cotyledons are frequently circled with a narrow peripheral necrotic border. The fetus was not autopsied. Sept. 21, titer of blood 1:1,280; titer of colostrum 1:2,560.

Dec. 9, 1922. Cow gave birth to full term calf. The placenta was discharged and found diseased. The disease probably started in one horn and extended into body of placenta. The lesions involve both chorion and cotyledons. *B. abortus* was later isolated from a guinea pig inoculated with a suspension from a uterine swab.

No. 660.—Black and white, born Apr. 16, 1919. Titer of blood: Mar. 15, 1920, 1:40; Dec. 10, 1:80; Jan. 17, 1921, 1:80; Feb. 14, 1:40; Apr. 19, 1:160. Sept. 26, heifer calved normally. The placenta was expelled but lost. Swab tested on two guinea pigs with negative result. Sept. 27, titer of blood 1:640.

Second normal calf born Jan. 29, 1923. A guinea pig inoculated with a suspension of uterine swab negative as to *B. abortus*. Mar. 9, blood titer 1:80.

No. 664.—Black and white, born Apr. 15, 1919. Titer of blood: Mar. 15, 1920, 1:640; Dec. 10, 1:80; Jan. 17 and Feb. 14, 1921, 1:80. On Oct. 10, heifer calved normally. The placenta was expelled. A small piece obtained appeared normal. Swab tested on a guinea pig with negative result. Titer of colostrum 1:160. Titer of blood, Oct. 11, 1:80.

Second normal calf born Dec. 3, 1922. The placenta was discharged. Guinea pig test of uterine swab negative. Feb. 2, 1923, blood titer 1:40.

No. 667.—Fawn and white, born May 20, 1919. Titer of blood: Mar. 15, 1920, 1:1,280; Dec. 10, 1:320; Jan. 27 and Apr. 19, 1921, above 1:320. Oct. 14, heifer aborted. The placenta was retained. Fetus weighs 21½ lbs. and is 66 cm. long. Some hairs on face and legs. Organs normal. Cultures remain sterile. Guinea pigs inoculated with suspensions of lung tissue and contents of fourth stomach and rectum negative for *B. abortus*. A guinea pig inoculated with suspension of uterine swab positive for *B. abortus*. Titer of blood 1:1,280; titer of colostrum 1:2,560. Sold.

No. 668.—Black and white, born Apr. 14, 1919. Titer of blood: Mar. 15, 1920, 1:80; Dec. 10, 1:320; Jan. 17 and Apr. 19, 1921, 1:320. Oct. 18, heifer aborted. Most of the placenta was retained. About one-third obtained for inspection. In this the cotyledons were necrotic and the chorion thickened with whitish plaques. The fetus appears fully developed. The stomachs free from fecal matter. This is largely stored in the rectum which is distended to a diameter of 3 to 3½ inches. The lungs are still airless. Transudates absent. *B. abortus* was isolated from fourth stomach, large intestine, rectum, and lungs. *B. abortus* not isolated from spleen, liver, and kidneys. A guinea pig inoculated with a suspension from uterine swab was positive for *B. abortus*. Titer of blood 1:160; of colostrum 1:2,560.

June 25, 1922. Aborted a second time. Fetus 46 cm. long, weighs 3,845 gm. Transudates in large serous cavities. *B. abortus* recovered from fourth stomach in cultures and from lungs, fourth stomach, and rectal contents through guinea pigs.

No. 671.—Fawn and white, born Feb. 5, 1919. Titer of blood: Mar. 25 and Apr. 21, 1920, 1:40; Dec. 4, 1:20; Apr. 14, 1921, 1:40. Oct. 24, heifer aborted. The placenta was retained. Fetus, male, weighs 34 lbs. Length 77 cm. Appears fully developed. Probably lived for some time after birth. Autopsy shows the following: A considerable quantity of blood, partly coagulated in peritoneal cavity. Clots attached to a ruptured mesenteric vessel and to stumps of umbilical arteries. Suffusions and petechiæ in mucosa of fourth stomach. Lungs partly inflated. *B. abortus* isolated from spleen, liver, heart's blood, colon, and rectum. Cultures from fourth stomach and lungs mixtures of several species. *B. abortus* was furthermore isolated from contents of fourth stomach, rectum, uterine swab, and from lung tissue through guinea pigs. Titer of colostrum 1:1,280. Oct. 29 and Nov. 10, titer of blood 1:1,280.

May 14, 1923. Heifer aborted a second time. Fetus is 23 inches long, and weighs 10 lbs. Transudates absent. Stomach contents normal. *B. abortus* isolated from contents of fourth stomach, from lungs, spleen, kidney, cecum, and rectum in cultures. May 25, blood titer 1:10,240.

No. 680.—Fawn, born Mar. 15, 1919. Titer of blood: Mar. 25, 1920, 1:20; Apr. 21, 1:40; Dec. 14, 1:80; Apr. 14, 1921, 1:80. Nov. 2, heifer calved normally. The placenta was expelled but lost. Swab tested on a guinea pig with positive result. Nov. 10, titer of blood 1:160.

A second normal calf was born Apr. 14, 1923. A guinea pig inoculated with washings of uterine swab was positive for *B. abortus*. May 25, blood titer 1:10,240. June 25, blood titer 1:10,240; titer of milk 1:640.

No. 686.—Black and white, born Feb. 5, 1919. Titer of blood: Mar. 25, Apr. 21, and Dec. 14, 1920, 1:20; Apr. 14, 1921, 1:80. Nov. 8, heifer gave birth to a full term dead calf. The placenta was expelled and appeared normal. Nov. 10, titer of blood 1:20.

Mar. 2, 1923. Heifer calved normally. The placenta was discharged but lost.

No. 688.—Fawn and white, born Feb. 22, 1919. Titer of blood: Mar. 20, 1920, 1:160; Apr. 21, 1:80; Dec. 14, 1:40; Apr. 14, 1921, 1:40. Nov. 18, heifer calved. Calf a little weak. The placenta was retained. Swab tested on guinea pigs with positive result for *B. abortus*. Titer of blood 1:640; titer of colostrum 1:2,560.

On Oct. 18, 1922, the cow calved normally. The placenta appeared normal to the unaided eye. Titer of blood and colostrum 1:2,560.

No. 689.—Black and white, born Feb. 10, 1919. Titer of blood: Mar. 25, 1920, 1:40; Apr. 21, 1:80; Dec. 14, 1:40; Apr. 14, 1921, 1:40. On Nov. 18, heifer calved normally. The placenta was expelled and about one-half obtained, which appeared normal. Suspension from uterine swab inoculated into a guinea pig with negative result. Nov. 18 and 23, titer of blood 1:1,280.

Second normal calf born Dec. 28, 1922. Blood titer 1:1,280. Placenta discharged but not recovered. A guinea pig inoculated with a suspension of uterine swab was negative for *B. abortus*.

No. 692.—Black and white, born Feb. 5, 1919. Titer of blood: Mar. 25 and Apr. 21, 1920, 1:40; Dec. 4, less than 1:20; Apr. 14, 1921, 1:40. Nov. 20, heifer gave birth to a full term dead calf. Placenta probably expelled but lost. Titer of blood 1:80. Nov. 21, suspension from uterine swab inoculated into a guinea pig with negative result. Nov. 27, bloody, whitish, thick, viscid discharge from vagina. Sold.

No. 693.—Black and white, born May 7, 1919. Titer of blood: Mar. 15, 1920, 1:20; Dec. 10, 1:40; Jan. 27 and Apr. 19, 1921, 1:40. Nov. 22, heifer aborted. The fetus was alive. Swab tested on guinea pigs with positive result. Nov. 23, titer of blood 1:1,280.

May 29, 1923. Heifer calved normally. The placenta was normal.

No. 695.—Black and white, born Feb. 21, 1919. Titer of blood: Mar. 25, Apr. 21, and Dec. 14, 1920, 1:80; Apr. 14, 1921, 1:80. Nov. 26, heifer calved normally. Probably entire placenta expelled. Only that from non-gravid horn obtained for examination, which appeared normal. Swab tested on guinea pigs with negative result. Dec. 21, titer of blood 1:80.

Apr. 27, 1923. Heifer calved normally. The placenta was expelled but lost.

No. 696.—Fawn and white, born Mar. 21, 1919. Titer of blood: Mar. 25, 1920, 1:40; Apr. 21 and Dec. 14, 1:20; Apr. 14, 1921, 1:20. Nov. 27, heifer

calved normally. The placenta was expelled but lost. Nov. 28, swab tested on guinea pigs with negative result. Dec. 21, titer of blood 1:640.

No. 701.—Black and white, born Mar. 22, 1919. Titer of blood: Mar. 25, 1920, 1:20; Apr. 21, 1:40; Dec. 14, 1:20; Apr. 14, 1921, 1:40. Dec. 12, heifer calved normally. The placenta was expelled. That from the non-gravid horn appeared normal. Swab tested on guinea pigs with negative result. Dec. 21, titer of blood 1:320.

Sept. 8, 1922. Cow aborted. The placenta was retained. Agglutinin titer of blood and colostrum 1:2,560. Fetus measures 72 cm. in length. Edemas and transudates absent. *B. abortus* isolated directly in pure culture from lungs and contents of fourth stomach, and through guinea pigs from uterine swab and fourth stomach, rectum, and lungs of fetus.

No. 706.—Black and white, born May 4, 1919. Titer of blood: Mar. 15, 1920, 1:80; Dec. 10, 1:40; Jan. 27 and Apr. 19, 1921, 1:40. Dec. 19, heifer aborted a live fetus. The placenta was expelled and presented the usual lesions of chorion and cotyledons. *B. abortus* present in films. A guinea pig inoculated with suspension of uterine swab was positive for *B. abortus*. Dec. 21, titer of blood 1:320.

No. 707.—Black and white, born Apr. 2, 1918. Titer of blood: Mar. 25 and Apr. 21, 1920, 1:40; Dec. 14, 1:80; Apr. 14, 1921, 1:40. Dec. 27, heifer gave birth to full term calf. Placenta retained. It was removed 3 days later, but decomposition and subsequent freezing prevented a diagnosis. Jan. 1, 1922, a suspension of uterine swab was inoculated into two guinea pigs with negative result. Titer of blood: Dec. 30, 1921, 1:80; Jan. 11 and 25, 1922, 1:40.

Second, normal calf born Feb. 3, 1923. Placenta discharged later and found to be normal. A guinea pig inoculated with a suspension from a uterine swab was negative for *B. abortus*. Mar. 9, blood titer 1:20.

No. 708.—Black and white, born June 13, 1919. Titer of blood: Mar. 15, 1920, 1:20; Dec. 10, 1:40; Jan. 27, 1921, 1:20; Apr. 19, 1:40. Dec. 18, heifer calved normally. The placenta was expelled but lost. Swab tested on guinea pigs with negative result. Dec. 21, titer of blood 1:40.

Feb. 8, 1923. A second normal calf born. The placenta was discharged. Blood titer 1:40.

No. 710.—Black and white, born Feb. 5, 1919. Titer of blood: Mar. 25, 1920, 1:40; Apr. 21 and Dec. 14, 1:20; Apr. 14, 1921, less than 1:20. Dec. 3, heifer gave birth to full term calf. The placenta was expelled but lost. Film from swab was suspicious of *B. abortus*. Swab tested on guinea pigs with positive result. Dec. 7, titer of blood 1:1,280; titer of mixed milk 1:160.

No. 713.—Black and white, born June 4, 1919. Titer of blood: Mar. 15, 1920, 1:20; Dec. 10, 1:80; Jan. 27 and Apr. 19, 1921, 1:40. Dec. 8, heifer calved normally. The placenta was expelled but lost. Swab tested on guinea pigs with negative result. Dec. 21, titer of blood 1:40. A second normal calf born Dec. 7, 1922. Placenta normal. Mar. 9, blood titer 1:20.

No. 714.—Black and white, born May 6, 1919. Titer of blood: Mar. 15, 1920, 1:160; Dec. 10, 1:80; Jan. 27, 1921, 1:80; Apr. 19, 1:40. Dec. 9, heifer

calved normally. The placenta was expelled but lost. Swab tested on guinea pigs with negative result. Dec. 21, titer of blood 1:40.

A second normal calf was born Apr. 6, 1923. The placenta was discharged but lost. A guinea pig inoculated with washings of uterine swab negative. May 25, blood titer 1:40.

No. 756.—Black and white, born May 8, 1919. Titer of blood: Mar. 15, 1920, 1:80; Dec. 10, 1:40; Jan. 27, 1921, and Apr. 19, 1:40. Jan. 17, 1922, heifer gave birth to full term calf. The placenta was expelled during the morning and found diseased in two foci, one in the unoccupied horn, the other in the central zone of placenta. In both the peripheral zone of large and the whole of small cotyledons was necrotic. Films and sections of the exudate contain epithelial cells filled with *B. abortus*. Titer of blood 1:1,280. Second, normal calf born Jan. 2, 1923. Placenta normal. Blood titer 1:1,280.

No. 820.—Fawn and white, born Feb. 14, 1919. Titer of blood: Mar. 25, 1920, 1:40; Apr. 21, 1:80; Dec. 14, 1:320; Apr. 14, 1921, 1:320. Nov. 8, heifer calved normally. The placenta was expelled and appeared normal. Nov. 10, titer of blood above 1:320.

Aborted in second pregnancy, Oct. 6, 1922. Placenta retained. Blood titer 1:2,560. Fetus about 6 months old. Marked edema and abundant transudates in large serous cavities. *B. abortus* isolated from lungs and digestive tract of fetus directly and from uterine swab, lungs, and digestive tract of fetus through guinea pigs.

No. 821.—Black and white, born Feb. 22, 1919. Titer of blood: Mar. 25, Apr. 21, and Dec. 14, 1920, 1:40; Apr. 14, 1921, 1:40. May 9, 1922, heifer calved normally. The placenta was expelled but lost. June 8, titer of blood 1:40. A second, normal calf was born Apr. 2, 1923. The placenta was discharged and lost. May 25, titer of blood 1:160.

No. 822.—Black and white, born Mar. 1, 1919. Titer of blood: Mar. 25, Apr. 21, and Dec. 14, 1920, 1:80; Apr. 14, 1921, 1:80. May 18, 1922, heifer calved normally. The placenta was expelled but lost. June 8, titer of blood 1:80. A second, normal calf was born Mar. 31, 1923. The placenta was retained. A guinea pig inoculated with washings of uterine swab was negative. May 25, titer of blood 1:80.

No. 823.—Fawn and white, born Mar. 9, 1919. Titer of blood: Mar. 25, 1920, 1:20; Apr. 21, 1:80; Dec. 14, 1:40; Apr. 14, 1921, 1:40. Oct. 28, heifer calved normally. The placenta was expelled but lost. Nov. 10, titer of blood 1:20.

Aborted in second pregnancy, Aug. 31, 1922. Placenta retained. Blood titer 1:1,280. Fetus about 6 months old. *B. abortus* isolated directly in cultures from fourth stomach, rectum, lungs, and spleen of fetus; through guinea pigs, from uterine swab, rectum, lungs, and fourth stomach of fetus.

No. 824.—Black and white heifer, born Mar. 11, 1919. Titer of blood: Mar. 25, 1920, 1:20; Apr. 21 and Dec. 14, 1:40; Apr. 14, 1921, 1:20. Dec. 31, heifer

calved normally. The placenta was expelled and appeared normal with the exception of four cotyledons. Jan. 2, 1922, suspension from uterine swab inoculated into a guinea pig with positive result. Jan. 11, titer of blood above 1:80.

Jan. 22, 1923. Heifer aborted during second pregnancy. Placenta retained. Fetus 28 inches long. Subcutaneous edema. General fetal pneumonia. *B. abortus* isolated from fourth stomach, rectum, lungs, and spleen in pure cultures. Miscellaneous species in cultures from kidney and liver. A guinea pig inoculated with a suspension from uterine swab was positive for *B. abortus*.

No. 825.—Black and white, born Mar. 13, 1919. Titer of blood: Mar. 25, 1920, 1:40; Apr. 21, 1:160; Dec. 14, and Apr. 14, 1921, 1:40. Dec. 6, heifer calved normally. Expelled placenta appeared normal. Dec. 21, titer of blood 1:40. Sold.

No. 826.—Black and white, born Mar. 14, 1919. Titer of blood: Mar. 25, 1920, 1:40; Apr. 21, 1:320; Dec. 14, and Apr. 14, 1921, 1:40. Nov. 1, heifer calved normally. The placenta was expelled but lost. Nov. 2, titer of colostrum 1:40. Dec. 21, titer of blood 1:80.

Second normal calf born Feb. 3, 1923. The placenta was expelled and found normal.

No. 827.—Black and white, born Mar. 15, 1919. Titer of blood: Mar. 25, 1920, 1:40; Apr. 21 and Dec. 14, 1:20; Apr. 14, 1921, less than 1:20. May 7, 1922, heifer calved normally. The placenta was expelled but lost. June 8, titer of blood 1:40.

A second normal calf was born Apr. 24, 1923. The placenta was normal. May 25, blood titer 1:40.

No. 828.—Black and white, born Mar. 21, 1919. Titer of blood: Mar. 25, 1920, 1:40; Apr. 21, 1:80; Dec. 14, and Apr. 14, 1921, 1:40. Feb. 15, 1922, heifer calved normally. The placenta was expelled and appeared normal. Mar. 10, titer of blood 1:40.

A second normal calf was born Mar. 16, 1923. The placenta was expelled but not recovered. May 25, blood titer 1:20.

No. 829.—Black and white, born Mar. 22, 1919. Titer of blood: Mar. 25, 1920, 1:20; Apr. 21, less than 1:20; Dec. 14, and Apr. 14, 1921, 1:20. Feb. 7, 1922, heifer calved normally. The placenta was expelled but lost. Titer of blood 1:20.

A second normal calf was born Mar. 24, 1923. The placenta was discharged, lost. Agglutinin titer of blood 1:80.

No. 830.—Black and white, born Mar. 23, 1919. Titer of blood: Mar. 25, 1920, 1:20; Apr. 21, 1:40; Dec. 14, 1:20; Apr. 14, 1921, 1:40. Nov. 30, heifer calved normally. The placenta was discharged and about two-thirds obtained which appeared normal. Dec. 21, titer of blood 1:80.

Second normal calf born Jan. 8, 1923. The placenta was normal. Feb. 2, titer of blood 1:40.

No. 831.—Black and white, born Mar. 27, 1919. Titer of blood: Mar. 25 and Apr. 21, 1920, 1:80; Dec. 14, 1:40; Apr. 14, 1921, 1:20. On Oct. 28, heifer

calved normally. The placenta was expelled but lost. Nov. 10, titer of blood 1:40.

A second normal calf was born Dec. 26, 1922. Placenta discharged but lost. Blood titer 1:40. Result of guinea pig test of uterine swab negative.

No. 832.—Fawn and white, born Apr. 7, 1919. Titer of blood: Mar. 15, 1920, 1:320; Dec. 14, 1:80; Apr. 14 and Nov. 10, 1921, 1:40. On Nov. 12, heifer calved normally. The placenta was expelled but lost. Nov. 18, titer of blood 1:40. Sold.

No. 833.—Fawn and white, born Apr. 7, 1919. Titer of blood: Mar. 15 and Dec. 10, 1920, 1:40; Jan. 17, 1921, 1:40; Apr. 19, 1:20. On Nov. 4, heifer gave birth to full term calf. The placenta from gravid horn was expelled but the rest retained. Nov. 10, titer of blood 1:1,280. Sold.

No. 834.—Black and white, born Apr. 9, 1919. Titer of blood: Mar. 15 and Dec. 14, 1920, 1:80; Apr. 19, 1921, 1:20. Jan. 5, 1922, heifer calved normally. The placenta was expelled and appeared normal. Jan. 11, titer of blood 1:40.

Second normal calf born Dec. 3, 1922. Placenta discharged and lost. Guinea pig test of uterine swab positive for *B. abortus*. Feb. 3, 1923, titer of blood 1:40. June 25, titer 1:80.

No. 835.—Fawn and white, born Apr. 9, 1919. Titer of blood: Mar. 15, 1920, 1:20; Dec. 14, 1:40; Apr. 14 and 19, 1921, 1:40. Dec. 16, heifer calved normally. The placenta was expelled but lost. Mar. 8, 1922, titer of blood 1:80.

May 4, 1923. Heifer calved normally. The placenta was expelled but lost.

No. 836.—Fawn and white, born Apr. 10, 1919. Titer of blood: Mar. 15, 1920, 1:20; Dec. 14, 1:40; Apr. 14, 1921, 1:40. Nov. 23, heifer calved normally. The placenta was expelled and appeared normal. Titer of blood 1:40.

Feb. 23, 1923. Second normal calf born. Placenta expelled but not recovered. A guinea pig inoculated with a suspension of a uterine swab was negative for *B. abortus*.

No. 837.—Black and white, born Apr. 13, 1919. Titer of blood: Mar. 15, 1920, 1:320; Dec. 14, 1:40; Apr. 14, 1921, 1:40. Dec. 2, heifer calved. Dystocia. Calf delivered dead. The placenta was expelled and appeared normal. Dec. 21, titer of blood 1:40.

A second, normal calf was born Apr. 9, 1923. The placenta was normal.

No. 839.—Fawn and white, born Apr. 29, 1919. Titer of blood: Mar. 15, 1920, 1:80; Dec. 10, 1:40; Jan. 17, 1921, 1:20. July 22, heifer probably aborted. There was a discharge from uterus. Os was open. Titer of blood 1:40. Sold.

No. 840.—Black and white, born May 12, 1919. Titer of blood: Mar. 15 and Dec. 10, 1920, 1:320; Apr. 19, 1921, 1:160. July 20, heifer probably aborted. Removed from pasture on account of swollen udder and discharge from vagina. Heifer was in œstrum. Titer of blood 1:320. Sold.

No. 841.—White and black, born May 15, 1919. Titer of blood: Mar. 15, 1920, 1:40; Dec. 10, 1:80; Jan. 27, 1921, 1:40; Apr. 19, 1:20. Dec. 5, heifer calved normally. The placenta was expelled and appeared normal. Dec. 21,

titer of blood 1:40. Second, normal calf born Nov. 19, 1922. The placenta was expelled but lost. Blood titer 1:20.

No. 842.—Black and white, born May 27, 1919. Titer of blood: Mar. 15, 1920, 1:160; Dec. 10, 1:40; Jan. 27 and Apr. 14, 1921, 1:80. Dec. 26, heifer calved normally. The placenta was expelled but lost. Jan. 25, 1922, titer of blood 1:80. Sold.

No. 843.—Fawn and white, born Aug. 31, 1918. Titer of blood: May 1 and Nov. 30, 1920, less than 1:20; Jan. 17, 1921, 1:20; Feb. 14, 1:40. May 17, heifer calved normally. The placenta was expelled but lost. Titer of blood: May 1, less than 1:20; Nov. 18, 1:20. May 29, 1922, heifer aborted. The fetus was lost. June 6, 1922, titer of blood 1:640.

No. 846.—Fawn and white, born Oct. 5, 1919. Titer of blood: Mar. 25 and Apr. 21, 1920, 1:20; Nov. 22, 1:40; Jan. 11 and Apr. 11, 1921, 1:40. June 11, heifer gave birth to full term calf. The placenta was retained. June 12, blood titer 1:20.

No. 847.—Fawn and white, born May 21, 1918. Titer of blood: Apr. 6, 1920, 1:320; Jan. 7, 1921, above 1:320. Jan. 27, heifer calved normally. The placenta was expelled and appeared normal. Titer of the blood and of the colostrum 1:320. Feb. 2, titer of mixed milk 1:40. May 1, titer of blood 1:320. Dec. 18, cow calved normally. The placenta was expelled but lost. Dec. 21, titer of blood 1:320.

No. 848.—Fawn, born May 29, 1918. Titer of blood: Apr. 6, 1920, 1:40; Oct. 13, 1:20. Oct. 25, heifer calved normally. The placenta was expelled but lost. Titer of blood: Mar. 29, 1921, 1:20; May 1 and 17, and July 28, 1:40. Feb. 2, 1922, cow calved normally. The placenta was expelled but lost. Feb. 3, suspension from uterine swab inoculated into a guinea pig with negative result. Feb. 7, titer of blood 1:20. Sold.

No. 849.—Fawn and white, born June 9, 1918. Titer of blood: Apr. 6 and Nov. 22, 1920, 1:80; Jan. 17, 1921, 1:80; Feb. 14, 1:40. Mar. 14, heifer calved normally. The placenta was expelled but lost. Titer of blood: Mar. 29, May 1 and 17, 1:80; July 28, 1:40. Sold.

No. 850.—Fawn and white, born Dec. 10, 1918. Titer of blood: Mar. 22, 1920, 1:40; Apr. 21, 1:80; Nov. 22, 1:40; Jan. 11 and Apr. 11, 1921, 1:40. July 16, heifer calved normally. The placenta was expelled and appeared normal. Titer of blood and of colostrum 1:80. July 28, titer of blood 1:40. Aug. 2, 1922, cow calved normally. The placenta was expelled but lost. Titer of blood 1:80. June 12, 1923, calved normally a third time.

No. 851.—Fawn and white, born Feb. 3, 1919. Titer of blood: Mar. 25 and Apr. 21, 1920, 1:20; Dec. 14, 1:40; Apr. 11, 1921, 1:40. Apr. 28, 1922, heifer was considered sterile and was slaughtered. The uterus was about twice normal size and contained a partly mummified fetus. Guinea pig tests were negative as to *B. abortus*. Cultures from the soft, cheesy, fetal mass contain a few miscellaneous colonies. Diagnosis remains doubtful.

No. 950.—Fawn, born Oct. 5, 1919. Titer of blood: Mar. 15 and Dec. 10, 1920, 1:80; Jan. 27, 1921, 1:80. May 8, 1922, heifer aborted. Placenta retained. Fetus weighs 16 lbs. and is 68 cm. long. Organs normal and more or less autolyzed. *B. abortus* was isolated from fourth stomach, large intestine, and lungs. Guinea pigs inoculated with suspensions of contents of fourth stomach, rectum, and of lung tissue were positive for *B. abortus*. Sold.

No. 1027.—Fawn, born Apr. 21, 1919. Titer of blood: Mar. 15, 1920, 1:20; Dec. 10, Jan. 17, 1921, and Apr. 19, above 1:320. Mar. 25, 1922, heifer gave birth to full term calf. Dystocia at parturition. Calf injured. Killed soon after birth. The placenta was expelled but lost. Sold.

The Vaccination of Cows: The Effect of the Subcutaneous Injection of Living Cultures of Bacillus abortus Made within 2 Months after Parturition on the Udder and on the Following Pregnancy.

In view of the results obtained from the inoculation of heifers at least 2 months before the beginning of the first pregnancy, it seemed probable that inoculations made within 4 to 8 weeks after calving and at least 6 to 8 weeks before the beginning of the next pregnancy would have the same effect as on the virgin heifer. To test this assumption, ten cows were selected which had passed through the first pregnancy in a normal way and without indications of carrying *Bacillus abortus*. After treatment these cows were placed among older cows in the milking barns, so as to be exposed to the average dangers of infection prevailing in the herd. The vaccine used on all cows alike was prepared from the same culture of *Bacillus abortus* used on most of the vaccinated heifers (No. 281). It had been under cultivation 2 years and 7 months. 4 day growths on sealed agar slants were suspended in normal salt solution and the density brought down to that of a 24 hour bouillon culture of the typhoid bacillus. Of this each animal received subcutaneously 5 cc., about 5/12 of an agar slant.

The animals were to be kept under observation for at least one pregnancy period. The temperature following the injection was usually highest on the day after, reaching 40°C. in some cases and subsiding to normal in about 4 days. The local swelling appeared next day and increased for 1 or 2 days, then subsided. The tissue involved was from 4 to 12 square inches in extent and $\frac{1}{2}$ inch thick.

Three of the heifers were under observation for several months only. Two were killed on account of suspected tuberculosis and the third

died of an acute disease. Of the seven remaining, six calved normally and one was sterile probably as a result of the vibronic (?) disease of the placenta in the first pregnancy. From none of the six was *Bacillus abortus* isolated from uterine swabs after delivery, although in one the placenta was adherent after a normal parturition.

In two the udder became infected with *Bacillus abortus*. One of these was the sterile cow (585). The other was a cow with normal pregnancy. In two cases there was a marked rise in agglutinins in the milk as the udder ceased secreting (887, 884), but guinea pig tests were negative. The concentration of agglutinins in the colostrum immediately after birth and their prompt dilution is seen in several cases.

In general the result showed the possibility of injecting living cultures into lactating cows without damage, provided the vaccination is done several months before the beginning of the next pregnancy. In fact the vaccination probably protected one or more of them from placental disease.

Notes on the Individual Cows.—

No. 845.—Guernsey cow. Gave birth to full term calf Apr. 23, 1921. Vaccinated May 7. Bred repeatedly. The agglutinin titers for the blood and milk are given in the following table. It will be noted that the blood titer to *B. abortus* rose in 10 days after vaccination and subsided later. The milk titer (except colostrum) remained below 1:20. Cow gave birth to a full term calf Aug. 21, 1922, but the placenta was retained. A small piece obtained later in a decomposed state. Three guinea pigs were inoculated with a suspension of uterine fluid collected on a swab. One died of septic disease next day. The others were chloroformed after 6 weeks and found normal.

TABLE XIV.
Vaccinated Cow 845.

Date of bleeding	Agglutinin titer.	Date of milk sample.	Agglutinin titer.
<i>1921</i>		<i>1921</i>	
May 4	1:20		
" 10	1:20		
" 17	1:320	May 11	<1:20
" 31	1:320	" 17	<1:20
		" 24	<1:20
		" 31	<1:20
		June 7	<1:20
		" 21	<1:20
June 22	1:320		
		July 11	<1:20
		Sept. 26	<1:20
Nov. 8	1:80		
		Nov. 16	<1:20
<i>1922</i>		<i>1922</i>	
Jan. 25	1:80	Jan. 25	<1:20
Apr. 7	1:160		
Aug. 21	(Calved.)		
" 21	1:160	Aug. 21	1:80

No. 887.—Holstein cow. First pregnancy ended normally, Mar. 30, 1921. Cow vaccinated May 7. Second pregnancy ended normally June 11, 1922, although the titer of the blood agglutinins had been relatively high throughout this period. The placenta was normal. The milk agglutinins remained low except for the period before the second calving when the quiescent udder stored agglutinins. When the milk secretion was fully established, 4 days after calving, the milk titer had fallen to normal. This cow was sold and further study ceased. Three guinea pigs which received into the peritoneal cavity milk from the quiescent udder failed to develop the characteristic lesions of *B. abortus*.

TABLE XV.
Vaccinated Cow 887.

Date of bleeding.	Agglutinin titer.	Date of milk sample.	Agglutinin titer.
<i>1921</i>		<i>1921</i>	
May 1	1:20	Apr. 21	1:10
" 10	1:20	May 10	<1:10
" 17	1:640	" 17	1:10
		" 24	1:20
" 31	1:2,560	" 31	1:20
		June 7	1:20
		" 21	1:20
June 22	1:2,560	July 11	1:20
		Sept. 26	1:80
Nov. 8	1:320	Nov. 16	<1:20
<i>1922</i>		<i>1922</i>	
Jan. 25	1:640	Jan. 25	1:40
Apr. 7	1:640	Apr. 5	1:640
		" 26	1:2,560
June 11	(Calved.)	June 11	1:640
		" 12	1:160
" 15	1:640	" 15	1:40

No. 872.—Jersey cow. Gave birth to a normal calf, Mar. 31, 1921. Vaccinated May 7. Bred July 25. Gave birth to a second normal calf, Apr. 28, 1922. The placenta was normal in appearance. The blood titer following vaccination rose and remained high during the entire period of gestation. The milk titer, low for 6 months after vaccination, became high thereafter. On Feb. 16, about 2½ months before birth of the second calf, *B. abortus* was isolated from the milk of three quarters through guinea pigs. A guinea pig inoculated with suspension from a uterine swab taken soon after delivery was negative for *B. abortus*.

TABLE XVI.
Vaccinated Cow 872.

Date of bleeding.	Agglutinin titer.	Date of milk sample.	Agglutinin titer.
1921		1921	
		Apr. 21	1:10
May 1	1:20		
" 10	1:20	May 10	<1:10
" 17	1:1,280	" 17	1:10
		" 24	1:20
" 31	1:2,560	" 31	1:20
		June 7	1:20
		" 21	1:10
		July 11	1:10
		Sept. 26	1:20
Nov. 8	1:640		
		Nov. 16	1:20
1922		1922	
Jan. 25	1:1,280	Jan. 25	1:320
		Feb. 16	R.F. 1:1,280
			R.H. 1:1,280
			L.F. 1:320
			L.H. 1:320
Apr. 7	1:1,280		
" 28	(Calved.)	Apr. 28	1:5,120
" 28	1:1,280		
		May 4	R.F. 1:320
			R.H. 1:320
			L.F. 1:160
			L.H. 1:160

No. 884.—Holstein cow. Calved normally for the first time Mar. 22, 1921. Vaccinated May 7. Bred Aug. 11. Gave birth to a second calf May 26, 1922. The calf was full term and placenta normal. The vaccination was followed by a high blood titer during the whole period of the second pregnancy. The milk titer was normal, except during the dry period when agglutinins were stored in the colostrum. Two separate tests of the milk on guinea pigs proved negative. A suspension of a uterine swab taken soon after parturition was injected into a guinea pig with negative result.

TABLE XVII.
Vaccinated Cow 884.

Date of bleeding.	Agglutinin titer.	Date of milk sample.	Agglutinin titer.
<i>1921</i>		<i>1921</i>	
		Apr. 21	<1:20
May 1	1:20		
" 10	1:80	May 10	<1:20
" 17	1:1,280	" 17	1:80
		" 24	1:160
" 31	1:2,560	" 31	1:160
		June 7	1:80
		" 21	1:40
June 22	1:2,560		
		July 11	1:40
		Sept. 26	1:20
Nov. 8	1:320		
		Nov. 16	1:20
<i>1922</i>		<i>1922</i>	
Jan. 25	1:640	Jan. 25	1:40
		Apr. 5	1:640
Apr. 7	1:640		
		" 26	1:1,280
May 26	(Calved.)		
" 28	1:640	May 28	1:40

No. 865.—Holstein cow. First calf born Mar. 25, 1921. The placenta appeared normal. Cow vaccinated May 7. Bred Aug. 10. A second calf born May 20, 1922. The placenta was expelled but lost. A uterine swab taken immediately after birth of calf was suspended in salt solution and the fluid injected into two guinea pigs. These were found negative as to *B. abortus* after the usual period.

The table shows a high content of the blood in agglutinins to the end of the second pregnancy but the milk remained at the normal low level. Further observation was stopped because the cow was slaughtered on account of traumatic pericarditis.

TABLE XVIII.
Vaccinated Cow 865.

Date of bleeding.	Agglutinin titer.	Date of milk sample.	Agglutinin titer.
<i>1921</i>		<i>1921</i>	
		Apr. 21	1:20
May 1	1:40		
" 10	1:40	May 10	<1:20
" 17	1:1,280	" 17	1:20
" 31	1:2,560	" 31	1:20
		June 7	1:20
		" 21	1:20
June 22	1:1,280		
		July 11	1:10
		Sept. 26	1:10
Nov. 8	1:1,280		
		Nov. 16	<1:20
<i>1922</i>		<i>1922</i>	
Jan. 25	1:320	Jan. 25	1:20
Apr. 7	1:1,280		
May 20	(Calved.)		
" 22	1:640	May 22	1:20

No. 591.—Guernsey cow. Gave birth to a full term calf and discharged the placenta normally, Apr. 13, 1921. The placenta had a few small adventitious cotyledons and sections showed dense cell infiltration and necrosis of the villi of these structures. Cow vaccinated May 7. The blood titer follows the usual curve. The milk titer rose slightly following the blood curve. When the titer was slightly above 1:80, three guinea pigs were inoculated with centrifuged milk but with negative result as to *B. abortus*. A full time calf was born Sept. 30, 1922. The placenta was discharged and found normal.

TABLE XIX.
Vaccinated Cow 591.

Date of bleeding.	Agglutinin titer.	Date of milk sample.	Agglutinin titer.
<i>1921</i>		<i>1921</i>	
May 1	1:20	Apr. 21	1:20
" 10	1:40	May 10	<1:20
" 17	1:1,280	" 17	1:20
		" 24	1:40
" 31	1:2,560	" 31	1:40
		June 7	1:40
		" 21	1:40
June 22	1:1,280		
		July 11	1:40
		Sept. 26	1:80
Nov. 8	1:320		
		Nov. 16	1:80
<i>1922</i>		<i>1922</i>	
Jan. 25	1:320	Jan. 25	1:80
		Apr. 5	1:40
Apr. 7	1:320		
Sept. 30	(Calved.)		
Oct. 2	1:1,280	Oct. 2	1:640

No. 585.—Holstein cow. Gave birth to a full term calf Mar. 25, 1921. The fetal membranes presented certain hitherto not observed lesions.

The main changes are in the amnion and the allantois. The former membrane is thickened to about 1 mm. throughout. Both where it is free and where united with allantois, the subepithelial zone is densely infiltrated with a layer of cells, largely necrotic and disintegrated. Here the epithelium has disappeared. To the naked eye this layer appears in the form of whitish plaques, set in the membrane. The chorion is involved where it is in contact with the amnion. The

membrane is edematous and filled with exudate cells. Portions of villi are filled with nuclear débris. The chorionic epithelium wherever *in situ* is free from bacteria. Sections and smears of chorionic tissue and cotyledons show dense masses of fine, tortuous, bacterial filaments wherever necrosis of villi and exudation are found. Subsequent studies have made it quite probable that the disease was due to *Vibrio fetus*, for *B. abortus* could not be demonstrated and the agglutinin titers of both blood and milk to this bacillus were low. Moreover, the lesions of the amnion resembled those associated with vibronic disease and the masses of fine filaments in the disease foci resembled the non-motile race of *Vibrio fetus*.

The cow was vaccinated May 7 and repeatedly bred thereafter without success. The agglutinin titer of the blood towards *B. abortus* rose in the usual way and continued high. The milk titer was found normal 2 months and fairly high 4½ months after vaccination. It remained high and *B. abortus* was isolated repeatedly from the milk both by direct culture and through guinea pigs.

TABLE XX.
Vaccinated Cow 585.

Date of bleeding.	Agglutinin titer.	Date of milk sample.	Agglutinin titer.
1921		1921	
		Apr. 21	1:20
May 1	1:40		
" 10	1:40	May 10	<1:20
" 17	1:1,280	" 17	1:40
		" 24	1:40
" 31	1:2,560	" 31	1:40
		June 7	1:20
June 22	1:1,280		
		July 11	1:40
		Sept. 26	1:320
Nov. 8	1:1,280		
		Nov. 18	1:640
		Dec. 7	R.F. 1:320
			R.H. 1:640
			L.F. 1:160
			L.H. 1:640
1922		1922	
		Jan. 6	R.H. 1:1,280
Jan. 25	1:1,280	" 25	1:640
		Apr. 5	1:320
Apr. 7	1:640		

No. 879.—Holstein cow. Passed through her first pregnancy normally. Calf born Mar. 22, 1921. Cow vaccinated May 7. Later on she was found to react to tuberculin and was killed. The blood titer shows the usual rise while the milk titer remained low to the end of the test about 4½ months after inoculation.

TABLE XXI.
Vaccinated Cow 879.

Date of bleeding.	Agglutinin titer.	Date of milk sample.	Agglutinin titer.
1921		1921	
May 1	1:40	Apr. 21	<1:10
" 10	1:40	May 10	<1:10
" 17	1:640	" 17	<1:10
		" 24	1:20
" 31	1:2,560	" 31	1:20
		June 7	1:20
		" 21	1:10
June 22	1:1,280	July 11	1:20
		Sept. 26	1:20

No. 854.—Jersey cow. Gave birth to first calf Apr. 13, 1921. Conditions as to calf and placenta normal. Vaccinated May 7. The cow died of an acute disease. The blood and milk titers are given below. As long as samples were available, the milk titer remained low.

TABLE XXII.
Vaccinated Cow 854.

Date of bleeding.	Agglutinin titer.	Date of milk sample.	Agglutinin titer.
1921		1921	
May 1	1:20	Apr. 21	1:10
" 10	1:40	May 10	<1:10
" 17	1:640	" 17	1:10
		" 24	1:10
" 31	1:2,560	" 31	1:20
		June 7	1:20
June 22	1:1,280		

No. 875.—Holstein cow. Gave birth to a first calf normally, Apr. 17, 1921. Vaccinated May 7. The blood titer given below shows the usual prompt rise after inoculation with later decline. The milk agglutinins rose slightly as a result of the rise in blood agglutinins. The cow was killed as a doubtful reactor later on and the records therefore are not complete.

TABLE XXIII.
Vaccinated Cow 875.

Date of bleeding.	Agglutinin titer.	Date of milk sample.	Agglutinin titer.
1921		1921	
		Apr. 21	1:10
May 1	1:40		
" 10	1:40	May 10	<1:10
" 17	1:1,280	" 17	1:20
		" 24	1:40
" 31	1:2,560	" 31	1:80
		June 7	1:40
		" 21	1:40
June 22	1:1,280		
		July 11	1:20
		Sept. 26	1:20
Nov. 8	1:320		
		Nov. 16	1:20

Discussion and Summary of the First Experiment.

The small number of animals, both vaccinated and controls, which have been under observation—187 in all—do not furnish statistical results of much weight. The value of the investigation rests on the thoroughness with which individuals, both vaccinated and controls, have been studied. In the investigations reported by others the vaccinations were carried out on herds at a distance and not under daily inspection. The results were gathered largely by owners and attendants. In the present study attention has been given not only to abortions but also to placental disease attending full term pregnancies. There is theoretically little difference between these and actual abortions for the former shade gradually into the latter. In the following table the three groups of heifers are presented statistically, so far as this is possible with material in which every case differs more or less from every other in details.

TABLE XXIV.
Summary of First Experiment.

No. investigated.	No. remaining available.	Full term pregnancies.		Abortions.	Per cent aborted.	Per cent aborted and with placenta diseased.
		Normal.	With placenta diseased.			
First Control Group.						
72	1st pregnancy.					
	68	44	4	20	29.4	35.3
	2nd pregnancy.					
	38	29	1	8	21.0	23.7
	3rd pregnancy.					
17	17	0	0	0	0	
Vaccinated Group.						
53	1st pregnancy.					
	48	34	6	8	16.7	29.2
	2nd pregnancy.					
	38	33	1	4	10.5	13.1
	3rd pregnancy.					
15	14	0	1 (vibrionic).	0	0	
Second Control Group.						
62	1st pregnancy.					
	59	43	4	12	20.3	27.1
	2nd pregnancy.					
40	30	3	7	17.5	25.0	
Total Control Animals.						
134	1st pregnancy.					
	127	87	8	32	25.1	31.4
	2nd pregnancy.					
78	59	4	15	19.2	24.3	

Viewed statistically the injection of living cultures does not seem to have given results above those registered for the second group of controls. The first group however comes nearer to the conditions surrounding the vaccinated group. The heifers of the first control group were born in 1917, those of the vaccinated group in 1917 and 1918, and those of the second control group late in 1918 and in 1919. In the period intervening more attention was given to this disease by the management and it is probable that the intensity of the infection had been slowly decreasing as a result.

The superiority of the vaccinated group comes to the surface in the second pregnancies. During these the heifers come in contact with older cows in the milking barns and here the "coefficient of infection" is more uniform than during the earlier segregation. It will be noted that in the vaccinated group no heifer which gave birth to a full term calf with normal membranes aborted in the second pregnancy. Three heifers aborted twice and a fourth, which aborted the second calf, had a diseased placenta with the first full term calf. Four which aborted in the first pregnancy had normal calves in the second. In the first control group, four with first calf normal aborted the second. Four aborted twice and four, aborting the first time, completed a normal second pregnancy. In the second control group, five with first pregnancy terminating normally aborted in the second; two aborted twice. Of two aborting in the first pregnancy, one was normal in the second and the other had a diseased placenta. In the third pregnancies of those cows which have been retained in the herd, no abortions or diseased placentas have been noticed either in the vaccinated or the first control group. The vaccination thus tends to protect the second pregnancies when the infection is more concentrated in the milking barns. Highly susceptible heifers probably do not get sufficient protection, since they tend to abort twice. The vaccination also tends to bring to the surface early those heifers which may become chronic aborters and which are not benefited by the treatment.

Further discussion will be found following the data of the second experiment.

SECOND VACCINATION EXPERIMENT.

General History of the Animals.

When the opportunity presented itself to deal with groups of heifers more homogeneous with reference to external conditions than those included in the first experiment, another test was undertaken to determine if possible whether repeated injections of heated cultures of *Bacillus abortus* would induce enough immunity to enable the heifer to carry the first fetus to full term. To this end, 35 heifers were vaccinated 4 times with cultures killed by heat. This group will be designated Group A. To control properly and evaluate the results of the treatment of this group, other groups were associated with it in the experiment. Group B consisted of 24 heifers, 10 of which were vaccinated with living cultures, as in the first experiment, and 14 reserved as controls. This group therefore checked the first in two directions, first by a comparison with the effect of living virus, and second by showing the prevalence of the disease among unprotected heifers.

With two exceptions (Nos. 750 and 754) all heifers in Groups A and B were bred to the same bull to eliminate certain undetermined variables due to the use of different bulls.

In addition to the 14 untreated control heifers of Group B, there are included two additional groups of untreated controls, C and D, 10 and 14 in number respectively, which were pastured together and which are distinguished as two groups because in September, 1922, Group C was pregnant, Group D not. At this time they came in contact with Groups A and B on account of a defective fence separating Groups A and B from C and D. Individuals of Groups C and D were bred to different bulls.

The various shifts of these groups from the winter of 1921-22 to November, 1922, are sketched in Fig. 1. They had nothing to do with the experiment except in so far as the individuals of the various groups were kept together and bred at nearly the same time. The following is a compact statement of these shifts.

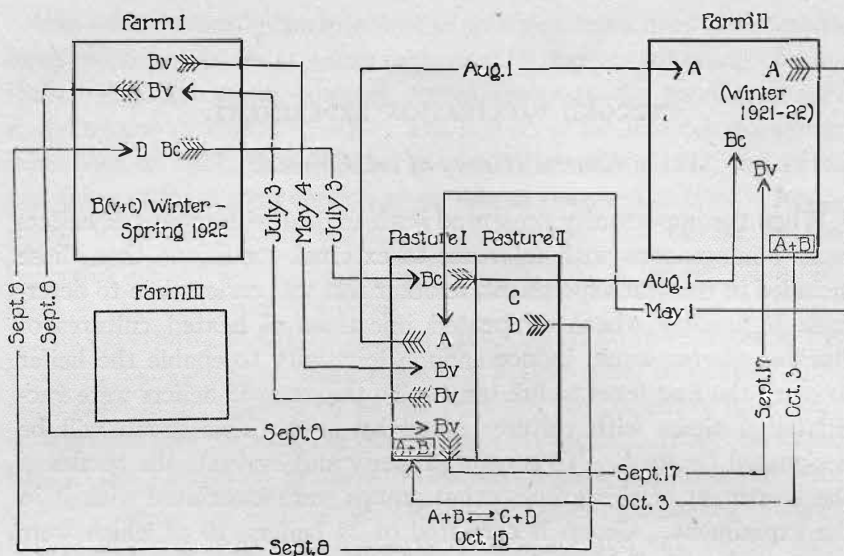


FIG. 1.

Group A.....	35 head treated with heated cultures.
“ Bv.....	10 “ “ “ living “
“ Bc.....	14 “ controls.
“ C.....	10 “ “
“ D.....	14 “ “

Groups A and B.—From November 15, 1921, the heifers of Group A were wintered on Farm II. On February 24, 1922, a young Holstein bull, No. 1, which had previously served only a few cows, was taken to Farm II and stabled in a separate paddock. On April 10, this bull was allowed to run with the heifers.

During the winter and spring of 1921–22, Group B was kept on Farm I. On May 1, 1922, Group A was placed on Pasture I, and on May 4, the vaccinated heifers of Group B (Bv) were placed on this pasture with Group A. On May 4, controls of Group B (Bc) were pastured on Farm I with Bull No. 1. On July 3, Group Bc was taken from Farm I to Pasture I and mixed with Group A. Group Bv was removed from Pasture I to Farm I and turned out with Bull No. 1. On August 1, all heifers on Pasture I were moved to Farm II. On September 8, Group Bv was moved from Farm I to Pasture I. On

September 12, Bull No. 1 was placed on Farm II. On September 17, Group Bv was taken from Pasture I to Farm II and turned out with Groups A and Bc. This was the first time that all of the treated and certain control heifers were together. On October 3, Bull No. 1 was taken to Farm III.

On October 3, all heifers were changed from Farm II to Pasture I. On October 15, Groups A, Bc, and Bv broke through a fence into the adjoining Pasture II on which 38 unbred and 9 pregnant heifers were pastured. Groups C and D belong to these. On November 6 and November 19, some individuals of Group A (721, 724, 726 to 729, 731, 734, 736, 738, 740, 742, 744, 746, 749, 751, 752) were taken from Pasture II to Farm III. On November 29, all remaining heifers of Groups A, Bc, and Bv were moved from Pasture I to Farm II and kept there until about 1 month before parturition when they were driven about $2\frac{1}{2}$ miles to Farm III.

Control Groups C and D.—During the summer of 1922, there were 10 pregnant and 38 unbred heifers on Pasture II adjoining Pasture I. The 10 pregnant heifers represent Group C. One of these (1007) aborted August 19.

Group D comprises 14 of the 38 unbred heifers on Pasture II. They were removed to Farm I on September 8, the same day that Group Bv was removed from Farm I to Pasture I. This group was pastured with Bull No. 1 to September 12 and Bull No. 4 to October 21. This latter bull had been with cows. Group D had been exposed to infection on August 19 when one of Group C aborted on pasture, as stated above.

On October 15, Groups A and B mingled with the remainder of this group through a broken fence. The individuals of Group C had been bred to different herd bulls on another farm. They were taken directly from pasture to Farm III about 1 month before parturition.

Following frequent inspections a number of heifers from the different groups which showed signs of premature calving or abortion were removed from Farm I to Farm III. Nevertheless a number calved and aborted on Farm I and remained there 2 or 3 days before removal to Farm III.

Vaccination of Group A with Heated Cultures.

In the preparation of the vaccine it seemed best, in view of the fact that the bacteria were to be killed by heat, to use as fresh and therefore as virulent a strain as possible. A culture prepared from a guinea pig spleen which had been inoculated with a salt solution suspension of a uterine swab taken immediately after an abortion was used for the first vaccine and subcultures of the same for later vaccines.

The second point to be secured was the sterility of the cultures. These were prepared on agar in sealed bottles and the growth film washed off in normal saline and distributed into tubes which were sealed in the flame and then immersed in a water bath at 61° to 63°C. for $\frac{1}{2}$ hour. Tubes of agar were inoculated with several drops each of the concentrated suspension, sealed, and incubated. For the three first vaccines, two guinea pigs were inoculated with 1 to 2 cc. of the heated suspension. The guinea pig test was omitted for the later vaccines, since the strain used had become well adapted by this time to the agar medium.

The suspension was reduced in all cases to one equal to 3 times the 2.4 density of the Gates instrument. This final suspension was equivalent to a 24 hour bouillon culture of the typhoid bacillus.

To obviate large subcutaneous swellings each dose was divided into two equal portions and a portion injected on each side of the neck. The resulting local swellings varied considerably from animal to animal and are recorded under each case, to be compared with the resulting agglutinin titer and the outcome after breeding.

The relatively feeble protection offered by heated cultures as compared with living cultures makes it necessary to inject the vaccine repeatedly and at intervals which will permit the preceding injection to expend its full energy. To meet these conditions, 8 to 10 cc. of the standard suspension were injected subcutaneously 4 times.

The first injection was carried out in two operations. On December 12, 1921, 14 head received 10 cc., and 21 head 8 cc. on December 19. The other injections were done on the entire group of 35 heifers on January 13, February 13, and April 24, 1922. In these four injections this group received about 8 times as many bacteria as were contained in the largest dose of living bacteria administered as vaccine to

Group Bv. The heifers were bred between the second and the third injections.

In none of the 35 head was the agglutinin titer previous to the first vaccination high enough to indicate infection with *Bacillus abortus*. From three to five blood tests were made in each case. The limiting titer ranged from 1:20 to 1:40, rarely to 1:80.

All animals were tested February 23, 1922, 10 days after the third vaccination. The titer of one animal was 1:80; of 5, 1:160; of 17, 1:320; of 7, 1:640; and of 5, 1:1,280. 7 days after the fourth injection, on May 1, the titer of 3 was 1:320; of 16, 1:640; of 13, 1:1,280; of 2, 1:2,560; and of 1, 1:5,120. $6\frac{1}{2}$ to 7 months later, the titers had rearranged themselves as follows: 1 was 1:40; 5, 1:80; 15, 1:160; 4, 1:320; 2, 1:640; 3, 1:1,280; and 1, 1:2,560. These figures indicate that after the third injection the largest number stood at 1:320. After the fourth injection the titer had risen somewhat. All stood at 1:320 or above. 91.4 per cent stood at 1:640 or above, and 48.5 per cent at 1:1,280 or above. About half a year later, 4 of those with high titers had aborted. Of the rest, the titers had dropped, as might have been anticipated unless actual infection had taken place. The larger number stood at 1:160. Those at 1:640 and above, 6 in all, carried their calves to full or nearly full term, but in 2 *Bacillus abortus* was found in the uterus and the placenta was in part diseased. In the others the udder was probably the seat of infection. In one case a diseased placenta was associated with a titer of 1:160.

Of the 35 vaccinated animals, all became pregnant and 34 have discharged calf or fetus at the time of completing this report. 5 aborted or discharged the calf prematurely, the rest gave birth to full term calves. Of these, 4 had the fetal membranes locally diseased and *Bacillus abortus* present, making 9 cases in which the pregnant uterus was invaded. Taking the cases of premature expulsion only, the percentage is 14.7. Adding the cases of infected placenta, the percentage would be 26.4.

Of special interest is the lateness of the abortion, as bearing upon (1) the disease of the entire group and (2) the portal of entry of the virus. The first abortion occurred on pasture, September 12, 3 months and 7 days before maturity. This may perhaps have served as one

source of infection. The 3 other abortions occurred a little over 2 months later. Thus, No. 728, due December 11, aborted November 11; No. 742, due December 19, aborted November 10; No. 748, due December 5, aborted November 9. The 5 cases of 'nearly full term pregnancy, giving birth to living fetuses from which *Bacillus abortus* was isolated, are as follows:

No. 720,	due Dec. 22,	calf discharged Dec. 9,	placenta diseased.
" 723,	" " 31,	" " 31,	" "
" 730,	" Jan. 12,	" Jan. 7,	" normal.
" 732,	" " 14,	" " 12,	" diseased.
" 736,	" Dec. 15,	" Dec. 4,	" "

Of these, No. 730 was 5 days premature, the calf and placenta normal, but the guinea pig inoculation with washings of a uterine swab positive for *Bacillus abortus*. The blood titer was high. The breeding record of No. 723 indicated that the calf was 28 days overdue. The record is probably wrong. No. 732 was only 2 days premature, No. 736, 11 days, and No. 720, 13 days.

The advanced stage of pregnancy and the localized placental lesions point to a late infection, possibly from the earlier abortions. In any case it is difficult to explain any of these cases as due to infection originating in the bull at the time of breeding. The lesions in nearly all cases were relatively recent, as shown by the microscopic examination of fresh films of the placenta.

Of the remaining cases of normal pregnancy, the dates of calvings show that all had the opportunity to be infected from the earlier abortion occurring on pasture in September and the three occurring early in November. There were 3 normal calvings in the last week of November, 21 in December, and 4 in January.

In the cases treated with heated cultures, twice before and twice after they were bred, a persisting high blood titer may be regarded as a sign that *Bacillus abortus* had invaded either the udder or the uterus or both organs.

In the following table a few data are presented in tabular form, giving (1) the agglutinin titer before and after vaccination, and after calving or abortion, (2) the termination of the pregnancy

and the condition of the placenta when obtained, and (3) the determination of the presence or absence of *Bacillus abortus* in the uterus and fetal membranes through the inoculation of guinea pigs.

When, after premature expulsion of the fetus, *Bacillus abortus* was not isolated from washings of uterine swabs or from fetuses, a high blood titer was taken as demonstrating infection with *Bacillus abortus* (No. 737).

TABLE XXV.
Summary of Vaccinations with Heated Cultures. Group A.

Case No.	Agglutinin titer of blood.			Local reaction to vaccine.	First pregnancy.	Placenta, condition of.	Test for <i>B. abortus</i> .*
	Before vaccination.	After vaccination.	After calving.				
720	l.	h., h.	h.	Severe.	Normal, Dec. 9, 1922.	Locally diseased.	m +, g.p. +
721	l.	h., m.	m.	Mild.	Full term, Dec. 14, 1922.	Normal.	g.p. —
722	l.	h., m.	m.	"	" " " 17, 1922.	"	"
723	l.	h., m.	h.	"	" " (calf dead), Dec. 31, 1922.	Locally diseased.	m +
724	l.	h., m.	m.	"	" " Dec. 8, 1922.	Normal.	g.p. —
725	l.	h., m.	m.	Severe.	" " " 11, 1922.	"	"
726	l.	h., m.	m.	Mild.	" " (calf dead), Dec. 17, 1922.	"	(Calf organs normal.)
727	l.	h., h.	h.	Severe.	" " Dec. 17, 1922.	"	"
728	l.	h.	h.	"	Aborted (8 mos. 7 days), Nov. 11, 1922.	Retained.	g.p. +
729	l.	h., m.	m.	"	Full term, Jan. 11, 1923.	Expelled, lost.	g.p. —
730	l.	h., m.	h.	Mild.	" " " 7, 1923.	Normal.	g.p. +
731	l.	h., m.	m.	"	" " Dec. 7, 1922.	"	"
732	l.	h., l.	m.	"	" " Jan. 12, 1923.	Locally diseased.	g.p. +
733	l.	h., h.	h.	Severe.	" " " 13, 1923.	Normal.	"
734	l.	m., l.	l.	None.	" " Dec. 24, 1922.	"	"
735	l.	h., m.	m.	Mild.	" " Nov. 26, 1922.	"	g.p. —
736	l.	h., h.	h.	Severe.	Nearly full term, Dec. 4, 1922.	About 1/2 diseased.	m +, g.p. +
737	l.	h.	h.	Mild.	Aborted (fetus lost), Sept. 12, 1922.	Not obtained.	"
738	l.	h., m.	m.	"	Full term, Nov. 28, 1922.	Normal.	g.p. —
739	l.	h., m.	m.	"	" " Dec. 9, 1922.	"	"
740	l.	h., m.	m.	Severe.	" " " 17, 1922.	"	"
741	l.	m., l.	l.	"	" " " 24, 1922.	"	"

742	l.	h.	h.	Mild.	Aborted (7 mos. 29 days), Nov. 10, 1922.	Lost.	g.p. +
743	l.	h., l.	m.	"	Full term, June 17, 1923.	Normal.	
744	l.	h., m.	m.	Severe.	" " Dec. 9, 1922.	Expelled, lost.	
745	l.	h., l.	h.	None.	" " " 18, 1922.	Normal.	
746	l.	h., m.	h.	Severe.	" " " 12, 1922.	Expelled, lost.	g.p. -
747	l.	h., h.	m.	"	" " July 14, 1923.	Normal.	g.p. -
748	l.	h.	h.	Mild.	Premature calf (8 mos. 14 days), Nov. 9, 1922.	Retained.	g.p. +
749	l.	h., m.	m.	Severe.	Full term, Dec. 8, 1922.	Expelled, lost.	g.p. -
750	l.	h. h.		"			
751	l.	m., l.	l.	Mild.	Full term, Dec. 25, 1922.	Normal.	
752	l.	h., m.	m.	"	" " " 14, 1922.	Expelled, lost.	g.p. -
753	l.	h., m.	l.	Severe.	" " " 16, 1922.	Normal.	
754	l.	h., m.	m.	"	" " Nov. 23, 1922.	"	g.p. -

* m = microscopic diagnosis.

Brief History of Individual Heifers of Group A.—

No. 720.—Black and white heifer, born Dec. 4, 1919.

Agglutinin titers: July 6, 1920, less than 1:20; Jan. 27 and Dec. 12, 1921, not above 1:40.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23, 1922, 1:640; May 1 and Dec. 1, 1:1,280. Bred Mar. 15. Dec. 9. Heifer gave birth to a full term calf, on Farm I. The placenta was expelled. The small cotyledons of one horn were necrotic, similarly the periphery of the large ones. Films show cells packed with *B. abortus*. A uterine swab was tested on guinea pigs with positive result. Dec. 15. Agglutinin titer 1:2,560.

No. 721.—Fawn and white heifer, born Dec. 5, 1919.

Agglutinin titers: June 25 and Oct. 21, 1920, not above 1:40; Jan. 27, May 9, and Dec. 12, 1921, 1:20.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Very mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:640; Nov. 22, 1:160. Bred Mar. 8. Dec. 14. Heifer calved normally. The placenta was expelled and appeared normal. A uterine swab was tested on a guinea pig with negative result. Dec. 15. Titer of blood 1:160.

No. 722.—Fawn heifer, born Dec. 5, 1919.

Agglutinin titers: July 6, 1920, less than 1:20; Jan. 5 and Dec. 12, 1921, not above 1:40.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Very mild reactions.

Subsequent tests: Feb. 23, 1922, 1:160; May 1, 1:640; Nov. 15, 1:160. Bred Mar. 12. Dec. 17. Heifer calved normally. The placenta was expelled and about one-half obtained, which appeared normal. Dec. 26. Agglutinin titer 1:160.

No. 723.—Fawn and white heifer, born Dec. 6, 1919.

Agglutinin titers: July 6, 1920, 1:40; Jan. 27, May 9, and Dec. 12, 1921, not above 1:40.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Small, tender, local swellings.

Subsequent tests: Feb. 23 and May 1, 1922, 1:640; Dec. 1, 1:160. Bred probably Mar. 24, 1922, due Dec. 31. On this day, heifer gave birth to a full term dead calf, on Farm III. The placenta was expelled and one horn found diseased. The cotyledons and the intervening chorion showed characteristic lesions. Films from the exudate show cells packed with *B. abortus*. Jan. 2, 1923. Agglutinin titer 1:640.

No. 724.—Fawn and white heifer, born Dec. 6, 1919.

Agglutinin titers: July 6, 1920, less than 1:20; Jan. 5 and Dec. 12, 1921, 1:80.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:1,280; Nov. 15, 1:160. Bred Mar. 1. Dec. 8. Heifer calved normally. The placenta was expelled and appeared normal. A uterine swab was tested on guinea pigs with negative result. Agglutinin titer 1:160.

No. 725.—Black and white heifer, born Dec. 6, 1919.

Agglutinin titers: June 25, 1920, less than 1:20; Jan. 17 and Dec. 12, 1921, not above 1:80.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:640; Dec. 1, 1:160. Bred Mar. 4. On Dec. 11, heifer calved normally. About two-thirds of the placenta was obtained and appeared normal; the remainder was expelled but lost. Dec. 15. Titer of blood 1:160.

No. 726.—Black and white heifer, born Dec. 12, 1919.

Agglutinin titers: Jan. 27, May 9, and Dec. 12, 1921, 1:40.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Very mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:1,280; Nov. 22, 1:160. Bred Mar. 5. On Dec. 17, heifer gave birth to a full term, dead calf. The placenta was expelled and appeared normal. The autopsy showed the calf's lungs fully inflated, and the stomach contents normal. Only one kidney present. There was no evidence to support *B. abortus* infection in this case. Moreover, it was 5 days overdue. Dec. 26. Titer of blood 1:160.

No. 727.—Fawn and white heifer, born Dec. 12, 1919.

Agglutinin titers: July 6, 1920, less than 1:20; Jan. 5 and Dec. 12, 1921, not above 1:40.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23, 1922, 1:1,280; May 1, 1:5,120; Nov. 22, 1:640. Bred Mar. 9. Dec. 17. Heifer calved normally. The placenta was expelled and appeared normal. Dec. 26. Titer of blood 1:640.

No. 728.—Black and white heifer, born Dec. 15, 1919.

Agglutinin titers: Jan. 5 and Dec. 12, 1921, 1:20.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:1,280. Bred Mar. 4. Nov. 11. Heifer aborted on Farm III. The placenta was retained. Titer of blood 1:2,560. Fetus, alive when born, 40 inches long. Weight 43 lbs. Lungs inflated. No lesions or transudations. Cultures from lungs and fourth stomach

contaminated with a variety of bacteria, including *B. coli*, probably aspirated before fetus died. Cultures of kidney, liver, spleen, and rectal contents remain sterile. Guinea pigs inoculated with bits of lung tissue and contents of rectum fail to develop characteristic lesions. Two inoculated with washings of uterine swab yield pure cultures of *B. abortus*.

No. 729.—Black and white heifer, born Dec. 17, 1919.

Agglutinin titers: July 6, 1920, less than 1:20; Jan. 5 and Dec. 12, 1921, not above 1:80.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23 and May 1, 1922, 1:1,280; Nov. 22, 1:320. Bred Mar. 16. Jan. 11, 1923. Heifer calved normally. The placenta was expelled but lost. A uterine swab was tested on two guinea pigs with negative result. Jan. 12. Titer of blood 1:320.

No. 730.—Black and white heifer, born Dec. 19, 1919.

Agglutinin titers: July 6, 1920, less than 1:20; Jan. 5 and Dec. 12, 1921, not above 1:80.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Very mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:1,280; Dec. 1, 1:160. Bred Mar. 6 and Apr. 5. Jan. 7, 1923. Heifer calved normally. The placenta was expelled and appeared normal. A guinea pig inoculated with washings from uterine swab was positive for *B. abortus*. Jan. 12. Titer of blood 1:1,280.

No. 731.—Black and white heifer, born Dec. 19, 1919.

Agglutinin titers: July 6, 1920, 1:40; Jan. 5 and Dec. 12, 1921, not above 1:80.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:640; Nov. 15, 1:160. Bred Feb. 28. Dec. 7. Heifer calved normally. The placenta was expelled and appeared normal. Dec. 8. Titer of blood 1:320.

No. 732.—Black and white heifer, born Dec. 26, 1919.

Agglutinin titers: July 6, 1920, less than 1:20; Jan. 5 and Dec. 12, 1921, not above 1:80.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Very mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:640; Dec. 1, 1:80. Bred Mar. 14 and Apr. 7. Jan. 12, 1923. Heifer calved normally on Farm III. The placenta was expelled and found locally diseased over an area about 6 to 7 inches square. The small adventitious cotyledons are completely necrotic, the larger ones only on the periphery. Films show miscellaneous bacteria, including streptococci and *B. subtilis*. Sections show advanced lesions with complete loss of chorionic epithelium, infiltration and necrosis of villi, and vessels of chorion partly or wholly occluded by fibrin thrombi. Three guinea pigs inoculated with

suspensions of this diseased tissue and one inoculated with washings of a uterine swab all developed characteristic lesions with presence of *B. abortus* in the spleen. Titer of blood 1:160. Mar. 31. Titer 1:1,280.

No. 733.—Fawn and white heifer, born Dec. 27, 1919.

Agglutinin titers: July 6, 1920, less than 1:20; Jan. 5 and Dec. 12, 1921, not over 1:40.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:1,280; Dec. 1, 1:1,280. Bred Mar. 25. Jan. 13, 1923. Heifer calved normally. The placenta was expelled and appeared normal. Mar. 9. Titer of blood 1:640.

No. 734.—Red and white heifer, born Dec. 29, 1919.

Agglutinin titers: June 25 and Oct. 21, 1920, not over 1:20; Jan. 27, May 9, and Dec. 12, 1921, not over 1:40.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. There were no local reactions.

Subsequent tests: Feb. 23 and May 1, 1922, 1:320; Nov. 22, 1:40. Bred Mar. 12. Dec. 24. Heifer calved normally. The placenta was expelled and appeared normal. Dec. 26 and 29. Titer of blood 1:40.

No. 735.—Fawn and white heifer, born Dec. 31, 1919.

Agglutinin titers: June 25, 1920, less than 1:20; Oct. 21, 1:80; May 9 and Dec. 12, 1921, 1:40.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Very mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:640; Nov. 15, 1:160. Bred Feb. 24. Nov. 26. Heifer calved normally. The placenta was expelled and appeared normal. Nov. 27. A uterine swab was tested on a guinea pig with negative result. Dec. 8. Titer of blood 1:160.

No. 736.—Black and white heifer, born Dec. 31, 1919.

Agglutinin titers: June 25 and Oct. 21, 1920, not above 1:20; Jan. 27, 1921, 1:20; May 9 and Dec. 12, 1:40.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23, 1922, 1:1,280; May 1, 1:2,560; Nov. 22, 1:2,560. Bred Mar. 8. Dec. 4. Heifer gave birth to a nearly full term calf on Farm II. The placenta was retained and removed later. About one-half or more diseased. The chorion is thickened in one region. In the remainder the small adventitious cotyledons have necrotic borders; the large ones are partly covered with a soft, mucoid exudate. Many cells filled with minute bacilli in this exudate. A uterine swab was tested on two guinea pigs with positive result. Dec. 8. Agglutinin titer 1:2,560.

No. 737.—Black and white heifer, born Dec. 28, 1919.

Agglutinin titers: July 6, 1920, less than 1:20; Jan. 5 and Dec. 12, 1921, 1:40.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Very mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:640. Bred Mar. 4. Sept. 12. Heifer aborted, on Farm II. The fetus was lost. Sept. 13. Agglutinin titer 1:5,120.

No. 738.—Fawn and white heifer, born Jan. 7, 1920.

Agglutinin titers: June 25, 1920, less than 1:20; Oct. 21, 1:40; Jan. 27, May 9, and Dec. 12, 1921, not above 1:20.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Very mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:160; May 1, 1:640; Nov. 15, 1:160. Nov. 28. Heifer calved normally. The placenta was expelled and about one-third obtained, which appeared normal; the remainder was lost. A uterine swab was tested on guinea pigs with negative result. Dec. 9. Agglutinin titer 1:320.

No. 739.—Fawn and white heifer, born Jan. 7, 1920.

Agglutinin titers: June 25 and Oct. 21, 1920, Jan. 27, May 9, and Dec. 12, 1921, not over 1:40.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Very mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:640; Nov. 22, 1:160. Bred Mar. 1. Dec. 9. Heifer calved normally. The placenta was expelled and appeared normal. Dec. 15. Agglutinin titer 1:160.

No. 740.—Black and white heifer, born Jan. 16, 1920.

Agglutinin titers: June 25 and Oct. 21, 1920, not over 1:40; Jan. 27, May 9, and Dec. 12, 1921, 1:40.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23, 1922, 1:640; May 1, 1:2,560; Nov. 22, 1:320. Bred Mar. 13. Dec. 17. Heifer calved normally. The placenta was expelled and appeared normal. Dec. 26. Agglutinin titer 1:320.

No. 741.—Black and white heifer, born Jan. 13, 1920.

Agglutinin titers: June 25 and Oct. 21, 1920, not over 1:160; Jan. 27 and Dec. 12, 1921, 1:80.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23 and May 1, 1922, 1:320; Dec. 4, 1:80. Bred Feb. 24 and Mar. 19. Dec. 24. Heifer calved normally. The placenta was expelled and appeared normal. Dec. 26. Agglutinin titer 1:40.

No. 742.—Black and white heifer, born Jan. 13, 1920.

Agglutinin titers: June 25 and Oct. 21, 1920, 1:40; Jan. 27 and Dec. 12, 1921, not over 1:80.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Very mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:160; May 1, 1:640. Bred Mar. 12. Nov. 10. Heifer aborted, on Farm I. The fetus was lost. Nov. 11. A uterine swab was tested on guinea pigs with positive result. Nov. 15. Agglutinin titer 1:5,120.

No. 743.—White and black heifer, born Jan. 12, 1920.

Agglutinin titers: Oct. 21, 1920, Jan. 27 and May 9, 1921, 1:40; Dec. 12, 1:20.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Slight local reactions.

Subsequent tests: Feb. 23, 1922, 1:80; May 1, 1:640; Dec. 1, 1:80; Mar. 15, 1923, 1:640. Bred Mar. 13 and Apr. 5, 1922. Calved normally on June 17, 1923. Placenta normal. June 20. Blood titer 1:320.

No. 744.—Black and white heifer, born Jan. 16, 1920.

Agglutinin titers: June 25 and Oct. 21, 1920, 1:20 or less; Jan. 27 and May 9, 1921, 1:40; Dec. 12, 1:80.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23 and May 1, 1922, 1:1,280; Nov. 22, 1:320. Bred Mar. 5. Dec. 9. Heifer calved normally. The placenta was expelled but lost. Dec. 15. Agglutinin titer 1:320.

No. 745.—Black and white heifer, born Jan. 19, 1920.

Agglutinin titers: June 25 and Oct. 21, 1920, not above 1:40; Jan. 27, 1921, less than 1:20; Dec. 12, 1:40.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. No reaction.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:640; Dec. 1, 1:80. Bred Mar. 11. Dec. 18. Heifer calved normally. The placenta was expelled and appeared normal. Agglutinin titers: Dec. 26, 1:640; Dec. 29, 1:1,280.

No. 746.—White heifer, born Jan. 18, 1920.

Agglutinin titers: June 25 and Oct. 21, 1920, not above 1:20; Jan. 27, 1921, 1:20; May 9 and Dec. 12, 1:40.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reaction.

Subsequent tests: Feb. 23, 1922, 1:640; May 1, 1:1,280; Nov. 22, 1:320. Bred Mar. 12. Dec. 12. Heifer calved normally. The placenta was expelled but lost. A uterine swab was tested on a guinea pig with negative result. Agglutinin titer 1:640.

No. 747.—Fawn and white heifer, born Jan. 18, 1920.

Agglutinin titers: June 25 and Oct. 21, 1920, 1:20, Jan. 21, May 9, and Dec. 12, 1921, not above 1:40.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reaction.

Subsequent tests: Feb. 23, 1922, 1:640; May 1 and Dec. 1, 1:1,280; Mar. 15, 1923, 1:640. Bred Mar. 7, 1922. Full term calf, born July 14, 1923. Placenta was normal and guinea pig test of uterine swab negative.

No. 748.—Black and white heifer, born Jan. 22, 1920.

Agglutinin titers: June 25 and Oct. 21, 1920, 1:20; Jan. 27 and Dec. 12, 1921, not above 1:20.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Very mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:160; May 1, 1:640. Bred Feb. 26.

Nov. 9. Heifer gave birth to a small, undersized, premature calf, on Pasture II. The placenta was retained. Nov. 13. A uterine swab was tested on two guinea pigs with positive result. Nov. 15. Agglutinin titer 1:5,120.

No. 749.—Fawn and white heifer, born Jan. 24, 1920.

Agglutinin titers: Oct. 21, 1920, 1:20; May 9 and Dec. 12, 1921, not above 1:40.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reaction.

Subsequent tests: Feb. 23 and May 1, 1922, 1:640; Nov. 15, 1:160. Bred Feb. 24. Dec. 8. Heifer calved normally. The placenta was expelled but lost. A uterine swab was tested on two guinea pigs with negative result. Agglutinin titers: Dec. 8, 1:160; Dec. 29, 1:320.

No. 750.—Fawn and white heifer, born Jan. 27, 1920.

Agglutinin titers: June 25, Oct. 21, Jan. 27, 1921, May 9, and Dec. 12, not above 1:40.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23 and May 1, 1922, 1:1,280; Dec. 1, 1:640; Mar. 14, 1923, 1:640. Bred Mar. 28 and Dec. 21, 1922. Due to calve Sept. 29, 1923.

No. 751.—Black and white heifer, born Jan. 28, 1920.

Agglutinin titers: June 25, Oct. 21, Jan. 27, 1921, May 9, and Dec. 21, not above 1:40.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Very mild local reactions.

Subsequent tests: Feb. 23 and May 1, 1922, 1:320; Nov. 22, 1:80. Bred Mar. 19. Dec. 25. Heifer calved normally. The placenta was expelled and appeared normal. Dec. 26. Agglutinin titer 1:80.

No. 752.—Fawn and white heifer, born Jan. 30, 1920.

Agglutinin titers: June 25, Oct. 21, Jan. 27, 1921, May 9, and Dec. 12, not above 1:40.

Dec. 19, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Mild local reactions.

Subsequent tests: Feb. 23, 1922, 1:160; May 1, 1:640; Nov. 15, 1:160. Bred Mar. 1. Dec. 14. Heifer calved normally. The placenta was expelled but lost. A uterine swab was tested on a guinea pig with negative result. Dec. 15. Agglutinin titer 1:160.

No. 753.—Black and white heifer, born Jan. 30, 1920.

Agglutinin titers: June 25, 1:80; Oct. 21, 1:20; May 9, and Dec. 12, 1921, 1:40.

Dec. 12, 1921, to Apr. 24, 1922. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23, 1922, 1:320; May 1, 1:1,280; Dec. 1, 1:160. Bred Mar. 7. Dec. 16. Heifer calved normally. The placenta was expelled and about one-half obtained which appeared normal. Dec. 26. Agglutinin titer 1:80.

No. 754.—Black and white heifer, born Jan. 30, 1920.

Agglutinin titers: June 25 and Oct. 21, not above 1:20; Jan. 27, May 9, and Dec. 12, 1921, not above 1:40.

Dec. 12, 1921, to Apr. 24, 1923. Received subcutaneously four injections of heated vaccine. Severe local reactions.

Subsequent tests: Feb. 23, 1922, 1:640; May 1, 1:1,280; Nov. 15, 1:160. Bred Feb. 16. Nov. 23. Heifer calved normally. The placenta was expelled, and that from the non-gravid horn obtained which appeared normal. A uterine swab was tested on two guinea pigs with negative result. Dec. 8. Agglutinin titer 1:320.

Vaccination of Group Bv with Living Cultures.

This group of ten heifers was treated with a living vaccine, so that it might serve as a means of comparing the effect of living and of heated cultures under more or less similar conditions. The vaccine was the same strain used in the first experiment (page 17) and at this time $3\frac{1}{2}$ years under cultivation. After incubation for 2 days on agar slants, the vigorous growth was washed off with normal salt solution and diluted with the same fluid to a density equivalent to a 24 hour bouillon culture of the typhoid bacillus. Of this, 5 cc. was the dose used. This was equivalent to about 0.6 of an ordinary agar slant.

The following table (XXVI) presents in compact form the results. It will be noted that one of the ten had an undeveloped uterus. Of the remaining nine, all gave birth to normal calves and apparently normal placentas, but infection was present in the uterine cavity in three cases as shown by inoculation of guinea pigs with washings from uterine swabs taken at the latest a few hours after the birth of the calves.

TABLE XXVI.

Group Bv. Heifers Vaccinated Once with a Living Culture.

Case No.	Agglutinin titer of blood.			Pregnancy terminated.	Placenta, condition of.	Tests for <i>B. abortus</i> (swab).
	Before vaccina- tion.	After vaccination.	After calving.			
				1923		
920	1.	h., m.	m.	Apr. 17, full term.	Normal.	g.p. +
921	1.	m., h.	h.	May 20, " "	"	g.p. —
922		l., m.	m.	Apr. 14, " "	"	g.p. +
923	1.	m., m.		(Sterile.)	(Uterus unde- veloped.)	
924	1.	l., l.	l.	Apr. 16, full term.	Normal.	g.p. —
925	1.	m., l.	l.	" 14, " "	"	g.p. +
927	1.	m., m.	m.	May 1, " "	"	g.p. —
928	1.	h., h.	h.	" 3, " "	"	g.p. —
929	1.	h., h.	h.	" 10, " "	"	g.p. —
930	1.	h., h.	h.	Apr. 13, " "	"	g.p. —

Brief History of Individuals of Group Bv.—

No. 920.—Fawn and white heifer, born Sept. 7, 1920. Apr. 14, 1922, agglutinin titer 1:40. Vaccinated Apr. 25. Subsequent tests: Dec. 1, 1:640; Mar. 15, 1923, 1:320.

Apr. 17. Heifer calved normally. The placenta was expelled and lost except about one-third, which appeared normal. Agglutinin titer 1:320. Two uterine swabs were tested on guinea pigs with positive result in one. June 25, agglutinin titer of blood 1:320, of the milk of two quarters of the udder 1:320.

No. 921.—Fawn and white heifer, born Sept. 7, 1920. Apr. 14, 1922, agglutinin titer 1:80. Vaccinated Apr. 25. Subsequent tests: Dec. 1, 1:320; Mar. 15, 1923, 1:640.

May 20. Heifer calved normally. Only about one-third of the placenta was obtained which was found normal. May 25, agglutinin titer 1:1,280. A guinea pig inoculated with washings of uterine swab was negative for *B. abortus*.

No. 922.—Black and white heifer, born Sept. 7, 1920. Vaccinated Apr. 25, 1922. Subsequent tests: Dec. 1, 1:80; Jan. 2, 1923, 1:80; Mar. 15, 1:160.

Apr. 14. Heifer calved normally. The placenta was expelled and about two-thirds obtained, which appeared normal. A uterine swab was tested on a guinea pig with positive result. Agglutinin titer 1:160. June 20, titer 1:80. Titer of milk negative.

No. 923.—Black and white heifer, born Sept. 10, 1920. Apr. 14, 1922, agglutinin titer 1:80. Vaccinated Apr. 25. Subsequent tests: Dec. 1, 1:160;

Mar. 15, 1923, 1:160. On this date the heifer was regarded as sterile, and was slaughtered on Apr. 28. The uterus was very small, the horns impervious, cylindrical masses about the thickness of a lead pencil.

No. 924.—Black and white heifer, born Sept. 17, 1920. Apr. 14, 1922, agglutinin titer 1:40. Vaccinated Apr. 25. Subsequent tests: Dec. 1, Jan. 2, 1923, and Mar. 15, 1:40.

Apr. 16. Heifer calved normally. The placenta was expelled and appeared normal. A uterine swab was tested on a guinea pig with negative result. Agglutinin titer 1:40.

No. 925.—Black and white heifer, born Oct. 4, 1920. Apr. 14, 1922, agglutinin titer less than 1:20. Vaccinated Apr. 25. Subsequent tests: Dec. 1, 1:160; Mar. 15, 1923, 1:80.

Apr. 14. Heifer calved normally. The placenta was expelled and appeared normal. A uterine swab was tested on a guinea pig with positive result. Agglutinin titer 1:80. June 21, titer 1:80. Titer of milk less than 1:20.

No. 927.—Black and white heifer, born Oct. 7, 1920. Apr. 14, 1922, agglutinin titer 1:80. Vaccinated Apr. 25. Subsequent tests: Dec. 1, and Mar. 15, 1923, 1:160. A guinea pig test of uterine swab negative for *B. abortus*.

May 1. Heifer calved normally. The placenta was expelled and appeared normal. A uterine swab was tested on a guinea pig with negative result. Agglutinin titer 1:160.

No. 928.—Black and white heifer, born Oct. 13, 1920. Apr. 14, 1922, agglutinin titer 1:40. Vaccinated Apr. 25. Subsequent tests: Dec. 1, and Mar. 15, 1923, 1:1,280.

A full term calf was born May 3. The placenta was expelled and found normal. A guinea pig inoculated with washings of uterine swab negative for *B. abortus*. Agglutinin titer of blood 1:2,560. June 21, agglutinin titer of milk 1:640.

No. 929.—Red and white heifer, born Oct. 14, 1920. Apr. 14, 1922, agglutinin titer 1:40. Vaccinated Apr. 25. Subsequent tests: Dec. 1, 1:1,280; Mar. 15, 1923, 1:2,560.

May 10. Normal parturition. Placenta found normal. Agglutinin titer 1:2,560. Guinea pig test negative. June 21, agglutinin titer of milk 1:1,280.

No. 930.—Red and white heifer, born Nov. 16, 1920. Apr. 14, 1922, agglutinin titer 1:20. Vaccinated Apr. 25. Subsequent tests: Dec. 1, and Mar. 15, 1923, 1:2,560.

Apr. 13. Heifer calved normally. The placenta was expelled and appeared normal. A uterine swab was tested on a guinea pig with negative result. Agglutinin titer 1:2,560. June 21, agglutinin titer of milk 1:1,280.

Detailed History of the Control Groups.

Group Bc.—This group of fourteen heifers was with the vaccinated group in the winter of 1921–22 and then both groups were moved

about over the same pastures but at different times until reunited in September of 1922. One of the group remained sterile and at autopsy an undeveloped uterus was found. Of the remaining thirteen, six calved normally and seven aborted. Among these were three which dropped living but premature calves. A summary of the cases is given in Table XXVII.

TABLE XXVII.
Control Group Bc.

Case No.	Agglutinin titer of blood.		Pregnancy terminated.	Placenta, condition of.	Tests for <i>B. abortus</i> .
	Before calving.	After calving.			
907*	l.	l.	Normally.	Expelled, lost.	
908	l.	h.	In abortion, fetus lost.	Diseased.	Placenta + Swab +
909	l.	l.	Normally.	Normal.	
910	l.	l.	"	"	
911	h.	h.	In abortion.	Retained.	Fetus + Swab +
912	l.	l.	Normally.	Expelled, lost.	" -
913	l.	l.	"	" "	" -
914	l.	h.	Prematurely.	Retained.	" +
915	l.	h.	In abortion.	"	Uterine exudate +
916	l.	l.	Normally.	Expelled, lost.	Swab -
917	m.		(Sterile.)	(Uterus undeveloped.)	
918	l., m.	h.	Prematurely.	Retained.	Swab +
919	h.	h.	In abortion.	Diseased.	Placenta +
926	h.	h.	Prematurely.	Retained, diseased.	" + Swab +

* Owing to some uncertainty in the identity of the inoculated guinea pig, the test for *B. abortus* is not included.

Notes on Individual Cases of Group Bc.—

No. 907.—Black and white heifer, born June 16, 1920. Agglutinin titers: Apr. 26, 1921, 1:20; Apr. 14, 1922, 1:80; Dec. 1, 1:40; Jan. 2, 1923, 1:40. Pasture bred.

Mar. 4. Heifer calved normally. The placenta was expelled but lost. Owing to some error the guinea pig inoculated with washings of the uterine swab was not identified later. The low agglutinin titers and the normal, full term

pregnancy make it fairly certain that this heifer was not infected. Mar. 9, agglutinin titer 1:40.

No. 908.—Black and white heifer, born June 19, 1920. Agglutinin titers: Apr. 26, 1921, 1:40; Apr. 14, 1922, 1:80. Pasture bred.

Nov. 18. Heifer aborted. Fetus lost. A portion of the placenta obtained was diseased. Bits of this organ were washed in running water to remove putrefactive organisms, ground up with sand, and suspended in normal salt solution. Two guinea pigs injected with this developed characteristic lesions. The same was true of two guinea pigs inoculated with washings from a uterine swab. *B. abortus* was isolated from the four animals. Nov. 19, agglutinin titer 1:1,280.

No. 909.—Black and white heifer, born Aug. 20, 1920. Agglutinin titers: Apr. 26, 1921, Apr. 14, 1922, Dec. 1, and Jan. 2, 1923, 1:40. Bred May 8, 1922.

Feb. 18, 1923. Heifer calved normally. The placenta was expelled and about two-thirds obtained, which appeared normal. Feb. 20, agglutinin titer 1:40.

No. 910.—Black and white heifer, born June 25, 1920. Agglutinin titers: Apr. 26, 1921, and Apr. 14, 1922, 1:20; Dec. 1, less than 1:20; Jan. 2, 1923, 1:20. Pasture bred. Feb. 19, heifer calved normally. The placenta was expelled and appeared normal. Agglutinin titer 1:40.

No. 911.—Black and white heifer, born June 26, 1920. Agglutinin titers: Apr. 14, 1922, 1:40; Dec. 1, 1:1,280. Pasture bred.

Dec. 29. Heifer aborted. The placenta was retained. The fetus weighed 25 lbs. and was 30 inches long. The large cavities were free from transudates, and the organs normal. The lungs were partly inflated. *B. abortus* was isolated from a culture of cecum contents. Cultures from fourth stomach, lungs, spleen, kidneys, and liver sterile. Guinea pigs inoculated with washings of uterine swab positive for *B. abortus*. Those inoculated with contents of fourth stomach, rectum, and bits of lung tissue negative. Jan. 2, 1923, agglutinin titer 1:2,560.

No. 912.—Black and white heifer, born July 22, 1920. Agglutinin titers: Apr. 26, 1921, 1:40; Apr. 14 and Dec. 1, 1922, 1:20; Jan. 2, 1923, 1:40. Pasture bred.

Mar. 4. Heifer calved normally. The placenta was expelled but lost. Suspension from uterine swab tested on guinea pigs with negative result. Mar. 9, agglutinin titer 1:40.

No. 913.—Black and white heifer, born Aug. 4, 1920. Agglutinin titers: Apr. 14, 1922, 1:40; Dec. 1, and Jan. 2, 1923, 1:20. Pasture bred. Feb. 13, heifer calved normally. The placenta was expelled but lost. Suspension from uterine swab was tested on a guinea pig with negative result. Mar. 31, agglutinin titer 1:80.

No. 914.—Black and white heifer, born Aug. 10, 1920. Agglutinin titers: Apr. 14, 1922, 1:40; Dec. 1, and Jan. 2, 1923, 1:20. Pasture bred. Feb. 18, heifer gave birth to a premature, living calf. The placenta was retained. Suspension from uterine swab was tested on a guinea pig with positive result. Agglutinin titer 1:1,280.

No. 915.—Fawn heifer, born Aug. 20, 1920. Agglutinin titer, Apr. 14, 1922, 1:40. Pasture bred.

Heifer aborted Nov. 28, and was slaughtered Dec. 1. The uterus on this date was about twice normal size. The left horn was twice the diameter of the right. The mucosa was congested and the caruncles capped by a very viscid, opaque, yellowish mass, beneath which the reddened caruncle showed minute yellow dots. These are the depressions into which the chorionic villi fit, filled with the same exudate. This exudate, made up of polynuclear leucocytes and mucus, contained many cocci. *B. abortus* was not demonstrated microscopically but two guinea pigs inoculated with the exudate developed characteristic lesions from which *B. abortus* was isolated. The blood titer of the heifer on Dec. 1 was 1:2,560.

No. 916.—Black and white heifer, born Aug. 18, 1920. Agglutinin titers: Apr. 14, 1922, 1:40; Dec. 1, and Jan. 2, 1923, 1:20; Mar. 15, 1:40. Pasture bred. July 10, 1923, calved normally. Placenta expelled but lost. Titer of blood next day 1:40. Guinea pig test of uterine swab negative.

No. 917.—Red and white heifer, born Aug. 19, 1920. Agglutinin titers: Apr. 14, 1922, and Jan. 2, 1923, 1:40; Mar. 15, 1:320. Bred May 8, 1922.

Animal slaughtered Apr. 28, 1923, because sterile. Autopsy showed a very small uterus with undeveloped, impervious horns.

No. 918.—Black and white heifer, born Aug. 25, 1920. Agglutinin titers: Dec. 1, 1922, 1:40; Jan. 2, 1923, 1:160. Pasture bred. Feb. 4, heifer gave birth to a premature, living calf. The placenta was retained. Feb. 20, agglutinin titer 1:1,280. *B. abortus* isolated from uterine swabs through guinea pigs.

No. 919.—Black and white heifer, born Aug. 26, 1920. Agglutinin titers: Apr. 14, 1922, 1:80; Dec. 1, 1:1,280. Pasture bred.

Dec. 14. Heifer aborted a fetus weighing 18 lbs. and 27½ inches long. The peritoneal and the pleural cavity contained each about 100 cc. of a clear, reddish fluid. The fourth stomach contents normal. Lungs not inflated. Cultures from the digestive tract, liver, and spleen remained sterile. Cultures from lung and kidney contain miscellaneous bacteria. The placenta is diseased with exception of unexpanded horn. The chorion is yellowish white and opaque, the subchorionic tissue edematous. The cotyledons have a necrotic periphery. Bacilli, resembling *B. abortus*, in leucocytes of stained films. Infected epithelia not seen. Guinea pigs inoculated with bits of lung and contents of fourth stomach negative for *B. abortus*. Dec. 15, agglutinin titer 1:1,280.

No. 926.—Black and white heifer, born Oct. 4, 1920. Agglutinin titers: Apr. 14, 1922, 1:40; Dec. 1, less than 1:20; Jan. 2, 1923, 1:40; Mar. 15, 1:640. Pasture bred.

Mar. 21. Heifer gave birth to a premature, living calf. The placenta was retained, but a small piece was obtained for examination. Films show *B. abortus* in the exudate. Suspension from uterine swab was tested on guinea pigs with positive result. Agglutinin titer 1:1,280.

Group C.—Some details of the history of this group have been given on page 87. One of the ten was slaughtered because sterile, leaving nine. Four aborted and one dropped a full term, dead calf. The uterine cavity contained *Bacillus abortus*, although the placenta appeared normal. The remaining four calved normally. The following table and notes give a more detailed statement of each case.

TABLE XXVIII.

Control Group C.

Case No.	Agglutinin titer of blood.		Pregnancy terminated.	Placenta, condition of.	Tests for <i>B. abortus</i> .
	Before calving.	After calving.			
990	l.	h.	Aborted, fetus lost.	Retained.	Swab +
995	l.	h.	" " "	Mucopurulent exudate in uterus.	Exudate +
1000	l.	l.	Normally.	Normal.	
1001	l.	l.	"	"	
1002	l.	l.	"	"	Swab --
1003	m.		(Sterile.)		
1004	l.	l.	Normally.	Normal.	Swab --
1005	l.	m.	Aborted.		" +
					Fetus +
1006	l.	l., h.	Full term, calf dead.	Normal.	Swab +
1007	l.	h.	Aborted.*		

* Aborted a second time nearly 8 months later.

Notes on Individual Cases of Group C.—

No. 990.—Black and white heifer, born Aug. 10, 1919.

Agglutinin titers: Mar. 15, 1920, Nov. 22, Jan. 27, 1921, and May 9, 1:40. Bred Mar. 27, 1922.

Oct. 22. Heifer aborted. Fetus lost. The placenta, detached after 24 hours, in a state of advanced putrefaction and lesions no longer recognizable. Washings from a uterine swab tested on a guinea pig with positive result. Oct. 23, titer of blood 1:640.

No. 995.—Fawn heifer, born Nov. 23, 1919.

Agglutinin titers: July 6, 1920, less than 1:20; Jan. 5, 1921, 1:20. Bred Mar. 13, 1922.

Oct. 17. Heifer aborted. Fetus lost. Agglutinin titer 1:1,280.

The heifer was slaughtered Oct. 25. The uterus was still slightly larger than normal. The right horn about normal in size, the left $2\frac{1}{2}$ to 3 times the normal

diameter. In this horn there are a number of thick, opaque, yellowish white masses consisting chiefly of polynuclear leucocytes and mucus. Bacteria scarce, chiefly cocci. The caruncles of the uterus are still slightly swollen and the mucosa somewhat nodular and slightly congested. The right horn is normal excepting at its base where masses of exudate extend into it from the left horn. Although *B. abortus* was not detected in films, two guinea pigs inoculated with the exudate developed a characteristic spleen from which *B. abortus* was isolated.

No. 1000.—Fawn and white heifer, born Oct. 5, 1919. Bred Sept. 30, 1921, and Mar. 27, 1922. Agglutinin titer, Nov. 15, 1:40.

Jan. 4, 1923. Heifer calved normally. The placenta was expelled and about one-half obtained which appeared normal. Jan. 12, agglutinin titer 1:80.

No. 1001.—Black and white heifer, born June 26, 1919. Bred Apr. 14, 1922. Agglutinin titer, Nov. 22, 1:80.

Jan. 25, 1923. Heifer calved normally. The placenta was expelled and appeared normal. Feb. 2, agglutinin titer 1:40.

No. 1002.—Light fawn heifer, born July 17, 1919. Bred Feb. 12, 1922. Agglutinin titer, Nov. 15, 1:80.

Nov. 28. Heifer calved normally. The placenta was expelled and appeared normal. A suspension from a uterine swab was tested on two guinea pigs with negative result. Dec. 8, agglutinin titer 1:80.

No. 1003.—Fawn and white heifer, born July 27, 1919. Agglutinin titers: Mar. 15 and Nov. 9, 1920, 1:20; Nov. 22, 1922, 1:320. Repeatedly bred. Dec. 13, considered sterile and slaughtered.

No. 1004.—Black and white heifer, born Oct. 2, 1919. Agglutinin titer on Nov. 9, 1920, and Nov. 15, 1922, 1:20. Bred Feb. 18, 1922.

Nov. 20. Heifer calved normally. The placenta was expelled and appeared normal. A suspension from uterine swab was tested on two guinea pigs with negative result. Agglutinin titer 1:40.

No. 1005.—Black and white heifer, born Oct. 2, 1919. Agglutinin titer, July 6 and Nov. 9, 1920, less than 1:20. Bred Mar. 3, 1922.

Nov. 13. Heifer aborted a large fetus, weighing 56 lbs. This was normal with exception of a large amount of meconium in the fourth stomach. *B. abortus* was isolated from fourth stomach, cecum, rectum, liver, lungs, spleen, and kidneys in pure culture, and from lung, fourth stomach, cecum, and uterine swab through guinea pigs. Agglutinin titer 1:320, Nov. 15.

No. 1006.—Black and white heifer, born Oct. 16, 1919. Agglutinin titers: July 6, 1920, less than 1:20; Nov. 9, 1920, and Nov. 22, 1922, 1:40.

Jan. 11, 1923. Heifer gave birth to a full term dead calf. About one-half of the placenta was obtained which appeared normal. A uterine swab was tested on two guinea pigs with positive result. Agglutinin titer: Jan. 12, 1:40; Mar. 31, 1:640.

No. 1007.—Fawn heifer, born Nov. 5, 1919. Agglutinin titer, Jan. 5, 1921, 1:20. Repeatedly bred.

Aug. 19, 1922. Heifer aborted. No examination was made. Agglutinin titer was 1:1,280 on Aug. 23.

Group D.—This control group, including fourteen cases, was briefly described on page 89. The outcome of first pregnancies remains to be given. Four aborted, and one (No. 1048) dropped what was probably a full term calf but had localized disease of the placenta. *Bacillus abortus* was demonstrated present in four animals. From the fifth (No. 1036) material was not obtained. The evidence in this case rests in the high agglutinin titer. Table XXIX summarizes the chief data.

TABLE XXIX.
Control Group D.

Case No.	Agglutinin titer of blood.		Pregnancy terminated.	Placenta, condition of.	Tests for <i>B. abortus</i> .
	Before calving.	After calving.			
1035		h.	In abortion.	Diseased.	Swab + Placenta +
1036		h.	" "		
1037	h.	h.	" "	Diseased.	Swab + Placenta +
1038	l.	l.	Normally.	Normal.	
1039	m.	h.	"	"	
1040	l.	l.	"	Discharged, lost.	Swab —
1041	l.	l.	"	Normal.	" —
1042	l.	l.	"	"	
1043	l.	l.	"	Discharged, lost.	
1044	h.	h.	In abortion.	Not known.	Fetus +
1045	l.	l.	Normally.	Discharged, lost.	
1046	l.	l.	"	" "	Swab —
1047	l.	l.	"	Normal.	
1048	l.,m.	h.	Probably at full term.	Diseased.	Placenta + Swab +

Brief History of Individual Cases of Group D.—

No. 1035.—Black and white heifer, born Nov. 26, 1920. Apr. 12, 1923, heifer aborted.

The fetus was 30 inches long. The placenta was retained but a piece about a foot square was obtained. Odor penetrating and highly putrefactive. The chorion is not involved. The cotyledons are grayish, macerated. The amount

of disease no longer ascertainable. Films showed leucocytes filled with minute bacilli. Washings of a uterine swab injected into a guinea pig yielded the typical lesions and cultures of *B. abortus*. Apr. 13, agglutinin titer of blood 1:1,280.

No. 1036.—Red and white heifer, born Nov. 30, 1920. Feb. 23, 1923, heifer aborted. No material was obtained for examination. Apr. 13, agglutinin titer of blood 1:1,280.

No. 1037.—Black and white heifer, born Nov. 14, 1920. Apr. 13, 1923, agglutinin titer of blood 1:2,560. Apr. 14, heifer aborted a fetus 28 inches long. Only a small portion of the placenta obtained, the rest adherent. The four or five cotyledons are bright red with masses of grayish exudate lying on them. The smaller adventitious cotyledons have necrotic margins. The chorion is thickened, opaque, and wrinkled. Films show epithelial cells, swollen and filled with minute bacilli. Washings of a uterine swab injected into a guinea pig resulted in the typical abortion disease and cultures of *B. abortus*. Apr. 17, agglutinin titer of the blood 1:2,560.

No. 1038.—Black and white heifer, born Nov. 17, 1920. Apr. 17, 1923, agglutinin titer of the blood 1:80. Normal calf, born June 21. Placenta normal. June 25, blood titer 1:80.

No. 1039.—Red and white heifer, born Nov. 24, 1920. Apr. 17, 1923, agglutinin titer of the blood 1:320. Gives birth to full term calf July 3. Traction was necessary to deliver calf. Placenta normal. July 9, agglutinin titer of blood 1:1,280.

No. 1040.—Black and white heifer, born Nov. 23, 1920. Apr. 17, 1923, agglutinin titer of the blood 1:40. July 23, heifer calved normally. Placenta expelled but lost. Agglutinin titer of blood 1:40. Guinea pig test of uterine swab negative.

No. 1041.—Black and white heifer, born Dec. 5, 1920. Apr. 17, 1923, agglutinin titer of the blood 1:40. Heifer calved normally, June 28. Placenta normal. Agglutinin titer of blood, June 30, 1:40.

No. 1042.—Black and white heifer, born Dec. 16, 1920. Apr. 17, 1923, agglutinin titer of the blood 1:40. Heifer calved June 27. Calf delivered by traction; full term. Agglutinin titer of blood, June 30, 1:40.

No. 1043.—Black and white heifer, born Dec. 28, 1920. Apr. 17, 1923, agglutinin titer of the blood 1:20. June 15, full time, normal calf born. The placenta was discharged, but lost. A guinea pig inoculated with washings of a uterine swab dies of septicemia. June 20, blood titer 1:40.

No. 1044.—Red and white heifer, born Jan. 6, 1921. Apr. 17, 1923, agglutinin titer of the blood 1:640. May 28, heifer aborted a fetus on pasture. Discovered later with hind feet gnawed off. Fetus is 24 inches long and weighs 8 lbs. Lungs not inflated. No serous effusions. The stomachs contain a small amount of fluid thoroughly saturated with meconium. *B. abortus* was isolated in pure culture from contents of fourth stomach and rectum and from lung tissue. Two guinea pigs inoculated with contents of fourth stomach positive for *B. abortus*. Blood titer 1:10,240.

No. 1045.—Black and white heifer, born Jan. 14, 1921. Apr. 17, 1923, agglutinin titer of the blood 1:40. Full term calf born, June 19. Placenta discharged but lost. Blood titer 1:80.

No. 1046.—Black and white heifer, born Jan. 16, 1921. Apr. 17, 1923, agglutinin titer of the blood 1:40. Full term calf born, June 22. Placenta discharged but lost. June 25, blood titer 1:40. Swab test on guinea pig negative.

No. 1047.—Black and white heifer, born Jan. 31, 1921. Apr. 17, 1923, agglutinin titer of the blood 1:20.

June 24, 1923. Full term, normal calf born. The placenta was obtained and found normal. Washings of a uterine swab injected into a guinea pig resulted in a fatal peritonitis. June 25, blood titer 1:40.

No. 1048.—Black and white heifer, born Feb. 18, 1921. Apr. 17, 1923, agglutinin titer of the blood 1:80; May 3, titer 1:320.

June 3. Heifer dropped a full term calf, but retained the placenta. That portion representing the vacant horn obtained inverted. About 50 cc. of an opaque, yellowish, flaky, semifluid exudate imprisoned in it. The flakes consist of masses of leucocytes and groups of epithelia, filled with *B. abortus*. The cotyledons are ringed with a zone of necrotic villi. Chorion still thin, translucent, injected, and beset with minute flakes containing infected epithelial cells. June 5, blood titer 1:640.

DISCUSSION AND SUMMARY OF THE SECOND EXPERIMENT.

The results are best presented in tabular form (Table XXX), since the various details have been given on pages 87-89 and under each group.

TABLE XXX.
Summary of Second Experiment.

No. investigated.	No. remaining available.	Full term pregnancies.		Abortions.	Per cent aborted.	Per cent aborted and with placenta diseased.
		Normal.	With placenta diseased.			
Vaccinated Groups.						
Group A (heated vaccine).						
35	34	25	4	5	14.7	26.4
Group Bv (living vaccine).						
10	9	9	(3)*	0	0	(33.3)*
Control Groups.						
Group Bc.						
14	13	6	0	7	53.8	53.8
Group C.						
10	9	4	1	4	44.4	55.5
Group D.						
14	14	9	1	4	28.5	35.7
Summary of Controls.						
38	36	19	2	15	41.6	47.2

* Placenta regarded normal. B. *abortus* in uterine cavity.

In this experiment, only the outcome of first pregnancies is as yet available; nevertheless the results are sufficiently clear-cut to warrant

publication. The abortion rate among the control animals is so high that the data obtainable from second pregnancies may not add much to the value of the experiment.

13278 The 35 heifers vaccinated 4 times with cultures killed by heat have come out very well. The abortion rate is less than half as high as that of the 34 controls, and fully as low as that of the heifers of the first experiment treated with living vaccine. The experiment is worth repeating with the number of injections increased to 6 or 8 given monthly to maintain the immunity well towards the end of pregnancy. The precise place of killed cultures in the suppression of this disease is open to debate. The injection of such cultures brings about an agglutinin reaction whose height depends on the number of doses and their size. It interferes, therefore, with the agglutination test as an indication of actual disease unless accurate and extensive quantitative studies are made to provide standards for the reaction under various conditions. Provided with such standards the one administering vaccines might be able at certain times to distinguish agglutination reactions due to dead cultures from those due to actual infection. Barring the disadvantages resulting from the ambiguous agglutination test, the use of killed cultures as vaccine might be of use in herds still free from the infection but threatened with it through additions to the herd from without, as well as through various other still undefined avenues, such as feed, bedding, wild animals on pasture, etc. This method would be far more costly than the single injection of living virus. When the disease prevails in a herd there does not appear to be any advantage in killed over living vaccine, provided the latter is so chosen as to be not actually provocative of disease.

Turning to the group treated with living vaccine, the results are rather unexpected. None of the nine aborted. All gave birth to full term calves and the placentas so far as obtainable were normal on ordinary naked eye inspection. There was no evidence of retention in those animals which supposedly destroyed the placenta. The unexpected absence of abortions was matched by the rather surprising result of the inoculation of washings of uterine swabs taken immediately after parturition. In three cases *Bacillus abortus* was isolated. With one exception, such inoculations hitherto had yielded negative results when the fetal membranes were normal on inspection and the agglu-

tinin titer of the blood low. Further studies of this peculiar phenomenon were made upon the udder. Two of the animals (Nos. 922, 925) with infected uterus and low agglutinin titer were retested 67 days after parturition and the blood found still low. The titer of the milk was below 1:20, indicating absence of any focus of infection in the udder or elsewhere. On the other hand, the high blood titer of three other cases with normal parturition and placenta and negative guinea pig tests of uterine swabs (Nos. 928 to 930) was accounted for by the condition of the udder. In every case the limiting titer of the milk was high, between 1:640 and 1:1,280. These figures indicate infection of the udder and this may serve to account for the high blood titer and perhaps the protection of the pregnant uterus. The only tentative explanation of the presence of *Bacillus abortus* with normal placenta is a late infection of the chorion kept in check by the acquired immunity and hence giving little opportunity for the production of agglutinins. It is difficult to classify these cases since placental disease was not found nor were there evidences of retained placenta. From a practical viewpoint they were normal parturitions.

The three control groups taken together present a much higher abortion rate than in the first experiment and contrast quite unfavorably with the two vaccinated groups.

GENERAL SUMMARY.

A brief discussion of the individual groups has been given with the data on each group and it remains to survey both experiments together and interpret the results. A comparison of Table XXIV, page 85, with Table XXX, page 114, shows that the two experiments present somewhat different aspects. In the first, the vaccinated heifers had but a moderate advantage over the controls; in the second, this advantage was marked. Similar conditions were encountered by C. O. Jensen (page 16). They reflect the quite general experience of those who attempt to obtain precise results from vaccinations carried out under the contingencies of everyday conditions and therefore subject to a variety of undetermined and unmeasurable factors. Even in animals entirely in the hands of the experimenter certain agencies remain beyond adequate control and certain conditions beyond his knowledge. Among these are the specific and general resistance of the individual animal, variations in this resistance from time to time, the relative virulence of the strain or strains of virus in the herd, the frequency of exposure to the virus, and the quantity of virus actually taken into the system at any given time.

In the present investigation, the resistance of heifers seemed to vary somewhat. Some aborted twice, even after vaccination, others only once. Differences in the virulence of strains of *Bacillus abortus* are at present not exactly measurable, since no method has yet been worked out to provide standards. Slight differences in certain biological characters have been demonstrated in strains just isolated from the fetus or membranes directly or through guinea pigs. Their significance with reference to virulence has not been studied. On the whole, the evidence points to uniformity in the level of virulence.

The most uncertain factors are the quantity or dose of natural virus taken into the body at any one time and the frequency of the opportunities for taking it. There is here opportunity given for a great variety of conditions. To mention but one, a given large dose of virus introduced at one time may lead to placental disease. The same dose divided into a number of small ones and taken at spaced intervals may produce immunity.

In estimating the value of vaccinal resistance and in endeavoring to account for variations in the outcome of experiments designed to measure this value, we are justified in assigning to the frequency, intensity, and duration of the exposure to infectious material a highly important rôle. It may be of service, therefore, to consider somewhat in detail the elements of which it is made up. It has been stated that the most prolific source of infectious material is the aborting cow. Especially in the earlier stages of placental disease are the bacilli very numerous in the chorionic epithelium and the overlying exudate, which is largely made up of such infected epithelium and phagocytes loaded with bacilli. In older stages of placental disease the virus is less abundant. After expulsion of the fetus the uterine discharges due to the liquefaction and disintegration of retained portions of the placenta may contain living bacilli for some weeks. In two recent cases of abortion on pasture, the uterus was obtained after slaughter. The organ had contracted to nearly normal size and the mucosa and caruncles differed but little from the normal. There were, however, present flakes and viscid lumps of mucus and pus. From both cases *Bacillus abortus* was isolated through guinea pigs. Unfortunately the period between abortion and slaughter could not be determined. Another source is the udder of a certain proportion of infected cows in which *Bacillus abortus* has settled down and from which the bacilli are discharged continuously or intermittently in the milk.

How far the bull, unless diseased, is responsible for spreading the infection is not known. In the cases included in this report and numerous others the following facts may be cited as against the agency of the bull:

1. Many cases have been studied in which the expulsion of the fetus occurred near full term and yet the placental disease was relatively fresh. If *Bacillus abortus* is introduced by the male, the expulsion of the fetus should occur very early in pregnancy. In the series studied it occurred usually from the 5th to the 8th month. Earlier abortions were frequently due to *Vibrio fetus*.

2. The repeated examination of the agglutinin titer of the blood, as given in the protocols, has brought out the fact that this titer may be low until the heifer or cow is well along in pregnancy. The vaccinations have shown that a maximum titer may be expected at the

latest within a month after infection. If the infection takes place at the time of conception, we should expect a prompt rise in the agglutinin titer of the blood. These facts go far to eliminate the male in most cases.

To these different sources of virus—the cow during and several weeks after abortion, the milk of permanently infected cows, and the bull—may be added a small percentage of virgin heifers having *Bacillus abortus* in the ducts of the undeveloped udder. These sources will differ in importance in different herds and in the same from day to day.

In the herd under observation the young stock was kept in groups and separate from the cows until after the first calvings. To this rule there were certain accidental exceptions. In young stock kept strictly isolated the writers have seen the abortion rate fall to near zero. The source of infection in such groups, beside the bull and accidental contact with older cows, is probably some heifer which has acquired the udder infection before conception and which may abort quite early and form a focus of infection for the remainder of the group.

If the vaccinated young stock up to the time of calving is kept, more or less successfully, from contact with the older infected animals and is therefore more or less protected from the usual sources of infection, there still remains one possible source. The vaccine used, it might be claimed, can cause abortion among the heifers and so establish sources of infection equal to those represented by older infected cows. This question is of theoretical importance unless the vaccinated group contains unvaccinated pregnant animals. Vaccination, if practiced at all, should include all animals in the same group. It is worth while, however, to consider it here.

Among the 53 vaccinated heifers of the first experiment, two were pregnant when inoculated (Nos. 411 and 485). This condition was unknown at the time. No. 411 has passed through four pregnancies successfully. The milk was tested after the first and second calvings and found infected with *Bacillus abortus*. The agglutinin titer has remained high throughout. No. 485 aborted twice. The third calf was full term and the placenta normal but the agglutinin titer still high. No. 411 was treated with a vaccine 153 days under cultivation. No. 485 was vaccinated with a different strain isolated 22 days before

and only in its second transfer. This case may be regarded as due to the vaccine. It demonstrates the importance of eliminating all pregnant animals.

In addition to the production of abortion by accidental vaccination of pregnant animals, there is the other question bearing on the possible production of placental disease in certain animals even when not pregnant at the time of vaccination. If the culture has been isolated only recently, it may do so in highly susceptible animals by first establishing itself in the udder. Later during pregnancy it may enter the circulation from the udder and pass into the placenta. Assuming the high susceptibility of animals in which this mode of transfer is possible, we may go further and state that such animals will in any case become infected sooner or later with the natural virus.

In the following table the results of vaccination are given according to the total period of artificial cultivation of the strain used as vaccine.

TABLE XXXI.

Total age of strain used as vaccine.	No. vaccinated.	No. of normal calves.	Abortions.	Placenta diseased.
First experiment.				
First strain.				
32 days.	2	2	0	0
81 "	2	1	0	1
153 "	9	7*	1†	0
220 "	6	5	0	0
1 yr. 163 "	3	3	0	0
1 " 208 "	24	14	4	5
Second strain.				
22 days.	7	4	3*‡	0
First strain.				
2 yrs. 7 mos.	10 (cows).	6	0	0
Second experiment.				
First strain.				
3 yrs. 6 mos.	9	9	0	(3)§

* One pregnant when vaccinated.

† Probably vibronic disease.

‡ One infected (high blood titer) when vaccinated.

§ Placenta regarded normal, but *B. abortus* in uterine cavity.

This table does not present any data pointing definitely to the virus as disease-producing. On the contrary the larger number of cases of disease associated with the use of the older vaccine would make it appear as if it failed to protect. An examination of Table VII and the protocols, page 41, will disclose the fact that the breeding of many animals of this group was delayed and that some of the acquired immunity may have been lost. The large proportion of disease associated with the fresh second strain may be credited to the vaccination of one pregnant heifer and one heifer already infected and probably highly susceptible. If we deduct these, there is but one clear case left in this group.

A study of *Bacillus abortus* isolated from a large proportion of both vaccinated and control heifers which developed disease of the placenta did not show any differences in the two groups of cultures which might tend to demonstrate that the vaccine was responsible for disease. It is possible, however, that in the body of the cow the cultural modifications of the vaccine may disappear and the strain assume its original characters. If this does not occur there should be no difficulty in recognizing the vaccine strain if present.

To sum up the discussion as to the relative infecting power of the vaccinated and controls, it may be assumed that abortion may be produced by vaccination in pregnant heifers and that these may then become centers of infection for the non-vaccinated. In view of accidents of this type, the best plan is not to use a strain for vaccine until at least several months under cultivation and not to vaccinate if there is any doubt that the animal is not pregnant.

The Vaccination of Cows.—Perhaps the best method for bringing out whatever advantages vaccination may possess is to keep young stock segregated from older cows, where this is possible, until after the first calving and then apply vaccination at least 6 to 8 weeks before breeding again. The heifers are older, with more natural resistance upon which to base the specific resistance to be acquired by vaccination. Results obtained by vaccinating a group of ten cows soon after the first calving as given on page 74 are very promising.

CONCLUSIONS.

1. In one experiment comprising 134 control and 53 vaccinated heifers, the abortion rate, following vaccination with living cultures, was 16.7 and 11 per cent respectively for first and second pregnancies as compared with 25.1 and 19.2 per cent for the control groups.

In a second experiment comprising 35 heifers treated with heated cultures and 10 with living cultures, the combined abortion rate for the first pregnancy was 14.7 per cent as compared with 41.6 per cent for 38 controls. The rate for the 10 treated with living cultures was 0. The above estimates do not include those cases in which full term pregnancies were associated with infected or diseased placentas.

2. Vaccination with living cultures should be applied only in herds in which abortion in the first pregnancy is frequent and in which cows freshly introduced abort the first or second calf.

3. Vaccination with small doses of living bacilli (one agar slant or less) practiced 2 to 3 months before conception is not a dangerous procedure.

4. Heifers or cows injected when pregnant may, but do not necessarily abort.

5. A small proportion of virgin heifers have a high blood titer and they may be regarded as infected, the virus being localized in the udder.

6. When possible, segregation of heifers up to the first calving and vaccination before the second breeding is recommended, provided the agglutination test to eliminate infected heifers is employed.

7. The relative efficiency and danger of recently isolated strains of *Bacillus abortus* and those under cultivation for months and years when used as vaccines is not clear. Under the circumstances older cultures should be used.

8. There is probably no gain in two injections of living vaccine, since high agglutinin titers of the blood are established by single injections and in a fair proportion maintained during several pregnancies.

9. In all studies of the value of vaccination the presence or absence of disease of the placenta in all full term pregnancies should be determined.

10. In herds in which vibronic disease exists, this organism must be taken into consideration in determining the value of vaccination with *Bacillus abortus*.

11. Vaccination will probably have different effects in different herds. The deciding factors are (1) the relative susceptibility of the animals, which in turn is governed by the length of the period during which the disease has prevailed and consequently the extent to which it has eliminated chronic aborters, and (2) the concentration of the infectious material in the environment of the herd.

12. The use of vaccines does not in itself tend to eliminate the infectious agent from any herd, although it may greatly reduce the infectious material quantitatively by reducing the number of cases of placental disease.

13. Animals treated with living virus should not be herded with the unvaccinated unless the latter have passed through the disease or have a high agglutinin titer.

14. Cultures of *Bacillus abortus* exposed to the lowest temperature which will kill all bacilli and injected repeatedly may produce a relatively high degree of resistance.

BIBLIOGRAPHY.

1. Bang, B., *Z. Tiermed.*, 1897, i, 241.
2. Smith, T., *J. Exp. Med.*, 1919, xxix, 451.
3. Smith, T., *J. Exp. Med.*, 1918, xxviii, 701; 1919, xxx, 313; 1923, xxxvii, 341.
4. Smith, T., and Taylor, M. S., *J. Exp. Med.*, 1919, xxx, 299.
5. Smith, T., Little, R. B., and Taylor, M. S., *J. Exp. Med.*, 1920, xxxii, 683.
6. Lerche, *Deutsch. tierärztl. Woch.*, 1922, xxx, 281.
7. Gminder, A., *Berl. tierärztl. Woch.*, 1922, xxxviii, 184.
8. Smith, T., *J. Exp. Med.*, 1920, xxxi, 115.

9. Stockman, S., *J. Comp. Path. and Therap.*, 1914, xxvii, 237.
10. *Arb. Reichsgesundheitsamte*, 1920, lii, 375.
11. Hadley, F. B., *J. Am. Vet. Med. Assn.*, 1921-22, xiii, N.S., 26.
12. Schroeder, E. C., *J. Am. Vet. Med. Assn.*, 1921-22, xiii, N.S., 542.
13. Jensen, C. O., *Maanedsskr. Dyrlaeger*, 1921, xxxiii, 305.
14. Schermer, S., and Ehrlich, K., *Z. Infektionskrankh. Haustiere*, 1922, xxiv, 21.
15. Craig, J. F., and Kehoe, D., *J. Comp. Path. and Therap.*, 1922, xxxv, 256.
16. Smillie, E. W., Little, R. B., and Florence, L., *J. Exp. Med.*, 1919, xxx, 341.
17. Little, R. B., and Orcutt, M. L., *J. Exp. Med.*, 1922, xxxv, 163.

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