THE EFFECTIVENESS OF A VACCINATION PROGRAM AS AN AID IN CONTROLLING BRUCELLOSIS IN A PARTIALLY INFECTED DAIRY HERD

by

JAMES SPENCER NORWOOD

B. S., Texas Technological College, 1954

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Dairy Husbandry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1955

TABLE OF CONTENTS

INTRODUCTION

Brucellosis, otherwise known as Bang's disease or contagious abortion in cattle, is an infectious disease of cattle and man caused by the specific microorganism Brucellus abortus (57).

Brucellosis according to Smith et al. (57) reduces milk production in infected cows about 22 percent and causes a 40 percent reduction in the yearly calf crop. They reported that Brucellosis-free cattle calve on the average every 11.5 months, whereas infected cattle have an average calving interval of 20 months. They further stated that one out of every five cows that abort will become sterile. This disease cost cattlemen an estimated $58 million in 1954. (1).

Smith and co-workers (57) stated that infection of cattle, sheep and goats occurs readily through the mouth and that cattle can be easily infected through the mucous membrane of the eye. They also stated that infection can occur through abrasions in the skin, and probably through the normal skin. Infected animals usually develop a positive reaction 30 to 60 days after infection enters the body, however, some animals do not develop a positive reaction for several months after infection (57).

Abortion, death and expulsion of a premature fetus are the most prominent symptoms of Brucellosis, according to Smith et al. (57). These workers state that in pregnant animals Brucella organisms enter the uterus, fetal membranes and even penetrate the body of the fetus. When this occurs the organism induces an inflammation of the placentae and cotyledons thereby interfering with the blood circulation within the fetus, the final result being the death and premature expulsion of the fetus, or abortion. They also found that the Brucella organisms are liberated from the body of the infected cow in the fetal membrane at the time of calving and in the vaginal discharge for a few days before parturition and for about one month thereafter.

In the approved eradication programs, there are two methods used officially for locating Brucellosis infection in a herd (2), (a) the blood agglutination test is used to identify individual reactors, and (b) the milk ring test is an efficient means of screening herds and areas for infection at low cost in time and money.

Smith et al. (57) describe the blood agglutination test as the most reliable and practical single method of diagnosing Brucellosis in farm animals, however, they believe this test is less reliable in hogs, sheep and humans than with cattle. They further stated that in the blood stream of Brucellosis diseased animals there is a substance known as agglutinins, and when blood serum containing agglutinin is brought in contact with a suspension of Brucella organisms (called antigen) the organisms clump, known as agglutination, and settle out of suspension. This constitutes a positive reaction to the test.

The ring test as described by Roepke et al. (52) is conducted by adding one drop of stained antigen to 1 c.c. of milk in a small test tube (10 mm. or less inside diameter), then after thoroughly mixing the sample, it is incubated at 37° C. for 45 to 50 minutes or left at room temperature for 60 to 90 minutes. These authors believe that this is sufficient time for the cream line and ring to form. They find that in milk from an infected herd, the stained antigen agglutinates and collects on the fat droplets and as the cream rises to the top, forming a deep blue ring, leaving the skimmilk fraction white. In milk from negative herds, the antigen remains in the skimmilk fraction, giving it a light blue color and the cream ring remains uncolored.

Favorable results using strain 19, made from living Brucella abortus cultures of moderate virulence, in immunizing young heifers to Brucella infection by vaccination was first reported by Buck (14) in 1930. Buck's work subsequently was supplemented by Cotton, et al. (18).

Buck (14) listed two important qualities of strain 19 which set it apart from other known strains; namely, low virulence and high immunizing qualities. Since he first reported Brucella strain 19 as a valuable immunizing agent against Brucellosis in cattle, its use for that purpose has increased steadily until now it is the most important strain used in the United States in the preparation of Brucella vaccine (Birch, et al. 9).

Bradt (12) reported there are now four plans recommended by the United States Department of Agriculture, Animal Disease Eradication Branch, for the control and eventual elimination of Brucellosis. These four plans also have the endorsement of the National Brucellosis Committee and the United States Livestock Sanitary Association. The four plans as described by Bradt are as follows:

Plan A - Test and remove reactors for slaughter with or without calf vaccination.

Plan B - Test and retain reactors; vaccinate calves.

Plan C - Vaccinate calves without testing the herd.

Plan D - Vaccinate adult cattle. This plan is advised only to meet emergency conditions.

Under the present nation-wide Brucellosis eradication program, remarkable progress is being made and the percentage of reactors found among cattle tested has been reduced from 11 percent in 1934, when the eradication program was started, to 2.6 percent in 1954 (2).

More concerted and systematic effort is being made today to eradicate Brucellosis from individual herds and from areas than at any time in the past. The demand for cattle free from this disease, the problem of interstate shipment, and the regulations in many milk ordinances stipulating that all milk to be produced in Brucellosis-free herds have all contributed to the increased interest in control and eradicarion programs. Another stimulus to such programs was the development of calfhood vaccination as a means of immunizing cattle against infection. It was not until 1934 that calfhood vaccination was first approved by the United States Sanitary Control officials and the American Veterinary Medical Association. Since then the acceptance of this control measure has been rapid by cattle owners, and today its acceptance is almost universal, even to the extent of statewide compulsory vaccination in some states.

At Kansas State College a vaccination program for the control of Brucellosis has been practiced in the dairy herd since 1938. During this time most of the female replacements were raised in the college herd. The few additions which came from outside herds were brought in only after having passed a negative test for Brucellosis. Some animals were brought in for specific experimental purposes but these animals were isolated while they are in the herd. In this herd, non-infected animals were allowed to mingle freely with infected animals. No animals have been removed because of Brucellosis infection. Data have been kept on the periodic Brucellosis tests of all animals, abortions, retained placentae, breeding efficiency, and other pertinent associative occurrences. It seemed worthwhile to summarize these related facts for a herd representing a continuous period of 16 years under uniform management. The year to year variability, and the over-all results obtained, are of interest since some infection has existed in the herd throughout the period. During the period, the services of the staff of the Veterinary Division of Kansas State College have been available.

REVIEW OF LITERATURE

Comparison between different routes of administration of Brucella abortus strain 19 vaccine to calves has been made by various workers (6, 17, 41, 44, 50, 39, 55). Berman, et al. (6) vaccinated three groups of heifers subcutaneously with 5.0 ml. of strain 19 at the age of eight months of age respectively. During the third pregnancy, they exposed all three groups by the conjunctival route to 12 X 10^6 organisms of Brucella abortus, strain 2308. They reported no increment of resistance engendered by the revaccination.

Manthei, et al. (41) studied the duration of immunity of cattle to Brucellosis when vaccinated subcutaneously with 5.0 ml. of strain 19 vaccine. They vaccinated eighty animals between 6 and 8½ months of age, and 13 were vaccinated as yearlings or two year olds. Fifty nine unvaccinated cattle were used as controls. The majority of each group of animals were artificially exposed with virulent Brucella abortus by the conjunctival sac. They found that in the vaccinated animals which received artificial exposure, the resistance to infection was greatest among the animals vaccinated at the older ages and least among those vaccinated at younger ages. These authors concluded that resistance induced in cattle with strain 19 did not decrease with time.

Berman and Beach (5) compared the agglutinin response of animals vaccinated as calves with those revaccinated as young adults. They vaccinated 59 calves at eight months of age, and then revaccinated 22 calves at 14 months of age, and 21 at 20 months. They found a more rapid and greater development of agglutinins following revaccination than after the first vaccination, but a much more rapid recession of the agglutinin titer than in animals vaccinated for the first time at ages of 14 or 20 months. After the second vaccination, the recession in titer required about 4 months to fall below 1:100 in each of the revaccinated groups and the trend in the level of titer was essentially the same as that for the single vaccinated group, but one or two dilutions higher.

Haring and Traum (32) have shown the immunity produced by strain 19 when injected into cattle of various ages. They found that resistance produced by vaccination apparently was greater among animals which were adolescent, or mature, at the time of vaccination than among calves vaccinated when younger than 9 months. They also found that the titer recession to negative after vaccination with strain 19 was related to the age of the animal at vaccination, the younger animals became negative more rapidly. The persistence of agglutinins induced by strain 19 was found to be shortest in time among animals vaccinated at four to six months of age, and increased respectively among animals of six to eight months, eight to ten months, ten to twelve months, and cows and heifers over sixteen months of age. It was observed that animals which are vaccinated after showing a reaction usually continue to be reactors for several years; therefore vaccination of reactors is a disadvantage if the ultimate aim is a negative herd.

Haring (31) vaccinated all unbred heifers on a prison farm and thereafter vaccinated the majority of calves between the ages of 4 and 12 months of age in an infected herd where no new cows or heifers were introduced on the premises. As heifers came into milk on this farm, no attempt was made to protect them from infection from older cows. Seventeen unbred heifers between 12 and 32 months of age were given the full vaccine dose and the high agglutin titres caused by vaccination of such mature heifers persisted in many cases for two or three years. Titres of all of these older heifers, however, had dropped below 1:25 at the end of four years, except one cow which was infected when vaccinated. He found no significant persistence of agglutination titres among the animals vaccinated between 4 and 12 months of age. During a span of five years when this vaccination program was in operation, there were five abortions, five stillbirths, and 146 normal calves from vaccinated animals. The author believed that Brucella infection was not responsible for these abortions or stillbirths since no Brucella organisms could be found in the uterine fluid at the time of abortion. However, he isolated _Brucella abortus_ strain 19 from two adult-vaccinated cows, and another cow which was infected at the time of vaccination. Until new bulls were introduced into this herd, an average of 1.9 breedings was required to establish pregnancies.

Birch, et al. (9) vaccinated calves between 4 and 8 months of age with live _Brucella abortus_ strain 19. These animals, after reaching breeding age and becoming pregnant, were allowed to associate with infected animals. Systematic examinations of the milk and uterine fluid were made for the presence of brucella organisms at the termination of each pregnancy. In cases of abortion, the organs of the fetus were also included in the examinations.

These workers found a decided advantage of the vaccinates over the controls, or over the non-vaccinated animals, as expressed in a higher percent of live calves, a lower abortion rate, and a lower incidence of Brucellosis spread at calving time. The vaccinates were 46.66 percent resistant to Brucellosis as compared to 33 percent resistance in the control animals. They found that vaccination delayed the development of Brucellosis, the greatest number of infections occurring in the fourth pregnancy among the vaccinates, and in the first and second pregnancies among the controls. They also found that vaccination mitigated the effects of Brucellosis, vaccinates having 94.27 percent normal parturitions as compared with 76.92 percent normal parturition for the controls. These workers could find no consistent relationship between the peak of the titer following vaccination and the subsequent resistance of animals to Brucellosis.

Moore and Mitchell (45) vaccinated 45 non-pregnant and 68 pregnant cows in negative herds with 10 ml. of Brucella abortus strain 19, and each ml. of the suspension contained approximately 12,000 million viable organisms. They found that 8 percent or 17.7 percent of the cows vaccinated when non-pregnant and 41, or 60 percent of the cows vaccinated when pregnant had not become negative two years after vaccination. These authors believed that this response due to vaccination did not appear to be appreciably influenced by the age of the animal, or the months of pregnancy at the time of vaccination. They stated that, if these results are representative of the majority of herds, then pregnancy at the time of vaccination is conducive to rather persistent implantation of strain 19.

Moore and Mitchell (46) subcutaneously inoculated 188 non-pregnant and 181 pregnant animals between the ages of one and eleven years with 10 ml. of strain 19. They found that within two years after vaccination, 137 (70.74 percent) of the non-pregnant cows, and 113 (62.43 percent) of the pregnant cows had become negative. At a later date, however, 53 (39.84 percent) of the former and 37 (32.74 percent) of the later became infected. Of the 30 cows pregnant between six and nine months when vaccinated, four (13.33 percent) aborted. However, of the 181 pregnant cows that were vaccinated in various periods of pregnancy, 96 (85.71 percent) calved normally, and 16 (14.29 percent) aborted.

In a newly infected herd, McDiarmid (40) vaccinated 42 pregnant cows and heifers representing the Ayrshire and Friesian breeds with strain 19 vaccine. The gestation period of these animals ranged from 172 to 296 days. Thirty six live calves were produced from the 42 animals. The author concluded that results of this field trial justify vaccination of pregnant cattle when dealing with a severe outbreak of contagious abortion in a dairy herd.

Considerable interest has been shown by both owners of cattle and disease control officials in the intradermal method of inoculating cattle with Brucella abortus, strain 19 (Manthei et al.44).

An experiment was designed by Goode, et al. (26) to develop information on the relationship between blood serum agglutinin titers and udder infection in calf vaccinated animals. Their interpretation of the sero-agglutination reaction was as follows: Reactor (+ 1:100 or higher), Suspect (I 1:50 - I 1:100), and Negative (+ 1:25 or lower). They collected milk and blood samples from 637 vaccinated and 103 non-vaccinated cattle for bacteriological and serological studies. They isolated Brucella from the milk of 4.55 percent of the calf vaccinated cattle and from 21.35 percent of the non-vaccinated cattle. From animals classified as suspects (titers of I 1:50 to I 1:100 inclusive), they isolated Brucella from 0.51 percent of the vaccinated and 2.7 percent of the non-vaccinated, and also found that Brucella isolations were two to four times greater in the non-vaccinated than in the vaccinated cattle at each sero-agglutinin titer level, except for those animals with titers of I 1:400 or higher, where the percentage was approximately the same.

They found the most precipitous rise in percentage of infection began with the + 1:100 titer in non-vaccinated and + 1:200 titer in vaccinated cattle. They then concluded that the chagnostic sero-agglutinin titer level was one dilution higher for vaccinated than for non-vaccinated cattle, if the present interpretation of this test is used as the standard. Using this information they prepared a new interpretation of the sero-agglutination reactions which are as follows: Reactor (+ 1:200 or higher), Suspect (I 1:100 - I 1:200) and Negative (+ 1:50 or lower).

Using the alternate interpretation they found that infection rates in the three classifications of calf vaccinated animals (reactor, suspect, negative) were almost identical to those in the same classification of non-vaccinated animals as determined by the present interpretation.

In 1942 Rabstein and Cotton (50) compared the results of vaccinating heifer calves and cows by the intradermal and subcutaneous methods. They found the agglutinin response in animals inoculated with 0.2 ml. of strain 19 vaccine intradermally to be as great as in animals that received 5 ml. of the vaccine subcutaneously. Also, the agglutinin titers receded below the diagnostic level more rapidly in calves that were vaccinated intradermally. At the end of 3 months the calves vaccinated intradermally were completely negative, whereas 50 percent of those vaccinated subcutaneously still showed positive or suspicious titers. The authors concluded that the calves vaccinated intradermally probably produced a substantial degree of immunity as shown by a high opsonic reaction, together with a high agglutination titer.

McDiarmid (39) vaccinated three groups of Brucellosis-free, sexually mature Ayrshire heifers with strain 19 and compared the immunity produced when one group was vaccinated intradermally with 0.2 ml., another intracaudally with 1.0 ml., and a third group with 5.0 ml. subcutaneously. After the majority of heifers were 5 months in calf, an infective dose of <u>Brucella abortus</u> was given via the conjunctival sac. He found high degree of immunity, as measured by the number of abortions was demonstrated in all three vaccinated groups. The intradermal and intracaudal methods were equally effective as the subcutaneous method.

Manthei, et al. (44) compared immunity and agglutinin response in yearling heifers (12 to 15 months) vaccinated with strain 19 by the intradermal and subcutaneous routes. They found no difference in the degree of immunity to Brucellosis, or in the termination of gestation in cattle inoculated as yearlings with either 5.0 ml. of vaccine subcutaneously, or with 0.2 ml. subcutaneously, or with 0.2 ml. intradermally. They also found maximal vaccinal titers to occur between the 10th and 14th or 15th days following vaccination, regardless of vaccinal dose or method of administration. Maximum agglutinin titers were found to be similar whether the animals were inoculated with 5 ml. subcutaneously or with only 0.2 ml. intradermally, but those vaccinated subcutaneously with only 0.2 ml. had a considerably lower blood titer.

Sieiro (55) compared the immunity to Brucellosis in cattle resulting from strain 19 vaccination by intradermal and subcutaneous routes. He found that when injected with a test dose of 5 million organisms, 100 percent of the animals vaccinated intradermally were protected, whereas only 50 percent of the animals vaccinated subcutaneously were protected. Against a test dose of 50 million organisms, the results were 75 percent for intradermally vaccinated animals, and 11 percent for those subcutaneously vaccinated.

Cotton (17) made a study of the post-vaccination responses in two groups of calves, one vaccinated intra-cutaneously with 0.2 ml. of strain 19, and the other subcutaneously with 5.0 ml. of the same vaccine. Eight of the calves he used had high opsonocytophagic reactions prior to vaccination intra-cutaneously, and these failed to develop high agglutination titers. Eight other calves, also with high opsonocytophagic reactions, were vaccinated subcutaneously, and five of them did not develop titers. The author concluded that sufficient immunity develops in calves allowed colostrum and milk from positive dams until weaning to cause a considerable reduction in the efficiency of calfhood vaccination.

In order to determine the influence of dosage and route of inoculation upon the agglutinin response to Brucella abortus strain 19 vaccine, Campbell and Rodwell (16) inoculated three groups of adult cattle, one group subcutaneously (5.0 ml., 1.0 ml., 0.2 ml., or 0.04 ml.), another intracutaneously (0.2 ml.) and another intracaudally (1.0 ml., 0.2 ml., or 0.04 ml.). Maximal mean titers were reached in 14 days after inoculation, except in the group vaccinated with 1.0 ml. subcutaneously, and they reached maximum titer in 22 days. These workers found that the agglutinin response was closely related to dosage and to route of inoculation, with the most efficient route for agglutinin production being the intracaudal. They found no severe or persistent local reactions resulting from this route of inoculation. The authors stated that 1 ml. intracaudally administered is much more efficient than 5.0 ml. given subcutaneously. They also believed that it is more economical, and causes a transient diminution of milk yield at least no greater than that reported after administering 5.0 ml. subcutaneously. They stated that doses of 0.2 ml. or even 0.04 ml. injected intracaudally appear to be at least as efficient as 5.0 ml. given subcutaneously.

Hendrikse, et al. (33) reported that the conception rate of herds negative to the abortus Bang Ring (ABR) test is better than that of herds where the ABR reaction of the milk is positive. They found that the stronger the reaction, the lower the conception rate. The conception rate of heifers and cows vaccinated with strain 19, and that of non-vaccinated heifers and cows on farms not infected with Brucella abortus, showed slight differences which were not significant.

Holman and McDiarmid (34) found that daily milk yield of six cows inoculated with strain 19 Brucella abortus vaccine and of eight cows inoculated with two doses of 45/20 Brucella abortus vaccine given at an interval of three weeks, caused a severe lowering of milk yield following inoculation; this loss averaged 24 pounds per cow. They found the loss in production was most pronounced during the period from two to four days after inoculation, and was negligible 11 days after inoculation.

The status of a persistent reactor to the blood serum agglutination test, after vaccination with strain 19 presents one of the more serious and perplexing problems in the control and eradication of bovine brucellosis (49). Repeated blood tests by Morse and co-workers (49) on known vaccinated persistent reactors disclosed that the vast majority show fluctuating titers, but some eventually become negative. These workers slaughtered ten persistent reactor cows and made intense postmortem-bacteriological examinations. Six of the animals had been vaccinated with strain 19 as calves and four had been vaccinated as calves and re-vaccinated as adults. They found none of these animals to be harboring Brucella and apparently these animals did not represent any serious hazard to livestock or human health.

The following are reports on comparative tests on the protective value of Huddleson's mucoid vaccine (35) and strain 19 vaccines against Brucellosis. Edington, et al. (23) selected 21 heifers from a brucella negative herd. These heifers ranged in age from 8 to 15 months, one group was vaccinated subcutaneously at the upper portion of the area immediately posterior to the scapula with either 1 ml. of Huddleson's M vaccine while the other group received 5.0 ml. of strain 19 vaccine. The other group remained unvaccinated. Each animal was submitted to conjunctival exposure of 750,000 viable organisms of Brucella, strain 2308, when 4 to 6 months pregnant. The protective values of the vaccines was assessed on the basis of recovery of Brucella abortus by cultural methods from each heifer or aborted foetus at or near the time of calving. These workers recovered the organisms from 83.3 percent of the un-vaccinated controls, from 50 percent of the M vaccinated, and from 20 percent of the strain 19 vaccinated animals. Also, the M vaccinates animals showed a slight titer following vaccine administration, whereas the strain 19 vaccinated animals had considerably higher titers than the M vaccinated heifers. The authors stated that the values of the M vaccine may lie in the absence of agglutination titers in vaccinated animals, especially in adult cattle. Edington and King (24) reported similar results on 25 heifers. These animals were exposed to a dose of 1,500,000 organisms into the conjunctival sac of each eye, one half the dose being placed in each eye. They found that strain 19 vaccinated animals showed a 100 percent resistance, whereas the M vaccinated and control animals showed 87.5 percent and 33.3 percent resistance respectively. These authors believe that under the conditions of this test, strain 19 vaccine afforded a greater protective value than did the M vaccine. However, they also stated there is a definite indication that M vaccine is not entirely devoid of protective value.

Berman and Irwin (7) vaccinated a group of calves and sexually mature heifers with strain 19 and another similar group with Huddleson's M vaccine. During the first pregnancy they exposed the vaccinated and unvaccinated control cattle to Brucella abortus, strain 2308, by the conjunctival route. Half of the animals in each group received a challenge inoculum of 6×10^5 organisms, and the other half received 6×10^6 virulent organisms. They found strain 19 vaccinated animals to be significantly more resistant to induced infection than un-vaccinated controls, and M vaccinated animals were not significantly more resistant than the controls.

Five experiments have been performed by Bryan and Mansfield (13) involving 228 heifers vaccinated with either Bureau Animal Industry strain 19, or Huddleson's Mucoid vaccine. These animals were exposed to viable organisms of strain 2308 as a challenge inoculation during the midgestation period of the first pregnancy. They found Brucella abortus infections by cultural and guinea pig inoculation methods in 12 of 26 specimens (46 percent) from strain 19 vaccinates, 26 of 34 specimens (76 percent) from M vaccinates, and from 22 of 25 controls (88 percent). Also, positive Brucella serum agglutination tests (titers of 1:100 or higher) were obtained after challenge in 14 of 31 strain 19 vaccinates (45 percent), 28 of 34 M vaccinates (82 percent) and in 22 of 25 un-vaccinated controls (88 percent). The authors concluded that, under the conditions of these experiments, Huddleson's mucoid vaccine failed to provide a significant degree of resistance against Brucellosis, whereas B. A. I. strain 19 vaccine did induce a significant resistance of a relative type.

Twenty months after vaccinating calves 4 to 6 months old, subcutaneously or intravenously with Huddleson's M vaccine (mucoid growth phases of Brucella Suis), Schoenaers (53) found that vaccinated cattle were no more resistant than unvaccinated controls to infection by the conjunctival instillation of Brucella abortus cultures.

McDiarmid (38) compared the immunizing value in cattle of using dead antigen of two levels in an oily base and strain 19 Brucella abortus vaccine. After the animals were five months in gestation, the author injected doses containing 130 million virulent Brucella abortus organisms of strain 544 into the conjunctival sac of each animal. He found that dead vaccines consisting of whole organisms suspended in oily base failed to equal the efficiency of the living avirulent strain 19 in immunizing cattle against Brucellosis.

Live and Danks (37) compared the immunizing efficiency of ether killed Brucella abortus vaccine with live strain 19 vaccine in a herd of 73 cows and heifers which had been exposed to a natural outbreak of brucellosis. The ether killed Brucella abortus was combined with fallea and mineral oil. They found that it produced a higher and more persistent blood titer than the live strain 19 vaccine with no other undesirable effects being produced. The authors concluded that these preliminary trials indicate that ether killed Brucella abortus may be of value as an immunizing agent in an infected herd.

Sieiro and Rosenbusch (54) obtained superior anti-infectious values by vaccinating with a non-agglutinogenic strain of Brucella abortus than with agglutinogenic strains 19 and 37. However, they found the anti-abortive values to be zero for the non-agglutinogenic and agglutinogenic strain 19 of Brucella abortus, whereas the anti-abortive value was 12 percent for strain 37.

In a later experiment, Sieiro and Rosenbusch (56) compared the protective value of strain 19 and Rosenbusch non-agglutinogenic strain B by the number of animals that did not abort when inoculated with a virulent strain of Brucella abortus at two levels during the 5th and 7th month of gestation. They found the difference between strain 19 and strain B was slight; 50, and 47.6 percent, respectively did not abort from the lower dose. The authors suggested that strain 19 produced a slightly higher immunity. For this reason they proposed that both vaccines could be used in a control program by vaccinating calves with strain 19 and using strain B for re-vaccination of heifers, or for vaccinating adult animals.

In 1933, Cotton et al. (18) vaccinated a small number of cattle with vaccines of different degrees of virulence. The different Brucella abortus strains used in the preparation of the vaccines were; one of low virulence (strain 11), another of moderate virulence (strain 19) and a third of high virulence (strain 484). They found that in the intracutaneously vaccinated animals, strains 19 and 484 became localized in the udder, but not in those subcutaneously vaccinated. They concluded that the danger of infecting milk, and thus making vaccination a public health hazard, was great when intracutaneous adult vaccination was used.

Manthei (42) produced brucellosis infection in eight of twelve cattle by intrauterine insemination with semen containing virulent Brucella abortus. However, he could produce no infection in 12 susceptible cattle by the intracervial method of insemination. Manthei (42) states that these findings emphasize that only disease-free bulls should be used for supplying semen in the artificial insemination of cattle.

It is definitely known that sexually immature cattle resist infection by Brucella abortus, and, also, castrated cattle show little or no susceptibility to Brucella (60). Washko, et al. (60) divided 12 young female calves under one month of age into three groups. Group one was injected subcutaneously with gonadogen (Pregnant Mare's serum and human pregnancy urine). Group two was treated in a similar manner but doses of estrogenic substance were used (dioviovylin, ciba). Group three served as the untreated controls. These animals were exposed by the conjunctival route using 64 million to 800 million organisms two weeks after starting hormone injection. The authors succeeded in infecting the four female calves that were injected with gonad stimulating hormones whereas four control calves, which were not injected with hormones, failed to become infected as measured by the blood serum agglutinin response. The infected animals, when exposed to virulent Brucella abortus during their first pregnancy, failed to show increased resistance as a result of prepubertal infection, though a possible alteration in the abortion rate was observed.

Washko, et al. (59), using Brucella abortus organisms, infected 15 cows by way of the conjunctival, and three by the intramammary route. After slaughter of the animals they made cultures from many of the internal organs. They recovered the organism from widespread areas in the carcasses of 9 of the 18 cattle. In all cases, when they isolated the organism from the udder, the organisms were also recovered from tissues other than the mammary gland or the reproductive organs. The authors stated that these findings further emphasize the potential exposure to Brucella encountered by persons handling raw meat originating from cattle infected with Brucellosis.

Washko and Hutchings (58) found that vaccination with strain 19 at six months of age did not protect two heifers against infection with Brucella suis injected into the teat canal three months after calving. Both animals became infected and Brucella suis was recovered from their milk when the animals were living and from the tissues at autopsy.

In studying the treatment of Brucellosis, King et al. (36) used blood plasma, sulphadiazine, streptomycin, colloidal manganese and aureomycin, singly or in combination to treat 16 cows which had been naturally and experimentally infected with Brucella abortus and had high blood titers as well as viable Brucella abortus in their milk. They found that in all cases except when manganese was used alone, the milk was free of Brucella abortus for 2 to 10 days following treatment. However, they noted no apparent effect of any of the treatments on the blood agglutination titer.

EXPERIMENTAL PROCEDURE

Vaccination against Brucellosis was initiated in the dairy herd at Kansas State College in 1938. The following records have been kept on each individual animal:

1. Age at the time of vaccination.
2. Kind of vaccine used.
3. Route of administration
4. Blood titer levels as indicated by periodic blood agglutination tests.
5. Complete clinical reports of reproductive efficiency.

Data compiled from these records were statistically analyzed to determine the efficacy of the Brucellosis control program, and to compare the effectiveness of the various procedures.

Live and killed cultures of Brucella abortus, strain 19, vaccine were used for vaccinations. Some animals were vaccinated with killed organisms, followed by a re-vaccination with live organisms; other animals were vaccinated only once with live organisms, while others were vaccinated twice with live organisms varied from months to years. Systematic blood agglutination tests were conducted on most of the animals and blood titer levels were recorded. All blood tests were conducted by staff members of the School of Veterinary Medicine, Kansas State College.

The efficacy of vaccination in increasing the degree of immunity to Brucellosis was measured by the number of "breaks" which occurred following vaccination. A "break", as used in this study, is defined as the occurrence of a positive blood titer in an animal that had shown a negative blood titer for at least 1 year following vaccination. Records of the number of abortions were available for study. An abortion is defined as an expulsion of a dead fetus before the 8th month of pregnancy.

In this study, calfhood vaccination was considered to be the vaccination of animals 14 months of age or younger. Cattle older than 14 months at the time of vaccination were considered to be adult vaccinates.

An effort was made to determine the effects of variations in vaccination procedures on blood titer levels and the subsequent number of "breaks" and abortions that occurred in the herd. The value of these procedures were compared to determine whether under the existing conditions, any one procedure had been more effective than others as an aid in controlling Brucellosis.

The effects of the various procedures used will be discussed as follows:

1. Vaccination of calves and adults with killed organisms, followed by a re-vaccination with live cultures of <u>Brucella</u> <u>abortus</u>, strain 19.

2. Vaccination of calves and adults with one injection of live organisms.

3. Vaccination of calves and adults with live organisms, followed by a re-vaccination with live cultures of Brucella abortus, strain 19 vaccine.

4. Intradermal vaccination of calves and adults.

5. Variations in age of the animals at vaccination time.

The following factors also were studied to determine the effects of degree of infection as measured by blood titer level on reproductive efficiency:

1. Effect of the blood titer level on conception rate.
2. Effect of positive blood titer on the incidence of abortion.
3. Effect of blood titer on the number of retained placentae.
4. Effect of breed of cattle on susceptibility to infection, and incidence of abortions.

RESULTS AND DISCUSSION

In Tables 1 through 4 data are presented to show the effects of types of vaccines, number of vaccinations, and age of animals at the time of vaccination on blood titer levels and subsequent Brucellosis infection rates.

Effects of Vaccinating Animals with Both Killed and Live Organisms.

According to Live and Danks (37), vaccinations with dead organisms cause a higher and more persistent rise in blood agglutination titers than do vaccinations with live organisms. However, McDiarmid (38) found that the immunizing effect resulting from vaccinations with dead organisms was inferior to that resulting from vaccinations with live organisms. Thus, it appears that dead organisms stimulates the production of agglutinins in the blood without producing an immunizing effect equal to live cultures of strain 19. An effort was made to determine whether such reaction might affect the future blood titer level, and the subsequent incidence of "breaks" and abortions among calves which received live organisms a few months after having been vaccinated with killed organisms.

The blood of 64 percent of the calves thus vaccinated had returned to negative within one year (Table 1). Among the groups in which "breaks" and abortions had occurred, no significant differences in the number of "breaks" or in the abortion rates could be found ($P > .05$). The blood of 11 percent of these calves failed to return to negative within three years. This is a higher percentage than reported by Campbell and Rodman (16), who reported that 95 percent of the calves vaccinated once between four and eight months of age became negative within 12 months after vaccination. Similar data were also reported by Haring and Traum (32), and Rabstein and Cotton (50). Since the blood titer of the calves in this study remained positive indefinitely, it was impossible to differentiate between positive titer resulting from vaccination or from natural infection. Thus, the number of "breaks" in this group could not be determined.

Effects of Vaccinating Calves Once with Live Organisms.

The length of time required for the blood titer of calves to return to negative after one vaccination with live organisms, and the effects of

Table 1. Effects of vaccinating calves with killed organisms, followed by a re-vaccination with live organisms on blood titer level and subsequent number of "breaks" and abortions.

	Length of interval required for blood titer to return to negative following vaccinations with live organisms								
	90 days or less	91-120 days	121-240 days	241-365 days	366-480 days	481-660 days	661-1005 days	More than 1005 days	Never ret. to neg.
Number that returned to negative	6	2	27	27	6	12	12	6	12
Percent returning to negative	6	2	28	28	6	12	12	6	
Number of "breaks"	0	1	5	4		3	4		
Percent "breaks"	0	50	19	15		25	33		
Number of abortions	0		3		1	2	2		1
Percent abortions	0		12		17	17	17		8

these varying time intervals on subsequent immunity are shown also in Table 2.

The blood of 86 percent of these calves had returned to negative within one year. It will be recalled that the blood titer of only 64 percent of the calves vaccinated with both dead and live organisms had returned to negative within a year after vaccination. The difference between these two groups was significant. ($.05 > P > .01$).

The blood of less than one percent of the calves vaccinated once with live organisms failed to return to negative within three years. These results are more in keeping with work previously quoted (16, 32, 50) than results obtained when calves were vaccinated with both dead and live organisms. The difference between the two groups in the percentages of animals that failed to return to negative within three years was highly significant ($P < .01$).

There were no significant differences between the number of "breaks", and between the abortion rates observed, among animals which became negative within a year, and those which became negative at a later date, at least during the years the animals remained in the herd.

Effects of Vaccinating Animals Twice with Live Organisms.

The length of time required for the blood to return to negative after two vaccinations with live organisms, and the subsequent number of "breaks" and abortions are shown in Table 3. The number of animals in each group is so small that any possible differences between the groups could not be detected statistically.

However, the blood of only 66 percent of these calves had returned to

Table 2. Effects of vaccinating calves only once with live organisms of strain 19 on subsequent number of "breaks" and abortions.

	Length of interval required for blood titer to return to negative following vaccination with live organisms								
	90 days or less	91-120 days	121-240 days	241-364 days	366-480 days	481-660 days	661-1005 days	More than 1005 days	Never ret. to neg.
Number that returned to negative	23	18	32	33	7	2	5	4	1
Percent returning to negative	19	15	26	27	6	2	4	3	
Number of "breaks"	3	3	6	4	1	0	1	1	
Percent "breaks"	13	17	19	12	14	0	20	25	
Number of abortions	5	2	5	2	2	0	0	0	
Percent abortions	22	11	16	6	29	0	0	0	

Table 3. Effects of vaccinating calves with live organisms followed by a re-vaccination with live organisms on blood titer level and subsequent number of "breaks" and abortions.

	Length of interval required for blood titer to return to negative following the second vaccination with live organisms								
	90 days or less	91-120 days	121-240 days	241-365 days	366-480 days	481-660 days	661-1005 days	More than 1005 days	Never ret. to neg.
Number that returned to negative	5	3	2	2	3		1	2	3
Percent returning to negative	28	17	11	11	17		6	11	
Number of "breaks"	2				1				
Percent "breaks"	40				33				
Number of abortions	1	1						1	
Percent abortions	20	33						50	

negative within a year after the second vaccination. Even though the blood titer of three animals never returned to negative, this number represents 14 percent of the calves in this group. These results are similar to the reactions obtained with calves vaccinated with both dead and live organisms. Conversely, a greater portion of animals vaccinated twice with live organisms failed to return to negative than did animals vaccinated only once with live organisms. The number of animals which became negative and then returned to positive and aborted in each of the groups (Table 3) was too small to justify interpretation.

It might be postulated from these data that a single vaccination with live organisms is the most practical way of conducting a calfhood vaccination program. However, even with this method a few animals can be expected to have positive blood titers, years after vaccination. Also, the above data indicate that revaccination will result in an abnormally high percentage of the animals failing to show negative blood titers, years after vaccinations.

Data on the effects of different kinds (killed and live organisms) used, and the effects of frequency of vaccination on subsequent number of "breaks" and abortions are summarized in Table 4. No significant difference in the number of either "breaks" or abortions was found between groups ($P > .05$).

Effects of Age at the time of Vaccination.

In an endeavor to determine the effect of the age of the animal at the time of vaccination on subsequent number of "breaks" and abortions, and abortion rates, the calves were grouped according to age in months at time

Table 4. Effects of kind (killed or live) of vaccine, and frequency of vaccination on subsequent "breaks" and abortions.

Method of vaccination	Number of animals	Number of pregnancies	Number of animals showing "breaks"	Percent of animals showing "breaks"	Number of abortions	Percent of pregnancies terminating in abortions
One killed organisms vaccination, followed by one live organisms vaccination	98	238	17	17.3	8	3
One vaccination (live organisms)	138	305	30	16	22	7
One intradermal vaccination (live organisms)	11	27	3	27.3	3	11
Two calfhood vaccinations (live organisms)	18	58	3	16.7	3	5
Total and averages	265	628	53	20	36	5.7

of vaccination. This was done irrespective of the kind of vaccine, or the frequency of vaccination represented in each group. Such a grouping seemed justified, since no statistical differences were found between the merits of the various methods. The data in Table 5 indicate that the age at the time of vaccination affects the subsequent number of abortions. There is a statistically significant difference between the rate of abortions in animals vaccinated from four to eight months of age and the rate of abortions occurring in animals which were vaccinated at the age of 9 to 14 months. These results are in agreement with those reported by Haring and Traum (32) and by Manthei, et al. (41). Those workers all found that the resistance to Brucellosis produced by vaccination was greater in animals which were adolescent at the time of vaccination than in calves vaccinated younger than nine months.

Effects of Intradermal Administration of Vaccine.

The intradermal route of injecting vaccine was used on only 13 animals, with the remainder being vaccinated by the subcutaneous route. The blood of ten of the intradermally vaccinated animals had returned to negative within one year after vaccination. The blood of the remaining three returned to negative after two years. Three "breaks" and three abortions occurred within this group of 13 animals. No important difference was found when these results were compared with the number of "breaks" and abortions observed in the group of animals vaccinated subcutaneously with one injection of live vaccine. These findings are in agreement with these reported by McDiarmid (39) and Manthei, Mingle and Carter (44).

Table 5. Effect of age at time of vaccination on subsequent "breaks" and abortions.

Age of animals at time of vaccination	Number of animals	Number of animals showing "breaks"	Percent of animals showing "breaks"	Number of pregnancies	Number of animals which aborted	Percent of pregnancies terminating in abortions
4 months	4	1	25	13	1	8
5 months	26	5	19	83	6	7
6 months	48	10	21	132	11	8
7 months	66	19	29	138	13	9
8 months	56	6	11	101	6	6
9 months	37	4	11	79	2	3
10 months	12	4	35	35	1	3
11 to 14 months	16	4	25	47	2	4
Totals	265	53	20	628	42	7

Effects of Vaccinating Animals as Adults.

The number of animals that were vaccinated as adults was relatively small. However, of the 31 animals which were vaccinated after 14 months of age, the blood titer of 55 percent of them had not returned to negative within one year.

These results are in agreement with those reported by Campbell and Rodwell (16), Haring (30), Haring and Traum (32) and Manthei et al. (44). Those workers all found that a high percentage of the animals that are vaccinated after 12 months of age will still show positive blood titer a year after vaccination.

In view of the requirement that grade A milk be produced only by herds which are negative to Brucellosis, it would seem highly undesirable for dairymen to vaccinate their animals after they had reached sexual maturity.

Effect of Blood Titer Level on Conception Rate.

An effort was made to determine the effects of blood titer level at the time of breeding on conception rate as measured by the number of services per conception. With the exception of the groups of cows that showed a blood titer of 1:100 at the time of service, a higher number of services per conception was required for all groups of animals that showed a blood titer of 1:50 or above at the time of service than those that showed no blood titer (Table 6). No significant difference ($P > .05$) in the services per conception was found among the five groups that showed a blood titer of 1:50 or higher. However, a statistical difference ($P < .02$) was found when the conception rate of the group that showed no blood titer

Table 6. Effect of blood titer level on conception rate.

Blood titer level	Total number of pregnancies	Number of services for total pregnancies	Average number of services per pregnancy
No titer	529	1558	2.94
1:50	162	553	3.41
1:100	100	291	2.91
1:200	30	113	3.76
1:400	12	45	3.75
Over 1:400	31	122	3.93
Total and av. of groups 1:50 or more	325	1124	3.7

was compared with that of the group that showed a blood titer of 1:50 or higher at the time of service.

The blood agglutination titers shown in Table 6 represents both vaccinal and infectious reactions. In order to determine whether animals which become negative after vaccination and subsequently reacted had a lower conception rate than those which never became negative following vaccination, the number of services per pregnancy was determined for both groups. An average of 3.7 services per pregnancy was required for both groups, indicating that animals bred when showing a reaction to the blood test require more services than negative cattle, regardless of whether or not they had previously been negative after vaccination.

These results, showing that the presence of a blood agglutination titer at the time of service lowers the conception rate in dairy cattle, are in agreement with the results reported by Hendrikse, et al. (33). These investigators using the Ring test to locate infected herds, found that the conception rate of negative herds was higher than that in herds which showed a positive reaction to the Abortus Bang Ring test. To avoid the effects of vaccinal titers on conception rate it would seem desirable to vaccinate calves at six or seven months of age. Vaccination at these ages would allow about a year for the blood titers to return to negative before the animals reached breeding age. However, as previously stated, calves (in this study) vaccinated before nine months of age had a slightly lower degree of immunization as indicated by subsequent abortion rate than calves vaccinated from 9 to 14 months of age.

The Effect of Blood Titer Level on Abortion Rate.

During the conduct of the Brucellosis control program many abortions were directly attributed to Brucellosis infection because Brucella abortus organisms were isolated from the animal or aborted material. However, this was not done in all cases. Therefore, the data presented in Table 7 were tabulated to ascertain whether Brucellosis could be further incriminated by determining the effect of blood agglutination titers on the abortion rates. No real differences existed in abortion rates among the groups showing blood titer levels ranging from no titer to titers of 1:100. Although the abortion rates were higher in the groups with blood titer of 1:200 or more, the differences in rates among these groups were not significant. However, the difference in average abortion rates of groups that had blood titers of 1:100 or below, and groups that had blood titers of 1:200 or above, was highly

Table 7. Effect of blood titer level on the number of abortions.

Blood titer level	Number of pregnancies	Number of abortions	Percent pregnancies terminating in abortions
No titer	529	24	5
1:50	162	10	6
1:100	100	5	5
1:200	30	9	30
1:400	12	2	18
Over 1:400	31	6	19
Totals	864	56	6

Table 8. Effect of blood titer level on the number of retained placentae.

Blood titer level	Number of pregnancies	Number of retained placentae	Percent retained placentae
No titer	529	68	13
1:50	162	10	6
1:100	100	8	8
1:200	30	8	27
1:400	12	0	0
Over 1:400	31	4	13
Total and average	864	98	11

significant ($P < .01$). It was found that 88 percent of all the "breaks" that occurred in all the breeds represented blood titers of 1:100 or below. The fact that a low percentage of these animals aborted might indicate that "breaks" representing blood titers of 1:100 or below may not generally represent infection.

These findings are of particularly interest in view of the new interpretation of blood agglutination titers which has recently been announced for nation-wide control programs. This new interpretation is based on results of a study by Goode, et al. (26). These workers found a direct relationship between the blood titer level of cows and the presence of Brucella organisms in their milk. They interpreted this to mean that the absence of Brucella organisms indicate a lack of infection. They found that the most precipitous rise in the percentage of animals that had Brucella organisms in milk began with the 1:100 titer-group among non-vaccinated animals, and the 1:200 titer group among vaccinated animals. They concluded that the blood agglutination titer in vaccinated animals can be one dilution higher than the maximum allowed for non-vaccinated animals before infection is indicated.

Even though the data presented in Table 7 represent only vaccinated animals, the results of this study are in complete accord with the new interpretation. The abortion rate in animals with blood titer levels of 1:100 or below was considerably smaller than the rate in animals with blood titers of 1:200 or above.

Although all the abortions which occurred in the College herd were not positively determined to be due to Brucellosis infection, the much higher rate of abortion that occurred in animals with high blood titers would indicate that Brucellosis infection is probably responsible for most of the abortions in these animals.

The Effect of Blood Titer Level on the Number of Retained Placentae.

Data are presented in Table 8 to show the effect of the blood titer level on the incidence of retained placentae. Except for the small group which had blood titer levels of 1:200, the percent of retained placentae in the no-titer group was as high as that for any other group. The differences in the percentages of retained placentae among blood titer level groups were not significant ($P > .05$). This would tend to indicate that other factors in the herd might be as important in causing retained placentae as Brucellosis infections.

Breed Differences in Susceptibility to Brucellosis.

Data are presented in Table 9 which show the number of "breaks" and abortions which occurred within each of the breed. The differences among breeds in the number of animals that showed "breaks" and also in the abortion rates are not significant ($P > .05$).

SUMMARY AND CONCLUSIONS

Records were available pertaining to a Brucellosis control program in the Kansas State College dairy herd during a 16 year period, beginning in 1938. Records for individuals included all tests, vaccinations, breeding records

Table 9. Number of "breaks" and abortions occurring in four breeds.

Breed	Number of:		Animals with "breaks"		Pregnancies terminating in abortions	
	Animals	Pregnancies	Number	Percent	Number	Percent
Jersey	86	215	18	21	19	8
Holstein	91	237	19	21	14	6
Ayrshire	61	150	10	16	9	6
Guernsey	41	91	8	20	3	3
Total Av.	279	693	55	19.7	45	6.5

and other associative facts pertinent to the problem.

The results obtained in this herd are of special interest because the same general management and herd health policies have prevailed throughout the period and because the herd typifies a specific condition in that few cattle have been brought into the herd, and some known infection has existed in the herd during the entire period. Results of a systematic control program in a known infected herd seemed worthy of study. Under these conditions the following conclusions might be drawn from the analysis of these data:

Vaccinating animals with dead or live cultures of _Brucella abortus_, strain 19, vaccine does not increase the degree of immunization brought about by a later vaccination with live organisms. Further the number of animals that fail to return to negative after two vaccinations is significantly higher than the number that fail to return to negative after a single vaccination. Vaccinating animals twice would be a disadvantage to dairymen who are trying to develop a negative herd.

In order to produce the greatest degree of immunization, animals should be vaccinated after reaching nine months of age. However, the vaccination of animals after that age results in a lowered conception rate. Apparently the association of high blood titer and time of breeding is the cause of lowered breeding efficiency.

Due to the Grade A milk marketing requirements it would be disadvantageous for dairymen who are producing Grade A milk to adult vaccinate animals because such a high percentage of animals would fail to return to negative after vaccination.

A dairyman who calfhood vaccinates his animals and retains known infected animals in his herd, can expect about 20 percent of his vaccinated animals to again show a positive blood agglutination titer even though these animals showed a negative titer for at least a year following vaccination. However, only about ten percent of the animals that show such a positive titer will have any agglutination at dilutions greater than 1:100. Since the percentage of vaccinated animals that abort while they show blood titer levels of 1:100 or below is small, it is possible that such "breaks" may not represent a Brucellosis infection. However, a small percentage of vaccinated animals may become infected, as indicated by blood titers of 1:200 or higher, and a relatively high percentage of such animals will abort.

The number of retained placentae which occur in a vaccinated herd does not seem to be materially affected by Brucellosis infection. As many retained placentae can be expected to occur in animals which show positive blood titers.

The percentage of vaccinated animals which will subsequently show "breaks" and abortions is not affected by the breed of those animals.

ACKNOWLEDGMENT

The writer wishes to express his appreciation to Dr. G. B. Marion and Prof. F. W. Atkeson of the Dairy Husbandry Department, Kansas State College, for their advice in planning and carrying out this study, and for their suggestions and criticisms in preparing this thesis; and to Dr. Keith Huston for his assistance with the manuscript.

He wishes to acknowledge the aid of Dr. H. C. Fryer of the Statistical Laboratory, Kansas State College, in designing and statistically interpreting this study.

BIBLIOGRAPHY

(1) Agricultural Research Service.
All out on brucellosis. Guernsey Breeders Jour. 93(5) 516 p. March 1, 1955.

(2) Agricultural Research Service.
The expanded bovine brucellosis eradication program. A progress report. U. S. Dept. Agr. p. 1-5. May, 1955.

(3) Bang, B.
Infectious abortions in cattle. Jour. Compar. Path. and Ther. 19 (191)-202. Sept. 1906.

(4) Barner, R. D., F. H. Oberst and F. W. Atkeson.
The use of dead strain 19 vaccine to differentiate vaccinal from infection titers of brucellosis (Abortus) in cows. Jour. Am. Vet. Med. Ass'n. 122(913):302-304. April, 1953.

(5) Berman, D. T., and B. A. Beach.
Studies on repeated vaccination of cattle with brucella abortus strain 19. I. The agglutinin response of animals vaccinated as calves and revaccinated as young adults. Am. Jour. Vet. Res. 10 (36). July, 1949.

(6) Berman, D. T., B. A. Beach and M. R. Irwin.
Studies on repeated vaccination of cattle with brucella abortus strain 19. III. The response of vaccinated and revaccinated cattle to conjunctural exposure with a virulent strain of brucella abortus during the third gestation period. Am. Jour. Vet. Res. 13 (48):351-358. July, 1952.

(7) Berman, David T., and M. R. Irwin.
The immunogenicity for cattle of brucella abortus strain 19 and M vaccine (Huddleson). Jour. Am. Vet. Med. Ass'n. 125 (932):401-405. Nov. 1954.

(8) Birch, R. R., H. L. Gilman and W. S. Stone.
The pathogenicity of brucella abortus strain 19 for sexually mature cows. The Cornell Veterinarian. 33:198-203. 1943.

(9) Birch, R. R., H. L. Gilman and W. S. Stone.
The immunity created by vaccination of calves with brucella abortus. The Cornell Veterinarian. 35:110-122. April, 1945.

(10) Blake, Grant E., C. A. Manthei and E. R. Goode, Jr.
A milk plate test for the detection of brucellosis. Jour. Am. Vet. Med. Ass'n. 120(898):1-6. Jan. 1952.

(11) Bosgra, D.
Brucella abortus strain 19 vaccine dried in a frozen condition. (Abstract) Jour. Am. Vet. Med. Ass'n. 120(900):160. March 1952. (Original article not available).

(12) Bradt, C. G.
Brucellosis is being wiped out. Jour. of Dairy Sci. 37(9): 1141-1144. Sept. 1954.

(13) Bryan, H. S., and M. E. Mansfield.
Studies on bovine brucellosis immunization: Controlled experiments with B. A. I. Strain 19 and Huddleson's mucoid vaccines. Proc. 57th ann. meeting U.S. Livestock Sanit. Ass'n. pp 124-134. 1953.

(14) Buck, J. M.
Studies of vaccination during calfhood to prevent bovine infectious abortion. Jour. Agr. Res. 41:667-689. 1930.

(15) Buddle, M. B.
Immunity in cattle vaccinated with brucella abortus strain 19. By the subcutaneous and intracaudal routes during calfhood. The Australian Vet. Jour. 24(10):262-271, Oct. 1948.

(16) Campbell, A. D. and A. W. Rodwell.
The relationship of dosage and site of inoculation to the agglutinin response to brucella abortus strain 19 vaccine: A comparison of the subcutaneous, intracutaneous and intracaudal routes. Jour. Comp. Path. and Ther., 55(4)277-289. Oct. 1945.

(17) Cotton, C. M.
An intensive study of post-vaccination responses in groups of calves vaccinated intracutaneously and subcutaneously with strain 19 brucella abortus vaccine. Am. Jour. Vet. Res., 14 (52):337-342. July 1953.

(18) Cotton, W. E., J. M. Buck and H. E. Smith.
Efficiency and safety of abortion vaccine prepared from brucella abortus strains of different degrees of virulence. Jour. Agr. Res. (1933) Xlvi., 46:291-314.

(19) Cotton, W. E. and J. M. Buck.
The status of vaccination against Bang's disease. Jour. Am. Vet. Med. Ass'n. 84:329-340. 1934.

(20) Danks, A. G.
Brucella abortus strain 19 and orchetis in a bull. Cornell Veterinarian. 33:381-382. 1943.

(21) Deem, A. W. and Floyd Cross.
Abortion following the vaccination of pregnant cows with strain 19. Jour. Am. Vet. Med. Ass'n. 106:213-214. 1945.

(22) Driver, Fred C. and Martin H. Roepke.
Results of county-wide blood tests following two to four county-wide ring tests for brucellosis. Proc. Book Am. Vet. Med. Ass'n. 88th ann. meeting, p. 148-152. Aug. 1951.

(23) Edington, B. H., N. B. King and Norman Frank.
Comparative tests of the protective value of Huddleson's mucoid and strain 19 vaccine in cattle. II. Am. Jour. Vet. Res., 13 (49):441-445. Oct. 1952.

(24) Edington, B. H. and N. B. King.
A summary of results obtained in Ohio in comparative tests of Huddleson's brucella mucoid and strain 19 vaccines. Proc. 56th ann. meeting U. S. Livestock Sanit. Ass'n. 126-130 pp. 1952.

(25) Fedyushin, V. P. and L. A. Alexeyeva.
The ring reaction for the diagnosis of brucellosis in milk cows. (Abstract). Jour. Am. Vet. Med. Ass'n. 903:404, June 1952. (Original article not available).

(26) Goode, E. R., T. E. Amerault and C. A. Manthei.
Relationship of sero-agglutinin titers to udder infection in strain 19 vaccinated cattle. Agr. Res. Service. p. 1-15.

(27) Gregory, T. S.
A standardized rapid agglutination test for the quantitative estimation of brucella antibody in milk. Jour. Comp. Path. and Ther. 63 (3):171-178. July 1953.

(28) Hagan, W. A., and D. W. Bruner.
The infectious diseases of domestic animals. Sec. edition. New York: Comstock Pub. Co. 1951.

(29) Haring, C. M. and J. Traum.
Observations of pathogenic and antigenic effects of brucella abortus, U. S. Bureau of Animal Industry strain 19. Jour. Agr. Res. 55:117-128. 1937.

(30) Haring, C. M.
Vaccination against Bang's disease in an infected dairy herd with U. S. Bureau of Animal Industry brucella abortus strain 19. Jour. Am. Vet. Med. Ass'n. 92:52-60. 1938.

(31) Haring, C. M.
Results of vaccination with brucella abortus strain 19 in an infected herd. Jour. Am. Vet. Med. Ass'n. 94 (6):537-589. June 1939.

(32) Haring, C. M. and Jacob Traum.
The effect of brucella abortus strain 19 on cattle of various ages and its bearing on adult cattle vaccination. Proc. 47th ann. meeting U. S. Livestock Sanit. Ass'n. 47:42-46. 1943.

(33) Hendrikse, J., K. F. Joling and G. B. R. Willems.
On the fertilization results of artificial insemination in cattle and infection with brucella bang. (Abstract). Jour. Am. Vet. Med. Ass'n. 125:163. Aug. 1954. (Original article not available).

(34) Holman, H. H. and A. McDiarmid.
An observation on reduction in milk yield following vaccination of lactating cows with living vaccines prepared from brucella abortus. The Vet. Record. 57(28). July 14, 1945.

(35) Huddleson, Forest.
The mucoid phases of the genus brucella. Am. Jour. Vet. Res. 7(22):5-10. Jan. 1946.

(36) King, N. B., W. G. Venzke and B. H. Edington.
Studies on the experimental treatment of bovine brucellosis. Ohio Agr. Expt. Sta., Columbus. Am. Jour. Vet. Res. 13, 47:152-157. Apr. 1952. (Jour. Dairy Sci., 35,A63, Sept.1952).

(37) Live, I. and A. G. Danks.
The use of ether-killed brucella abortus in salive-in-oil emulsion in a herd of cattle with brucellosis. Am. Jour. Vet. Res. 12, 44:175-182. July, 1951.

(38) McDiarmid, A.
A comparison of the immunizing value in cattle of dead antigens and strain 19 brucella abortus vaccine. Vet. Record, 61(22); 305-308. May 28, 1949.

(39) McDiarmid, A.
Comparison of the immunity produced in cattle by the inoculation of brucella abortus strain 19 intradermally, intracaudally and subcutaneously. The Vet. Record. 62(25):361-364. June 24, 1950.

(40) McDiarmid, A.
The vaccination of pregnant cattle with strain 19 brucella abortus vaccine during an outbreak of brucellosis in a dairy herd. Vet. Record, 63:15:265-268. April 1951.

(41) Manthei, C. A., C. K. Mingle, and R. W. Carter.
Duration of immunity to brucellosis induced in cattle with strain 19 vaccine. Proc. Book Am. Vet. Med. Ass'n. 88th ann. meeting, p. 128-141. August 1951.

(42) Manthei, C. A.
Research on brucellosis of animals. Vet. Med. 47:179-180. May 1952.

(43) Manthei, C. A.
Evaluation of vaccinal methods and doses of brucella abortus strain 19. Proc. 56th ann. meeting U. S. Livestock Sanit. Ass'n. p. 115-125. 1952.

(44) Manthei, C. A., C. K. Mingle and R. W. Carter.
Comparison of immunity and agglutin response in cattle vaccinated with brucella abortus strain 19 by the introdermal and subcutaneous methods. Proc. 56th ann. meeting U. S. Livestock Sanit. Ass'n. p. 110-114. 1952.

(45) Moore, Thomas and Chas. A. Mitchell.
Vaccination of sexually mature cows with brucella abortus strain 19. I. Negative cows contained in negative dairy herds. Canadian Jour. Comp. Med. 12(10):278-282. Oct. 1948.

(46) Moore, Thomas and Chas. A. Mitchell.
Vaccination of sexually mature cows with brucella abortus strain 19 vaccine. Canadian Jour. Comp. Med. and Vet. Sci. 14(6):209-213. June 1950.

(47) Morse, E. V., E. P. Pope and C. A. Grota.
A comparison of the ring and capillary tube tests for bovine brucellosis in a county-wide area test. Jour. Am. Vet. Med. Ass'n. 120(901):209-212. April 1952.

(48) Morse, E. V., Edith Smith, R. N. Schmidt and Eleanor Schmidt.
A comparison of the ABR, capillary tube, milk whey and blood serum agglutination tests. Vet. Med. 47 (2):48-51. Feb. 1952.

(49) Morse, E. V., G. W. Robertstad, B. A. Beach and M. Ristic.
A bacteriological study of ten persistent reactor cows following vaccination with brucella abortus strain 19. Jour. Am. Vet. Med. Ass'n. 125(930):212-214. Sept. 1954.

(50) Rabstein, Melvin M. and Cornelia Cotton.
Bang's disease immunity tests and a new vaccination method. Proc. 46th ann. meeting U. S. Livestock Sanit. Ass'n. p.129-136. 1942.

(51) Roepke, Martin H., Lawrence B. Clausen and Andrew L. Walsh.
The milk and cream ring test for brucellosis. Proc. 52nd ann. meeting U. S. Livestock Sanit. Ass'n. p. 147-159. 1948.

(52) Roepke, Martin H., Katherine G. Paterson, Fred C. Driver, L. B. Clausen, Louis Olson and J. E. Wentworth.
The brucella abortus ring test. Am. Jour. Vet. Res. 11 (39):199-205. April 1950.

(53) Schoenaers, F.
Immunization of cows against brucellosis with Huddleson's mucoid vaccine. (Abstract). The Vet. Bul. 24 (4):169. 1954. (Original article not available).

(54) Sieiro, F. and C. Rosenbusch.
Comparative immunity experiments in cattle using live vaccine prepared from agglutinogenic and nonagglutinogenic brucella abortus strains. Am. Jour. Vet. Res. 13(49):476-485. Oct. 1952.

(55) Sieiro, F.
Immunity to brucellosis produced in cattle by strain 19 vaccine by the intradermal and subcutaneous routes. The Vet. Bul. (Abstract). 24(3):113. March 1954. (Original article not available).

(56) Sieiro, F. and C. Rosenbusch.
New studies on comparative brucella immunity with agglutinogenic and nonagglutinogenic vaccines. Am. Jour. Vet. Res. 15, 56:417. 1954.

(57) Smith, R. W., R. R. Birch, C. P. Bishop, C. R. Donham and R. L. West.
What is known about brucellosis. Proc. 52nd ann. meeting U. S. Livestock Sanit. Ass'n. p. 160-180. 1948.

(58) Washko, F. V. and L. M. Hutchings.
Susceptibility of cattle to brucella suis following vaccination with brucella abortus strain 19. Am. Jour. Vet. Res. 13(46):24-25. Jan. 1952.

(59) Washko, F. V., C. R. Donham, L. M. Hutchings and Ann Heimlich.
Recovery of brucella from tissues of cattle exposed to brucella abortus. Jour. Am. Vet. Med. Ass'n. 120(899):82-84. Feb. 1952.

(60) Washko, F.V., C. R. Donham and Ann Heimlich.
Response of cattle to brucella following sexual stimulation and brucella exposure during early calfhood. Am. Jour. Vet. Res. 13 (49):437-440. Oct. 1952.

THE EFFECTIVENESS OF A VACCINATION PROGRAM AS AN AID
IN CONTROLLING BRUCELLOSIS IN A PARTIALLY INFECTED DAIRY HERD

by

JAMES SPENCER NORWOOD

B. S., Texas Technological College, 1954

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Dairy Husbandry

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1955

Brucellosis, otherwise known as Bang's disease or contagious abortion in cattle, is an infectious disease of cattle and man caused by the specific microorganism Brucella abortus.

Records were available pertaining to a Brucellosis control program in the Kansas State College dairy herd during a 16 year period, beginning in 1938. Records for individual animals included all tests, vaccinations, breeding records, and other associative facts pertinent to the problem.

The results obtained in this herd are of special interest because the same general management and herd health policies have prevailed throughout the period, and because the herd typifies a specific condition in that few cattle have been brought into the herd, and some known infection has existed in the herd during the entire period. The efficacy of vaccination in increasing the degree of immunity to Brucellosis was measured by the number of "breaks" which occurred following vaccination. A "break", as used in this study, is defined as the occurrence of a positive blood titer in an animal that had shown a negative blood titer for at least one year following vaccination. An abortion is defined as an expulsion of a dead fetus before the 8th month of pregnancy.

In this study, calfhood vaccination was considered to be the vaccination of animals 14 months of age or younger. Cattle older than 14 months at the time of vaccination were considered to be adult vaccinates.

Three different procedures using organisms of Brucella abortus, strain 19, were used to vaccinate three groups of calves. One group was vaccinated with killed organisms followed by a revaccination with live organisms. Another group was vaccinated only once with live organisms while another group was vaccinated twice with live organisms. Of these groups, the blood of 64 percent, 86 percent, and 66 percent, respectively, had returned to negative within one year. The blood of 11 percent, one percent, and 14 percent, respectively, had not returned to negative within three years. The blood of the remaining animals returned to negative from one to three years after vaccination. It was found that there was a significantly higher percentage of the animals returning to negative within one year after a single vaccination of live organisms as compared to animals returning to negative within one year in either of the other two groups ($.05 > P > .01$). It was also found the much lower percentage of animals vaccinated once with live organisms that failed to return to negative within 3 years as compared to the other two groups was highly significant ($P < .01$).

No significant difference ($P > .05$) in the number of either "breaks", or abortions, was found between the different procedures (killed and live organisms) used.

A greater resistance to Brucellosis produced by vaccination was found among animals which were vaccinated at the age of 9 to 14 months than among those animals vaccinated from four to eight months of age as determined by the number of abortions.

No significant difference was found between animals vaccinated once subcutaneously, and animals vaccinated intradermally.

The blood of 55 percent of the animals vaccinated as adults had not returned to negative within one year.

No significant difference ($P > .05$) in the services per conception was found among the groups that showed a blood titer of 1:50, or higher. However, fewer services per conception were required for animals having a negative blood titer at the time of service as compared to animals having a blood titer of 1:50 or higher. The difference was significant ($P < .02$).

The differences in the percentages of retained placentae among blood titer level groups were not significant. ($P > .05$).

Animals with blood titers of 1:100 or less had a five percent abortion rate as compared with a 23 percent abortion rate for animals having blood titers of 1:200 or above. This difference was highly significant. ($P < .01$).

It was found that 88 percent of all the "breaks" that occurred in all the breeds represented blood titers of 1:100 or below. The fact that a low percentage of these animals aborted might indicate that "breaks" representing blood titers of 1:100 or below may not generally represent infection.

The differences among breeds in the number of animals that showed "breaks" and also in the abortion rates are not significant. ($P > .05$).